ON THE ROLE OF NUTRITION IN NORMAL HUMAN PREGNANCY

STELLINGEN

1. De veranderingen die zich tijdens een normale zwangerschap voordoen in een aantal parameters van de vitaminestatus dienen vooral te worden beschouwd als een aanpassing van het maternale metabolisme aan de zwangerschap.
   Dit proefschrift

2. De door Hall gepostuleerde causale samenhang tussen de daling van het serum foliumzuurgehalte en de toename in het circulerend plasmavolume tijdens de zwangerschap wordt noch door haar eigen, noch door onze resultaten bevestigd.
   Hall et al., Brit J Obstet Gynaecol 83, 132 (1976)
   Dit proefschrift

3. De nadruk op de hogere aanbevelingen van energie en nutriënten in de laatste maanden van de zwangerschap door veel voedingsraden is slechts ten dele gebaseerd op de fysiologie van de normale zwangerschap.
   Dit proefschrift

4. Om wille van de inzichtelijkheid verdient het de voorkeur bij enzymstimuleringstesten als maat voor de vitaminestatus het percentage verzadiging van het aanwezige apoenzym te vermelden, in plaats van de nu gebruikelijke stimuleringsratio.

5. Ten onrechte wordt bij het vermelden van de resultaten van enzymstimuleringstesten vaak de basale of gestimuleerde enzymaktiviteit niet opgegeven.

6. Matrixeffecten en onvolledige solubilisatie van de ligand in het assaysysteem worden vaak onvoldoende onderkend als potentiële foutenbronnen bij ligand-bindingsassays.

7. De UCV-tabel dient te worden uitgebreid met gegevens over het foliumzuurgehalte van voedingsmiddelen.

8. Zowel het door de Commissie Vitaminering van Levensmiddelen van de Voedingsraad uitgebrachte deeladvies inzake de toevoeging van vitamines aan voedingsmiddelen, alsmede het

advies inzake multi-vitamine preparaten, is meer gebaseerd op veronderstellingen dan op relevante onderzoeksgegevens.

9. Op basis van tot nu toe gerapporteerde klinische en methodologische studies kan vooralsnog geen voorkeur worden uitgesproken ten aanzien van het te gebruiken type binder bij de radioassay van vitamine B_{12}.
Kolhouse et al., New Engl J Med 299, 785 (1978)

10. In veel rapporten en publicaties waarin het β-caroteengehalte staat vermeld wordt het totaal caroteengehalte bedoeld.

11. Het gebruik van maaltijden in cafetaria's of kantines van voedingsinstituten is op zich geen garantie voor een prudente voeding.

12. In het nieuwe functiewaarderingssysteem (FW-18) van TNO dient het doen van toegepast onderzoek beter te worden gewaardeerd.

13. Het werk van een laboratorium verruimt de klinische blik van de geneesheer, waarvan echter de werkgelegenheid op dat laboratorium weer afhankelijk is.

14. Blootstelling aan traangas ontneemt burgers de mogelijkheid de situatie nog langer helder te beschouwen en is alleen al daarom ongeschikt als middel bij bestrijding van rellen.

15. Door haar steun te onthouden aan de zogenaamde Freeze-motie heeft het CDA de vredesbeweging aardig in de kou laten staan.

16. De kennis van het vaarreglement lijkt bij veel gemotoriseerde watersportliefhebbers omgekeerd evenredig met het motorvermogen van hun vaartuig.

Utrecht, 19 april 1983

Stellingen behorend bij het proefschrift "On the role of nutrition during normal human pregnancy".

H.van den Berg

STELLINGEN

1. Bij gezonde zwangeren hebben noch de maternale gewichtstoename, noch de geboortegewichtspercentiel van het kind een aantoonbaar verband met de energie-opname of de opname van macronutriënten uit de voeding.
   Dit proefschrift

2. Een normale zwangerschap vermindert de maternale ijzer- en foliumzuurvoorraad; terugkeer tot de uitgangswaarden duurt vele maanden.
   Dit proefschrift

3. Tijdens de zwangerschap hebben vrouwen die roken geen duidelijk ander voedselconsumptiepatroon dan vrouwen die niet roken.
   Dit proefschrift

4. Bij de behandeling van een sepsis in de neonatale periode dient het toedienen van vers plasma een even belangrijke plaats in te nemen als de behandeling met antibiotica.

5. Als weeën schaden.....kan weeënremming dat ook. Het overtuigend bewijs dat door weeënremming de baring zinvol kan worden uitgesteld is nog niet geleverd.
   Ch.H.Hendricks. In Preterm Labor. Ed. by M.G.Elder and Ch.H.Hendricks. London Butterworth 1981

6. Onderzoek naar de maternale ijzervoorraad tijdens de zwangerschap door middel van een beenmergpunctie is zinloos.
   B.Svenberg. Acta Obstet Gynecol Scand, Suppl 48: 1975

7. Een landelijke screening van het maternale serumgehalte op alpha-foeto-proteïne voor opsporing van foetale neurale buisdefecten is om economische maar ook volksgezondheids redenen niet gewenst. Gericht onderzoek bij de risicopopulatie in gespecialiseerde centra verdient de voorkeur.

8. Een oorzaak van habituele abortus kan gelegen zijn in een te gering genetisch verschil tussen de betreffende vrouw en haar

partner. Vermenging van rassen heeft dus mogelijk nog meer voordelen.

9. Bij het onderzoek naar het effect van prostaglandineremmers op de hoeveelheid menstrueel bloedverlies wordt een opmerkelijke voorkeur getoond voor de duurdere middelen.

10. De in-vitro fertilisatie zal op de kindersterfte in de derde wereld waarschijnlijk geen merkbare invloed hebben.

11. Het alom aanwezige antenatale verlangen naar een lichamelijk en geestelijk gezond kind steekt schril af tegen de wijze waarop postnataal ouders hun kinderen deze verworvenheden soms trachten te ontnemen.

12. De vrouwenborst is een middelpunt in de driehoek moeder, vader, zuigeling. Daarbinnen kunnen erotiek en borstvoeding elkaar zowel positief als negatief beïnvloeden.

13. Oogletsels bij squash kunnen worden vermeden door de bal voortdurend in het oog te houden.

14. Een zwangerschap dient zo mogelijk te eindigen in dezelfde sfeer en op dezelfde plaats als waar deze begon; sommige gynaecologen overwegen dan ook de coïtus onder continue bewaking en epiduraal analgesie in het ziekenhuis te laten uitvoeren.

15. Medisch handelen wordt soms een DES-illusie.

Utrecht, 19 april 1983

Stellingen behorend bij het proefschrift "On the role of nutrition during normal human pregnancy".

H.W.Bruinse

ON THE ROLE OF NUTRITION IN NORMAL HUMAN PREGNANCY

Effects of maternal nutrition, physiological and environmental influences on changes in vitamin status parameters during and after pregnancy

Proefschrift

ter verkrijging van de graad van doctor in de Geneeskunde
aan de Rijksuniversiteit te Utrecht, op gezag van de
Rector Magnificus Prof. Dr. O. J. de Jong,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 19 april 1983
des namiddags te 3.15 door

Hendrik van den Berg

geboren op 28 juni 1947 te Ede (Gld)

Promotores:

Prof. Dr. A. A. Haspels
Prof. Dr. J. F. de Wijn (Rijksuniversiteit Leiden)

ON THE ROLE OF NUTRITION IN NORMAL HUMAN PREGNANCY

The influence of maternal nutrition and biochemical parameters representing maternal health on birthweight

Proefschrift

ter verkrijging van de graad van doctor in de Geneeskunde
aan de Rijksuniversiteit te Utrecht, op gezag van de
Rector Magnificus Prof. Dr. O. J. de Jong,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 19 april 1983
des namiddags te 4.15 door

Hendrik Willem Bruinse

geboren op 19 april 1946 te Gorinchem

Promotores:

Prof. Dr. A. A. Haspels
Prof. Dr. J. F. de Wijn (Rijksuniversiteit Leiden)

Aan mijn ouders
voor Heleen,
Corine, Leontine, Wynke

Ter nagedachtenis aan mijn vader
aan mijn moeder
aan Vesi, Sandra en Kenya

VOORWOORD

Allen die door hun medewerking, kritiek of belangstelling hebben bijgedragen aan het onderzoek en het tot stand komen van dit proefschrift zijn wij dank verschuldigd, met name:

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CONTENTS

Introduction and justification of the study.

Pregnancy is characterized by many changes, both visible and invisible. Very early in pregnancy, a new "milieu interieur" develops. The purpose and significance of many of these changes are still mysterious. Such changes in pregnancy have too often been considered pathological if they were found to be outside the range of values found in healthy non-pregnant women. Changes during and after pregnancy - the latter a more or less forgotten field - should be studied first in healthy women who have had an uneventful pregnancy producing a normal healthy baby before conclusions about observations in pathological pregnancies can be made (WHO, 1965).

It can be assumed that nutrition influences the nutrient levels in the body, course and outcome of pregnancy. In industrialized countries, such as the Netherlands, it is unlikely that energy and protein intake before, during and after pregnancy are inadequate. Less is known about micronutrients such as vitamins and iron.

Nutritional habits change over time. For example, in Holland the consumption of various food groups has changed over the last two decades (de Wijn, 1976). Other habits, such as cigarette smoking, are changing as well. The last extensive study in Holland about nutrition and pregnancy was performed in the 1950's in Amsterdam (Van der Rijst, 1962).

A literature review reveals that in a high percentage of pregnant women, vitamin and iron levels in body fluids are lower than the range observed in non-pregnant women. The findings of our own study in Rotterdam were in line with the above (Van den Berg et al., 1978). There is no consensus about the interpretation of these observations. Are they pathological? What is the role of nutrition in the establishment of a "milieu interieur".

A large number of books and other publications have been written in this century about nutritional aspects related to pregnancy. Up to the end of the 1960's, attention was generally focused on pathological conditions of pregnancy. More recently,

the main interest concerns the relationship between maternal nutrition, course of pregnancy and intrauterine fetal growth. Todate our knowledge of the role of nutrition on human reproduction and visa versa is still scarce.

The maternal readjustment from the pregnant to the non-pregnant state is an almost forgotten area of study. Few studies about vitamins, iron and pregnancy explore the postpartum period, and if they do, it is for only 6 days and at most up to 6 weeks postpartum. Knowledge of the normalization of the vitamin and iron status after pregnancy might be helpful in estimating the "vitamin and iron" cost of pregnancy and in discriminating between a physiological or a pathological explanation of the hypovitaminemia and low iron levels during pregnancy.

As to birthweight, it seems proven that an extreme insufficiency of macro- and micronutrients reduces birthweight (Smith, 1947; Antonov, 1947). The situation in which this occurs is almost always an involuntary one. Only in the beginning of this century was a restricted diet and fluid intake prescribed to women with a contracted pelvis to reduce the growth of the child on medical grounds (Prochownick, 1901). The influence of energy intake on fetal growth is still unclear in the "normal" situation. Whether birthweight can be increased by supplementation of energy, proteins and/or micronutrients to the habital diet is still debated, even in countries where the nutritional situation is marginal.

Considering these facts and mutual interests, a prospective study was undertaken in 1978 by the Department of Clinical Biochemistry of the CIVO/TNO Institute (Dr.W.H.P.Schreurs) and the Department of Obstetrics and Gynecology of the University of Utrecht (Prof.Dr.A.A.Haspels).

Objectives of the study were:

- To describe the course of vitamin and iron status parameters during normal pregnancy and in the period up to 6 months after delivery.
- To investigate whether changes in vitamin levels during normal pregnancy may be explained by physiological adjustments of pregnancy.
- To investigate whether nutrition during pregnancy meets recommended daily allowances.
- To investigate whether nutrition during pregnancy and biochemical parameters, representing nutritional health during pregnancy, are related to the birthweight-centile of the newborn.

A few practical remarks for people who want to continue reading. Measuring a large number of parameters results in a large number of figures and tables. To keep this thesis more or less readable, most figures and tables are collected in a seperate "Figure and Table" appendix. However, sometimes figures and tables are used in the text. To avoid confusion, figures and tables in the appendix have been numbered with arabic numerals and figures and tables in the text with roman numerals. For example, Figure 5.4.2 will be found in the appendix and Figure 5.4.II is found in section 4 of chapter 5. The appendix has been divided into the same chapters and sections as the book.

CHAPTER 1.

Review of the literature on nutrition during pregnancy, the relationship between nutrition and course and outcome of pregnancy and changes in biochemical parameters representing the nutritional status.

1.1. Physiological, metabolic and hormonal changes during pregnancy and intrauterine fetal growth.

-Introduction.

Section 1.1. should not be considered an up-to-date and in-depth literature review of the topics described in it. It covers such a wide and complex area that books can be and have been written on almost all subjects mentioned. We had to remain rather superficial in this part because of the aforementioned reason and because we are not pretending to know more than we do. One may ask: why write it then? The answer is that nutrition and biochemical changes representing the nutritional status should be judged against a background of physiological and hormonal changes during pregnancy, and this section is meant for those who are not familiar with these changes.

Also, we have not strived for completeness in this section. Hormones, are discussed only when measured in this study or when, in our opinion, they are important in relation to metabolic changes.

1.1.1. Physiological and metabolic changes in pregnancy.

The healthy, pregnant woman resembles, from a medical point of view, in many aspects an ill, non-pregnant woman or as Thomson (1973) expresses it: "She gains weight extremely rapidly, ten-twelve kilo's on average in a few months. She may be sluggish and often complains of digestive upsets. Body temperature is slightly raised and pulse, respiration and basal metabolic rates are also increased, often in association with some enlargement of the thyroid gland. Hematological changes suggest anemia. The erythrocyte sedimentation rate is characteristic of a chronic infection and the reduced level of serum albumin, often accompanied by edema, suggests severe malnutrition. The blood osmolality falls to a level that should provoke diabetes insipidus. Glucosuria is frequent and there is evidence of aminoaciduria. Yet, the end of all this, often more than not, is the production of a perfectly healthy baby by a contented, healthy mother".

A number of changes that accompany pregnancy will be discussed. For an extensive, basal description, the reader is referred to Hytten and Leitch's standard work, "The Physiology of Pregnancy" (1971).

- Weight gain in pregnancy

Average weight gain in a normal first pregnancy of Western women aged 24 years, height 163 cm. and prepregnant weight 56 kg., is estimated to be about 12.5 kg. (Hytten and Leitch, 1971; Jacobson, 1975). This weight gain is thought to be composed of the following elements:

fetus 3400 g.
placenta 550 g.
amniotic fluid 800 g.
weight gain, uterus 970 g.
weight gain, breasts 400 g.
increased blood volume 1250 g.
increased extracellular-extravascular water 1600 g.
fat deposition 3500 g.

It is not possible to measure the increase in extracellular - extravascular water. The amount that is given is derived from measurements of total body water that increases by 7.6 l during pregnancy (Seitchik, 1967). Six liters of water are necessary for the other mentioned elements, which leaves 1.6 liters in the extracellular-extravascular space, probably in the ground substance of the connective tissue. This ground substance can accumulate water (Gersh and Catchpole, 1960), and when this happens, it swells, becoming softer and more elastic. The rest of this weight gain consists mainly of fat tissue. McCartney et al. (1959) supposed this, and Taggart et al. (1967) confirmed it by serial skinfold measurements.

The exact amount of weight gain considered desirable during a pregnancy is an individual matter and not a figure that can be stated for all women. The optimal weight gain of women with a normal weight is 10 kg, with a prepregnant weight less than 90%, 13.5 kg and 6.7 kg for those with a prepregnant weight of more than 135% (Naeye, 1979).

- Blood volume.

Plasma volume starts increasing from the sixth week of pregnancy onwards. The main increase takes place in the second trimester and reaches a plateau in the third trimester. Chesley (1972), reviewing many plasma volume measurements, found a mean increase of 42% (the mean increase in different investigations differs from 21 to 66%). The decrease in plasma volume, sometimes described in the third trimester, is probably caused by the fact that measurements were not made in a lateral position, but in a flat, posterior position. The lower values of plasma volume found in the posterior position can be explained by a compression of the vena cava inferior or by an incomplete dilution of the dye (Chesley and Duffus, 1971).

There is no agreement about plasma volume increasing more (Pritchard, 1965) or less (Hytten and Paintin, 1963) in tall than in short women. Neither is there agreement about a larger (Hytten et al., 1963) or a smaller (Lund and Donovan, 1967) increase in multipara than in nullipara.

There is a clear correlation between increase in plasma volume and birthweight (Hytten and Paintin, 1963). Croall et al. (1978) found in women who repeatedly gave birth to small-for-gestational age children a reduced non-pregnant plasma volume compared to matched controls.
According to Chesley (1972), erythrocyte volume increases from 17 to 40%. This large range is probably caused by the fact that not all investigators mentioned the use or non-use of iron supplementation. Hytten and Leitch (1971) found an increase in erythrocyte volume of 200 ml without and 400 ml with routine iron administration.

Due to the fact that plasma volume increases more than red cell volume during pregnancy, a hemodilution occurs.
Positive effects of this volume expansion are:

- a decrease of the blood viscocity by about 20% (Hamilton, 1950). This change in viscocity decreases the flow resistance and, subsequently, the cardiac force required to propel the blood.
- notwithstanding the fact that hemoglobin, protein and bicarbonate decrease in concentration per ml, the total circulating amounts increase, thereby increasing the buffer capacity for hydrogen ions.
- a blood flow through uterus and placenta, both non-privileged organs, is secured (McFadyen, 1979).
- heat production, which increases during pregnancy with 15-20%, can be more easily removed by the increased blood flow in the cutis (Ginsberg and Duncan, 1967).
- blood loss during parturition can be compensated for without hemodynamical problems. This blood loss is often underestimated. Ueland (1976) found in a careful study an average blood loss of 970 ml up to the fifth postpartum day.

- Heart.

There is agreement about the increase of 30-50% in cardiac output, but there is no agreement at what time in pregnancy this rise begins (Hytten and Lind, 1973; Walters and Lim, 1975). The heart rate increases with 10-15 strokes per minute, although it

is not clear whether this starts early in pregnancy or whether it rises gradually (Hytten and Leitch, 1971; Walters, 1979). The stroke volume can be calculated from the cardiac output and the heart rate. Due to the differences in measurements of cardiac output increase and heart rate increase, it will be clear that the increase in stroke volume differs greatly. An increase of 70 ml is given by Hytten and Lind (1973), whereas Walters (1979) calculates an increase from 84 ml to 98 ml.

- Blood pressure.

The systolic blood pressure decreases slightly in the first two trimesters (5-10 mmHg) compared with non-pregnant values. Diastolic blood pressure decreases with 10-15 mmHg; lowest values are measured the 22nd-24th week of pregnancy, after which a gradual increase takes place (Schwarz, 1964; MacGillavry et al., 1969; Christianson, 1976). Blood pressure in pregnancy increases with age (MacGillavry et al., 1969), and in the same age group, primigravidae have a higher blood pressure than multigravidae (Christianson, 1976). The peripheral venous pressure in the arm does not change as opposed to the pressure in the femoral vein, which increases gradually (McLennan, 1943). Central venous pressure does not change in pregnancy (O'Driscoll and McCarthy, 1966).

- Lungs.

The extra oxygen need during pregnancy increases gradually from 5 ml./min. at 10 weeks to 32 ml./min. in the last few weeks. The respiration rate probably does not change. The tidal volume, the volume of air inspired and expired at each breath, increases progressively by 40%, expiratory reserve volume decreases by about 15% and residual volume decreases by about 20%. As the oxygen carrying capacity of the blood increases by 18%, the arterio-venous oxygen difference is somewhat lower in pregnancy. The extra oxygen consumption required during pregnancy is easily compensated for by the changes in pulmonary function and changes in increased erythrocyte volume in the healthy, pregnant woman (Hytten and Leitch, 1971).

- Digestive tract.

The most striking change in the gastro-intestinal tract is the change in tonus and motility. The stomach empties itself probably slower, and there is probably a reduction in the acidity of the gastric juice due to lowered hydrochloric acid secretion (Hytten, 1980[a]). Davison et al. (1970) found a longer, although not significantly, emptying time of the stomach in pregnancy after a test meal, but the amount remaining in the stomach after 30 min. was significantly greater than in non-pregnant controls. In the small intestine, motility is also decreased (Parry et al., 1970). Whether absorption is increased is still uncertain. There may be an increased absorption for iron (section 1.3.10), but the problem in absorption studies is that results may represent the same phenomenon in non-pregnant persons, namely that increased absorption is found when the need for a nutrient is increased. The large intestine shares the general lack of motility and every clinician is acquainted with the general complaint of constipation during pregnancy. Constipation is, however, only partly explained by the decreased motility. Another augmenting factor is the increased water reabsorption in the colon (Parry et al., 1971).

- The liver.

The liver, an organ at the end of so many important metabolic pathways, is bound to influence and to be influenced by at least some of the metabolic changes in pregnancy; little, however, is known about this (Hytten, 1980[a]). Liver function tests are normal during pregnancy. Studies on carbohydrate physiology suggest that stores of liver glycogen and its metabolisation are not effected during pregnancy (Lind, 1980).

- Kidney.

The renal plasma flow (RPF) increases during pregnancy by about 200-250 ml/min or 30-50% above non-pregnant values (Sims and Krantz, 1958). The decrease in the last three weeks of pregnancy found by these investigators is caused by the measurements being made in the posterior position in which the uterus obstructs the

kidney veins. This phenomenon was demonstrated by Chesley and Sloan (1964), who found a 20% decrease of renal plasma flow when lateral position was changed into posterior position. The glomerular filtration rate (GFR) increases also by 30 to 50% (Sims and Krantz, 1958). The mechanism by which the GFR is increased is not exactly known. The GFR is closely related to the RPF, and its increase may be no more than the inevitable result of the rise in the latter. At the same time the filtration pressure at the glomerulus is likely to be greatly augmented by the reduced colloid-osmotic pressure of the plasma; however, the pattern of change in the GFR, rising in early pregnancy to a high plateau, is unlike that in colloid-osmotic pressure which falls progressively throughout pregnancy (Robertson, 1969). The increase of excretions of nutrients and metabolites as a result of changes in renal function will be discussed in the next chapter. For an extensive review about renal alterations in pregnancy, the reader is referred to Lindheimer and Katz (1977).

Metabolic changes in the pregnant woman.

The overall metabolic changes during pregnancy are characterized by a rise in basal metabolic rate (BMR), estimated to be 15% higher than in the non-pregnant state (Hytten and Leitch, 1971).

-Carbohydrate metabolism.

Glucose is the principal carbohydrate used for cellular nutrition. The circulating blood sugar is glucose, obtained from three sources:

a. Dietary carbohydrates. Gastro-intestinal enzymes convert these mainly into glucose.
b. Gluconeogenesis: conversion of non-carbohydrates precursors into glucose. These precursors include the glycogeneic amino acids glycine, alamine, glutamic acid, aspartic acid and arginine. Also, a number of products of the glucose metabolic

pathways may themselves be reconverted into glucose. These include succinic, fumaric, lactic and pyruvic acids. Fat hydrolysis to glycerol is a further source of glycogenetic glucose.

c. Glycogenolysis: the hydrolysis of glycogen stored in the liver.

Maintenance of blood sugar levels depends upon a balance between production, utilization and excretion of glucose. After a meal glucose concentration rises in the blood and returns to the "fasting" level: the level which exists about 16-20 hrs. of the day. How this fasting level is determined and maintained is open to questions (Lind, 1980). During pregnancy, fasting glucose levels are slightly reduced from the tenth week of pregnancy onwards (Lind et al., 1973). After an oral glucose load, the time to reach the maximal value in the blood is delayed and there is a slight delay in returning to the normal level (Lind, 1977; Abell and Beischer, 1979).

During normal pregnancy, glucose homeostasis is changed. The reasons for this change and the mechanisms by which these changes are achieved are not yet clear. A number of hormones, human placental lactogen, estrogens, progesterone, cortisol and glucagon are involved presumably by peripheral insuline resistance, although the latter does not seem to contribute much.

It is beyond the scope of this review to describe all possible mechanisms by which these changes are induced, and the reader is referred to Kalkhoff et al. (1978), Abell and Beischer (1979), Lind (1980) and Coelingh Bennink (1980).

Changes in insuline and glucagon secretion are described in section 1.2.

-Glucosuria.

Since every person excretes glucose, it is not easy to define glucosuria, and its incidence depends on how it is defined and what method is used to investigate it. Published incidences vary from 5% to 70% in pregnancy (Davison, 1975).

Is more glucose excreted during pregnancy than in the non-pregnant state? Lind and Hytten (1972) showed that normal healthy pregnant women excrete more glucose than postpartum, that there is a wide variation in the amount excreted and the pattern is inconsistent. In their study, they found that 10 out of 30 women having a normal oral glucose tolerance test excreted less than 100 mg/24 hrs., the remainder excreted more than 100 mg/24 hrs on some days, and 10 women excreted more than 1000 mg on at least 1 day. The increased glucosuria during pregnancy may be due to the greatly increased filtered load of glucose as a result of the rise in glomerular filtration rate or due to a change in reabsorption capacity of the proximal tube itself or possibly both factors are involved.

Davison and Hytten (1975) showed that tubular reabsorption during glucose infusion is less efficient in pregnancy. In the non-pregnant state, plasma flow to each glomerulus and the proportion filtered is much less than the possible maximum and all nephrons are working below their maximal capacity. Nephrons in the kidney, however, do not all have the same reabsorption capacity and intra-renal blood flow varies continuously. In pregnancy when renal plasma flow and glomerular filtration rate are increased by 50%, some nephrons probably cannot cope with the increased glucose load and glucose appears in the urine. The amount depends on the number of nephrons that cannot reabsorb glucose completely and the amount of blood distributed to these "insufficient" nephrons. This explains the inconsistent pattern of glucosuria seen in pregnant women. For an extensive review of glucosuria of pregnancy, the reader is referred to Davison (1975) and Lind (1977).

-Protein metabolism in pregnancy.

During pregnancy, an additional amount of protein is needed for the fetus and the maternal tissues. This amount is estimated to be 925 g. The mean extra daily requirement was calculated in Table 1.1.1.I (Hytten and Leitch, 1971).

TABLE 1.1.1.I Estimated extra protein requirements during pregnancy.

	Weeks of pregnancy			
	0-10	10-20	20-30	30-40
Protein g/day	0.64	1.84	4.76	6.1

Studies on protein metabolism during pregnancy have, of necessity, largely been confined to measurements of the nitrogen (N) balance. The earlier studies grossly overestimated N-retention (Macy and Hunscher, 1934), and the matter of storage of all this protein has not been taken into account. Hytten (1971) calculated that the average retention of 370 g N as quoted by Macy and Hunscher would be accompanied by about 10 liters of water. However, these calculations are based on observations of the water-protein ratio in the liver of the dog and extrapolation to pregnant women is questionable. Reviewing balance studies, Hegsted (1976) concluded that reported data obtained by such studies must be artifactual for one reason or another, and results obtained should be viewed with caution and skepticism.

In recently performed N-balance studies (Calloway, 1980), a positive N-balance was found far exceeding the need for mother and conceptus, although Johnstone et al. (1981) found that nitrogen retention in the period between 30-34 weeks of pregnancy was in agreement with the daily nitrogen retention for growth of the fetus and reproductive tissues during that time. An interesting aspect of Calloway's longitudinal studies is that the difference in N-retention between the first and third trimester of pregnancy is much less than would be expected when looking at the calculated increase in protein requirements (Table 1.1.1.I.). N-retention in balance studies only increases two-fold while the protein requirement increases, theoretically, eight- to ten-fold.

Naismith (1980[a], 1980[b]) showed evidence that in the first two weeks of pregnancy, in the rat, a substantial amount of protein is deposited in the maternal muscle; the anabolic

phase. This protein is withdrawn in the third and final week, the period of rapid fetal growth; the catabolic phase. These observations were made from carcass-analyses. A similar protein storage may exist in human pregnancy as Naismith (1980[a]) produced some evidence that the excretion of 3-methyl histidine, an amino acid that is not reutilized during normal muscle tissue turn-over and is excreted unchanged in the urine, is elevated in healthy, pregnant women in the third trimester. This observation, however, awaits further confirmation. Other evidence, showing the change in protein metabolism during pregnancy, was produced by Naismith (1977, 1980a) when he demonstrated that enzyme changes in the liver of the pregnant rat reduce its capacity to form urea, sparing protein for anabolic purposes. No data are available for the human pregnancy. It is an interesting speculation as this might explain the reduced blood urea level that until now has always been attributed to changed renal function and hemodilution. If these assumptions are true, the protein requirements in the last trimester may be much less than the calculated 6.1 g/day. Protein metabolism in pregnancy is far from solved.

-Lipid metabolism in pregnancy.

About 3.5 kg of fat is accumulated up to the 30th week of human pregnancy (Hytten and Leitch, 1971). Very little extra fat is deposited during the last 10 weeks (Table 1.1.1.II).

TABLE 1.1.1.II Fat deposition during pregnancy.

	Weeks of pregnancy				
	0-10	10-20	20-30	30-40	
Fat g/day	5.85	24.8	21.85	3.3	Total 3.825 g

This accumulated fat serves as an energy bank for the last part of pregnancy and lactation period.

Every aspect of lipid metabolism is affected by pregnancy. Plasma levels of free fatty acids (FFA), triglycerides, cholesterol and phospholipids are changed as well as are their transport kinetics. The two phases in pregnancy, an anabolic phase in which fat is stored and a catabolic one in which it is released, are demonstrated in the serum levels of FFA (McDonald-Gibson et al., 1975). Up to 30 weeks, there was, according to this study, a slight decrease of serum levels of FFA followed by a sharp increase until delivery. In the early puerperium, there is a sudden fall followed by a steep increase until the 10th week postpartum after which serum levels slowly decrease. Glycerol levels changed in a parallel manner, suggesting that the change in FFA represented true changes in adipose tissue lipolysis during these intervals. In late gestation, the rising plasma concentrations of FFA guarantee an important fuel supply for maternal and skeletal muscles, thus sparing glucose and other non-lipid nutrients from utilization by these tissues.

During pregnancy, there is an increase in the serum levels of triglycerides (200-400%), cholesterol (25-50%) and phospholipids (10-20%) (De Alvarez et al., 1959; Taylor and Akande, 1975; Svanborg and Vikrot, 1975).

The physiological meaning of these changes are not clear. Possible mechanisms inducing these changes are discussed in section 1.1.3. For an extensive review of this subject, the reader is referred to Kalkhoff et al. (1978).

1.1.2. Maternal hormonal changes which influence metabolism.

The altered metabolism at the tissue level as described in the previous paragraphs occurs presumably under endocrine influence.

-The thyroid gland.

The thyroid gland hormones are important in the regulation of many aspects of all metabolic pathways, particularly heat and

energy production.

In pregnancy, a physiological hyperthyroidism develops indicated by clinical findings, such as emotional upset, heat intolerance, tachycardia, a hyperdynamic circulation and an enlarged goiter, which may have a bruit over it. Also, laboratory findings of an elevated BMR and elevated thyroid hormone concentrations in the blood indicate a state of hyperthyroidism. However, thyroid function is basically normal in pregnancy, and the increase in BMR is caused by the metabolic requirements of the uterus and fetus and by the increased work of maternal heart and lungs (Hytten and Leitch, 1971).

The increased production of estrogens during pregnancy induces the increased synthesis of thyroxine-binding globulin in the liver; its concentration is doubled by the end of the first trimester (Dowling et al., 1960). Because of the increased number of binding sites, thyroxine (T4) and tri-iodothyroxine (T3) concentrations increase, although generally T3 is raised only in the third trimester (Finucane et al., 1976; Osathanoudh et al., 1976). Not all binding sites are saturated, and the T3-uptake will give results in the hypothyroid range. The free T4 and T3 concentrations in pregnancy are, however, not different from the non-pregnant state (Finucane et al., 1976; Osathanoudh et al., 1976).

The thyroid stimulating hormone (TSH) is either slightly raised or normal (Ramsay, 1980). TSH shows a normal rise after a bolus injection of thyroid releasing hormone (TRH) (Kannan et al, 1973).

-The pancreas.

Fasting insulin concentrations are higher in the second half of pregnancy when compared to the non-pregnant state (Lind et al., 1973). There is no consensus whether glucagon fasting levels are altered during pregnancy (Spellacy, 1975; Kühl and Holst, 1976). The insulin response after a glucose load changes during pregnancy. The peak value becomes progressively higher as pregnancy advances and the time to reach this value is delayed

(Lind, 1973). A depression of glucagon levels, which is more pronounced than in the non-pregnant state, is found only late in pregnancy (Kühl and Holst, 1976). As stated before about glucose metabolism, the reason and causes of the changes in glucose homeostasis are far from clear. A number of hormones are probably involved, but the question of whether the "insulin antagonism" or "tissue resistance" are hormone mediated is not yet solved. For further reading, the reader is referred to Coelingh Bennink (1980) and Lind (1980).

-The adrenal gland.

Adrenocorticotropic hormone (ACTH) levels rise progressively in pregnancy; a rise which is thought to be due to a ACTH production by the placenta (Rees et al., 1975). The ACTH increase is accompanied by an increase in total plasma cortisol. In addition to the increased amount of total cortisol in plasma, the mean, unbound cortisol is elevated with a loss of diurnal variation (Galvao-Teles and Burke, 1973). Transcortin or corticosteroid-binding globulin (CBG) rises steadily in pregnancy to twice the normal value (Doe et al., 1964).

Sex hormone-binding globulin is considerably raised during pregnancy and so testosterone levels are raised as well. However, unbound testosterone and testosterone production rate are reported to be normal (Gandy, 1977). Plasma levels of adrenaline and noradrenaline are the same during pregnancy as in the non-pregnant state (Lederman, 1977).

The pituitary gland.

-Prolactin.

Prolactin levels in plasma rise steadily during pregnancy to reach 10-20 times higher levels at term than in non-pregnant women (Rigg et al., 1977). The mechanism of this rise is probably direct stimulation by the estrogens produced by the feto-placental unit (Healy and Burger, 1977). Another observation, suggesting an estrogen stimulation effect, is that the maternal serum levels of prolactin are only slightly raised

in the case of a sulfatase-deficiency causing a hypoestrogenic situation in pregnancy (Selby et al., 1981). In molar pregnancy, however, prolactin values are more elevated than in normal pregnancy, and Mochizuki et al. (1976) have suggested that human placental lactogen (HPL), which is low in molar pregnancy, has an inhibitory effect on the prolactin production. During pregnancy, the normal daily pattern of secretion is maintained with episodic release and increase of prolactin levels during sleep (Boyer et al., 1975). The prolactin concentration in the amniotic fluid is much higher than the maternal and fetal serum concentrations (Josimovich, 1977[a]; Riddick, 1977).

The role of prolactin during pregnancy is far from clear. Together with HPL and estrogens, it has a preparatory effect on the mammary gland. It might be involved in the maintenance of amniotic water and salt balance (Josimovich, 1977[a]). It might also be involved in the maternal calcium balance by increasing the rate of hydroxylation of 25-hydroxy-cholecalciferol to 1.25-dihydroxy-cholecalciferol (Jacobs, 1980). Postpartum prolactin levels are normal after 2-3 weeks. During breast-feeding, prolactin levels remain significantly above the normal level up to 2 years, depending on the number of episodes of suckling per day (Delvoye et al., 1977).

-Growth hormone.

Growth hormone secretion is inhibited during pregnancy. In the non-pregnant state, growth hormone secretion increases during treatment with estrogens, so the reduction in pregnancy seems paradoxical. However, studies during molar pregnancies, in which HPL levels are low, show a normal growth hormone secretion.

Contrary to the situation in prolactin secretion, the inhibitory effect of HPL on the growth hormone secretion probably exceeds the stimulatory effect of the estrogens (Mochizuki et al., 1976).

1.1.3. The feto-placental unit.

Hormones produced by the feto-placental unit are the means by which the maternal physiological processes are changed and adapted to the needs of pregnancy. First, it was thought that only the placenta was involved in the production of hormones. Later, Diczfalusy (1962) suggested that the placenta acted together with the fetus, and the concept of the feto-placental unit was created.

For the steroid hormone production, fetus and placenta are interdependent, but the placenta is probably independent from the fetus for the protein-hormone production.

Protein hormones of the placenta.

-Human chorionic gonadotrophin (HCG).

HCG is produced by the trophoblast and can be detected very early in pregnancy.

The function of HCG during pregnancy is rather unclear, except that it stimulates the ovary and rescues early pregnancy. There may be some evidence that HCG plays a part in placental steroidogenesis (Klopper, 1980[a]). For further reading about HCG, the reader is referred to Fuchs and Klopper (1977).

-Human placental lactogen (HPL).

HPL is also produced by the trophoblast. At term the total secretion rate is about 0.5 g/100 g placental tissue/24 hrs. (Josimovich, 1977[b]). A correlation between placental weight and HPL level was found by Josimovich and Archer (1977), but not by Spellacy et al. (1971).

The role of HPL in pregnancy is far from clear. It may be connected in two ways with progesterone production: by stimulating the corpus luteum to secrete progesterone in the second half of pregnancy (Munzo, 1980) and by stimulating the biologically inactive 20α-dihydroprogesterone into progesterone in the placenta (Josimovich and Archer, 1977).

HPL has a preparatory effect on the mammary gland, together with prolactin and estrogens (Josimovich, 1977).
In early pregnancy, the augmented tissue glycogen storage and the suppressed fasting plasma glucose concentration can be explained, partly, on the basis of estrogen and progesterone action, i.e. reduced hepatic glucose production and enhanced peripheral glucose utilization (Kalkhoff, 1978). Later in pregnancy, this situation changes and contra-insulin events appear. HPL may diminish the maternal responsiveness to her own insulin and thus allow glucose and amino acids to be available for fetal use (Munro, 1980). On the other hand, the lipolytic effect of HPL increases the release of free fatty acids as a readily available maternal fuel (Josimovich, 1977). Or, as Klopper (1980) says:"although it is probably an over-simplification, one may think of HPL as the feto-placental signal by which the fetus obtains its nutrient requirement from the mother. HPL is the metabolic screwdriver which the fetus reaches out to reset carbohydrate controls in the mother". However, Nielsen (1979) describes an uneventful pregnancy with no detectable HPL. So HPL is not absolutely necessary for maintaining pregnancy and its functions may be taken over (partly or completely) by other hormones.

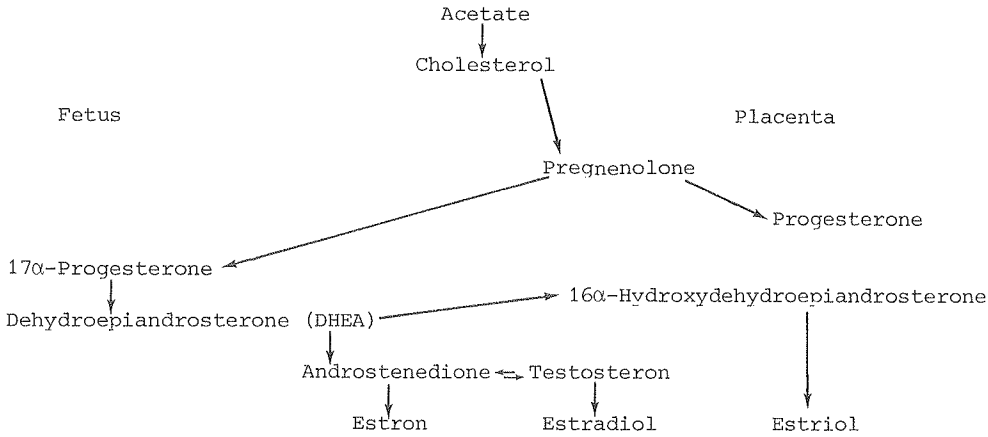
Four more other placental proteins are known at this time: Schwangerschaftsprotein 1 (SP1), pregnancy-associated plasma protein A (PAPP-A), pregnancy-associated plasma protein B (PAPP-B) and placental protein 5 (PP5). They are formed in the syncytiotrophoblast. Their functions are rather unclear at this time, and for further reading, the reader is referred to Klopper (1980^b).

Steroid metabolism in the feto-placental unit.

All steroid hormones of the ovary, testis and adrenal are synthesized in the feto-placental unit. The fetus synthesizes mainly the "primitive" forms of steroids such as pregnenolone and dehydroepiandrosterone. The placenta converts them from the

sulfated form into estrogens. To do this, three enzyme systems are necessary: sulfatase, 3β-hydroxysteroid dehydrogenase and aromatase. The fetus is missing these enzymes, thus giving the placenta its role in the steroidogenesis of the feto-placental unit (Klopper, 1980[a]) (Figure 1.1.3.I).

TABLE 1.1.3.I. Steroid metabolism in the feto-placental unit.



However, precursors of the estrogens can be found not only in the fetal, but also in the maternal blood. Sometimes, the placenta uses maternal precursors (DHEA) and sometimes not (pregnenolone sulfate) (Klopper, 1980[a]).

Unique in the pregnant situation is the fact that due to the 16-hydroxylation capacity of fetal liver and adrenal, estriol is made "de novo" by the feto-placental unit by a route not involving estron or estradiol.

Function of progesterone and estrogens in pregnancy.

Progesterone has in combination with estrogens and other hormones an effect on breast growth. With estrogens, it is required for uterine growth.
Although progesterone has growth-promoting effects on uterus and breasts, the net-effect on the body as a whole is catabolic.

Administration of progesterone leads to an increased urinary urea loss, without a fall in plasma urea. Progesterone increases protein breakdown. Increasing progesterone administration leads to an increased protein breakdown until a critical level is reached, beyond which no increase in progesterone will enhance urinary nitrogen excretion. This critical level is well below progesterone levels late in pregnancy, therefore, its catabolic effect is limited in pregnancy (Landau, 1973).

Concerning lipid metabolism, the suggestion is made that progesterone increases fat deposition (Fotherby, 1964), but this is not clear as the increase in fat deposition under progesterone treatment may also be due to increased appetite or reduced activity (Galetti and Klopper, 1964).

During pregnancy, there has to be a powerful mechanism for dampening down uterine contractions as pregnancy advances. Progesterone may exert the role of "uterine sedative" and this led to the "progesterone bloc" theory (Czapo, 1969).

Progesterone may also be involved in the increase in blood volume by reducing peripheral vascular tonus, but up to now, little is known about the action of progesterone on the cardio-vascular system (Walters, 1979), just as for the estrogens.

Estrogens are involved in a great diversity of biological processes, and there is hardly any organ that is not effected by estrogen action. There is an influence upon the genital system, breasts, skin, hypothalamic-hypophysian axis and the psyche. Estrogens effect metabolic processes; there is an anabolic effect on protein metabolism, and lipid metabolism is altered. Estrogens increase some carrier-proteins such as thyroxine-binding globulin, cortisol-binding globulins and sex hormone-binding globulins. Estrogens cause a water and sodium retention (Tausk, 1976).

In pregnancy, estrogens are involved in the growth of the uterus and the breasts. They create an anabolic metabolism, counter-acting the progesterone effect. They may be involved in the fat deposition in pregnancy by stimulating insulin secretion

(Kalkhoff, 1978). The extracellular-extravascular water retention is probably due to estrogen action and estrogens are probably involved in the increase in blood volume (Walters, 1979).

However, the physiological role of the enormous increase in estrogens during pregnancy is poorly understood, especially the rise in estriol. Women with a sulfatase deficiency have extremely low values of all estrogens. Yet they normally have an uneventful pregnancy, except they often have problems going into labor (France et al., 1973). Therefore, estrogens may be involved in the process called labor.

The role of both progesterone and estrogens on the maternal site has received almost all attention up to now. The placenta and fetus are full of progesterone and estrogens as well, but the role of these hormones in the fetus is absolutely unclear. The physiological role of progesterone and estrogens may be more on the feto-placental than the maternal side.

Reviewing all hormonal changes during pregnancy and its influence on metabolism, one would agree that there must be nutritional implications, but what they are, is virtually unknown at present.

1.1.4. Fetal development.

After fertilization, the egg spends some four to five days drifting down the tube and floating in the uterine cavity before it implants into the wall of the uterus. By that time, the blastocyst consists of around 150 cells. This blastocyst undergoes a series of changes which culminate in the formation of the placenta, and only a small proportion of the inner layer develops into the embryo. Eight weeks after fertilization the fetus, recognizably human now, is about 3 cm long. In the embryonic period, the growth velocity is relatively small. During this time, differentiation of the originally homogeneous whole into regions, such as head, arms and so forth, takes place, as does histogenesis, the differentiation of cells into

specialized tissues as muscle and nerve. There are good data for fetal length in the period 10-18 weeks and after 28 weeks, but no reliable data are available for the period between the eighteenth and twenty-eighth week of pregnancy. The peak in the velocity curves of growth in body length and weight of singleton children composed by Tanner (1978) is somewhat uncertain, see Figure 1.1.4.I.

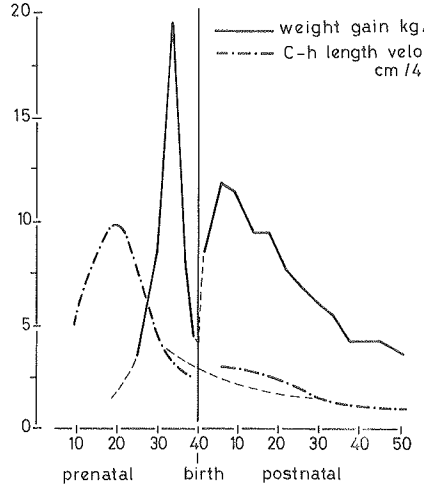


FIGURE 1.1.4.I Velocity curves for growth in weight and for growth in body length of singleton children in prenatal and early postnatal period (figure composed of curves derived from Tanner, 1978).

The high growth rate of the fetus compared with that of a child is largely due to the fact that cells are multiplying, a process that almost stops after the 30th week. Thereafter, late fetal and postnatal growth is, for most tissues, chiefly a period of development and enlargement of existing cells, whereas early fetal life is a period of cell division and addition of new cells (Tanner, 1978). During the last 10 weeks of fetal life, a considerable amount of energy in the form of fat is stored. Up to 26 weeks, the increase in fetal weight has been caused by protein accumulation, but from then on, fat begins to accumulate, both deep in the body and subcutaneously. This accumulation of fat is further illustrated in Table 1.1.4.I, composed by figures given by Southgate and Hey (1976) and

Widdowson (1979).

TABLE 1.1.4.I. Fetal accumulation of fat and protein.

Fetal age weeks	Fetal weight gram	FETAL FAT		FETAL PROTEIN	
		Total g	Mean increments g/day over 4 weeks period	Total g	Mean increments g/day over 4 weeks period
12	20	0.1	0.02	1.1	0.18
16	100	0.7	0.07	6.3	0.58
20	300	2.7	0.37	22.5	1.52
24	750	13.1	1.07	65	2.04
28	1350	43.2	2.11	123	2.41
32	2000	102	4.57	189	3.15
36	2700	230	8.79	277	4.46
40	3400	476		402	

It is evident that there is a rapid rise in fat deposition and added protein in the last 12 weeks. The fetus also has some carbohydrate in the form of glucose and glycogen, but this is small in quantity.

Assumed energy requirements of the fetus in the last intrauterine weeks differ greatly because lipid transport across the placenta in the human is unclear. Widdowson (1980) assumes that only 1-2% of the lipid in the fetus comes from essential fatty acids in the maternal serum and that most of the fat is synthesized from carbohydrate. She estimates the required energy for the fetus at 200-300 kcal/day in the last weeks. There is, however, a growing evidence that the fetus derives almost all its fats from the transfer of maternal, free fatty acids (Hull and Elphick, 1979; Hytten, 1980[b]). This would decrease the required energy by almost 100 kcal/day. Moreover, it would be almost impossible for all fat to be synthesized from carbohydrate since the net quantity of glucose transferred by the placenta is estimated to be not more than about 30 g/day (Hytten, 1979).

The only proteins that cross the placenta are immuno-globulins, almost entirely the IgG fraction. All other fetal proteins are built up from amino acids transferred from the

mother. This transfer is an active process; fetal levels are higher than maternal for every amino acid and the placenta contains levels higher than either circulation. The regulation of the placental uptake and transfer is not understood. Young (1979) reviewed this subject recently.

The amount of some minerals in the fetus at different gestational ages is shown in Table 1.1.4.II, derived from Widdowson (1979). As can be seen from Table 1.1.4.II, fetal demands for all minerals are greater towards the end of gestation.

TABLE 1.1.4.II. Accumulation of minerals in the fetus during pregnancy (after Widdowson, 1979).

Fetal age weeks	Weight kg	Na(g)	K(g)	Cl(g)	Ca(g)	Mg(g)	P(g)	Fe(mg)	Cu(mg)	Zn(mg)
12	0.02	0.06	0.03	0.06	0.03	0.002	0.03			
16	0.1	0.26	0.16	0.29	0.36	0.02	0.25	5.0		
20	0.3	0.75	0.48	0.84	1.38	0.05	0.90	17.4	1.05	5.4
24	0.75	1.63	1.22	1.92	4.44	0.13	2.59	45.9	2.74	13.3
28	1.35	2.74	2.22	3.00	8.86	0.26	5.34	91.2	5.21	23.5
32	2.0	3.85	3.29	4.32	13.7	0.41	8.46	141	8.08	33.8
36	2.7	4.89	4.65	5.14	20.1	0.58	12.5	208	11.0	44.0
40	3.4	5.56	6.14	5.85	28.1	0.76	16.4	278	14.0	52.6

Why the fetus is growing i.e. which the growth promoting substances are, is still not clear. These substances may be of maternal, placental or fetal origin. As to the maternal side, Kastrup et al. (1978) found a positive correlation between maternal somatomedin levels and the weight and length of the fetus. No specific placental substances have been discovered which induce fetal growth. Human placental lactogen (HPL) has often been suggested but never proved to be related to fetal growth (section 1.1.3). A number of fetal substances has also been suggested but fetal insulin is probably the only factor that has been proved to be related to fetal growth (section 1.4). For a review on the subject is referred to Vorherr (1982).

Summarizing, we see that although the peak of the velocity for growth in length falls around the 20th week, the net requirements of fat, protein and minerals are almost neglectable at that time. In the last 12 weeks of pregnancy, the fetus really starts accumulating fat, protein and minerals.

1.2. Nutrition during pregnancy.

1.2.1. Determining nutrient requirements in pregnancy.

For optimal fetal growth and development while maintaining normal maternal metabolism, an adequate nutrient supply during pregnancy is essential. To secure an adequate supply of oxygen and nutrients to the fetal compartment, changes occur in the maternal organism, the so-called maternal adaptive mechanism or "physiological adjustments of pregnancy" (WHO, 1965). The total amount of additional nutrients and energy required for fetal growth, growth of maternal tissue and milk production are taken together in the so-called "physiological cost of pregnancy" (WHO, 1965).

Hytten and Leitch (1971) estimated the total energy cost of pregnancy for a standard woman - age 24 years; height 163 cm; prepregnancy weight 56 kg and a basal metabolism of 1,400 kcal (5.9 MJ) - with an average weight gain of about 12.5 kg at 75,000 kcal (315 MJ). This additional energy cost estimate for the different stages of pregnancy is indicated in Table 1.2.1.I.

From this data it may be concluded that the energy needs of pregnancy are spread fairly evenly over most of the gestational period. It is remarkable that the extra energy needed between the 10th and 30th week is even greater than that during the last 10 weeks, the period in which fetal energy needs are maximal. The relatively high energy needs between 10 and 30 weeks can be accounted for by maternal fat storage to provide an "energy bank" of about 36,000 kcal (150 MJ). The energy stored is released during the last 10 weeks of pregnancy, the period of maximal fetal growth when maintenance costs are also high. So fat storage in the first half of pregnancy followed by fat mobilization in the second half of pregnancy is the mechanism to spread energy needs over the whole period and provides a buffer against sudden food shortage. The stored energy is probably also used to fulfil additional energy needs during lactation. This

physiological adjustment of energy metabolism is controlled by a change in hormonal balance (Naismith, 1980[b]).

TABLE 1.2.1.I Energy requirements for pregnancy (Hytten and Leitch, 1971).

	Weeks of pregnancy				Cumulative total	
	0-10	10-20	20-30	30-40	G	Kcal(MJ)
Protein (g/d)	0.64	1.84	4.76	6.1	925	5180(21.7)
(kcal/d)*	4(0.02)	10(0.04)	27(0.11)	34(0.14)		
Fat (g/d)	5.9	24.8	21.9	3.3	3825	36337(153)
(kcal/d)*	56(0.23)	236(1.0)	208(0.9)	31(0.13)		
Additional (ml/min)	5.6	11.9	20.8	31.7		26244(110)
O_2-consumption (kcal/d)*	20(0.08)	62(0.26)	111(0.46)	186(0.78)		
Total additional energy (kcal/d)*	79(0.33)	308(1.28)	345(1.44)	252(1.05)		67761(284)
Metabolizable energy (kcal/d)* (total energy + 10%)	87(0.36)	339(1.4)	380(1.6)	277(1.15)		74539(310)

* in parentheses: MJ/d

Data from developing countries (Tafari et al., 1980) on weight gain and birthweight suggest, however, that birthweights around 3,000 g and a mean weight gain of 9,200 g may also be observed in pregnant women with a mean energy intake far below the WHO-FAO allowances (section 1.2.2.). This may be accounted for by a reduction in energy output such as a decrease in voluntary activity. Well-controlled studies on energy expenditure during pregnancy are not available as yet.

Also, for some of the micronutrients the physiological cost of pregnancy has been calculated based upon changes in maternal body composition and data about chemical composition of fetal tissues (Hytten and Leitch, 1971; Widdowson et al., 1964; 1980). The calculated total iron needs amount to 500-600 mg per pregnancy (Table 1.2.1.II). About 30 g calcium is deposited in the fetus and this amount is, therefore, accepted as the physiological cost (FAO/WHO, 1965). For most other micronutrients, including vitamins, such information is hardly available.

TABLE 1.2.1.II Iron requirements for pregnancy (FAO/WHO, 1970).

	First half of pregnancy (mg)	Second half of pregnancy (mg)	Total (mg)	Net cost of pregnancy (mg)
Expansion red cell mass	-	500	500	-
Blood loss during delivery	-	-	-	250
Fetal iron	-	290	290	290
Fetal iron in placenta	-	25	25	25
Basal losses (skin,etc.)	110	110	220	-
Total	110	925	1035	565

Nutrient requirements based upon calculated changes in body composition, nutrient losses and fetal nutrient accumulation, the so-called factorial approach, have some principal weaknesses, e.g. limited knowledge of body composition and incomplete understanding of absorption and retention for most nutrients (Beaton, 1979; Sandstead, 1981).

Another approach to determine nutrient requirements is the epidemiologic one in which the health of free-living individuals and populations are considered in relation to nutrient intake. The health of a population is assessed by clinical and laboratory findings. This approach does have its drawbacks as well: accurate quantitation of dietary intake is nearly impossible (section 1.2.3.) and conversion of dietary intake into nutrient intake requires extensive knowledge of nutrient composition of the foods consumed. Most food composition tables provide only average figures, while nutrient composition may vary strongly depending upon season, geography, food processing, etc. This is especially true for micronutrients. Another, more general problem in the assessment of nutrient needs using clinical and laboratory indices is that any criterium used, e.g. the incidence of some pregnancy related pathology, optimal growth rate, birthweight or response of some biochemical parameter, results in its own specific requirement. So the estimated requirement depends on the sensitivity and specificity of the parameter used (Caster and Meadows, 1980). This may be illustrated for vitamin B6 requirement in pregnancy. Using plasma pyridoxal-5'-phosphate (PLP) levels as an index of

vitamin B6 status, between 5-10 mg vitamin B6/day are required to maintain plasma PLP levels within the range considered acceptable for non-pregnant subjects. With the tryptophan loading test, even higher amounts are required to "normalize" vitamin B6 status during pregnancy (section 1.3.6.).

Lechtig (1976) has pointed out that in populations with maternal malnutrition other unfavorable conditions may exist that affect course and outcome of pregnancy like infections and, in general, a bad socio-economic environment. Therefore, not every statistical correlation reflects a cause-effect relationship. These nutrition surveys may provide more useful estimates of nutrient requirements when they involve nutritional intervention to determine the consistency of an observed association. Study design and execution are extremely important in such observational and experimental studies (Bergner and Susser, 1970) (section 1.2.5.). Estimated requirements for some vitamins, e.g. folacin and vitamin B6, are based upon this epidemiologic approach (section 1.3.).

Experimental determination of nutrient requirement of subjects in a controlled environment, like balance studies and depletion-repletion studies, is the third basic approach to the definition of nutrient requirements (Beaton, 1979). Both for practical and ethical reasons such studies are difficult to perform during pregnancy. The balance technique has its limitations and should be interpreted with caution because retention appears to increase with intake even above the requirements (Hegsted, 1976).

Because of the intrinsic drawbacks of all methods for determining nutrient requirements in pregnancy, it is not surprising that conflicting results have been obtained. Balance studies (King et al., 1973) have demonstrated protein accumulation during pregnancy far above the amount found by body composition studies. The additional iron requirement in pregnancy, calculated using the factorial approach, i.e. about 3 mg/day, is not very different from that of non-pregnant, menstruating women. Epidemiologic evidence suggest, however, that many pregnant women tend to become iron depleted, with or

TABLE 1.2.2.I Recommended daily dietary allowances for pregnant (second and third trimester) and non-pregnant females.

	Energy kcal(MJ)	Protein g	Vit.A* µg	Vit.D µg	Vit.B1 mg	Vit.B2 mg	Vit.B6 mg	Niacine mg	Folacin µg	Vit.B12 µg	Vit.C mg	Ca g	Fe mg
Neth. Nutrition Council (1978):													
Non-pregnant (20-35 years, moderately active)	2000(8.4)	55	850	-	0.8	1.3	-	-	-	-	50	0.8	10
Pregnant (20-40 years)	2200(9.2) 2300(9.6)	60-65	950	-	1.1	1.6	-	-	-	-	75	1.3	15
WHO (1974):													
Adult women	2200(9.2)	29	750	2.5	0.9[1)]	1.3[2)]	-	14.5[3)]	200[4)]	2.0	30	0.4-0.5	14-28[6)]
Pregnant	2550(10.7)	38	750	10	1.0[1)]	1.5[2)]	-	17	400[4)]	3.0	30	1.0-1.2	14-28[6)]
DHSS (UK,1969):													
Non-pregnant	2200(9.2)	55	750	2.5	0.9	1.3	-	15	-	-	30	0.5	12
Pregnant	2400(10.1)	60	750	10	1.0	1.6	-	18	-	-	60	1.2	15
NAS/NRC (USA,1980):													
Non-pregnant	2000(8.4)	44	800	5	1.0	1.2	2.0	13	400[5)]	3.0	60	0.8	18
Pregnant	2300(9.6)	74	1000	10	1.4	1.5	2.5	15	800[5)]	4.0	60	1.2	18[7)]

* 1 µg retinol = 6 µg β-carotene = 12 µg carotenoids
1) 0.4 mg/1000 kcal (4.2 MJ)
2) 0.55 mg/1000 kcal (4.2 MJ)
3) 6.6 mg/1000 kcal (4.2 MJ)
4) expressed as free folacin
5) total folacin
6) depending of the type of diet
7) 30-60 mg supplemented iron is recommended
-) no recommendations given

without anemia, while they continue to ingest the same diet. These apparent discrepancies may probably be accounted for by the size of preconceptional body stores. The factorial approach assumes adequate nutrient stores of women when entering pregnancy and determines the amount of a specific nutrient needed to maintain an adequate nutritional status, while the epidemiologic approach reflects depletion of maternal stores during pregnancy. When maternal stores are marginal or already depleted at conception, the estimated nutrient need may be higher because maternal stores have to also be repleted ("maintenance" versus "therapeutic" requirements, Beaton, 1979; Luke and Petri, 1980).

The ultimate goal in defining nutrient and energy requirements in pregnancy is: what is a desirable birthweight and weight gain, and what is an optimal biochemical response. Infant development would be a better indicator, but as already mentioned, each clinical or biochemical criterium has its own requirement.

1.2.2. Recommended allowances for pregnant women.

Table 1.2.2.I. summarizes the recommended daily allowances both for pregnant and non-pregnant women given, by respectively, the Netherlands Nutrition Council (1979), the FAO/WHO (1974), the Department of Health and Social Security (DHSS) of the U.K. (1969) and the National Academy of Science/National Research Council (NAS/NRC, 1980). This table shows the recommendations for women between $\pm$ 20 and 40 years of age, moderately active, while those for pregnant women apply to the second and third trimester. These recommendations are based upon the average physiological need of a specific nutrient to which a certain amount is added such that the recommended intake can be considered sufficient for the "maintenance of health in nearly all people" (WHO/FAO, 1974) or "to afford a margin of sufficiency above average physiological requirements to cover variations among essentially all individuals in the general

population" (Food and Nutrition Board of the NAS/NRC, 1980). The main purpose of recommended daily allowances is to evaluate the intakes of population groups not of individuals. Data about variation in individual needs, as well as the variation in individual nutrient intake, are scarce (Harper, 1974; Egger and Hermus, 1980). In practice these recommendations are always a compromise reached after interpretation and extrapolation of the available data (Harper, 1974). Differences in recommendations between the various countries originate from different approaches to define nutrient requirements, (section 1.2.1) while existing dietary and cultural patterns also play a role (Saris, 1980).

1.2.3. Methods for measurement of food consumption.

It is not easy to measure food consumption of free-living individuals. The available methods can roughly be divided into two categories:

a. Interview methods (recall, dietary history)
b. Record methods (precise weighing; weighed inventory etc.).

The different methods will not be discussed in detail here because excellent reviews on this subject are available (Marr, 1971; Van der Haar and Kromhout, 1978). For comparison of methods, information about validity and reproducibility of the respective methods is needed. Validity has been defined as "the true accuracy of a method as a measurement of the variable it is supposed to measure" (Keys, 1965). Accuracy describes the deviation of the estimated value from the "true" value. Since no absolute reference method is available, such comparisons are extremely difficult (Marr, 1971; van Staveren and Hulshof, 1980). All methods seem at best semi-quantitative and have their own disadvantages. Record and weighing methods almost certainly modify dietary habits and the more tedious procedures involved may limit cooperation. Interview techniques also have several drawbacks, especially distortion of the memory of the respondent (Thomson, 1958; Rush, 1975). Nearly all methods estimate the

"actual" nutrient intake over a certain period (1-7 days), while the dietary history methods give information on the "usual" nutrient intake. From the available data, it seems that dietary history methods sometimes overestimate, while recall methods underestimate average food consumption as obtained with record methods. A possible explanation for the overestimation of the food intake by the dietary history may be that the amounts of small or infrequently used foods are overestimated (Van der Haar and Kromhout, 1978).

An important question in food consumption surveys is: How many days, and which days are required for an accurate estimate? The intra-individual variation seems to be as large as the inter-individual variation (Beal, 1967). In long-lasting surveys, seasonal effects may play a role, especially in the case of vitamins (Chapell, 1955; Van der Rijst, 1962). The method to be preferred in nutrition surveys should depend on the goals and aims of that particular study and the population involved. Also practical criteria have to be considered, e.g. the available capacity of trained dieticians, budget, etc. (Van Staveren and Hulshof, 1980). The ultimate choice is, therefore, always a compromise between that what is desired and what is feasible. However, "large numbers of inaccurate estimates are no substitute at all for a few accurate measurements" (Thomson, 1958).

1.2.4. Reports on food consumption in pregnancy.

Table 1.2.4.I summarizes a number of reports about nutrient intake of women at different stages of pregnancy. This summary is far from complete, but is intended to show estimated average nutrient intake of pregnant women living in industrialized countries. Data about Dutch gravidae, reported by Den Hartog et al. (1953) and Van der Rijst (1962) are also included.

Referring to the accuracy and reliability of food consumption surveys (section 1.2.3) comparison of reported data

TABLE 1.2.4.I Mean daily nutrient intakes by pregnant women.

Authors	Subjects	Energy (Kcal)	Protein (g)	Fat (g)	Vit.A (RE)	Vit.D (ug)	Vit.B_1 (mg)	Vit.B_2 (mg)	Vit.C (mg)	Niacin (mg)	Iron (mg)	Ca (g)	Period	Method
Darby et al., USA, 1953	White women, middle or lower income class n=278	2140	75	-	1950[1)]	1.6[1)]	1.5	2.5	66[1)]	12	13	1.1	1st.Tr.	Dietary record
	n=1222	2200	75	-	1980[1)]	1.7[1)]	1.5	2.5	120[1)]	12	14	1.1	2nd.Tr.	
	n=1665	2020	70	-	1770[1)]	1.6[1)]	1.4	2.3	55[1)]	11	13	1.0	3rd.Tr.	
Den Hartog et al., Holland, 1953	Rural population n=270	2770	78	-	840	-	1.4	1.6	114	14	14.5	1.0	2nd.Tr.	Dietary history
Thomson, UK, 1958	Primigravidae, wives of skilled manual workers n=109	2521	78	-	2430	-	1.2	1.9	65	11	-	1.1	3rd.Tr.	Weighed record
Gräfe, DDR, 1961	Normal pregnancy n=89	2753	77	122	2580	-	1.57	1.85	79	15	15	0.8	5th.M.	7-day record
		2755	77	120	2400	-	1.63	1.97	95	16	15	0.9	8th.M.	
Van der Rijst, Holland, 1962	499 healthy urban dwellers (Amsterdam)	2620	79	112	1250	-	1.32	-	123	-	14	0.85	1st.Tr.	3-day recall
		2720	80	116	1280	-	1.30	-	120	-	16	1.1	2nd.Tr.	
		2620	76	109	1340	-	1.30	-	122	-	16	1.0	3rd.Tr.	
Hankin, Australia, 1964	174 healthy women	2312	58	97	2350	-	1.0	1.7	81	13	11	0.8	2nd.Tr.	4-day record
		2327	80	95	2350	-	1.0	1.9	92	14	11	0.95	3rd.Tr.	
Lunell, Sweden, 1969	58 healthy women	2035	65	98	790	2.5	1.3	1.7	91	11.5	14	1.0	1st.Tr.	24 hrs. recall
		2185	70	104	680	3.6	1.2	1.8	103	10	13	1.2	2nd.Tr.	
		2137	73	92	680	2.3	1.5	2.2	116	11	14	1.3	3rd.Tr.	
Smithells, UK, 1977	168 healthy women (all social classes)	2010	70	90	1000	1.9	0.99	1.53	68	12	11	0.9	1st.Tr.	Weighed 7-day record
Darke, UK, 1980	435 healthy women (between 1968 and 1971)	2152	70	98	1270	2.3	1.04	1.60	55	14	12	1.0	3rd.Tr.	Weighed 7-day record
Papoz, France, 1980	156 healthy women (Paris)	2307	82	108	670	-	1.40	1.83	172	-	13	0.9	1st.Tr.	Recall
		2121	79	100	660	-	1.32	1.85	173	-	12	0.9	3rd.Tr.	

should be done carefully. Data reported in Table 1.2.4.I are not necessarily representative or reliable. Surveys including data from populations with a high incidence of vitamin and mineral supplementation, e.g. some of the more recent studies from the U.S., have been left out. As might be expected, mean nutrient intake in these well fed populations meets the allowances (section 1.2.2). From the U.K. studies, a socio-economic gradient is apparent. Lower intake levels, together with a higher incidence of poor nutritional status, were observed in the lower social classes (Thomson, 1958; Smithells et al., 1977).

-Dietary vitamin intake.

Most reports only give information on vitamin A (retinol), B1 (thiamin), B2 (riboflavin), C and sometimes niacin intake, while data on vitamin D, B6 and folacin are more limited. Vitamin E and K and the other water-soluble vitamins (Biotin, Pantothenic acid and Vitamin B12) are missing in nearly all surveys. This may be explained by the limited knowledge about content of these vitamins in food products, because dietary supply is supposed to be adequate or simply because no allowance has been established. Data about vitamin B6 and folacin intake, not included in Table 1.2.4.I, are summarized below because they were mostly reported separately.

-Folacin intake of pregnant women.

The term folacin is used to describe a number of compounds exhibiting the same biological activity as folic acid (pteroylglutamic acid) (section 1.3.8). Both oxidized, reduced, mono- and polyglutamate forms of folic acid have been identified and may occur in food products (Butterworth, 1963). The availability of the different folates in humans has not been established completely. Approximately three fourth of the folacin in unrestricted American diets is present in the form of polyglutamates (Butterworth, 1963). These are available nutritionally only after hydrolysis in the intestinal mucosa by a specific conjugase (section 1.3.8). Folacin content of food

products is commonly determined by microbiological assay with Lactobacillus caseï, the test organism that responds to the greatest number of folacin derivatives. The amount of folacin measured without pretreatment of the sample with conjugase is commonly referred to as "free folacin", comprising mainly the easily absorbable reduced monoglutamates. The term "total folacin" is used to describe folacin content after conjugase treatment to make the polyglutamate forms of the vitamin available to the test organism (Hurdle, 1973).

A complicating factor in folacin assays is the instability of most folacin derivatives. Many folates are readily oxidized, and ascorbate, or another reducing agent must be present during extraction and assay. Unfortunately, most food tables list folacin content of raw foods determined after conjugase digestion, but without using ascorbate at any stage (Toepfer et al., 1951; McCance and Widdowson, 1960). It is obvious that these figures are, therefore, unrealistic. Löwenstein (1966) e.g. reported a mean total folacin intake of, respectively, 92, 82 and 83 µg/day in a population of Canadian gravidae in the first, second and third trimester. Moscovitch and Cooper (1973) determined, however, folacin intake in about the same Canadian population and found a mean "free folacin" intake of 206 µg/day using L-caseï as the test organism and including ascorbate in the complete assay. Löwenstein et al. (1966) used data from Toepfer et al. (1951) for calculation of folacin intake. Chanarin et al. (1960) determined the folacin content of 111 separate, 24 hour food collections of pregnant women. Contents varied between 50 and 300 µg, with an average of 160 µg for "free" folacin and between 200 and 1,600 µg with a mean content of 676 µg for "total" folacin. Martinez and Roe (1976) calculated a mean "free" folacin intake of about 200 µg per day, using data on "free" folacin content of Butterfield et al. (1972). These folacin intake levels are considerably higher than those reported by Alperin (1966) and Pritchard (1969) who found intake levels between 30 and 50 µg/day. Also in more recent reports from, respectively, Denmark (Elsborg and Rosenquist, 1979) and France (Papoz et al., 1981) relatively low "free"

folacin intake levels were calculated for healthy women at different stages of pregnancy. In the Danish study, a mean daily intake of 82 µg/day (range 30-174 µg) was observed, in the French study 58 ± 1 µg/day (x ± SE). Although a season dependent folacin intake has been supposed, no significant differences between summer and winter have been found (Rolschau et al., 1979; Martinez and Roe, 1976).

-Vitamin B6 intake of pregnant women.

As for folacin, methods for determination of vitamin B6 content of food products are complex and insufficiently standardized. Vitamin B6 content of food products is mostly determined using a microbiological method with Saccharomyces carlsbergensis as the test organism. In food products pyridoxin, pyridoxal and pyridoxamine and their respective phosphate esters may be present and are equally active. The estimated vitamin B6 intake, reported in most studies with healthy pregnant women living mainly in western, industrialized societies, varies between 1 and 2 mg/day: 1.5 mg/day (Coursin and Brown, 1961); 1.3 mg/day (first trimester, Smithells, 1977); 1.5 mg/day (Vir et al., 1980); 1.8 mg/day (first trimester) and 1.7 mg/day (third trimester) (Papoz et al., 1980).

-Mean vitamin intake in pregnancy in relation to recommended daily allowances (RDA).

Considering the data presented in Table 1.2.4.I the conclusion seems warranted that the mean intake of vitamin A, thiamin, riboflavin, C and niacin of pregnant women living in most western countries is adequate, i.e. meets the recommended allowances. This does not necessarily mean that the intake is adequate for all individuals because data about variation of the mean intake and mean requirement are commonly not available (section 1.2.2). The mean intake for vitamin B6 and folacin, however, seems inadequate in many cases, i.e. remains below recommended allowances. From available data, estimated mean vitamin D intake also usually falls below the accepted standards. The contribution of endogenous synthesis from its

provitamin in the skin, depending on solar exposure is difficult to quantify, but undoubtedly yields a significant contribution (section 1.3.2).

-Nutrient intake at different stages of pregnancy.

Consistent trends in nutrient intake in the course of pregnancy are not obvious for most nutrients from data reported in literature. Most studies refer to only one specific stage of pregnancy, e.g. early pregnancy or near term, or have a cross-sectional (transversal) design, i.e. different women at different stages of pregnancy. Only few longitudinal studies have been reported, determining dietary intake from the same group of women at different stages of pregnancy. The cross-sectional studies from Darby et al. (U.S.A., 1953) and Van der Rijst (Holland, 1962) suggest a higher energy intake in the second trimester compared with the third trimester, with a mean difference of 200 kcal (0.8 MJ). In both studies, a wide range in estimated nutrient intake is observed with no significant differences in the intake of most micronutrients between the trimesters.

Most micronutrients, including the vitamins, showed a significant different intake level between summer and winter. A recent longitudinal study on dietary behaviour during pregnancy was reported from France (Papoz et al., 1981). In the first six months of pregnancy they found a slight but significant increase in energy and micronutrient intake, followed by a small decline in the third trimester (Table 1.2.4.I). A similar trend in nutrient intake in pregnancy was reported by Lunell et al. (Sweden, 1969).

Interesting information about changes in food consumption during pregnancy were reported by Beal (1971). In a longitudinal study covering twenty years (1946-1966), records of nutritional intake (dietary history) were obtained from 23 single pregnancies, from 23 women during two pregnancies, from 6 during three pregnancies and from 2 during four pregnancies. Although the validity and especially the reproducibility over such a long period may be questioned, some conclusions were drawn. Energy

intake increased with approximately 100 kcal (0.4 MJ) above preconceptional intake levels until the second trimester and then decreased with about 150 kcal (0.6 MJ) in the third trimester, primarily due to a decreasing fat intake. The percentage of energy from protein increased throughout pregnancy. Between preconceptional nutrient intake and intake during pregnancy, a significant correlation was found for all nutrients studied (energy, carbohydrates, fat, protein and calcium); a correlation that decreased as pregnancy progressed. High preconceptional intakes tended to decrease during pregnancy and low intakes to increase, especially in protein and calcium. This remarkable observation was not explained by the authors. The lower energy intakes were found for women who were overweight for their height. A similar inverse relationship between prepregnancy body size and energy intake was observed by Papoz et al. (1981). The women who were followed during successive pregnancies showed a consistent food consumption pattern in respective pregnancies.

1.2.5. Pathology related to nutrition during pregnancy.

Animal research has revealed that, during maternal malnutrition, phyisological and psychological prenatal development of the fetus is not optimal. Malnutrition may involve all nutrients or may be limited to a specific nutrient. The duration and severity of malnutrition influence the outcome for the fetus and possibilities of later recovery. Malnutrition during generations worsens outcome and prognosis. For a review of animal experiments, the reader is referred to Osofsky (1969), Winick (1969), Brasel and Winick (1972) and Rosso (1980).

Corresponding data concerning the human situation are hard to interpret. An absolute deficiency of a specific nutrient never exists, meaning it is not possible to investigate specific effects as in animal research. Malnutrition alone does not exist. Malnutrition goes with poverty, scanty education, bad hygiene and low social stimulation and control. Effects of a

deficiency of a specific nutrient are very difficult to interpret because of this mixture of factors.

There are two models possible to investigate the relationship between nutrition and existence of pathology during pregnancy and that between nutrition and intra-uterine development.

The first model is the observational study: to analyze nutrition during pregnancy, leading to the evaluation of differences in cases of pathological development as compared to the non-pathological group. The hypothesis that a certain pathological condition stems from a dietary deficiency (lack of a particular nutrient) can be proven in follow-up studies if correction of the diet leads to the elimination of this problem.

The second model is the intervention study: one group is supplemented with the nutrient in question, while another is given a placebo. The purpose of these studies is to prove that dietary supplement decreases the risk of eventual nutrition related pathology. The idea that supplementing the diet may worsen subclinical pathology or may induce new pathology is hardly ever taken into consideration. In the ideal situation, these models are not used next to each other, but the observational study is followed by the intervention study. During this century, a number of observational studies have been performed.

One of the first was by Smith (1916). He classified a group of pregnant women by their nutritional status (good, moderate or poor), according to their dietary history, length to weight ratio and physical investigation. In the group with a poor nutritional status, he found a higher number of intra-uterine death and more children with a birthweight below 2.7 kg. This study was followed by many others, the results of which (i.e. the relation between nutrition in general or nutritional status and birthweight, perinatal mortality, prematurity, toxemia, breast-feeding and congenital anomalies) will be discussed in this section. The results of the intervention studies will be

discussed in section 1.2.6.

-Birthweight.

The studies of Smith (1916) and Woodhill (1955) show that women of poor nutritional status seem to have more term babies below 2.7 kg - the worse the diet, the smaller the baby. However, in the large Vanderbilt study, no clear relationship between birthweight and energy intake during pregnancy could be demonstrated (McGanity et al., 1954). Thomson (1959[a]), in a careful study in Aberdeen, found a positive correlation between birthweight and energy intake per kg bodyweight during the 20th week of pregnancy. On the other hand, he observed a relationship between birthweight and maternal weight at that time of pregnancy. This correlation is much stronger than the correlation between birthweight and energy intake, and Thomson concluded that maternal weight determines both energy intake and birthweight and that the relationship between energy intake and birthweight is not a causal one. This relationship could not be demonstrated in other studies, either (Van der Rijst, 1962; Hankin et al., 1964).

However, Eastman and Jackson (1968) have demonstrated a distinct connection between weight gain during pregnancy and birthweight. Rush (1972) showed that the influence of maternal height, age and parity on birthweight disappears when prepregnant weight and weight gain are held constant. His conclusion is that the nutritional status, both at the beginning and during pregnancy (weight gain), has a greater effect on birthweight than the past nutritional status of the mother, of which height is a rough expression. Rush opposes Thomson's conclusion by citing the latter's use of the maternal weight at 20 weeks. According to Rush, maternal weight at this time is dependent on the alimentation during the first half of pregnancy.

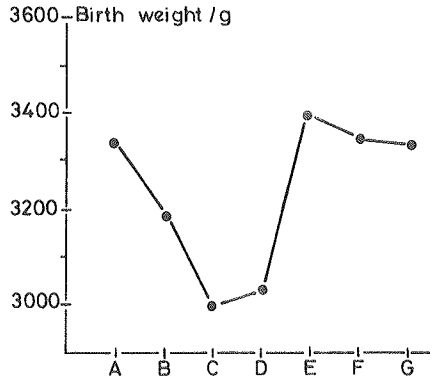
Women clinically suspected of intra-uterine growth retardation in an otherwise uneventful pregnancy have a significantly lower energy intake than a control group

(Papiernik et al., 1976). Papoz (1980) found no correlation between energy intake and birthweight or even between energy intake and weight gain during pregnancy. Yet she did find a significant relationship between the increase in energy intake in the first six months of pregnancy and weight gain.

The existence of a possible relationship between energy intake and birthweight will be difficult to prove, because overweight women have a tendency to eat less during pregnancy in contrast with underweight pregnant women (Beal, 1971; Papoz et al., 1981).

If the nutritional situation suddenly deteriorates and the intake of both macro- and micronutrients falls drastically, birthweight decreases significantly (Smith, 1947; Antonov, 1947). Figures of the Dutch famine in the winter of 1944-1945 have been worked out by Stein (1975): see Figure 1.2.5.I.

FIGURE 1.2.5.I Birthweight before, during and after the Dutch famine of 1944-1945.



A. Birthweight before famine.
B. Exposed to famine during third trimester.
C. Exposed to famine during second and third trimester.
D. Exposed to famine 6 months in the middle of pregnancy.
E. Exposed to famine in the first and second trimester.
F. Exposed to famine in the first trimester.
G. Conception after famine, no exposure.

The mean decrease of birthweight was 327 grams, or 9% in the group that was exposed to famine during the last two trimesters. In Leningrad, the mean decrease was about 540 grams. This figure shows that reestablishment of normal energy intake in the last weeks of pregnancy after a long exposure to famine has only a small effect (group D). The "over-shoot" of group E may be an expression of a certain selection. It is difficult to assess how much of the decrease in birthweight in these circumstances may

be attributed to the low energy intake; the quick reestablishment of birthweight after nutrition was normalized makes it plausible that shortage of food was an important factor to the lowered birthweight.

Summarizing: there is a general agreement that, if nutrition (energy intake) falls below a certain minimum, birthweight is influenced in a negative way. Likewise, there is consensus that the nutritional status before pregnancy (prepregnant weight) influences birthweight. However, it is still controversial whether nutrition during pregnancy influences birthweight. Two theories exist:

Thomson's: maternal weight determines both energy intake and birthweight.

Rush's : energy intake determines maternal weight gain which determines birthweight.

-Perinatal mortality.

Smith (1916) observed more cases of intrauterine death in pregnant women with a poor nutritional status than in the better fed group. In the Vanderbilt study, there were 72 cases of perinatal death (McGannity et al., 1954). In these cases an increased protein and albumin level during pregnancy and an increased vitamin C level after pregnancy were found. Overweight was over-represented in this group. Deficiencies in nutrition were not present. In other studies, numbers are too small to justify conclusions (Woodhill, 1955; Thomson, 1959; Van der Rijst, 1962). Smith (1947) did not find an increased perinatal mortality in the winter of 1944-1945 in Holland, but Stein et al. (1975) found an increased, although not significant, number of intrauterine deaths in pregnancies exposed to famine in the first trimester. From the Health Statistics, it is clear that perinatal mortality did not change much during the war in Holland, but a distinctly higher mortality of children in the first three months of life was observed in the period 1944-1945 (Posthuma, 1953). An indication that nutrition during pregnancy may influence perinatal mortality is the observation that perinatal mortality decreased from 3.8% in 1940 to 2.8% in 1945

in the United Kingdom. A possible explanation for this decrease may be the food rationing, established in those years, and the fact that overfeeding, existing before 1940, disappeared (Duncan et al., 1952).

-Prematurity.

Over the years, a problem has developed in examining the relationship between nutrition, or the nutritional status during pregnancy, and prematurity because of the different defining criteria: a weight less than 2500 grams (Smith, 1916; Woodhill, 1955), a weight less than 2250 grams (Smith, 1947), a length less than 47 cm (Antonov, 1947), a length less than 43.2 cm (Woodhill, 1955), a duration of pregnancy less than 38 completed weeks (Woodhill, 1955; Thomson, 1959; McGanity et al., 1954; Van der Rijst, 1962) and a duration of pregnancy less than 37 completed weeks (Rush et al., 1980; Papoz et al., 1981). Also, a distinction between prematurity and dysmaturity was not always made. Smith (1916) and Woodhill (1955) found an increased incidence of birthweights below 2500 g in women with a poor nutritional status before pregnancy or who had been poorly fed during pregnancy. An increased incidence of birthweights below 2250 grams in Holland in 1944-1945 and of children with a length at birth below 47 cm in Leningrad were found (Smith, 1947; Antonov, 1947). In the Vanderbilt study (McGanity et al., 1954), signs of malnutrition were found more often in the group of women delivering before 38 weeks. Vitamin C intake and vitamin C blood levels were lower in this group compared to the group that delivered at term. Due to possible connections between prematurity and other obstetrical problems, McGanity et al. were reluctant to assume a relationship between malnutrition and prematurity. Thomson (1959) and Van der Rijst (1962) did not find a relationship between energy intake and duration of pregnancy less than 38 weeks. Fedrich and Adelstein (1978) determined that one of the factors clearly associated with prematurity was maternal prepregnant weight.

Summarizing: there seems to be a relationship between

nutritional status before pregnancy and premature delivery, although no relationship has been established between prematurity and nutrition during pregnancy.

-Toxemia.

During the Dutch winter of 1944-1945, Smith (1947) observed a decrease in the incidence of toxemia (Table 1.2.5.I).

TABLE 1.2.5.I. Incidence of toxemia before the war, in 1944, during the famine of 1944-1945 and after the war.

	Before the war	End 1944	1944-1945	After the war
Toxemia not defined	3.2%	3.4%	1.9%	3.8%
RR syst. 140 or albumiuria ++ or edema ++ or convulsions	2.2%	2.4%	1.1%	2.2%
Albumiuria ++ or edema ++ or convulsions	0.8%	0.75%	0.6%	1.1%

Holmer (1949) also noticed a decrease of toxemia during the Second World War in Holland. A suggested explanation for this decrease has been the lesser use of salt - an explanation doubted, however, by de Wijn (1969). A lower energy intake in pre- eclampsia was observed in the Vanderbilt study (McGanity et al., 1954). In these cases, a higher serum protein level was found before toxemia developed. The assumption that toxemia might develop due to protein deficiency did not seem likely (McGanity et al., 1954). The reduction of the energy intake in this group might be explained by the dietary advice given in cases of "pathological" weight gain. Woodhill (1955) reported a distinct decrease in the incidence of toxemia when better nutritional conditions exist during pregnancy; 35% toxemia in the "poor diet" group and 4% in the "good diet" group. An increase in the incidence of "pre-eclamptic" toxemia, parallel to the energy intake, was found by Thomson (1959). When the daily intake was about 2250 kcal (9450 J), this incidence was 2.9% and 13.3% when the daily intake was over 2500 kcal (10,500 J). Hankin et al. (1964) could not confirm Thomson's findings.

Van der Rijst (1962) found a slight, but not significant, increase in the incidence of toxemia in women with a higher appraised alimentation during pregnancy.

Summarizing: poor nutrition and (too) many calories may be correlated with toxemia. The causal relationship is not clear; neither are the reasons behind the decrease of toxemia incidence during the war. In studies about toxemia, one encounters the same problem as in prematurity: namely that studies, because of the differences between criteria used in defining toxemia, are hardly comparable to each other.

-Congenital anomalies.

The number of congenital anomalies in some groups studied were too small for any conclusions to be drawn about a relationship between nutrition and congenital anomalies (Smith, 1947; Van der Rijst, 1962). In the Vanderbilt study - in which more than 2000 women were observed - congenital anomalies are not mentioned.

Stein et al. (1975) seemed to find in children exposed to famine in the first trimester a higher, although not significant, incidence of central nervous system anomalies. This observation may go well with the observations that peri-conceptional vitamin (Smithells et al., 1980) or folacin supplementation (Laurence et al., 1981) reduced the incidence of recurrence of neural tube defects in a high risk group. In the last study, this was significantly proven in a double-blind, randomized, controlled trial. However, this does not mean that in these women a vitamin, or more specific, a folacin deficiency exists. It is even most unlikely since the net folacin need of the fetus in early pregnancy, compared to the maternal reserve, is almost neglectable. A speculative explanation may be that folacin transport to the fetus in early pregnancy is hampered, for instance, by a partial enzyme deficiency, and that, only by overloading the system, enough folacin can reach the fetus.

The intake of even modest amounts of alcohol during early pregnancy may be teratogenic and cause the fetal alcohol syndrome. The principal features of this syndrome are central

nervous system dysfunction, growth retardation (pre- and postnatal) and certain facial characteristics (Clarren and Smith, 1978; Hinckers, 1978).

Summarizing: no clear relationships have been demonstrated between nutrition in general and congenital anomalies, with the possible exception of the "nutrient" alcohol, although even here the role of co-variables is undetermined (Hinckers, 1978).

-Lactation.

During the winter of 1944-1945, almost no decrease in number of women breast-feeding was observed. A small increase was seen in the upper social class, but the lower social class showed a slight decrease (Smith, 1947). Women feeding themselves well during and after pregnancy, breast-fed their children for a longer period. In the group "very poor diet" only 12% lactated as long as 6 months postpartum, while 92% of the women did so in the "good to excellent diet" group (Woodhill, 1955). Unfortunately it was not mentioned why lactation was stopped, so the assumed causal relationship in this study may be doubted. In the group of women with the best estimated nutrition, a higher, but not significant, percentage of women who fully breast-fed their children was found (Van der Rijst, 1962).

Summarizing: studies on the relationship between breast-feeding and nutrition during and after pregnancy are scarce and as breast-feeding is strongly dependent on motivation, social and cultural background, no judgement will be passed on this relationship.

1.2.6. Intervention studies during pregnancy.

During pregnancy, two models of an intervention study are possible. The first possibility is that a situation is created in which a shortage of food develops. This intervention is involuntary and goes with circumstances which may influence the parameters one wants to examine. In the industrialized world,

this situation exists almost only in war time; in the third world, crop failure, prolonged draught, etc. may cause this involuntary intervention. Research in these situations is almost always in retrospect, and data are difficult to collect. Thereby, it always concerns a shortage of both micro- and macronutrients. In spite of these problems, valuable information may be collected.

The second possibility is that a pregnant woman voluntarily participates in a study whereby micro- or macronutrients, or both, are added to their normal diet, and the parameters studied are compared to those of pregnant women not receiving the supplement. Research in which healthy, pregnant women voluntarily received a diet without certain components has never been done. Restriction of food happened only on medical grounds (Prochownik, 1901).

TABLE 1.2.6.I. The intake of micro- and macronutrients before, during and after the famine in the Western part of Holland in 1944-1945.

A. Official ration to pregnant women in Western Holland.

B. The amount distributed to pregnant women in The Hague and Leiden.

		Sept. 1944	Jan/Febr. 1945	April 1945	July 1945
Calories/day	A	2099(8816 J)	1144(4805 J)	1427(5993 J)	2546(10693
	B	1925(8085 J)	731(3070 J)	912(3830 J)	-
Protein g/day	A	62	34	35	78
	B	61	33	39	
Fat g/day	A	51	29	42	91
	B	50	11	14	
Carbohydrates		331	177	215	344
Calcium (mg)		1075	649	517	
Iron (mg)		16,3	9,6	10,7	
Vit.A (IU)		1260	445	766	
Thiamin (mg)		1,1	1,0	0,6	
Niacin (mg)		9,2	4,0	4,1	
Riboflavin (mg)		1,2	0,5	0,5	
Vit.C (mg)		59	34	53	

The results and conclusions of the involuntary intervention "studies", the winter of 1944-1945 in Western Holland and the

siege of Leningrad, have been discussed in section 1.2.5.

In Table 1.2.6.I, the decrease in intake of macro- and micronutrients is shown for comparison with the intake in the voluntary intervention studies.

Voluntary intervention studies during pregnancy.

The best known intervention studies will be discussed in chronological sequence. In the studies of Balfour (1944), People's League of Health (1942) and Dieckmann (1944) only minerals and vitamins were supplemented. These studies will be discussed briefly, because they are regularly referred to in literature. In other studies, minerals and vitamins were often given as a supplement to the control group.

-1937-1939 Northern England and South of Wales (Balfour, 1944).
Purpose of the study: to evaluate the influence of supplemental vitamin A, D and B-complex and calcium, phosphorus and iron on stillbirth-, neonatal death-, maternal death rate and toxemia.
Design of the study: the main criterion for admission to the supplemented group was poverty (n = 11,600). The control group (n = 8,100) was composed of pregnant women of higher social classes.
Critique: due to this selection criterion, groups were not comparable (e.g. 21% primigravidae in the supplemented group, 41% in the non-supplemented group). The intake of supplement was not controlled.
Conclusion: no conclusions can be drawn from this study.

-1937-1939 London (People's League of Health, 1942).
Purpose of the study: to evaluate the influence of supplemental vitamin A, B, C and D and the minerals, iron and calcium, on "toxemia", prematurity (not defined) and birthweight.
Design of the study: before the 24th week of pregnancy, supplement was given alternately to healthy, pregnant women. The control group received no tablets at all. Two groups of 5,000 were formed in 10 London hospitals.

Results of the study: in the treated group, pre-eclampsia and prematurity were significantly less frequent. Prematurity was defined as a pregnancy ending before the 40th week. Birthweight was not found to be significantly different.
Critique: the intake of tablets was not controlled. Prematurity is too vaguely defined. The groups on which the conclusions about the incidence of pre-eclampsia are based, are rather small, and it is not mentioned whether they are comparable concerning maternal age, social class and frequency of antenatal visits.
Conclusion: no conclusions can be drawn from this study.

-1940-1941 Toronto (Ebbs et al., 1941).
Purpose of the study: to evaluate the influence of supplement on the course of pregnancy, delivery and puerperium (described as good, fair, poor or bad) and on toxemia and pre-eclampsia, stillbirth, prematurity, birthweight, endometritis, mastitis, lactation and diseases of the child during the first 6 months of life. Supplement consisted of extra milk, one egg and an orange daily, and weekly, canned tomatoes and half a cheddar cheese together with iron and vitamin D.
Design of the study: according to a dietary history, women were divided into a "poor diet" and "fair-to-good diet" group. A part (n = 90) of the "poor diet" group received the above mentioned supplement; the other part (n = 120) received capsules of corn oil as a placebo. The "fair-to-good" group received only dietary advice.
Results of the study: women who were supplemented or who had a good diet were healthier during pregnancy and less obstetrical problems were encountered. The incidence of abortion, stillbirth and premature delivery in the "poor" diet, placebo group was significantly higher. The incidence of diseases of children during the first 6 months in this latter group was increased as well.
Critique: the groups are not comparable, concerning parity and obstetrical history (for instance, 9.5% stillbirth rate in the

placebo group compared to 4.7% and 2.2%, respectively, in the supplemented and "good diet" group). Prematurity is not defined and the number of drop-outs is not mentioned.
Conclusion: the results may be doubted due to the incomparability of the groups, and in our opinion, no conclusions may be drawn from this study. It is remarkable, however, that there is no difference in birthweight, including premature infants, between the 3 groups.

-1942 Chicago (Dieckmann, 1944).
Purpose of the study: to evaluate the influence of supplemental minerals and/or vitamins on the "clinical course of pregnancy", the puerperium, the birthweight and the Apgar score of the child.
Design of the study: 4 groups of women of low income class were formed. The first group served as a control group (n = 175), the second group received calcium, phosphorus and iron (n = 179), the third group received vitamin A and D (n = 98) and the fourth group received both minerals and vitamins (n = 102).
Results of the study: some differences were noted, e.g. a significantly larger increase in maternal weight gain in all groups in which something was added to the diet, but the authors conclude that generally no conclusions can be drawn. No effect on the fetus, attributable to the changes in diet, was noted.
Critique: no explanation for the difference in numbers in the 4 groups is given, although the groups were selected at random. It is not clear whether groups are comparable.
Conclusion: no conclusions may be drawn from this study.

-1963-1971 Montreal (Higgins et al., 1973; Rush et al., 1976).
Purpose of the study: to evaluate the influence of supplement on perinatal mortality and prematurity. The supplement consisted of milk, eggs, oranges, multivitamin pills and iron. Dietary advice was also given. Later, in retrospect, birthweight was evaluated as well (Rush et al., 1976).
Design of the study: the supplemented group was composed of

women from families with a minimal income. Part (n = 1,164) received both supplement and dietary advice, others (n = 472) only dietary advice. Due to these measures, the daily energy intake increased from 2,251 to 2,782 kcal (9,425 to 11,648 J), and protein intake increased from 68 to 100 grams per day. The control group was composed of women from another clinic in the same hospital.
Results of the study: a significant lower perinatal mortality and prematurity rate was found in the supplemented group. The mean birthweight in the supplemented group was 40 grams higher than in the control group.
Critique: the study took 8 years and changes in time are not evaluated. In their article, Higgins et al. (1973) uses numbers carelessly. Only 1,541 children are born out of 1,636 "completed" cases in the study group. Out of 7,694 children born in the control group, 1,245 children are chosen and compared with the 1,605 (?) children of the study group. The composition of the control group and the comparability with the study group are not mentioned, and the dietary intake of the control group was not assessed. The intake of the supplement was not controlled. Only in the last 2 years of the study was smoking evaluated and advised against in the supplemented group.
Conclusion: due to the inaccurate handling of numbers, no conclusions can be drawn from this study. In retrospect, matching is not justified when an important factor such as smoking is largely missing, and no conclusions can be drawn concerning birthweight.

-1966 Hyderabad, India (Iyenger. 1967).
Purpose of the study: to evaluate the influence of nutrition on birthweight during the last 4 weeks of pregnancy.
Design of the study: during the last 4 weeks of pregnancy women were admitted into the hospital, received the normal hospital food (2,100 kcal (8,820 J) and 60g protein) and were supplemented either with 350 Kcal (1,470 J) + 30 g protein (n = 12) or with 350 Kcal (1,470 J) alone (n = 13). The control group (n = 16) was composed of women of the same social class visiting

the antenatal clinic. The mean food intake in this group was 1,400 Kcal (5,880 J) and 40 g protein.
Results of the study: a significant increase in birthweight was found in the hospital admitted, supplemented group.
Critique: the groups are rather small and are not well defined. The supplemented group stayed 4 weeks in the hospital in bed, by which an uncomparable situation is created.
Conclusion: no conclusions can be drawn from this study.

-1967-1973 Taiwan (Quentin-Blackwell, 1973; Rush et al., 1980; McDonald et al., 1981).
Purpose of the study: to evaluate the influence of energy and protein supplement on birthweight, lactation and development of the child.
Design of the study: 2 randomised groups of multiparous pregnant women were formed. First, one pregnancy was only observed. During the next pregnancy, one group was supplemented with 800 Kcal (3,360 J) and 43 g protein per day together with minerals and vitamins, and the other group received only the latter. Participation in the study was on a voluntary basis. Before the study was undertaken, energy intake in the area was estimated to be between 1,600 and 2,000 Kcal (6,720 and 8,400 J). A nutrition analysis during the first trimester revealed, however, that the mean intake of the first group was 1,130 Kcal (4,746 J) and of the second group 1,200 Kcal (5,040 J) per day.
Results of the study: mean birthweight of males (n = 41) of mothers who received energy and protein supplement was 79 g heavier than that of males of the other group (n = 47). Mean birthweight of females was 34 g more. These differences are not significant. In a recent article about this study, McDonald et al. (1981) found a correlation between the energy supplement and birthweight. Unfortunately, their conclusions were based on faulty information about the energy content of the supplement, an error described in the addendum of their article.
Critique: only women who received more than 50% of the (voluntary) supplement were evaluated. Because the participation was voluntary, the problem of self-selection is encountered.

Especially in small groups, this may be important, and is difficult to interpret.
Conclusion: this is a carefully designed study, although numbers are rather small. Energy and protein supplement during pregnancy did not increase birthweight significantly.

-1969-1973 Guatemala (Habicht et al., 1973; Lechtig et al., 1975a, 1975b, 1975c).
Purpose of the study: to evaluate the influence of two forms of supplement (high energy, high protein and low energy, no protein) during and after pregnancy on birthweight, neonatal mortality rate and neurological development of the child.
Design of the study: supplement was given free of charge and on a voluntary basis. It could be used "ad libitum". One village received as supplement 163 Kcal (685 J), 11 g protein, 0.7 g fat, 27 g carbohydrates, vitamins and minerals per 180 ml porridge. The other village received 59 Kcal (248 J), 15.3 g carbohydrates, minerals and vitamins (per 180 ml). The mean intake in this area was 1,500 Kcal (6,300 J) and 40 g protein. Medical care was given free as well.
Results of the study: division of the suppleted groups into more (A) or less (B) than 20,000 Kcal (84,000 J) total supplement during pregnancy revealed the following results: in group A (n = 170), a mean birthweight of 3,105 gram was observed and in group B (n = 235), 2,994 gram. This difference of 111 gram is significant ($P < 0.01$). The difference could not be explained by other parameters such as maternal weight, age, parity, socio-economic status and duration of pregnancy. The incidence of a birthweight below 2,500 g differed significantly as well. Group A had a 9% incidence, group B 19% ($P < 0.05$). The neonatal mortality rate did not differ significantly.
Critique: as soon as it became clear that extra protein had no effect, the original purpose of the study was abandoned and only the effect of energy supplement on birthweight, neonatal mortality rate and development was evaluated. As in the Taiwan study, the self-selection problem is encountered. Only 405 (62%) of the 651 born children were evaluated. Because the original

protocol was abandoned, it was not possible to evaluate negative effects of the supplement (see the next study). An advantage of this and the Taiwan study is that the energy value of the extra food is exactly known since it had to be consumed on the spot. Conclusion: in this study, it seems proven that energy supplement during pregnancy influences birthweight in a positive way, although the gain is only marginal (about 28 gram/10,000 Kcal or 42,000 J).

-1970-1973 New York (Rush et al., 1980).

Purpose of the study: to evaluate the influence of energy and protein supplement on birthweight and neurological development. Design of the study: women having a high risk for a low birthweight child were admitted to the study. High risk factors were considered: prepregnant weight below 50 kg, low weight gain during the present pregnancy, at least one previous low birthweight infant and a history of protein intake less than 50 gram in the 24 hrs. preceding registration. Three groups were formed at random. Group A (n = 248) received an energy (460 kcal or 1,932 J) and protein (40 g) supplement. Group B (n = 256) received an energy (320 Kcal or (1,344 J) and low protein (6 g) supplement. Group C (n = 264) received only minerals and vitamins, which were given to group A and B as well.
This randomized, partly double-blind, controlled study is well designed, and the researchers have tried to avoid all possible pitfalls that accompany such an enormous study.
Results of the study: birthweight of children of group B (balanced protein-energy supplement) was 44 gram more than the control group. Children of group A (high protein-energy supplement) weighed 42 gram less than the controls. Differences did not reach significance. In group A, however, more cases of early prematures, neonatal death and low birthweight were found, the last especially when pregnancy had not reached 37 weeks. The incidence of early premature in group A is on the verge of significance. However, the increase in low birthweight infants among prematures in group A is highly significant. The only

positive effect of both forms of supplement was found in the group of heavy smokers (n = 49). When this group was compared with a smoking control group (n = 19), birthweight was found to be significantly higher, although duration of pregnancy was also longer.
Critique: the supposition that protein deficiency might exist in the group was not true. About one third of the women were admitted into the study because a 24-recall diet analysis revealed a protein intake in the previous 24 hrs. of less than 50 gram. This could not be reproduced later, and the protein intake of the population studied proved to be normal. Further, it is not mentioned how the duration of pregnancy was calculated; an average birthweight (group C) in week 31-32 of pregnancy of 2824 gram is too high and probably points to an inaccuracy in duration of pregnancy (Rush et al., 1980, page 71).
Conclusion: a well designed study showing that protein-energy and energy supplement in a non-protein deficient, pregnant population have no effect on birthweight. The conclusion that protein supplement might be dangerous, since significantly more growth retarded prematures were observed, may be a bit hasty. The right conclusion must be that protein supplement to a non-protein deficient group, might be dangerous. Evaluation of eventual negative effects of supplement is a must in every study, although it is almost always forgotten.

-1974 Philadelphia (Osofsky, 1975).
Purpose of the study: to evaluate the influence of protein and mineral supplement on the course of pregnancy with the emphasis on toxemia, birthweight and neurological score directly after birth.
Design of the study: a low income group of pregnant women was observed and served as a control group (n = 118). Later another group of pregnant women (same social class, n = 120) received extra protein and minerals.
Results of the study: except significantly lower incidence of toxemia, no differences are found between both groups.

Critique: the amounts supplemented are not mentioned. The intake of supplement was not controlled. Study and control group were not studied during the same period.
Conclusion: no conclusions can be drawn from this study.

-1977-1978 Bogota (Mora et al., 1979).
Purpose of the study: to evaluate the influence of energy and protein supplement on birthweight and neurological and psychological development of children up to the age of 3 years.
Design of the study: pregnant multiparae living in a poor urban environment were randomly selected. At least 50% of their children should have signs of malnutrition. They and their family were given supplement to their habitual diet of about 1600 Kcal (6720 J) and 35.5 g protein. This daily diet was considered to be deficient. Supplement consisted of 856 Kcal (3595 J) and 38.4 g protein and extra vitamin A and iron. The controls received no supplement at all; medical care was given free to both groups. Four hundred thirty-three living singletons were born. Intra-uterine death (10) and twins (6) were not evaluated.
Results of the study: in the supplemented group, the mean birthweight of boys was 95 g heavier than the controls ($P < 0.05$). Difference in birthweight of girls was 6 g. The difference is found independent of the duration of pregnancy.
Critique: only 407 of the 433 children are evaluated; 26 "disappeared", no reason was given; 110 children were not weighed immediately postpartum, and the weight of 86 children was taken 15 days postpartum. This problem was "solved" by making a postpartum, weight curve up to the fifteenth day to calculate birthweight in retrospect. It is not mentioned if these forgotten children were divided equally among the two groups, but as supplement was given daily, it is most likely that almost all forgotten children are found in the control group. Intake of supplement was not controlled. It is not possible to evaluate eventual disadvantages of supplement when leaving intra-uterine death and 26 other children (prematures that died early?) out of the study.

Conclusion: due to the inaccuracy in measuring birthweight, no conclusion regarding the relation supplement and birthweight can be drawn.

The reason for describing the intervention studies in such detail is that, in the literature concerning nutrition and pregnancy, these studies are always referred to, and, because quite a number of conclusions drawn from these studies might be doubted.

Summarizing: we find that in the older intervention studies, in which mostly vitamins and minerals (Balfour, 1942; People's League of Health, 1942; Dieckmann, 1942), but sometimes also macronutrients (Ebbs et al., 1941) were supplemented, the emphasis is on the pathology of pregnancy, e.g.toxemia, prematurity and neonatal mortality. No conclusions can be drawn from these studies as too many methodological mistakes are made.

In the more recent studies (Taiwan, New York, Bogota and Guatemala), the emphasis was on the child and, more specifically the child's birthweight. Only Lechtig et al. (1975) were able to show a positive effect of energy supplement on birthweight: about 28 gram per 10,000 supplemented Kcal (42,000 J) in pregnancy. Mora et al. (1979) showed this effect only for boys, but in our opinion, these results might be doubted due to the procedures used. The Taiwan study did not show a positive effect and the New York study showed a negative effect of protein supplement. This protein supplement increased significantly the incidence of low birthweight for prematures. As has been said, the conclusion of this study should be that protein supplement can be dangerous in a population with a normal protein intake. It might also be possible that in a protein deficient population, protein supplement has an adverse effect. However, conclusions about this effect can not be drawn from the studies in Guatemala and Bogota as intra-uterine death and very early neonatal death are omitted or not included in the study population. Of all these studies, only the Guatemalan shows an effect of energy supplement on birthweight, although a modest

one.

The purpose of the more recent studies is also the evaluation of prenatal nutrition on subsequent neurological and psychological development of the children. As this subject is beyond the scope of this literature review, we have not mentioned any result. A short remark will do. In the Taiwan and New York study, no differences in development were found. In the Guatemala study (Klein et al., 1977), the development of supplemented children seemed better than controls, but it should be mentioned that, in the latter study, supplement (voluntarily) was given postnatally as well, making effects of prenatal supplement no longer evaluable.

1.3. Vitamin and iron status during pregnancy.

1.3.1. Vitamin A.

Vitamin A is the generic name for all β-ionone derivatives other than provitamin A carotenoids, exhibiting qualitatively the biological activity of retinol (according to the Committee on Nomenclature of the International Union of Nutritional Societies (IUNS) and the American Institute of Nutrition (AIN)(1977). The term retinoids is sometimes used to describe the natural forms of vitamin A and the synthetic analogs, with or without the biological activity of retinol. The diet contains vitamin A either as the preformed vitamin or as a provitamin compound, such as β-carotene. For calculation of the vitamin A activity of a diet, the term " g retinol equivalent" has been introduced (1 retinol equivalent = 1 µg retinol = 6 µg β-carotene = 12 µg other provitamin A carotenoids = 3.33 IU retinol). In the intestinal mucosal cells, β-carotene is converted into retinol, and after esterification with long chain fatty acids, mainly palmitic acid, these retinylesters are incorporated in chylomicrons. These are transported via the lymph into the bloodstream and are ultimately taken up by the liver (Goodman, 1980). Vitamin A is primarily stored in the parenchymal cells of the liver, concentrations varying between 100 and 300 µg/g wet liver. These liver stores can protect the organism from developing a vitamin A deficiency for about one year (Sauberlich, 1976). From liver stores vitamin A is released as a complex with Retinol-Binding Protein (RBP). In blood this retinol-RBP complex is again complexed by another protein, prealbumin (PA). Formation of this retinol-RBP-PA complex prevents its removal from the organism by glomerular filtration and renal catabolism of RBP. Only a small amount, less than 5%, of circulating retinol seems to be uncomplexed with RBP. Part of this retinol is esterified and associated with lipoproteins; another part exists as free retinol and arises probably by breakdown, or dissociation, of the retinol-RBP complex. Vitamin

A toxicity, i.e. the membranolytic action of retinol, is associated with excess free retinol. Retinol bound to RBP is not toxic (Dingle et al., 1972). The biochemical functions of vitamin A have not yet been fully elucidated. Besides the well-known function in the visual process, vitamin A is also involved in the reproductive function, bone growth and maintenance and differentiation of epithelial tissues. Retinoic acid can replace retinol for normal body growth as well as normal differentiation of epithelial tissues, but not in the visual process and the reproductive function.

-Vitamin A metabolism in pregnancy and during fetal growth

During pregnancy additional vitamin A is apparently necessary for a number of special metabolic processes, such as fetal development and storage in the liver, maternal formation of colostrum and storage for lactation and, possibly for hormone synthesis. At birth, livers of normal infants contain rather low concentrations of vitamin A (<50 µg/g), even though the mothers were well nourished (Iyengar and Apte, 1972; Montreewasuwat and Olson, 1979). These low, but rather constant fetal retinol concentrations suggest a strictly controlled vitamin A transfer into the fetus. Vitamin A is transported across the placenta as the retinol-RBP complex (Takahashi et al., 1975 and 1977). At the end of the gestational period, the fetal liver is able to synthesize RBP. According to Ismadi and Olson (1975), over 90% of the retinol in fetal and cord serum is complexed with RBP and PA. Both RBP- and PA-level are low at birth, about 50% of maternal values. Maternal RBP- and PA-levels are slightly lowered or about the same as for non-pregnant controls (Vahlquist et al., 1975).

-Hormonal effects on serum retinol concentrations (effects of the use of oral contraceptives)

Epidemiological studies have revealed higher serum retinol levels in women using oral contraceptives compared to controls. Serum levels are elevated by 30-50% during oral contraceptive

use (Wynn, 1975; Prasad et al., 1975) even in women with marginal or poor vitamin A status (Ram and Bamji, 1979). Yeung et al. (1975) have shown that this effect is due to the estrogenic rather than the progestagenic component of the anticonceptive pills. There seems to be no risk for a toxic effect as all "released" vitamin A is complexed with RBP nor for development of vitamin A deficiency since the liver depletion rate is only slightly affected and no significant changes in metabolism or excretion have been observed (Supopak and Olson, 1975; Nonavinakere et al., 1981). More recently Vahlquist et al. (1979) studied the effect of sex hormones on the vitamin A transporting proteins in humans. They confirmed that oral contraceptives induced a significant increase of RBP which was correlated with the increase of retinol. The increase was higher with synthetic hormones than with natural estrogens. Prealbumin levels also increase during oral contraceptive use. Besides contraceptive hormones, other hormones also affect vitamin A metabolism. A relationship has been established between vitamin A metabolism and thyroid function: vitamin A deficiency produces biochemical hyperthyroidism in rats (Morley et al., 1978). Corticosteroids induce a decrease in the serum retinol level (Clark and Colburn, 1955; Atukorala et al., 1981).

-Biochemical parameters for assessment of the vitamin A status

Tests mostly used for the assessment of the vitamin A status, are measurement of retinol content in serum and liver, using spectrophotometric, colorimetric or fluorometric methods (Pitt, 1981). It has been recognized by many investigators that retinol levels in serum do not represent body (i.e. liver) stores. Only in two extreme situations, i.e. when liver stores have been largely depleted or when the liver has become saturated with vitamin A, does serum retinol level reflect vitamin A status. The ingenious experiments with rats, described by Underwood and co-workers (1979), have shown that vitamin A homeostasis in serum is primarily determined by the vitamin A needs of extrahepatic tissues. Protein deficiency, Zinc-deficiency, hormonal changes and chronic and acute diseases can also produce

(secondary) changes in serum retinol level.

-Serum retinol concentrations during pregnancy

Although older literature about changes in serum retinol concentrations is rather inconsistent, generally a decreasing trend was observed (Rodriguez and Irwin, 1972). In a more recent report from Kübler and Moch (1975), a slight decrease in serum retinol level throughout pregnancy in a group of healthy German gravidae was described. Al-Nagdy (1971), Dawson et al. (1969) and Morse et al. (1975), however, found no significant changes during pregnancy. Gal and Parkinson (1974), studying the effect of nutrition and other factors on serum retinol levels, described a rather variable pattern during pregnancy. They observed a slight decrease in serum retinol level in early pregnancy followed by an increase until a few weeks before delivery when there is again a tendency for the levels to fall. During labor low retinol levels were found; some days after delivery, retinol levels were high again. In the older studies of Darby et al. (1953) an increase postpartum was also found up to levels even higher than found for non-pregnant females, although they observed decreasing levels in the course of pregnancy. Gal and Parkinson (1974) and also Kübler and Moch (1975) found evidence for a seasonal variation for retinol and carotene levels. In the study from Van der Rijst (1962) such seasonal effects were not observed.

Changes observed before, during and after pregnancy, found in most of the studies, suggest that not only nutritional factors are involved, but that also other (i.e. hormonal) factors may play a role.

Carotene levels show an upward trend during pregnancy in most studies (Gal and Parkinson, 1974; Kübler and Moch, 1975; Baker et al., 1975). Dawson et al. (1969) and Morse et al. (1975) found, however, no significant changes in carotene levels during pregnancy,while Metcoff et al. (1976) observed a progressive fall. Retinol and carotene levels in the fetal circulation and in cord blood are in general lower than in the maternal circulation (Ismadi and Olson, 1975; Baker et al.,

1975).

-Pathology related to vitamin A deficiency in pregnancy

Animal experiments have shown profound effects of vitamin A deficiency on reproductive efficiency. In addition to high fetal mortality, vitamin A deficiency in the rat produces severe congenital malformations of the skeleton (Takahashi et al., 1975; Giroud, 1968). In human pregnancies, there are no indications for vitamin A related pathology. This is not surprising as substantial reserves are available in the liver. Only after these reserves are exhausted severe complications are to be expected. There is one report which describes low retinol levels in women with pre-eclamptic toxemia (Basu and Arulanantham, 1973). Their group consisted of Indian women of low socio-economic status and because serum protein levels were also significantly lower, the author suggested a relationship between serum protein and retinol level. A significant (positive) correlation between maternal serum retinol level in the second and third trimester and birthweight was reported by Kübler and Moch (1975).

-Vitamin A requirement in pregnancy

The many experiments that have been conducted to determine the requirement for vitamin A, have been reviewed by Rodriguez and Irwin (1972). The recommended daily allowance (RDA) of 950 µg for pregnant women, set by the Netherlands Nutrition Council (1978), is 100 µg above that recommended for non-pregnant women. The WHO (1974) does not differentiate between pregnant and non-pregnant women: the recommended intake for both groups in 750 µg. However, during lactation the recommendation increases to 1200 µg per day. The daily allowance during pregnancy by the NAS/NRC (1980) is 1000 µg, 200 µg above their non-pregnant recommendation to compensate for fetal vitamin A storage. Large doses of vitamin A, however, have a teratogenic effect in animals and probably also in humans. Bernhardt and Dorsey (1979) reported renal anomalies in a baby born to a mother with hypervitaminosis A.

1.3.2. Vitamin D.

Vitamin D plays an essential role in calcium and phosphate homeostasis. Pioneering work by the group of De Luca (1979) has contributed much to our understanding of vitamin D metabolism. Vitamin D needs of the organism can be met both by dietary intake and endogenous synthesis from the provitamin 7-dehydrocholesterol, a process taking place in the epidermal layer of the skin and dependent on solar (ultraviolet light) exposure. Because of this endogenous production which, depending on the extent of solar exposure, can completely meet body needs, and also because of its working mechanism, vitamin D is now considered more a hormone than a vitamin.

In the liver, vitamin D is hydroxylated to 25-hydroxyvitamin D (25-OHD). This 25-OHD is the main circulating metabolite of vitamin D and is considered as a prohormone susceptible to further hydroxylation in positions 1 and 24; the latter hydroxylations taking place exclusively within the kidneys. Studies by Weisman et al.(1979) have indicated that also the human placenta can synthesize both 1,25-dihydroxyvitamin D (1,25-DHCC) and 24,25-dihydroxyvitamin D (24,25-DHCC). In a state of hypocalcemia, renal 1α-hydroxylation is activated through the action of parathyroid-hormone (PTH) and plasma levels of 1,25-DHCC increase. 1,25-DHCC stimulates intestinal calcium absorption and bone mineral mobilization, the latter effect requiring also the presence of PTH. In the eucalcemic and hypercalcemic state, 25-OHD is no longer hydroxylated at the 1α -position, but is converted to 24,25-DHCC. The exact role of 24,25-DHCC has not yet been well-established. Corvol et al. (1978) demonstrated that picogram amounts of 24,25-DHCC stimulated synthesis of proteoglucans in isolated rabbit chrondrocytes indicating a physiological role of 24,25-DHCC is skeleton formation.

-Vitamin D metabolism and oral contraceptives

Plasma concentrations of vitamin D binding globulin, the specific plasma carrier protein for 25-OHD, are significantly

increased during oral contraceptive therapy (Haddad et al., 1976). However, 25-OHD plasma or serum levels are not affected (Schreurs et al., 1981). No effects of oral contraceptives on plasma levels of the dihydroxyvitamin D metabolites have been reported.

-Vitamin D metabolism during pregnancy

Pregnancy induces massive shifts in calcium; about 30 g of calcium, ± 2.5% of total maternal calcium stores, is translocated from the mother to the fetus (Haeney and Skillman, 1971; Pitkin, 1975). This change in calcium homeostasis is regulated by vitamin D. Due to maternal hypocalcemia, which develops in the course of pregnancy, the 1,25-DHCC concentration in the maternal circulation is elevated, inducing enhanced intestinal calcium absorption. The increased maternal calcitonin levels probably protect the maternal skeleton against excessive bone resorption (Stevenson et al., 1979; Kovarik et al., 1980). Bone mineral mobilization requires the synergistic action of PTH. Conflicting results have been reported on serum PTH levels during pregnancy and lactation, but in the third trimester levels are increased (Cushard et al., 1972; Dent and Gupta, 1975; Hillman et al., 1978; Steichen et al., 1980). Overall mineral content of the maternal skeleton shows no significant change during normal human pregnancy (Christiansen et al., 1976). Next to PTH,other factors may be involved in the regulation of vitamin D metabolism during pregnancy. Estrogens (Castillo et al., 1977), prolactin (Spanos, 1978) and growth hormone (Spanos, 1978) have all been demonstrated to induce 1α -hydroxylase activity in animal models. Levels of estrogens, prolactin and the growth hormone resembling hormone human placental lactogen (HPL),are increased during pregnancy. In the lactation period, however, only prolactin levels remain elevated while 1,25-DHCC concentrations are high as well, suggesting an important role for prolactin (Lund and Selnes, 1979). Maternal serum 1,25-DHCC levels are increased during pregnancy (Kumar et al., 1979; Lund and Selnes, 1979; Steichen et al., 1980), but

24,25-DHCC levels are slightly lower compared with non-pregnant females (Hillman et al., 1978; Reiter et al., 1979).

Fetal 1,25-DHCC levels are lower than maternal levels (Steichen et al., 1980). It is uncertain whether this fetal 1,25-DHCC is derived from the maternal circulation. Weismann et al. (1976) found that after administration of labelled 1,25-DHCC to pregnant rats, only 0.5% of the administered dose accumulated in the fetus. Midgett and Quinby (1980) reported, however, considerable fetal acquisition of vitamin D metabolites, including 1,25-DHCC. Wieland et al. (1980) found a positive relationship between fetal and maternal 1,25-DHCC levels, contrary to Steichen et al. (1980) who found no correlation. 1α-Hydroxylation in fetal renal homogenates has been demonstrated in many species (Weismann et al., 1976; Lester et al., 1978; Sunaga et al., 1979). As already mentioned, the human placenta is able to synthesize 1,25-DHCC and 24,25-DHCC. In comparison with the maternal circulation, the 1,25-DHCC/24,25-DHCC ratio in the fetal circulation of the rat is clearly shifted toward higher 24,25-DHCC levels (Lester et al., 1978) suggesting an independent control mechanism of vitamin D metabolism by the feto-placental unit. The relatively high 24,25-DHCC levels in the fetal circulation may play an important role in the development of the fetal skeleton (Corvol et al., 1978). Fetal vitamin D binding protein (DBP) is about half the maternal level (Bouillon et al., 1977).

-Vitamin D status parameters during pregnancy

Serum alkaline phosphatase activity and calcium concentration are the classical, indirect vitamin D status parameters. In pregnancy maternal serum calcium levels show a steady decrease. These changes are thought to reflect a decrease in protein bound calcium relative to the hypoalbuminemia (Pitkin, 1975). Alkaline phosphatase activity in serum increases during pregnancy, especially in the third trimester due to an increasing production of the "heat-labile" isoenzyme from the placenta. Determination of serum alkaline phosphatase activity has,

therefore, sometimes been used as a parameter of placental function rather than a vitamin D status parameter. Determination of serum 25-OHD concentration is now the most frequently used vitamin D status parameter, reflecting both exogenous and endogenous vitamin D supply (Avioli and Haddad, 1977). Determination of the active dihydroxy-metabolites is also possible but these assays are complex and time consuming.

Reports about 25-OHD serum levels in the course of pregnancy show no consistent trend. Dent and Gupta (1975) observed no significant differences in 25-OHD serum levels between pregnant and non-pregnant women. Significantly lower 25-OHD serum levels at the end of pregnancy were reported by Turton et al. (1977), Weisman et al. (1978) and Reiter et al. (1979). The latter authors measured serum 25-OHD content at various stages of pregnancy, in a cross-sectional study design, and observed a progressive fall from the second trimester until term. Measurements were significantly below non-pregnant control levels. A recent report from the National Institute of Nutrition in Hyderabad, India (1980) described higher 25-OHD levels during pregnancy as compared with non-pregnant females. Also, Fairney et al. (1977) reported slightly higher values in recently delivered women. Some of the observed differences may be explained by the fact that results were not adjusted for seasonal effects. The lowest 25-OHD levels are found at the end of the winter (February-March), the highest levels (about twice the lowest levels) in the summer (August-September) (Holmberg and Larsson, 1980). Differences in dietary vitamin D intake and vitamin D supplementation have hardly any effect on the course of 25-OHD levels during pregnancy (Hillmann and Haddad, 1974; Turton et al., 1977; Paunier et al., 1978). However, Hillmann and Haddad (1976) reported afterwards a small but significant correlation between 25-OHD levels and dietary vitamin D intake during the winter period. Since the maternal DBG concentration increases during pregnancy (Bouillon et al., 1977; Barragry et al., 1978) the decrease observed by some investigators can not be explained by a lower serum binding capacity.

Very low 25-OHD serum levels were reported in pregnant Asian

women living in England (Heckmatt et al., 1978; Brooke et al., 1981). Babies born to these Asian immigrants have a higher risk of developing neonatal rickets (Goel et al., 1976). Although inadequate dietary intake may be involved, the main reason for this condition seems to be that these immigrants, living in countries with a colder climate, are less prone to expose their skin to the sun for climatological and cultural reasons (Dent and Gupta, 1975; Brooke et al., 1981).

In cord blood, 25-OHD levels in general are 10-20% lower than the corresponding maternal values. Between paired maternal and cord 25-OHD levels, a significant correlation is reported (Hillman and Haddad, 1979; Shimotsuji et al., 1979; Birbeck and Scott, 1980). Paunier et al. (1978) observed a higher maternal-cord serum concentration gradient at the higher maternal 25-OHD levels, suggesting a regulating role for the placenta.

-Vitamin D related pathology in pregnancy

No specific maternal pathology in pregnancy has been related to vitamin D deficiency, although Rosen et al. (1974) demonstrated low maternal and neonatal 25-OHD levels in about 50% of premature babies developing neonatal hypocalcemia. Maternal vitamin D deficiency may also be an etiologic factor in primary dental hypoplasia (Heckmatt et al., 1979; Purvis et al., 1973). Hillman and Haddad (1975) determined serial concentrations of 25-OHD in cord blood of premature and term infants. In premature infants low 25-OHD levels were found until a postconceptual age of 36-38 weeks. Supplementary vitamin D given orally or intravenously did not increase 25-OHD concentrations in premature infants.

-Vitamin D requirement in pregnancy

Both the NAS/NRC (1980) and the WHO (1974) recommend a daily allowance of 10 µg (400 IU) vitamin D during pregnancy and lactation, for non-pregnant females recommendations are, respectively, 5 and 2.5 µg. This increased requirement is based

upon the physiological stress on calcium metabolism during pregnancy. The observed correlation between dietary vitamin D intake and 25-OHD serum levels in winter months suggests that, in periods with decreased solar (ultraviolet) exposure, dietary intake may be a limiting factor and the pregnancy induced higher vitamin D demands are difficult to meet (Hillman and Haddad, 1976). Although it has been suggested that vitamin D toxicity during gestation may be responsible for the infantile hypercalcemic syndrome, the evidence is far from conclusive (Forbes, 1979).

1.3.3. Vitamin E.

Vitamin E is the generic name for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol (Committee on Nomenclature IUNS/AIN, 1977). Tocopherols are especially present in products of vegetable origin (green leavy plants, oils of seeds). Tocopherols differ in number and position of the methyl groups attached to the tocol molecule; the biological activity decreases in the order $\alpha > \beta > \gamma > \delta$. This difference in biological activity is shown mainly by a difference in retention of the molecule in the tissues.

The organism is able to store considerable amounts of vitamin E, primarily in liver and adipose tissues. With an adequate nutritional status, these stores are sufficient for at least one year (Horwitt, 1974). Vitamin E functions in at least two metabolic roles:

1. as a fat-soluble antioxydant, and
2. in a specific role interrelated with the metabolism of selenium.

Vitamin E and selenium, integral components of the enzyme glutathione peroxidase, are both involved in the maintenance of the functional integrity of (sub)cellular membranes by defending these membranes against oxidation of vital phospholipids. Vitamin E deficiency diseases in experimental animals are now well-established and include reproductive failure, myopathies,

circulatory disorders and encephalomalacia (Scott, 1980). In humans the role of vitamin E is more controversial. Cases of well-established vitamin E deficiency have generally been limited to premature infants and patients with malabsorption syndromes. The requirement of vitamin E is related to the PUFA (polyunsaturated fatty acid) content of the diet. Selenium shows a vitamin E sparing effect (Scott, 1980). Vitamin E requirements may be higher for persons exposed to certain environmental pollutants (e.g. ozone and nitrites).

-Vitamin E metabolism in pregnancy and the effect of oral contraceptives

Maternal serum vitamin E levels rise during pregnancy up to 50% of non-pregnant values (Darby et al., 1957; Vobecky et al., 1974; Takahashi et al., 1978; Jagadeesan and Prema, 1980a). This increase in serum vitamin E level parallels the increase in serum lipid concentration (i.e. cholesterol), the main increase occurring in the second and third trimester. Vitamin E supplements during pregnancy hardly affect serum vitamin E levels (Baker et al., 1975), confirming the observations from Horwitt et al. (1972) and others, that serum vitamin E levels are also controlled by other metabolic processes and are not only a reflection of vitamin E intake and body stores.

The effect of oral contraceptive use on serum vitamin E levels is controversial. Horwitt (1974) and Jagadeesan and Prema (1980a) observed no significant consequence, but Yeung and Chen (1975) and Smith et al. (1975) did report a small increase.

Vitamin E levels in cord blood are considerably lower than in maternal blood. Leonard et al. (1972) and Mino and Nishino (1973) observed a significant correlation between maternal and cord serum levels. Haga and Lunde (1978) and Jagadeesan and Prema (1980b) did not find such a relationship. However, there was a strong correlation between serum vitamin E and the β -lipoprotein concentration (Haga and Lunde, 1978) in both maternal and cord serum. The lower vitamin E cord serum level may, therefore, reflect limited transport capacity rather than impaired placental transfer. The lower transport capacity in the

fetal circulation may be due to an inability of the fetus to synthesize enough carrier proteins (Jagadeesan and Prema, 1980b).

-Biochemical parameters for assessment of the vitamin E status

Determination of the serum content of α-tocopherol is the most frequently used parameter for assessment of the vitamin E status in humans. However, since vitamin E levels in the circulation are related to serum lipid levels (Horwitt et al., 1972), it has been suggested to express results in terms of amount of tocopherol per unit plasma or serum lipids (Combs, 1981). During pregnancy the increase in serum vitamin E levels parallel that in serum lipid content and the vitamin E/serum lipids ratio does not change significantly (Takahashi et al., 1978; Jagadeesan and Prema, 1980a). A more functional test is the measurement of erythrocyte fragility to resist hemolysis by H_2O_2 (Sauberlich et al., 1976). In animal experiments analysis of liver and adipose content is a valuable test (Combs, 1981).

-Vitamin E related pathology in pregnancy

A relationship between vitamin E status and outcome of pregnancy was suggested in some older studies. Vobecky et al. (1974) reviewed these studies and concluded that the data were equivocal. Their own data indicate an association of low α -tocopherol serum levels in pregnancies ending in stillbirth; however, the number of observations was limited. Jagadeesan and Prema (1980a) found no difference in the mean plasma vitamin E level of normal pregnant women and women with pre-eclampsia. Tapp and Anfield (1975) suggested a relationship between vitamin E deficiency and the incidence of sudden infant death syndrome (SIDS). SIDS death is more frequently found in infants with a complicated perinatal course such as prematurity, intra-uterine malnutrition and slight neurological disturbances. Vitamin E concentrations in term and preterm infants are, however, not significantly different (Petrich et al., 1976; Haga and Lunde, 1978). The finding of similar vitamin E (and selenium) levels in preterm compared with normal term infants casts some doubt upon

the etiology of the vitamin E responsive hemolytic anemia in preterm newborns.

-Birthweight

Tateno and Oshima (1973) reported a significant relationship between cord vitamin E serum level and birthweight but this was not confirmed by Vobecky et al. (1976) and Jagadeesan and Prema (1980b). Also, from experimental rat studies no correlation of birthweight to vitamin E intake is obvious (Martin and Hurley, 1977).

1.3.4. Thiamin.

Thiamin, in the form of its diphosphate, serves as a coenzyme in a number of reactions involved in carbohydrate and intermediary metabolism, especially the oxydative decarboxylation of α-keto acids such as pyruvate and α-ketoglutarate. About 80% of the thiamin stored in the body (± 30 mg) is present as the diphosphate (ThDP). The mono- and triphosphate forms (ThMP and ThTP) as well as free thiamin have also been demonstrated. Although excess dietary thiamin is excreted in the urine, a considerable quantity is degraded. At least 20 urinary thiamin metabolites have been identified up to now (Ariaye-Nejad et al., 1970). Because thiamin is particularly involved in carbohydrate and intermediary metabolism (Krebs cycle), thiamin requirements are mainly related to the energy release from carbohydrates and fats. Thiamin deficiency is characterized by cardiomyopathy and disturbances of the peripheral nervous system. Clinical signs may be observed within a few weeks in subjects maintained on a deficient diet. Less specific symptoms may be seen in a marginal thiamin deficiency, i.e. tachycardia, nystagmus, anorexia and fatique.

-Thiamin metabolism during pregnancy and fetal development

Thiamin metabolism seems to be unaffected during pregnancy, although the lowered thiamin excretion and the incidence of

increased transketolase stimulation ratios (see below) may reflect an increase in thiamin requirement. Thiamin readily crosses the placenta (Kaminetzky et al., 1974) and a significant relationship between maternal and cord blood thiamin status parameters has been observed (Tripathy, 1968). Studies with pregnant rats have shown that the fetus has a high ability to remove thiamin from the maternal circulation, even at the expense of maternal stores (Leclerc, 1978).

-Biochemical parameters for assessment of the thiamin status during pregnancy

Urinary thiamin excretion, thiamin blood levels and the erythrocyte transketolase (ETK) stimulation test have all been described as parameters of the thiamin status. During thiamin deficiency blood pyruvate and lactate concentrations are increased in the fasting state. Measurements of blood pyruvate and lactate in the fasting state, or after oral glucose loading, (carbohydrate metabolic index, Horwitt and Kreisler, 1949) have also been used to assess the thiamin status, but are no longer regarded as sufficiently specific or sensitive (Sauberlich et al., 1976). Urinary thiamin excretion is also considered to be inadequate (Kaufmann and Guggenheim, 1977) because it reflects recent dietary intake rather than thiamin status.

-Thiamin excretion

Thiamin excretion falls during the second and third trimester of pregnancy (Toverud, 1940; Darby et al., 1953). The variation between mean individual excretions is, however, large (Van der Rijst, 1962).

-Thiamin blood level

Only limited information is available regarding thiamin blood levels during pregnancy. Baker et al. (1975) found reduced blood levels in about 50% of a group of unsupplemented mothers at parturition. In a previously performed pilot-study, low blood levels were found in about 11% of a small group of Dutch parturient women (Van den Berg et al., 1978). Cord blood levels

are normally two to three times higher than maternal blood levels (Baker et al., 1975).

-Erythrocyte Transketolase (ETK) stimulation test

Measurements of the transketolase (ETK) activity in erythrocytes and the in-vitro stimulation with ThDP (αETK) is a functional test of the thiamin status (Sauberlich et al., 1976). Increased ETK stimulation ratios (> 1.20), indicating a marginal or deficient thiamin status, have been reported in 15-50% of cases during pregnancy (Tripathy, 1968; Heller et al., 1975; Kübler and Moch, 1975; Van den Berg et al., 1978; Vir et al., 1980). No relationship with the number of previous pregnancies, history of oral contraceptives, smoking or alcohol consumption has been found by Vir and Love (1980) in contrast to Kübler and Moch (1975) who observed higher mean stimulation ratios for women with higher ($\geq$ 2) parity and cigarette and alcohol consumption. Heller et al. (1975) observed that a deficiency of thiamin is established early in pregnancy and remains constant until delivery. This was concluded from the constant percentage of abnormal values throughout pregnancy. In a longitudinal study Vir et al. (1980) found that, from the 17 subjects in their group of pregnant women (n = 60) with too high ETK stimulation ratios (>1.20) in the second trimester, only five still revealed increased stimulation ratios during the third trimester. When the pregnant group was compared with non-pregnant controls, they found an increase in basal ETK activity during the second trimester followed by a decrease below non-pregnant levels during the third trimester. Since mean ETK stimulation ratios remained constant throughout pregnancy, this would imply (hormone induced?) changes in the levels of apoenzyme. A decrease in ETK apoenzyme level at the end of pregnancy was also described by Van den Berg et al. (1978) and Dirige et al. (1978). The level of enzyme activity is not related to the occurence of thiamin deficiency (Dirige et al., 1978). Low ETK apoenzyme levels have also been reported in women using oral contraceptives (Ahmed et al., 1975). However, Vir and Love (1978) and Lewis and King (1980) could not confirm this effect.

-Pathology related to thiamin deficiency in pregnancy

Despite the relatively high incidence of abnormal biochemical parameters reported in some of the aforementioned studies, only few reports are available in which a relationship between thiamin deficiency and any complication in pregnancy is indicated. In some older studies evidence was presented that thiamin deficiency was responsible for nausea, vomiting and eclampsia. Chauduri (1971) reported higher blood pyruvic acid levels after dextrose loading in pre-eclampsia and eclampsia. After intramuscular thiamin supplementation with 100 mg, a positive response was found: both toxemic features improved and blood pyruvic acid levels were normalized. However, this finding may be doubted since only 5 patients were treated and the parameter used to assess the thiamin status, i.e. blood pyruvic acid levels, is not very specific (Sauberlich et al., 1976). The positive response observed in these cases after supplementation with very high doses may reflect a therapeutic action of thiamin rather than a "nutritional" effect, i.e. repletion of depleted stores. The same may be true in the treatment of neurological complications that occasionally accompany hyperemesis gravidarum, a complication that has also been related with thiamin deficiency during pregnancy (Endtz, 1970). With the exception of Kübler and Moch (1975), no relationship between maternal thiamin status parameters and birthweight has been found. Thiamin blood levels of premature or small for gestational age babies are not different from those found in normal term babies (Baker et al., 1977).

1.3.5. Riboflavin.

Riboflavin in the form of mono- and dinucleotides (respectively, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) functions as a coenzyme in oxidation-reduction reactions, e.g. in the respiratory chain and oxidative phosphorylation, attached to a group of enzymes called flavoproteins.

Riboflavin has been demonstrated in all tissues principally

as a nucleotide. In blood, riboflavin occurs mainly in the erythrocytes. Riboflavin is stored in the body only to a limited extent; any excess of dietary riboflavin intake is promptly excreted in the urine. At present, no quantatively important degradation products have been reported.

Clinical signs normally associated with riboflavin deficiency are cheilosis, angular stomatitis, fissuring and atrophic papillae on the tongue.

-Riboflavin metabolism during pregnancy and fetal development; the effect of hormones on riboflavin metabolism

Although hyporiboflavinosis is by no means rare during pregnancy, its incidence seems much smaller compared to some other water soluble vitamins, e.g. folacin and vitamin B6. There is no evidence that hyporiboflavinosis or riboflavin deficiency is related with any obstetrical complication or pathology, or with length of pregnancy and pregnancy outcome.

No change or adjustment in riboflavin metabolism during pregnancy is indicated.

Riboflavin readily crosses the placenta (Kaminetzky et al., 1974), and the higher riboflavin concentration in fetal blood indicates an active transport mechanism. Lust et al. (1954) found free riboflavin to be the predominating form in the fetal circulation, while flavin adenine dinucleotide (FAD) is the main form in the maternal blood. So placental conversion of FAD into free riboflavin and a different transplacental transport between these molecules may be involved. From plasma of pregnant cows and blood of other species, e.g. chickens, a specific high-affinity riboflavin binding protein has been isolated (Merill et al., 1979). In non-pregnant animals higher riboflavin levels in blood can be induced by estrogen administration which causes increased riboflavin binding (Rivlin, 1975). In humans no such specific binding proteins have been identified.

The effects of synthetic estrogens (OCA) on riboflavin metabolism and riboflavin status parameters are somewhat obscure. Newman et al. (1978), Prasad et al. (1975) and Bamji (1979) found lowered riboflavin excretion, and an increase in

erythrocyte glutathione reductase (EGR) stimulation ratios, but Lewis and King (1980) and Vir and Love (1979) found no effects.

Biochemical parameters for the assessment of riboflavin status in pregnancy

-Riboflavin excretion

Urinary riboflavin excretion, spontaneous or after a riboflavin overload is the parameter used in older studies (Oldham et al., 1950 and Brzezinsky et al., 1952). In general, lowered riboflavin excretion is found during pregnancy. Urinary excretion is, however, influenced by many factors, kidney function and nitrogen balance, and reflects recent intake from the diet rather than riboflavin status (Heller et al., 1974). Nowadays, riboflavin excretion as a parameter of riboflavin status has been replaced by other tests in the majority of reports, e.g. riboflavin blood level and the EGR stimulation test.

-Riboflavin concentration in blood

Erythrocytes normally have higher riboflavin levels than plasma (Baker et al., 1969). Whole blood riboflavin levels in pregnant women at the end of pregnancy are generally lower than those of non-pregnant women but usually still within the accepted range (Baker et al., 1975; Clarke, 1969 and Knobloch et al., 1979).

Higher levels are found in the neonate; neonatal-maternal ratios between 1.2 and 2 have been reported (Baker et al., 1975; Van den Berg et al., 1978 and Clarke, 1971). Knobloch et al. (1979) calculated the mean corpuscular riboflavin concentration (MCRC) in paired maternal and cord blood samples and found no significant difference, indicating that the observed differences in whole blood riboflavin content can be accounted for by a different blood cell volume.

-Erythrocyte glutathione reductase (EGR) stimulation test

Measurement of the EGR activity and *in-vitro* stimulation with

FAD (α EGR) is a functional test of the riboflavin status (Glatzle et al., 1970). Using this test, Heller et al. (1974) found an increase in the incidence of marginal or deficient riboflavin status in the course of pregnancy. At the end of pregnancy, 42% of the women demonstrated too high stimulation ratios. A comparable incidence (30%) was found in our previous study in a small group of parturient women (Van den Berg, 1978). Considerably lower incidences (< 10%) were reported by a.o. Hunt et al. (1976), Jacob et al. (1976) and Kübler (1981). Vir et al. (1981) found in a longitudinal study, no evidence for a significant change in EGR stimulation ratio during normal pregnancy.

In all these studies, cut-off points for αEGR based on non-pregnant reference groups were used. Smithells et al. (1976) found a mean EGR stimulation ratio of 1.23 in about 1,300 normal, pregnant females during the first trimester. From their data a socio-economic gradient in riboflavin status was apparent. Recently, Bates et al. (1981) reported a mean EGR stimulation ratio of 1.19 $\pm$ 0.08 for a healthy pregnant population (n = 59) in Cambridge (U.K.) (mean riboflavin intake 2.2 $\pm$ 0.7 mg/day). About 20% of the values were above the upper normal limit (1.30). We (Van den Berg et al., 1978) questioned the validity of the EGR stimulation test to assess riboflavin status in pregnancy because we found a slight increase in EGR activity, indicating elevated EGR apoenzyme levels, and suggested a relative rather than a true erythrocyte FAD deficiency as total riboflavin blood levels were normal.

Dirige et al. (1978) found constant EGR apoenzyme levels throughout pregnancy. High EGR apoenzyme levels can be found during iron-deficiency anemia (Ramachandran and Iyer, 1974), so iron deficiency may probably be a modulating factor in the performance of the EGR stimulation test. A coincidence between low hemoglobin levels and increased EGR stimulation during pregnancy was reported by Decker et al. (1975). From their data it can not be concluded whether the increased stimulation ratios were due to an increase in apoenzyme level since only the stimulation ratio, but not the EGR activity, was reported.

-Pathology related to riboflavin deficiency in pregnancy

Clinical signs of riboflavin deficiency have been observed during pregnancy in undernourished populations (Bamji, 1976; Bates et al., 1981). An increased incidence of hyperemesis gravidarum and prematurity was reported by Brzezinsky et al. (1952). More recent studies, mostly performed in developed countries, did not reveal any relationship between riboflavin deficiency and pregnancy complications or outcome (Heller et al., 1974).

The increased incidence of elevated αEGR values among women with low hemoglobin levels has already been mentioned (Decker et al., 1975). When these women were supplemented with iron (300 mg FeSO4/day), a small, but significant further decrease in hemoglobin levels was observed, but not when iron and riboflavin were administered together (Decker et al., 1977). Clarke (1973) reported lower blood riboflavin levels in anemic women treated with iron and folic acid supplements during pregnancy when compared with untreated anemic mothers or when riboflavin was included in the supplements. The lower blood riboflavin concentration in the anemic pregnant women treated only with iron supplements was thought to result from the increased red cell volume following treatment with iron.

1.3.6. Vitamin B6.

The term vitamin B6 is used as the generic name for all 2-methyl-pyridine derivatives exhibiting qualitatively the biological activity of pyridoxine (Committee on Nomenclature IUNS/AIN, 1977). B6-Vitamers absorbed from the diet are rapidly converted in the body into the coenzyme forms, pyridoxal-5'-phosphate (PLP) and pyridoxamine-5'-phosphate (PMP) (McCoy and Colombini, 1972).

Total body stores of vitamin B6 have been estimated to be between 20 and 150 mg with a total turnover rate of about 3% per day (Tillotson et al., 1966; Shane, 1978). About half of the total vitamin B6 content is stored in muscle as PLP bound to the

enzyme glycogenphosphorylase; muscle PLP is, however, only available in a state of muscle protein breakdown (Black et al., 1978). Both PLP and PMP are stored in liver, brain and kidneys. Protein binding of PLP to tissue proteins plays an important role in the regulation of tissue PLP content, protecting PLP against hydrolysis and subsequent oxidation to 4-pyridoxic acid, the main excretion product of vitamin B6 (Li et al., 1974). In plasma both phosphorylated and non-phosphorylated B6-vitamers have been demonstrated.

Vitamin B6 is, via its coenzyme PLP, involved in many enzymatic reactions, especially in amino acid metabolism, i.e. racemization, transamination, decarboxylation and desulphydration reactions. An interaction between PLP and cytosolic and nuclear glucocorticoid receptors has been recently demonstrated. Evidence was obtained that binding of PLP to steroid-receptor complexes influences receptor conformation and inhibits binding of these complexes to the nuclei (Cake et al., 1978); Cidlowski and Thanassi, 1979).

Vitamin B6 metabolism during pregnancy and fetal development

Much of the recent knowledge about adaptations or changes in vitamin B6 metabolism during (human) pregnancy is based upon studies in which qualitative and quantitative changes in blood and urinary B6 excretion were studied. When interpreting such data, it should be kept in mind that the amount of vitamin B6 in the circulation represents only 1%, or less, of the total body stores (Shane, 1978). More data about vitamin B6 metabolism during pregnancy are available from animal experiments done with pregnant rats and mice. Contractor and Shane (1971) described in-vivo labelling studies with 14C-pyridoxine in pregnant rats. Patterns of labelling in the different tissues were not different from those found with non-pregnant rats, indicating that no significant changes occurred in maternal vitamin B6 metabolism. Compared with the rat, the human placenta has a much lower PLP content (Klieger et al., 1969). This may be explained by the lower pyridoxine kinase activity in human placental tissue (Contractor and Shane, 1969). Also, the enzyme pyridoxine

5'-phosphate oxidase has been demonstrated in the placenta (Dempsey, 1978). Both enzymes are involved in PLP formation from pyridoxine and pyridoxal.

Pyridoxine kinase and pyridoxine-5'-phosphate oxidase activity have also been demonstrated in fetal liver. The specific kinase activity increases during fetal development while fetal oxidase activity is kept on a low level. The low fetal oxidase activity is thought to be the rate limiting factor in fetal PLP synthesis (Shane and Contractor, 1980). Although the fetus is able to synthesize its own PLP evidence has been presented that the PLP in the fetal circulation is derived from the maternal circulation and placental PLP synthesis. This was concluded from loading studies in which the maximum maternal PLP blood levels, after a vitamin B6 load, always preceded the PLP peak in the fetal circulation (Contractor and Shane, 1970). The observed difference between arterial and venous cord blood PLP content (with the higher PLP concentrations in the venous blood) suggests a transplacental PLP transport (Cleary et al., 1975). The PLP concentation of cord blood is on average 3-5 times higher than that in the maternal circulation. A significant correlation was found between paired maternal and cord blood PLP values (Lumeng et al., 1976).

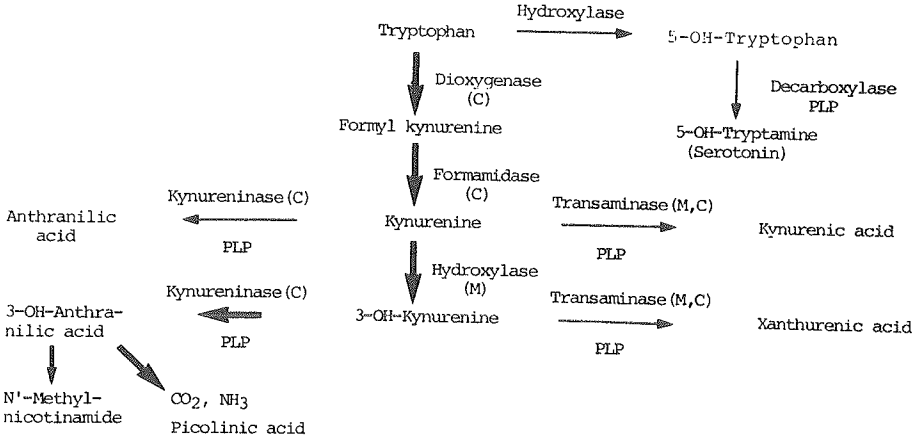
-Hormonal effects on vitamin B6 metabolism

During vitamin B6 deficiency, tryptophan metabolism is disturbed, leading to increased excretion of xanthurenic acid and other tryptophan metabolites after an oral load of tryptophan (Henderson and Hulse, 1978). High urinary excretions of xanthurenic acid after tryptophan loading were also reported for women using oral contraceptives and in post-menopausal women treated with synthetic estrogens (Rose, 1966 and 1978; Haspels et al., 1975). Also during pregnancy, xanthurenic acid excretion is increased after an oral load of tryptophan (Wachstein, 1964). The disturbed tryptophan metabolism and the relatively large amounts of pyridoxine required to restore xanthurenic acid excretion to normal (see below) were regarded for many years as an indication of vitamin B6 deficiency in

pregnancy and during oral contraceptive use.

However, it has become clear that disturbance in tryptophan metabolism can arise from hormonal changes and may not necessarily indicate a vitamin B6 deficiency per se (Leklem et al., 1975; Vir and Rose, 1980). Estrogens cause an induction of the enzyme tryptophan 2,3-dioxygenase resulting in an increased tryptophan catabolism (Braidman and Rose, 1971). Due to the ability of the PLP-dependent enzyme kynureninase, the increased flux of tryptophan into the tryptophan-niacin catabolic route results in an accumulation of such intermediates as xanthurenic acid (see Figure 1.3.6.I). The enzyme induction caused by estrogens is possibly mediated via corticosteroids. Mason et al. (1969) suggested a competition of estrogensulphate esters with PLP for binding on the coenzyme binding sites of PLP-dependent enzymes. All these effects result in an increased vitamin B6 requirement.

FIGURE 1.3.6.I Metabolic pathway for the degradation of tryptophan through the Kynurenine-niacin pathway. The heavy arrows indicate the major pathway under normal physiological conditions. The PLP dependence of some of the enzymes and their subcellular localization in the cytosol (C) or mitochondrion (M) are indicated.



Lumeng et al. (1974) and Shane and Contractor (1975) reported significantly lower PLP plasma levels in women using oral contraceptives. However, the lowering effect of oral contraceptives decreased in magnitude after 3 months of therapy. Using the EGOT or EGPT stimulation tests (see below) as parameters for the assessment of vitamin B6 status, such a

"pill-effect" can usually not be demonstrated (Shane and Contractor, 1975; Vir and Love, 1980). Recently, Roepke and Kirksey (1979) reported significant lower PLP plasma levels in pregnant and lactating women who had used oral contraceptives for at least 30 months before conception. These women were compared with pregnant and lactating women who did not use oral contraceptives or did so for only a short period before conception. Haspels et al. (1978) and Wolf et al. (1980) demonstrated that natural estrogens induced abnormalities of tryptophan metabolism similar to those induced by synthetic estrogens.

-Biochemical parameters for assessment of the vitamin B6 status during pregnancy

Both direct and indirect parameters for assessment of the vitamin B6 status are available (Sauberlich et al., 1976). The direct parameters include measurement of vitamin B6 content in blood and urine. An indication of intracellular tissue content and action is obtained, using the indirect, and more functional tests. Among the latter are the tryptophan loading test (see before) and the EGOT and EGPT stimulation tests.

In the enzyme stimulation test, the activity of the PLP dependent glutamate-oxaloacetate transaminase (GOT) or glutamate-pyruvate transaminase (GPT) is measured in hemolysates of erythrocytes before and after in-vitro stimulation with excess PLP.

-The tryptophan loading test.

Much of the older research about the vitamin B6 status during pregnancy is based upon this test (Wachstein, 1964). Increased xanthurenic acid excretion after an oral tryptophan load was frequently observed, especially in the third trimester. Supplementation with at least 10 mg vitamin B6 is needed to "normalize" tryptophan metabolism (Coursin and Brown, 1961). As already indicated, this test does not reflect vitamin B6 deficiency, per se, and may be less suitable for evaluation of

the vitamin B6 status in pregnancy.

-Enzyme stimulation tests.

Both the EGOT and EGPT stimulation test are considered specific and valid indicators of vitamin B6 status in humans, a judgement based upon depletion-repletion studies (Cinnamon and Beaton, 1970; Sauberlich et al., 1972). The EGOT activity in hemolysates is considerably higher than the EGPT activity and that is probably the reason why the EGOT stimulation test is used more frequently. Lumeng et al. (1976) and Shane and Contractor (1975) have questioned the sensitivity and reproducibility of these tests. Conflicting results and differences in reference values may at least partially, be explained by different methodology and insufficient standardization (Bayoumi et al., 1976). Using the EGOT stimulation test, Heller et al. (1973) found elevated stimulation ratios in 40-60% of (German) women with uncomplicated pregnancies. At all stages of pregnancy, high percentages were observed, even in 33% of women who were less than 6 weeks pregnant. Their cut-off point of 1.66 for αEGOT was based upon non-pregnant reference values ($x \pm 2$ S.D.). We found comparable percentages of abnormal values in a previous pilot-study of parturient women (Van den Berg et al., 1978). Lower percentages were reported by Kübler and Moch (1975) (17% >2.00) and Lumeng et al. (1976) (5% > 1.50). Using the EGPT stimulation test, the latter authors found 15% elevated (> 1.25) stimulation ratios. Hunt et al. (1976) and Shane and Contractor (1975) found abnormal values in less than 5% of their pregnant test subjects. In their longitudinal study on vitamin B6 status during pregnancy, Vir et al. (1980) observed 40-50% abnormal values (> 1.15) in a group of 60 healthy pregnant Caucasian women both in their second and third trimester as well as 3 days postpartum. Individual variation was large. Of the 30 women with abnormal values in the second trimester, 22 were examined again in the third trimester and 12 of these 22 were reexamined 3 days postpartum. Only 8 of these 22 subjects showed biochemical deficiency and 5 of the 12 women examined postpartum.

The interpretation of enzyme stimulation tests can be complicated by secondary influences on the erythrocyte apoenzyme content. Changes in apoenzyme content can mask vitamin B6 deficiency or indicate an apparent vitamin B6 deficiency. The first situation may occur when apoenzyme levels are lowered, the latter when apoenzyme levels are increased. Apoenzyme levels may be affected by hormones (Rose, 1978), during vitamin supplementation (Lumeng, 1978) and chronic vitamin deficiency (Bamji, 1970 and Pandit and Chakrabarti, 1972).

Bamji (1976) reported increased EGPT apoenzyme levels in a small group (n = 15) of pregnant women during their third trimester. Dirige et al. (1978) found, however, no evidence for a change in EGPT apoenzyme content in the course of pregnancy.

-Pyridoxic acid excretion.

Determination of 4-pyridoxic acid, the main vitamin B6 metabolite in urine, is sometimes used as a parameter to assess vitamin B6 status. Like most other urinary vitamin status parameters, pyridoxic acid excretion reflects recent dietary intake rather than vitamin (tissue) stores, at least, when vitamin absorption and metabolism are unimpaired (Linksweiler, 1978). During pregnancy no significant change in pyridoxic acid excretion occurs both with and without pyridoxine loading (Contractor and Shane, 1970).

-Vitamin B6 in blood.

Both phosphorylated and non-phosphorylated B6 vitamers have been demonstrated in human blood (Shane, 1978). Besides pyridoxal, albumin-bound PLP is the main circulating metabolite and is equally distributed between plasma and erythrocytes under normal conditions (Bhagavan et al., 1975). Plasma PLP is derived exclusively from the liver, while erythrocyte PLP is synthesized within the red cell in which both pyridoxine kinase and pyridoxamine-5'-phosphate oxidase activity has been demonstrated (Lumeng et al., 1974; Anderson, 1980). Earlier methods for estimation of vitamin B6 blood levels were mainly microbiological assays, but they have been replaced in most

laboratories by radioenzymatic assays (Chabner and Livingstone, 1978) or high performance liquid chromatography (HPLC) methods (Schrijver et al., 1981). Plasma PLP levels are now thought to be a sensitive and reliable indicator of vitamin B6 status in humans (Shane, 1978; Lumeng et al., 1978). A progressive decrease in plasma PLP content has been reported during pregnancy (Hamfelt and Tuvemo, 1972; Lumeng et al., 1976 and Shane and Contractor, 1975). For non-pregnant subjects, mean PLP plasma levels between 5 and 15 ng/ml (18-60 nmol/l) are found. PLP levels are decreased to 1-5 ng/ml (4-18 nmol/l) at the end of pregnancy. Plasma PLP levels fall at this time even after supplementation with 10 mg/day (Hamfelt and Tuvemo, 1972; Cleary et al., 1975 and Lumeng et al., 1976).

Plasma dilution, an estrogen induced increase in maternal tissue retention, fetal sequestration and a disturbed vitamin B6 metabolism have all been proposed as explaining factors for the fall in plasma PLP content. A more quantitative approach establishing to what extent these effects can account for the observed decrease in plasma PLP, is unknown and is one of the objectives of this study.

Pathology related to vitamin B6 deficiency in pregnancy

Dietary deprivation of vitamin B6 in adults may result in electro-encephalographic abnormalities, depression and confusion, cheilosis and seborrheic dermatitis and, in infants, epileptiform convulsions, microcytic hypochromic anemia and distress have been reported. Clinical vitamin B6 deficiency in man is, however, extremely rare. Both in earlier and more recent reports evidence has been presented that vitamin B6 inadequacy may affect course and outcome of pregnancy. Reports on B6-related pathology in pregnancy are summarized below:

-Toxemia.

Significantly lower PLP levels in maternal and cord blood were reported in pre-eclampsia (Brophy and Siiteri, 1975). Also, the placental PLP content was found to be reduced in toxemic

subjects (Klieger et al., 1969). This could be explained by the reduced pyridoxine kinase- and pyridoxamine-5'-phosphate oxidase activity measured in these placentas (Gaynor and Dempsey, 1972). Pyridoxine supplementation had no positive effect upon the incidence of pre-eclampsia (Hillman et al., 1963). It is not unlikely that the coincidence of a disturbed vitamin B6 metabolism and the hypertensive disorders of pregnancy reflect a common etiology rather than a cause-effect relationship.

-Hyperemesis gravidarum.

A beneficial effect of pyridoxine supplementaion in cases of hyperemesis gravidarum was first reported by Willis et al. (1942) and has been confirmed in some, but not in all, studies (McGanity et al., 1942; Reinken and Gant, 1974 and Hemminki and Starfield, 1978). It is unknown to what extent the low PLP plasma levels in these women are caused by an inadequate dietary vitamin B6 supply in their first weeks of pregnancy because of vomiting and abdominal distress.

-Pregnancy depressions.

Depressive syndromes, both during pregnancy and in the postpartum period as well as in women using hormonal contraceptives, have been associated with a disturbed tryptophan metabolism (relative 5-hydroxytryptamine deficiency). Treatment of these patients with vitamin B6 gave positive results (Adams et al., 1974). Pullkinen et al. (1978) described a significant correlation between the depth of depression and serum vitamin B6 content. Livingstone et al. (1978) assessed vitamin B6 status by means of the EGOT stimulation test in 24 women with postpartum depression and in 20 women not depressed postpartum. No evidence for vitamin B6 deficiency in women suffering from postpartum depression was found.

-Gestational diabetes.

Coelingh Bennink and Schreurs (1975) and also Spellacy et al. (1977) reported a significant improvement in the oral and

intravenous glucose tolerance test after treatment of women with gestational diabetes with massive doses of vitamin B6 (50-100 mg/day). In a more recent study no relationship was found between the degree of xanthurenic acid excretion and the glucose level during the oral glucose tolerance test in groups of patients treated either with a placebo of with vitamin B6. However, the incidence of abnormal glucose tolerance was significantly lower in a group of pregnant women treated with vitamin B6 when compared to a group treated with placebo (Coelingh Bennink, 1980).

-Vitamin B6 and intrauterine growth

Kaminetzky et al. (1973), Kübler and Moch (1975) and Reinken and Dapunt (1978) reported a relationship between marginal vitamin B6 status and low birthweight. The latter authors found a positive correlation between serum PLP concentration in the second month of pregnancy and birthweight. Also, the decrease in PLP level between the second and fourth month of pregnancy correlated with birthweight. Although no significant difference in birthweight could be demonstrated between infants born to mothers with marginal vitamin B6 status (low blood vitamin B6 levels or high stimulation ratios) and mothers with adequate vitamin B6 status, Roepke and Kirksey (1979) and Schuster et al. (1981) reported lower Apgar scores in infants whose mothers were vitamin B6 deficient. Hamfelt and Tuvemo (1972), Heller et al. (1973) and Vir et al. (1980) found, however, no evidence for a relationship between vitamin B6 status and pregnancy outcome.

Vitamin B6 requirement in pregnancy

To prevent the fall of plasma PLP content during pregnancy below non-pregnant standards (i.e.< 4 ng/ml), supplemental doses of 2-10 mg/day are required (Hamfelt and Tuvemo, 1972; Cleary et al., 1975 and Lumeng et al., 1975). Plasma PLP levels, after an initial increase, decreased at the end of pregnancy, even at the high supplementation level (10 mg/day) (Hamfelt and Tuvemo,

1972). Supplemental vitamin B6 doses of 2.5-10 mg/day are required to keep the EGOT stimulation test within the non-pregnant reference range (Lumeng et al., 1976). Suppression of xanthurenic acid excretion after tryptophan loading requires even higher supplemental doses (Coursin and Brown, 1961; Wachstein, 1964). Although Khera (1975) reported no teratogenic effects in rats after giving high doses of pyridoxine during pregnancy, Contractor and Shane (1975) opposed the use of vitamin B6 supplements in pregnancy because of the possibly adverse effects of supplementation on synthesis of fetal PLP enzymes, leading to a high vitamin B6 requirement after birth. Greentree (1979) dissuaded prenatal vitamin B6 supplements because of the possible suppression of prolactin levels inhibiting breast milk secretion (Foukas, 1973). In, recently reported, intervention studies (e.g. Ejderhamn and Hamfelt, 1980) no evidence for such an anti-lactogenic effect was obtained after supplemental pyridoxine (2-6 mg/day) during pregnancy.

Clinical evidence correlating vitamin B6 deficiency with pregnancy complications is limited, and clinical trials of routine pyridoxine supplementation in pregnancy have failed to indicate any difference in outcome. For these reasons, the allowance for vitamin B6 in pregnancy, accepted by the NAS/NRC (9th Ed., 1980), is increased by only 0.6 mg/day above the 2.0 mg/day accepted for non-pregnant females, providing for the additional protein allowance during pregnancy.

1.3.7. Vitamin B12.

The term vitamin B12 should be used as the generic name for all corrinoids, i.e. compounds containing a corrin nucleus, exhibiting qualitatively the biological activity of cyanocobalamin (Committee on Nomenclature IUNS/AIN, 1977). Vitamin B12 can be absorbed only when bound with Intrinsic Factor, a mucoprotein secreted by the parietal cells of the stomach. It is the only water soluble vitamin that is stored to

an appreciable extent; body stores have been estimated to be 1-10 mg with a daily turnover of about 0.1-0.2% (WHO, 1970). This relatively slow turnover rate is attributed to the effective enterohepatic recycling of the vitamin from bile and other intestinal secretions. Vitamin B12 coenzymes, methyl- and 5'-deoxyadenosyl-cobalamine, are involved in the transfer of labile C_1 (methyl)-groups in the nucleic acid and amino acid metabolism.

A close interrelation exists between folacin and vitamin B12 metabolism. Only a small number of enzyme reactions involving vitamin B12 coenzymes have been identified at present. Vitamin B12 deficiency is characterized by hematological and neurological symptoms.

-Vitamin B12 metabolism during pregnancy and fetal development; hormonal effects

Serum vitamin B12 levels fall progressively during pregnancy, followed by a spontaneous increase to pre-pregnancy values within 3-5 weeks after delivery. Low levels of serum vitamin B12 in pregnancy may be associated with folacin deficiency (Lawrence and Klipstein, 1967).

Factors thought to be involved in the progressive decrease of serum vitamin B12 level in pregnancy are hemodilution and active transfer to the fetus. Vitamin B12 is readily transported across the placenta (Luhby et al., 1961; Kaminetzky, 1974). Inadequate dietary intake does not seem to play a role as indicated by the supplementation studies of Metz et al. (1965). Tracer studies from Luhby et al. (1961) suggested decreased gastrointestinal absorption, but other studies indicate normal absorption even in patients with megaloblastic anemia in the puerperium (Chanarin, 1969). Chanarin (1969) calculated the total vitamin B12 content of the fetus at term to be about 50 µg, less than 2% of the maternal stores, so fetal sequestration of maternal stores is unlikely to explain the fall in maternal vitamin B12 level.

The fall in serum vitamin B12 most likely represents a change in vitamin B12 metabolism. The investigations from

Shojania and Wylie (1979), who studied the effect of oral contraceptives on vitamin B12 metabolism, suggest that metabolic change is on the level of vitamin B12 serum binding. Low serum vitamin B12 levels in women using oral contraceptives have been found (Wynn, 1975). Urinary methylmalonic acid excretion and vitamin B12 absorption (using the Schilling test) were normal. They did find a significant lower total serum vitamin B12 binding capacity in their group of oral contraceptive users. The decreased binding capacity was found to be related to lower transcobalamin I levels. Transcobalamin I is the major vitamin B12 carrier in plasma, binding about 90% of the circulating vitamin B12, but it is not essential for vitamin B12 transport to the tissues. TC-II plays a major role in vitamin B12 transport. A study on the changes in transcobalamin levels during pregnancy was, recently, reported by Fernandez-Costa and Metz (1982). Unsaturated TC-I and TC-II rose steadily in the course of pregnancy and fell again in the puerperium. TC-II decreased in the second trimester, increased sharply in the third trimester, but fell again in the puerperium. These changes seem much more complex than those observed in oral contraceptive users. Cord blood transcobalamins showed a greater saturation than maternal transcobalamins.

-Biochemical parameters to assess vitamin B12 status in pregnancy

Determination of the serum vitamin B12 concentration has been found a useful and reliable parameter for evaluation of the vitamin B12 status (Sauberlich et al., 1976). Vitamin B12 levels in serum decrease progressively with a mean decrease of about 100-150 pg/ml (75-115 pmol/l) (Okuda et al., 1956; Löwenstein et al., 1960; Chanarin, 1969; Metz, 1965). This pattern is not changed by vitamin B12 supplementation (Metz, 1965). At the end of the third trimester, low serum vitamin B12 levels (< 150 pg/ml) have been reported in 10-30% of women (Chanarin, 1969). Cord blood levels are almost 2-3 times higher than maternal levels (Baker, 1975; Van den Berg, 1978). Kalamegham and Krishnaswamy (1977) studied the physiological significance of

low serum vitamin B12 late in pregnancy. They measured methylmalonic acid (MMA) excretion after a valine load in 37 women. Most patients with true vitamin B12 deficiency excrete abnormal amounts of MMA after valine loading due to failure of the vitamin B12 (cobamide-) coenzyme dependent isomerization reaction of methylmalony-CoA to succinyl-CoA (Gompertz, 1967) In their group of 37 women, 23 had vitamin B12 levels below 150 pg/ml, 8 women below 100 pg/ml, indicating moderate (< 150 pg/ml) or severe (< 100 pg/ml) vitamin B12 deficiency. However, only one women showed abnormal MMA excretion after a valine load; so the fall in serum vitamin B12 level during pregnancy does not seem to reflect an unsatisfactory maternal vitamin B12 status.

The excretion of some histidine metabolites (aminoimidazole carboxamide (AIC), urocanic acid and formiminoglutamic acid (FIGLU)) has been reported in vitamin B12 deficiency, secondary to an induced folacin deficiency (Van Roon-Djordjevic and Van Straalen, 1972), but has not been used, to our knowledge, for evaluation of the vitamin B12 status in pregnancy.

-Vitamin B12 related pathology in pregnancy

Vitamin B12 deficiency caused by dietary inadequacy is rare except perhaps in strict vegetarians. Pernicious anemia (PA) has a negligible incidence during pregnancy (PA is accompanied by infertility, Chanarin, 1969). Recently Schorah (1980) reported low serum vitamin B12 levels in women who gave birth to children with neural tube defects.

Whiteside et al. (1968) explained birthweight and duration of pregnancy, using serum folacin, vitamin B12 and iron level measured at the twenty-sixth week as the independent variables in multiple repression analysis. The contribution of vitamin B12 serum level was negligible. Baker et al. (1977) reported significantly lower serum vitamin B12 levels in cord blood of small for gestational age (SGA) (< 2,500 g) babies compared with normal weight, term infants. Maternal values were not different between both groups.

1.3.8. Folacin.

Folacin is the generic name for folic acid and related compounds exhibiting qualitatively the biological activity of folic acid (Commiitee on Nomenclature IUNS/AIN, 1977). In literature the term folate is also frequently used to describe this group of compounds. The biochemically active forms are all derivatives from the reduced form of folic acid, tetrahydrofolic acid (THF). The naturally occurring folates, including the enzymatically active forms, are mainly polyglutamates with 1-7 glutamic acid molecules attached to the pteroyl-molecule.

There is still a controversy about the availability of these polyglutamate forms in man. In the intestinal lumen and within the mucosal cells, an enzyme folate conjugase has been demonstrated which splits the polyglutamates into the readily absorbable, short chain glutamates ("free folate", Hurdle, 1973; see section 1.2.4).

More than half of the body stores of folacin (about 10-20 mg) are in the liver, stored primarily as 5-methyltetrahydrofolic acid polyglutamates. In plasma only 5-methyltetrahydrofolic acid (monoglutamate) has been demonstrated.

Folacin coenzymes are involved in the transfer of C_1-fragments, i.e. substituted THF derivatives (CHO-, CH_3-) functioning as C_1-donors, and THF as C_1-acceptor, especially in protein (amino-acid) and nucleic acid metabolism. Therefore, folacin requirements are increased during conditions with high metabolic rates and rate of cell synthesis, e.g. in pregnancy.

Megaloblastic anemia is the most pronounced and best documented clinical symptom of folacin deficiency. Oval macrocytes and hypersegmented neutrophils are usually found in the peripheral blood long before megaloblastic changes in bone marrow cells appear. These changes are, however, not specific for folic acid deficiency.

-Folacin metabolism in pregnancy

A progressive decline of serum and red cell folacin concentrations during pregnancy has been described. Prepregnancy

values can be maintained in the course of pregnancy only after supplementation with folic acid. The decreasing serum and red cell folacin levels are thought to reflect depletion of maternal stores caused by increasing fetal demands, blood volume expansion, increased urinary excretion and changes in maternal folacin absorption and metabolism. The level in fetal serum and red cells are almost invariably higher than in maternal blood (Avery and Ledger, 1970; Baker et al., 1977; Van den Berg et al., 1978).

Placental transfer of folacin to the fetus seems to be an efficient proces, enabling normal intra-uterine hemopoiesis even in situations of maternal deficiency. Landon and Hytten (1975) studied placental folacin transport, using ^{3}H-labelled folic acid. Surprisingly, they found only a small percentage of the intravenously administred dose in the fetus after 24 hours; most of the label was found in the urine and maternal stores, as well as in the placenta. The observed lack of transfer to the fetus may possibly occur because a small unique tracer dose does not equilibrate with the body stores. Fetal hepatic folacin stores seem to be related to fetal weight. Inadequate hepatic folacin stores in situations of intra-uterine growth retardation were reported by Iyenger and Apte (1972), Vaz-Pinto et al. (1975) and Loria et al. (1977). At term, the fetal liver contains about 300 µg folacin.

Although shunting of folacin across the placenta into the fetus against a concentration gradient has not been fully explained, protein binding seems to be involved. Kamen and Caston (1975) identified a specific folacin binding protein in cord serum. Some of the earlier studies (Grossowicz et al., 1966) indicated that formyl folate derivatives form an important constituent of fetal blood, suggesting the existence of a selective transport mechanism. 5-Methyl-THF, the main circulating form in maternal plasma, is taken up by the placenta and converted into a formyl-derivative which is subsequently trapped in the fetal circulation. Folacin binding proteins have also been identified in the blood of pregnant women,

concentrations increasing in the course of pregnancy (Da Costa and Rothenberg, 1974; Colman and Herbert, 1976; Areekul et al., 1977). In an excellent study, Fernandez-Costa and Metz (1979) investigated the role of these serum folacin binders. They found that a specific high affinity, low capacity binder (specific FBP, described by Colman and Herbert, 1976) was primarily involved in conservation of folacin in the maternal stores while a so-called unspecific FBP (low affinity, high capacity) was involved in delivering folacin to the sites of utilization, i.e. the fetus rather than maternal stores. In cord blood, high concentrations of specific FBP were found concentrating folacin across the placenta.

So folacin levels in maternal and fetal circulation depend partly on specific protein binders whose concentrations seem to be hormone regulated (Markkanen et al., 1974).

The effect of plasma volume expansion on serum folacin concentration was studied by Hall et al. (1976). They calculated total serum folacin, using measured plasma volumes, and found total folacin to be constant throughout pregnancy. However, in smokers and in twin pregnancies a significant decrease was observed at the end of pregnancy. This decrease of folacin levels was caused mainly by the lower plasma volume expansion observed among smokers since no significant differences between serum folate concentration in primigravid singleton pregnancies according to smoking habit was found. Increased plasma folacin clearance in pregnancy was described by Fleming (1972) and Landon and Hytten (1971). It was found to be independent of creatinine clearance suggesting that it was the result of altered renal tubular function rather than of an increased glomerular filtration rate (Fleming, 1972). Fleming also observed a significant correlation between serum folacin concentration and urinary folacin clearance in his group of pregnant women. This correlation seems to be based upon observations of different women at different stages of pregnancy, so this relationship may reflect a coincidence in falling serum folacin levels and increasing clearance rates rather than a causal relationship. Folacin losses via the urine

may increase up to 15 µg/day (range 1.8-15 µg/24 hrs.). In older literature, impaired folacin absorption is frequently mentioned as one of the causes of low serum folacin in the mother (Giles, 1966), but more recent studies indicate normal absorption (Landon and Hytten, 1972). McLean et al. (1970) did not observe any difference on gastrointestinal absorption between mono- and polyglutamate folate forms during pregnancy.

Finally, folacin deficiency in pregnancy may be explained, at least partly, by increased liver enzyme activity. Folacin is known to be a cofactor for microsomal enzyme systems (Hagerman, 1964), and prolonged treatment with enzyme-inducing agents has been shown to be associated with lower than normal levels of serum and red cell folacin (Maxwell et al., 1972; Davis et al., 1973).

-Folacin and oral contraceptive use

Several investigators have reported low serum and red cell folacin levels in women using oral contraceptives (Shojania et al., 1969; Prasad et al., 1975; Smith et al., 1975; Ahmed et al., 1975; Martinez and Roe, 1977). As in pregnancy this decrease in serum folacin occurs in spite of an increase of specific folate-binding protein in plasma of oral contraceptive users (Da Costa and Rothenberg, 1974). Bamji et al. (1979) found evidence that oral contraceptives reduced the half-life of the labile folacin pool, which is in equilibrium with dietary folacin, but increased the half-life of the stable pool of folacin in liver. This may explain the observed increase in urinary folacin excretion and decrease in serum folacin content.

Streiff (1970) suggested oral contraceptive induced inhibition of folacin conjugase impairing folacin absorption, which was not confirmed by other investigators (Stephens et al., 1972).

Biochemical parameters to assess the folacin status in pregnancy

Determination of serum and red cell folacin by microbiological or radioisotopic methods are the most frequently used biochemical parameters for evaluation of the folacin status. Folacin excretion (with or without an oral folacin load) and formiminoglutamic acid (FIGLU) excretion after a histidine load have also been used.

Peripheral blood and bone marrow examinations are more functional tests and are used mostly to confirm biochemical findings. The sequence of deficiency signs observed by Herbert (1962) in his classic experimental depletion study is summarized below:

Day from start of study	Deficiency sign
Day 10-20	Decreasing serum folacin concentration
Day 22	Serum folacin concentration, < 3 ng/ml
Day 35	Megaloblastic staining in bone marrow aspirate
Day 50	Hypersegmentation in peripheral blood smear
Day 100	Increased FIGLU excretion
Day 120	Red cell folacin, < 20 ng/ml
Day 135	Actual megaloblastic anemia

-Serum and red cell folacin.

Serum folacin concentrations have invariably been found to decrease even in uncomplicated pregnancies, unless folic acid is supplemented. Literature has been reviewed by Rothman (1970) and Rodriguez (1978). Using a microbiological assay (with Lactobacillus-casei as the test organism), Chanarin et al. (1968) found a progressive decrease from a mean value of 6.1 ng/ml in the first weeks of pregnancy to a mean value of 4.5 ng/ml at term.

The incidence of low serum folacin concentrations during

pregnancy is high. Percentages of 50 and higher have been reported for uncomplicated pregnancies when no folacin supplements were used (Lowenstein et al., 1966; Willoughby et al., 1966; Hansen, 1967; Herbert, 1968; Chanarin et al., 1968; Avery and Ledger, 1970; Daniel et al., 1970; Baker et al., 1975; Huser et al., 1975; Areekul et al., 1977).

Very low serum folacin concentrations are seen in megaloblastic anemia of pregnancy (Rothman, 1970). However, in most instances low serum folacin is not associated with hematological abnormalities (Chanarin, 1968).

Determination of whole blood, or the red cell, folacin content is generally considered to be a better index for folacin status in pregnancy (Hansen, 1967; Rothman, 1970 and Hershko et al., 1976). Red cell folacin content also decreases during pregnancy, especially in the third trimester, but to a lesser extent than serum folacin (Chanarin, 1968; Hansen, 1967; Hamfelt and Tuvemo, 1972). Some investigators reported higher red cell folacin levels at term than those found for non-pregnant reference subjects (Avery and Ledger, 1970; Rolschau e al., 1979; Ek and Magnus, 1981). Because of the relatively slow turnover of red cells, red cell folacin concentration reflects folacin stores at the time of generation, i.e. 2-3 months earlier. Willoughby and Jewell (1968) found the lowest red cell folacin values in a non-supplemented group of pregnant women, 6 weeks postpartum. Very low blood folacin levels are usually associated with megaloblastic changes in the bone marrow (Hansen and Rybo, 1967).

In megaloblastic anemias caused by vitamin B12 deficiency, a discrepancy in values for serum and red cell folacin can be observed. Due to an impaired ability to incorporate folacin into the blood corpuscles very low red cell folacin levels in vitamin B12 deficiency are found while serum folacin is usually within the normal range. Therefore, serum folacin has a greater differential diagnostic value than red cell folacin in megaloblastic anemia (Hansen, 1967; Forshaw and Harwood, 1971).

The reported incidence of low red cell or whole blood

folacin in uncomplicated (and unsupplemented) pregnancies varies between 5 and 35% (Hansen, 1967; Streiff and Little, 1967; Cantli et al., 1971). Differences in observed incidence of low serum and red cell folacin levels in pregnancy can be attributed to social and geographical differences between populations studied with the consequent variation in folacin intake and folacin requirement. Methodological differences in folacin assays and the interpretation of the results (i.e. the cut-off point used) are also likely to contribute to the observed variance.

-Urinary formiminoglumatic acid (FIGLU) excretion after histidine loading

FIGLU excretion after an oral histidine load is thought to reflect a biochemical folacin deficiency. FIGLU is an intermediate product of histidine metabolism. THF functions as the formimino (C_1)-acceptor in the histidine-glutamic acid pathway, and during folacin deficiency, FIGLU accumulation occurs followed by an increased excretion in the urine. Stone et al. (1967) found that FIGLU excretion was proportional to the degree of megaloblastosis in the marrow and was, therefore, the most sensitive and reliable index of folacin deficiency. The high incidence of positive FIGLU tests in cases with folacin-related pathology (e.g. abruptio placentae, toxemia) and in twin pregnancies was thought to confirm its use. Chanarin et al. (1963) and Chisholm and Sharp (1964) found the FIGLU test to be unreliable as a folacin-status parameter in pregnancy, because histidine absorption and metabolism actually change in pregnancy and FIGLU excretion is not related with hematologic findings.

Pathology related to folacin deficiency in pregnancy

A number of pathological conditions and pregnancy complications have been associated with folacin deficiency. Some of the associated disorders will be mentioned here only briefly. More information is available from excellent reviews on this subject

(Rothman, 1970; Rodriguez, 1978).

-Megaloblastic anemia

The reported incidence of megaloblastic anemia in pregnancy varies between 20 and 50% in developing countries, especially in India and in some African countries. In industrialized societies rates of 1-5% have been reported, although incidences may be higher in low-income groups (Rothman, 1970; Herbert, 1970; Lowenstein et al., 1968). In Holland, Verloop (1966) estimated the occurrence rate to be between 1 and 3°/oo, similar to the low percentages observed in Scandinavian countries (Hansen and Rybo, 1967). The frequency of megaloblastic anemia seems to be related to parity (Giles, 1966; Lowenstein, 1968) and multiple pregnancies and seems to be season-dependent since the highest frequencies are found in early spring (Gatenby, 1956; Girdwood, 1969; Chanarin, 1969).

-Toxemia, abruptio placenta, abortion and stillbirth

The relationship of these obstetric complications and folacin deficiency is much more controversial. It is based mainly upon the association between these complications and megaloblastic anemia as well as the high incidence of elevated FIGLU excretion and low serum folacin levels in these patients (Hibbard, 1964; Stone et al., 1967; Streiff and Little, 1967). Other investigators were unable to confirm these relationships (see Rodriguez, 1978) and it seems most likely that some common etiology exists between the biochemical abnormalities and these pathologic conditions rather than a causal relationship. No beneficial effect of folacin supplementation upon the incidence of these conditions has been found in well-controlled studies (Hemminki and Starfield, 1978).

-Congenital malformations of the central nervous system

Smithells and coworkers (University of Leeds) found significant lower serum and red cell folacin levels in the first trimester of pregnancy of mothers who gave birth to infants with neural tube defects (Smithells et al., 1976). In earlier studies no

relationship between maternal folacin status and fetal cerebral malformations was found (Giles, 1966; Scott et al., 1970). Neural tube defects (NTD) have a relatively high incidence in England, the risk of NTD being higher in the lower social classes and also "geography-dependent". The incidence of NTD in the Netherlands is about four times lower than in England (Niermeyer, 1980). Periconceptional vitamin supplementation had a preventive effect on the recurrence of NTD (Smithells et al., 1980). These results were critisized by Stone (1980) because data were biased by differing geographical risks of NTD for study and control groups. However, a double-blind controlled study was rejected by hospital research ethics committees (Smithells and Sheppard, 1980). Retrospective studies of the effect of dietary counselling on the recurrence risk of fetal NTD have indicated a beneficial effect of an adequate dietary intake (Laurence et al., 1980). Recently the same group of investigators published the results of a double-blind, randomized, controlled trial of folacin treatment before conception and during early pregnancy of women who had had a pregnancy complicated by fetal NTD. No recurrences occurred in the treated group (n = 44) but 4 recurrences occurred in the placebo group (n = 51), a significant difference. In treatment group the women received 2x2 mg folic acid per day starting from the time they stopped using (oral) contraceptives (Laurence et al., 1981).

-Folacin status and intrauterine growth

Lower serum folacin levels were described for babies weighing less than 2500 g (Hibbard and Kenna, 1974; Baker et al., 1977) irrespective of whether the babies were preterm or small for gestational age (SGA) babies. Some authors also reported lower birthweights in megaloblastic anemia of pregnancy (see Rothman, 1970), but this was not confirmed by others (a.o. Chanarin, 1968). Gandy and Jacobson (1977) found a strong correlation between low maternal serum folacin and the incidence of small-for-date babies and also significantly lower birthweights of infants with erythroblastosis. Preterm infants show more

megaloblastic changes in their peripheral blood compared with term infants and respond to small doses (100-200 µg) of folic acid (Strelling et al., 1979). Whiteside et al. (1968) studied the effect of maternal serum folacin concentration on the infant's birthweight and found a considerable influence. From these data it seems that "folacin deficiency" in the rapidly growing fetus becomes manifest by a diminished growth rate before the development of a macrocytic anemia (Gandy and Jacobson, 1977). In their review of controlled clinical trials of the effects of routine administration of iron and vitamins, Hemminki and Starfield (1978) concluded that folacin and other vitamin supplementation in developed western countries had no beneficial effect on birthweight.

In populations with poor diets (and probably in all respects a poor socio-economic environment), a positive effect of folacin supplementation on birthweight was reported by Iyenger and Rajalakshmi (1975) and Baumschlag (1970). Recently Rolschau et al. (1979) found a significant increase of about 13% in birthweights of babies born to mothers who were supplemented with 5 mg folic acid from the 23rd week of pregnancy. Their experimental group consisted, however, of only 20 healthy Danish women and a control group (n = 16) matched for parity, smoking habit, age and prepregnancy weight. The investigators attributed this surprising observation to the performance of their study in spring while folacin intake in winter and spring in Denmark is supposed to be low.

Folacin requirements in pregnancy

Estimates of nutrient requirements depend how requirements are defined (Beaton, 1979) and the criterium used to assess whether these requirements are met (section 1.2.1). According to Cooper (1970) a clear megaloblastic anemia can be prevented by a daily intake of 50 µg folic acid (80-90 µg food folacin). Alperin (1966), Löwenstein et al. (1966) and Pritchard et al. (1969) stated that at least 400 µg folacin per day were required to reverse megaloblastic changes in the bone marrow and to elicit

an optimal hematological response in folacin depleted women in the third trimester of pregnancy.

Hansen and Rybo (1967) and Chanarin (1968) determined the amount of folacin required to maintain whole blood folacin levels throughout pregnancy; 100 µg per day was found to be sufficient. In populations with a higher incidence of folacin deficiency, supplementation with 200-300 µg/day during the last trimester may be required (Willoughby and Jewell, 1968).

Based on these and other data (see also the comprehensive review on folacin requirements by Rodriguez (1978)), the WHO Expert Group (1970) recommend a daily dietary intake of 400 µg "free" folacin throughout pregnancy. The recommended daily allowance given by the NRC/NAS-USA (9th Ed., 1980) is about the same, i.e. 800 µg total folacin throughout pregnancy, or twice the amount recommended for non-pregnant females.

1.3.9. Vitamin C.

The term vitamin C should be used as the generic name for all compounds exhibiting qualitatively the biological activity of ascorbic acid (Committee on Nomenclature IUNS/AIN, 1977). Ascorbic acid and its reduced form, dehydroascorbic acid, have the same biological activity. Fruits and vegetables are the main sources of ascorbic acid. Using radioisotopic studies, total body stores for healthy adults have been estimated between 1000 and 1500 mg with a daily turnover of about 2-3% (Kallner et al., 1979; Ginter, 1979). Contrary to the other water soluble vitamins vitamin C has no direct coenzyme function but is involved in a number of hydroxylation reactions, e.g. in amino acid metabolism (Proline, Tyrosine, Tryptophan).

Vitamin C is also involved in collagen synthesis, steroid metabolism (adrenal cortex hormones), catecholamine metabolism, the incorporation of iron in ferritin and the interconversions in folic acid metabolism (THF-formation from folic acid). Although there have been reports that vitamin C is effective in the prevention against infections and in detoxification

reactions, the evidence is still equivocal.

Vitamin C deficiency for a long period will result in the development of scurvy. Nowadays scurvy, is uncommon. Weight loss and hemorrhage of the gums are among the non specific clinical signs of marginal vitamin C status.

-Vitamin C metabolism during pregnancy and fetal development

Based upon the observed fall in plasma and leucocyte vitamin C levels, vitamin C requirement is thought to be greater in pregnancy. Urinary excretion of a test dose of ascorbic acid is reduced during pregnancy and lactation (Toverud, 1934). Most of the older literature on vitamin C requirement of man, including the pregnancy period, was reviewed by Irwin and Hutchins (1976).

The concentration of ascorbic acid in fetal blood is higher than in maternal blood (Khattab et al., 1970; Baker et al., 1975 and Morse et al., 1975). This fetal-maternal gradient was thought to reflect selective retention of ascorbic and dehydroascorbic acid in fetal and maternal tissues (Raiha, 1958). Dehydroascorbic acid, not ascorbic acid, would preferentially cross the placenta (Raiha, 1958; Khattab et al., 1970). Studies on perfused human placentas have shown that the maternal-fetal gradient is maintained by the placenta (Hensleight and Krantz, 1966).

Recently Streeter and Rosso (1981) reported on transport mechanisms for ascorbic acid in the human placenta. At very high ascorbic acid concentrations, the vitamin crosses the placenta by simple diffusion; at lower concentrations, the predominant form of transport is carrier mediated and energy dependent. It was suggested that both forms of the vitamin are transported at the same rate across the placental membranes. Studies in guinea pigs have indicated that large doses of vitamin C during pregnancy may result in an increased demand for vitamin C in the offspring, caused by induced increase in the rate of catabolism in the neonatal period (Norkus and Rosso, 1981).

-Vitamin C and hormonal contraceptives

A relationship has been proposed between vitamin C metabolism and (sex-)hormones (Dodds, 1969). Women, before the menopause, have generally higher vitamin C blood levels than men. Changes in vitamin C plasma levels and vitamin C excretion during the menstrual cycle have been reported (Irwin and Hutchins, 1976). Leucocyte vitamin C levels are, however, not affected (McLeroy and Schendel, 1973).

Women on oral contraceptive therapy show significantly lower plasma and leucocyte vitamin C levels compared with controls (Rivers and Devine, 1972; McLeroy and Schendel, 1973). Vitamin C supplementation (50-200 mg/day) had a significant effect on vitamin C concentration in the leucocytes of control subjects (non-users), but no effect in women using oral contraceptives. Saroja et al. (1971) suggested that estrogens induce an increase in ceruloplasmin content. Ceruloplasmin shows in-vitro ascorbate oxidase activity. Increased vitamin C excretion and a shift of ascorbic acid in the tissues have also been suggested to explain the observed changes after oral contraceptive treatment, but have not yet been confirmed (Rivers and Devine, 1972).

-Biochemical parameters to assess vitamin C status during pregnancy

In general, there is a relationship between leucocyte and plasma ascorbic acid levels, although the extent of correlation may depend on age and sex of the subjects. Leucocyte levels are believed to reflect total body stores better than plasma levels and are less affected by changes in recent dietary intake (Sauberlich et al., 1976). Both for technical and practical reasons, determination of plasma or serum vitamin C level is mostly used.

Vitamin C concentration in serum (plasma) decrease during pregnancy by 10-20% (Martin et al., 1957; Van der Rijst, 1962; Mason and Rivers, 1971; Vobecky et al., 1974 and Morse et al., 1975). Dietary intakes of vitamin C above 80 mg/day are required to maintain serum vitamin C levels on a constant level during

pregnancy (Martin et al., 1957). Also leucocyte vitamin C content decreases during pregnancy especially in the second trimester. However, this decrease was associated with a leucocytosis since total leucocyte ascorbic acid content of blood was unchanged (Schorah et al., 1978). Both serum and leucocyte vitamin levels were found to be season dependent; the lowest levels are found in early spring (Martin et al., 1957; Schorah et al., 1978). Besides a seasonal variation, Schorah et al. (1978) also reported that leucocyte concentrations were negatively associated with smoking and social class.

-Pathology related to vitamin C deficiency in pregnancy

In some older studies a low vitamin C serum level was reported in patients with abortions and in pregnancies ending with stillbirths. Vobecky et al. (1974) found no relationship between low vitamin C plasma levels and pregnancy outcome. Schorah et al. (1978) found no relationship between maternal vitamin C status and birthweight.

1.3.10. Other vitamins.

From the remaining vitamins (Niacin, Biotin, Pantothenic acid and vitamin K) few data are available about requirements and metabolism in pregnancy. With the exception of niacin, all these vitamins are thought to be present in our food in abundant amounts and dietary deficiencies are very rare. Dietary niacin deficiency, pellagra, is still endemic in some countries in the Middle-East and Africa where diets are based on maize. In Central America where maize is lime-treated, pellagra is rarely reported to occur (WHO, 1965).

-Pantothenic acid

Pantothenic acid plays an essential role in intermediary metabolism, being an integral part of coenzyme A. Cohenour and Calloway (1972) and Srinivasan and Belavady(1976) studied

pantothenic status in pregnancy. The former authors reported a reduction in blood levels in early pregnancy; the latter authors observed no significant differences between pregnant and non-pregnant women. Baker et al. (1975) reported even higher level in parturient women. Cord blood values were higher than maternal blood levels, indicating an active transport across the placenta (Srinivasan and Belavady, 1976).

The mean pantothenate blood level in SGA babies (< 2500 gr) was significantly lower compared with that for term, normal weight babies (Baker et al., 1977).

Oral contraceptive treatment had no significant effect on pantothenate blood and urine levels (Lewis and King, 1980).

-Biotin

Biotin is involved as a coenzyme in many carboxylation reactions. Although dietary biotin deficiencies are unknown, a biotin dependency in some inborn errors of metabolism was observed. Bhagavan et al. (1969) observed a progressive decrease in biotin blood levels in the course of pregnancy. Also Baker et al. (1975) reported lower blood biotin levels in parturient women. Cord blood levels were about twice the maternal value.

A relationship of marginal deficiency and the occurence of sudden infant death syndrom (SIDS) has been suggested by Johnson et al. (1980), who showed that liver biotin concentrations of SIDS victims were lower than those of infants in the same age group, who died from known causes.

-Niacin

Niacin is incorporated in the nicotinamide adenine dinucleotides (NADH and NADPH) functioning as coenzymes in many redox reactions in respiratory chain and oxidative phosphorylation. Niacin is metabolized within the organism, N-methylnicotinamide being the main urinary metabolite. Determination of the urinary excretion of this (and other) metabolite(s) is considered a better status parameter than niacin blood levels (Sauberlich et al., 1976). Darby et al. (1953) reported increased

N-methylnicotinamide excretion during the third trimester of pregnancy. Both an Expert Committee of the WHO (1974) and the NRC/NAS (1980) concluded that there is no evidence that niacin requirements are increased in pregnancy. Niacin requirement is related with the energy intake. The daily niacin intake recommended by the WHO (1974) is 6.6 niacin equivalents/1000 kcal (Table 1.2.2.I).

-Vitamin K

Vitamin K plays an important role in the blood clotting proces. Methods for direct estimation of blood or urinary vitamin K level have not been described. A prolonged prothrombin clotting time may indicate vitamin K deficiency and most commonly occurs in malabsorption syndromes or after prolonged antibiotic therapy (Sauberlich et al., 1976). Data about vitamin K requirement and metabolism in pregnancy are not available.

Summarizing: from the extensive literature on maternal vitamin status during normal human pregnancy it can be concluded that for most water soluble vitamins a significant fall in vitamin blood or serum levels occurs, even when vitamins are supplemented. For the fat soluble vitamins, retinol and 25-OH-vitamin D, none, or only a slight decrease is apparent, while serum vitamin E levels invariably increase. However, a considerable variation in reported data is present, probably due to different socio-economic and environmental factors, e.g. a different habitual dietary intake, smoking and drinking habits, but not at least as a result of different biochemical methodology in assessing the vitamin status.

The changes in some vitamin status parameters induced by pregnancy, are very similar to those observed during vitamin depletion in the non-pregnant state and, therefore, the fall in vitamin blood levels, or the increased incidence of abnormal results with the enzyme stimulation tests, are frequently interpreted as a depletion of maternal stores due to increasing

fetal needs. Plasma dilution, and in the case of folacin increased urinary excretion, are considered causative factors as well (Hall et al., 1976; Fleming, 1972; Metcoff, 1976). An hormone mediated effect on vitamin metabolism during pregnancy is suggested by comparable effects on some vitamin status parameters of hormonal contraceptives. Generally, vitamin supplementation during pregnancy has only minor effects on most parameters; unphysiological doses are needed to keep parameters within the non-pregnant range, suggesting a change in vitamin metabolism rather than dietary insufficiency. Cord blood vitamin levels are generally higher than corresponding maternal ones, but are highly correlated. Placental vitamin transfer mechanisms are still poorly understood, although for vitamin C a carrier mediated active transport mechanism has been established (Streeter and Rosso, 1981). A relationship between a marginal maternal vitamin status and some complications of pregnancy, like toxemia, hyperemesis gravidarum as well as pregnancy outcome, was found in some older studies, but such a causative relationship was not confirmed in more recent, well-organized studies. Also, vitamin supplementation studies failed to indicate therapeutic or prophylactic benefit (Hemminki and Starfield, 1978). The only well established relationship between maternal vitamin deficiency and pregnancy related pathology is that between folacin deficiency and megaloblastic anemia of pregnancy. However, studies reported by Smithells et al. (1976), Baker et al. (1977), Rolschau et al. (1979) and Laurence et al. (1981), as well as the studies from Heller et al. (1973, 1974) are indicative for a probably non-optimal vitamin status, interfering with fetal development and intrauterine growth. Although our knowledge about fetal and maternal vitamin needs is still far from complete, vitamin needs are assumed to be higher during pregnancy compared with the non-pregnant state. Therefore, recommended daily allowances (RDA) set by most Nutrition Councils, like the Expert Committee of the WHO/FAO, the Food and Nutrition Board of the NAS/NRC and the Netherlands Nutrition Council are higher for pregnant women, especially in the second half of pregnancy. The advised higher vitamin

recommendations are to allow for the higher energy and protein intakes, but are sometimes dysproportionally higher, like for folacin and vitamin D.

1.3.11. Iron.

Every organism needs iron, not too much or too little. It can regulate iron absorption according to its need and possesses the possibility to store iron, thus neutralising its toxicity. In humans, iron is divided over the body as follows: 70% in hemoglobin, 20% in ferritin (mainly in the liver, spleen and bone marrow) and hemosiderin, 3% in myoglobins, 0.3% in celhemin, cytochromes and katalase and 0.1% in transferrin (Van Eyk and Van der Heul, 1978). The total amount of iron in humans is estimated at 3-4 gram, or 50 mg/kg bodyweight in males. Menstruating women have only 38 mg/kg bodyweight, and the iron store in this group is estimated to be 400 mg (Van Eyk and Van der Heul, 1978). However, measurements of iron stores by phlebotomy show even lower mean iron stores in menstruating women: 254 mg (Pritchard and Scott, 1964) and 210 mg (Walters, 1973).

Humans use iron economically, losing about 1 mg/day through epithelial shedding. Two ml of blood equal 2 mg iron, so in menstruating women, there is an average daily loss of about 2 mg.

It is extremely difficult to define iron deficiency. If it is the situation of increased iron absorption, every blood donor or every postmenstrual woman should be considered iron deficient. If it is the situation of decreased iron stores, established by bone marrow biopsy or ferritin measurement, what criteria should be used? Or do we consider a person iron deficient when serum iron or transferrin saturation percentage is below a certain value, or only when the hemoglobin content decreases and the erythrocyte changes.

The prevalence of iron deficiency in a population is largely dependent on the criteria used. Twenty-four percent of American students were considered iron deficient by absence of stainable iron in a bone marrow biopsy (Scott and Pritchard, 1967); a percentage also found in Sweden (Hallberg, 1968).

A nutrition survey in ten North American states resulted in 22% males with iron deficiency when hemoglobin was used as the

criterion, while in another nutrition survey using serum iron as criterion, only 2% of females were found to be iron deficient (Crosby, 1977). During puberty, 10% of boys were iron deficient, however, without an iron deficiency anemia. This deficiency disappeared after some years (De Wijn and Pikaar, 1971). This "ironhunger" has to be considered physiological as it probably does not lead to iron deficiency anemia (Verloop, Liem and De Wijn, 1970). Elwood and Hughes (1970) investigated iron deficiency associated disabilities (fatigue, weakness, etc.) and found, much to their amazement, almost all complaints still existing after correction of the iron stores. They concluded: epidemiologically, iron deficiency causes problems when the hemoglobin content decreases below 5 mmol/l.

Problems concerning description and definition of iron deficiency, from a clinical and epidemiological point of view, are extensively discussed by Garby (1973).

-Iron requirements during pregnancy

Extra iron is needed during pregnancy for fetus, placenta and the increase of red cell volume. Only the iron contained in the red cells that are not lost during parturition, returns to the iron stores after normalization of the red cell volume.

Estimations of the extra amount of iron needed in pregnancy vary in different publications, Table 1.3.11.I.

TABLE 1.3.11.I Iron requirements during pregnancy.

Source	Fetus and placenta	Increased red cell volume	Blood loss
FAO/WHO, 1970	315 mg	500 mg	250 mg
Pritchard, 1970	265 mg	500 mg	250 mg
Hytten and Leitch, 1971	300 mg / 75 mg	250 mg without Fe 400 mg with Fe suppl.	150 mg

The amount of iron in the "term" fetus was calculated by Widdowson (1951, 1979) to be about 278 mg (range 200-374 mg). Mishel (1957) and McCoy et al. (1961) measured the iron content of the placenta. They found, respectively, 11.1 mg/100 g placenta (with blood) and a mean total placental value of 75.7

mg (mean placenta weight, 560 g). Therefore, about 625-815 mg extra iron is needed during pregnancy, of which 525-565 mg disappears during parturition. Assuming that the loss of iron due to epithelial shedding remains the same during pregnancy, this increased iron need could be met by an iron absorption of 3.2-3.9 mg/day. This would leave the iron content of the body even higher after pregnancy than in its prepregnant state since not all extra, formed red cells are lost during parturition. If absorption would remain the same as before pregnancy (about 2 mg, the amount needed by menstruating women), 345-535 mg of iron are needed from the body stores. However, the mean iron store is only 210-234 mg (Pritchard and Scott, 1964; Walters, 1973); so an iron deficiency, or a reduced accumulation of iron, in fetus and placenta is likely to occur.

These considerations are based on the presumption that the increments in iron need are equally divided during pregnancy. This is not so. In the first 20 weeks, about 109-134 mg iron are needed for fetus, placenta and increased red cell volume, requiring an iron absorption of 2 mg per day (Widdowson, 1951; Mishel, 1957; Hytten and Lind, 1973). In the second half of pregnancy, the need increases rapidly: 319 mg for fetus and placenta and 175-400 mg for the increased red cell volume. The absorption must be 4.5-6.1 mg per day to meet this increased demand.

-Iron absorption during pregnancy

Hahn et al. (1951) investigated iron absorption during pregnancy with radioactive ^{59}Fe in a cross-sectional study. Radioactivity was measured in the erythrocyte two weeks after administration of labeled iron, assuming that all radioactive absorbed iron was used in the erythrocytes. On this basis, they calculated absorption percentages during the different stages of pregnancy (Table 1.3.11.II).

From these semiquantitative estimates, iron absorption seems to increase during pregnancy and utilization decreases with increasing dosage, although in absolute values, larger amounts are absorbed. If these percentages are a mirror of reality,

absorption during pregnancy would be 914 mg when the diet contains 18 mg of iron/day. If the intake would be 120 mg/day, the total absorption would be 1830 mg. An intake of 18 mg/day would be more than sufficient.

TABLE 1.3.11.II. Iron absorption during pregnancy according to Hahn (1951).

	Amount of administrated labeled ^{59}Fe			
Duration of pregnancy	1.8-9 mg	18 mg	39 mg	120 mg
15 weeks	11%	10%	6.5%	2.2%
15-24 weeks	30%	19.5%	12.5%	6.5%
25-40 weeks	40%	26%	16.5%	8.0%

TABLE 1.3.11.III. Iron absorption during pregnancy according to Svendberg (1975).

		ABSORPTION PERCENTAGE	
Duration of pregnancy	Test meal	100 mg Fe^{++} + ^{59}Fe non-supplemented	100 mg Fe^{++} + ^{59}Fe supplemented
12 weeks	1.5%	6.5%	6.7%
24 weeks	5.8%	9.2%	6.0%
36 weeks	14.6%	14.3%	8.6%

However, Svendberg (1975) reached a different conclusion after his longitudinal absorption studies. He calculated the non-heme iron absorption (Table 1.3.11.III) an average Swedish diet, containing 1-2 mg heme iron, 10 mg non-heme iron and 5-6 mg fortification iron (since 1970, 6.5 mg iron has been added to every 100 mg flour in Sweden). He also studied the absorption of a test dose of 100 mg ^{59}Fe in pregnant women with or without iron supplement of 200 mg $FeSO_4$. The observation that iron absorption early in pregnancy is below that in the non-pregnant state (3.2%) was not confirmed in a later study (Svendberg, 1975). Svendberg concluded that a diet containing 17 mg iron per day will result in a deficit of 600 mg iron at the end of pregnancy. However, according to the absorption percentages given, a woman with iron supplementation would absorb about 2300 mg during pregnancy. In 65% of the supplemented group, no stainable iron in bone marrow was found in the last trimester. This may cast doubt on the given absorption values and also on

the value of this method of iron storage assessment during pregnancy, an idea already expressed by Beaton in 1974. Another objection to Svendberg's study is that only percentages of non-heme iron (absorbed less readily than heme iron, (Björn-Rasmussen et al., 1974) are given. Absorption of heme iron is estimated in Svendberg's calculation to be 20-50%, which may produce large differences in absorbed iron.

From these studies, and those of Heinrich et al. (1968) and balance studies of Apte and Iyengar (1970), it may be concluded that iron absorption during pregnancy increases, the percentage of increase varies considerably and it is still not possible to say if a pregnant woman with 15 mg iron in her diet is "at risk". It is not clear if there is a mechanism by which more iron than necessary is absorbed in the first half of pregnancy, producing a reserve for the second half. Hahn's study indicates this; Svendberg's study does not.

Iron absorption is dependent on many factors: increased erythropoiesis for instance. Erythropoiesis is dependent on circulating erythropoiëtin, which is increased during pregnancy (Manasc and Jepson, 1969). Erythropoiëtin production is hormone dependent. For an extensive review of factors influencing iron absorption, the reader is referred to Conrad (1970) and for a review of erythropoiëtin and factors influencing its production to Fischer (1970). Apart from these physiological intrinsic factors, the composition of the diet is important. Some animal proteins are able to stimulate iron absorption two- to four-fold (Cook and Monson, 1975, 1976).

-Parameters of the iron status

Table 1.3.11.IV shows the most commonly used parameters of the iron status and shows the changes that are seen in iron deficiency anemia and in pregnancy. For an extensive review the reader is referred to Hytten and Leitch (1971) and Hytten and Lind (1973). Ferritin, as a parameter of iron storage, will be discussed more extensively. As can be seen form this table, changes during a normal pregnancy resemble those occurring in iron deficiency (anemia). Outside pregnancy, it is already

difficult to define iron deficiency. During pregnancy, the interpretation of the different parameters is also blurred by physiological changes. The larger increase of plasma volume relative to red cell volume causes a fall in the hemoglobin and hematocrit values. Serum iron values decrease about 35% during pregnancy (Verloop, 1959). However, serum iron is a difficult parameter to interpret because of the large diurnal variation of up to 70% (Van Eyk and Van de Heul, 1978).

TABLE 1.3.11.IV. Changes in iron status during pregnancy, and in iron deficiency.

Parameter	Normal values in non-pregnant women	Changes during pregnancy	Changes due to iron deficiency (anemia)
Hb mmol/l	7.6 - 9.7	↓	↓
Ht l/l	0.37 - 0.46	↓	↓
MCV fl	85 - 105	→	↓
MCH amol/l x 10^3	1.750 - 2.230	→	↓
MCHC mmol/l	20 - 23	→	↓
Fe in serum µmol/l	14 - 33	↓	↓
Transferrin g/l	2.2 - 4.2	↑	→
TIBC µmol/l	46 - 73	↑	↑
Ferritin ng/ml	30 - 150	↓	↓

Transferrin, a β-globulin, increases, and as a result, the total iron binding capacity (TIBC) increases (Spetz and Brody, 1967), probably under the influence of estrogens (Jacobi et al., 1969).

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) remain almost constant during pregnancy (Hytten and Leitch, 1971).

Ferritin is the protein in which the largest part of the iron reserve is stored. Ferritin leaks into the circulation probably by normal cell decay (Van Eyk et al., 1978).

Since immunoradiometric (IRMA) assays for ferritin have become more sensitive and reliable (Beamish, 1971; Addison,

1972) and serum values appeared to correlate well with iron reserve (Jacobs et al., 1972; Lipschitz et al., 1974; Cook et al., 1974), serum ferritin level measurement became the simplest and least invasive technique for measuring the iron reserve. Levels not in accordance with stored iron are measured only during infections, liver disease and increased red cell turnover (Lipschitz et al., 1974). Walters et al. (1973) calculated by means of repeated phlebotomy that 1 ng serum ferritin was equivalent to 8 mg depot iron.

At serum ferritin concentrations is below 10 ng/ml in the non-pregnant state, a low transferrin saturation and iron deficient erythropoiesis are found (Jacobs et al., 1972). In the literature, comparison of serum ferritin levels is difficult since different ferritin standards and antibodies with different specificities are used, and international standarization has not yet been achieved.

Serum ferritin levels decrease during pregnancy (Table 1.3.11.V). A recovery of serum ferritin levels occurs postpartum, although values are found below those of the first trimester of pregnancy up to 3 months (Van Eyk et al., 1978) and 6 months (Puolakka et al., 1979) after delivery.

If serum ferritin levels really represent iron reserve, it seems that a depletion of iron stores occurs during pregnancy. The decrease is too large to be explained by dilution. The slow recovery - still partial even after 6 months - points even more to depletion since the physiological influence of pregnancy on the serum ferritin level is no longer expected.

TABLE 1.3.11.V. Ferritin levels during pregnancy.

	Duration of pregnancy	Ferritin ng/ml			
Author	12	20	28	36	40
Kelly, 1977	-	23.8	14.9	13.9	17.3
Fenton, 1977	96	-	-	-	13
Taft, 1978	-	21.5	8.1	5.2	5.1
Van Eyk, 1978	52	26	20	18	10
Kanishige, 1980	97.4	-	22.2	14.7	27.6

-Relationship between maternal and fetal iron parameters

Fletcher and Suter (1969) reinjected the ^{59}Fe-labeled plasma of a pregnant women, and labeled iron could be demonstrated in the fetus after about 12 minutes. The mechanism by which iron is transported from maternal plasma to placenta and fetus is not clear (Van Eyk et al., 1978).

The total amount of iron in the fetus increases during pregnancy (Widdowson, 1951, 1979) and correlates with fetal weight (Widdowson, 1951; Osgood, 1955; Chang, 1973).

Whether the maternal iron store determines the fetal reserve is controversial (Bowering et al., 1976; McFee, 1973). Measurement of serum ferritin, representing the iron store, was thought to solve this problem. In most studies no relationship between maternal serum ferritin levels and those of cord blood was found (Rios et al., 1975; Hussain et al., 1977; Van Eyk et al., 1978; Jansson et al., 1979). Fenton (1977) found significantly lower cord blood values when maternal levels were below 12 ng/ml. Only Kaneshige (1980) found a clear correlation. Whether ferritin levels in cord blood represent the fetal iron store is doubtful. Fetal serum ferritin levels increase during the first 24 hrs., remain constant for about a week and decrease slowly after this time (Rios et al., 1975). Measurements at 24 hrs. postpartum may be more reliable and representative for the fetal iron store than measurements from cord blood. Jansson et al. (1979) found an increase in ferritin level of cord blood relative to duration of pregnancy and to birthweight, which confirmed the observations of Widdowson (1951), Osgood (1955) and Chang (1973). How well serum ferritin levels reflect iron stores in the fetus is not yet known.

Summarizing: the iron store of the fetus is dependent on the duration of pregnancy and on its birthweight and is probably independent of maternal iron stores.

-Pathology caused by iron deficiency in pregnancy

In the obstetrical literature, anemia during pregnancy is correlated with an increased maternal death rate, toxemia, puerperal infections, prematurity, an increased perinatal death

rate, dysmaturity, intra-uterine hypoxia and fetal distress (McFee, 1973). However, some of these complications are only encountered in very severe anemia. Others may be doubted. In Kuala-Lumpur, an increased maternal death rate was found due to heart failure and shock from hemorrhage in women with levels below 3.1 mmol/l Hb (Llewellyn-Jones, 1965). The study (Chaudhuri, 1970), correlating anemia and pre-eclampsia, is not well designed and uses ill-defined groups. A low hemoglobin level is not very likely in pre-eclampsia, in which a hemo-concentration (increased Hb and Ht) due to vasoconstriction is almost always observed (Pritchard, 1975; Asali, 1977). An increased puerperal morbidity was observed at levels below 5 mmol/l. No difference in puerperial morbidity was found when anemia was moderate (Scott, 1961). When Hb was below 4.0 mmol/l, 18% of the newborns had a birthweight below 2000 gram (Llewellyn-Jones, 1965), and in Kenya, McGregor (1963) found 42% of birthweights below 2000 gram at Hb levels below 4.7 mmol/l. No differentiation between prematurity and dysmaturity was made in these studies. Perinatal death rate is increased two- to three-fold, mostly due to prematurity (McGregor, 1963). Llewellyn-Jones (1965), however, found a six-fold increase in the intra-uterine death rate, but no increase in the rate of postnatal death.

In a prospective collaborative study in Australia, Singapore, India, Thailand and New-Guinea, Beischer et al. (1970) observed a hypertrophy of the placenta in 17% of the cases of pregnant women with a Hb level below 5.0 mmol/l. An increased perinatal death rate was not found if anemia was present with hypertrophy of the placenta. The mean birthweight in these "anemic" pregnancies (< 5 mmol/l Hb) was slightly below that of the control group, a difference that could be explained almost totally by the increased prematurity rate. It seems that the decreased oxygen content of the blood may be compensated for by a hypertrophy of the placenta.

Ratten and Beischer (1972) found more often fetal distress and subnormal estriol levels in a group of 568 women with a Hb below 5.7 mmol/l. Anemia was caused by iron deficiency in half

of these cases; two perinatal deaths, probably due to intra-uterine hypoxia, were encountered in this group. In the remaining half, anemia had other causes (thallasemia, chronic kidney disease and folic acid deficiency) and 19 cases of perinatal death were found.

Summarizing: iron deficiency anemia in pregnancy is related to an increased maternal mortality at Hb levels below 3.7 mmol/l. Fetal problems develop when the oxygen carrying capacity of the blood become insufficient. In a normal pregnancy the oxygen-need increases by 15%. This increase is amply compensated for by the 18% increase of the oxygen carrying capacity of the blood (Hytten and Leitch, 1971). If the Hb falls below 5.5 mmol/l, problems arise for the fetus because of the decreased supply of oxygen, resulting more frequently in premature delivery and/or fetal distress. Birthweight in this situation is reduced by about 140 gram, a reduction than can almost completely be explained by the shorter duration of pregnancy (Beischer et al., 1970). Perinatal mortality seems increased in severe anemia. However, if anemia is only due to iron deficiency, this may even be doubted (Ratten and Beischer, 1972).

-Iron supplementation during pregnancy

Everyone agrees about treating severe anemia during pregnancy since this condition may be harmful to mother and child. Whether or not iron should be supplemented during pregnancy is still controversial. Opinions about iron supplementation maybe divided into two groups. One group considers the iron stores to be insufficient and strongly advises extra iron during pregnancy (Evers, 1965; FAO/WHO, 1970; Pritchard and Scott, 1970; McFee, 1973; Svendberg, 1975; Kelly et al., 1977; Puolakka, 1979); the other regards changes in iron status parameters as physiological and considers the iron stores less at risk, due to the fact that a woman using a normal diet can compensate for the increased needs by increasing absorption. No iron supplement is advised and iron is only given as "treatment" when an iron status parameter, mostly Hb, falls below a certain level (Paintin et

al., 1966; Hytten and Leitch, 1971; Taylor and Lind, 1976).

In Holland, the administration of iron supplement as a routine was at first advised by De Vries (1957) and Verloop et al. (1959); later, they changed their opinion and advised iron administration only if Hb fell below 6.8 mmol/l. Evers (1962, 1964 and 1965) advised routine administration of iron during the last 4 months of pregnancy.

The most important arguments of the supporters of routine iron administration are:

- the iron status is at risk - a deficit of 600 mg might develop (Svendberg, 1975)
- the fall of some iron status parameters during pregnancy is less when iron is routinely supplemented (Evers, 1962; Svendberg, 1975; Kelly et al., 1977; Puolakka, 1979)
- the number of blood transfusions decreased significantly during the puerperium (Pritchard and Scott, 1970)
- the number of premature deliveries may decrease (Evers, 1964) and less cases of toxemia may develop (Chaudhury, 1970)
- postpartum iron stores (ferritin levels) were found to be higher in supplemented groups (Puolakka et al., 1979), an observation that was not confirmed by Van Eyk et al. (1978).

The most important arguments of the opponents of routine iron administration are:

- the extra iron needed is overestimated, and a healthy woman may compensate for the increased demand by an increased absorption (Hytten and Leitch, 1971)
- the "recovery" of iron status parameters when abundant iron is supplied is caused by a pharmacological reaction to iron administration thereby increasing the level of erythropoietin (Paintin et al., 1966; Bowering et al., 1976). Iron supplementation in an amount covering the increased need did not cause this "recovery" (Paintin et al., 1966)
- changes in red cell concentration influence blood viscocity (Hamilton, 1950). Routine iron administration increases red cell volume without a parallel increase in plasma volume. Viscocity will, therefore, decrease less, resulting in more

cardiac work which may prove unfavorable (Taylor and Lind, 1976). These authors found that macrocytosis of erythrocytes developed during routine administration of iron. This could have an adverse effect on blood flow

- a positive effect of routine iron administration in healthy women on duration of pregnancy, birthweight and perinatal mortality and morbidity has never been proven in well-designed studies (Hemminski and Starfield, 1978)
- the intake of iron tablets in the first trimester of pregnancy is associated with an increased abortion risk and, therefore, should not been given routinely during that period (Nelson and Forfar, 1971).

Summarizing: it is doubtful, whether iron routinely supplemented during pregnancy is useful, and it should definitely not be given during the first trimester. There is no reason for routine supplementation of iron if the purpose is to improve the course of pregnancy, fetal outcome or puerperial complications, as this has never been proven. Serum ferritin levels after pregnancy, however, seem to indicate that pregnancy, at least partly, depletes maternal iron stores; whether this can be prevented by routinely iron supplementation remains controversial.

Concerning "treatment", it is probably the most simple way to start treatment when the Hb falls below a certain level during pregnancy. The level that is used in the obstetrical unit of the University Hospital in Utrecht is 6.8 mmol/l. Besides this Hb level, a peripheral blood film is useful in discriminating between a "dilution" and an iron deficiency anemia (Taylor and Lind, 1976).

1.4. Factors influencing intrauterine growth.

Intrauterine growth is determined by three elements: fetus, placenta and mother. In each of these, there may be a cause for non-optimal intrauterine development. However, what is optimal intrauterine development? Theoretically, there has been optimal intrauterine development if a mature fetus is born without congenital abnormalities and grows along its birth centile after a short extrauterine adaptation. The fetus has developed undisturbed, within its genetic growth potential, and the environment has influenced its development neither in a positive nor in a negative way. This definition is, however, not very workable as it regards individual growth only in retrospect and, thereby, it is difficult, if not impossible, to establish an individual genetic growth potential. Practically, one often uses "the optimal birthweight": the weight whereby the chance for a fetus to survive without morbidity due to an intrauterine or intra-partum cause is largest. The optimal birthweight has been established in retrospect according to a large number of fetal weights and outcomes, but it has the disadvantage to be dependent on numerous socio-biological circumstances. For example: the optimal birthweight in Ghana from 1954-1956 was between 2500 and 2950 gram (Hollingworth, 1965), while in the United Kingdom in 1958 it was between 3500 and 4000gram (Butler and Alberman, 1969). In all studies optimal birthweight is higher than mean birthweight (Hytten and Leitch, 1971). The classification, fetus, placenta and mother, is just a practical one, and it will be clear that some factors are involved in more than one of these three elements.

Fetus

-Chromosomal and other congenital influences

About 40% of the variation in birthweight can be explained by genetic factors (Morton, 1955; Polani, 1974). For instance,

birthweight of boys is always higher than that of girls (Thomson et al., 1968; Kloosterman, 1969). Chromosome anomalies upset development and lower the birthweight. The effect of autosome abnormalities is more marked than that of anomalies of the sex chromosomes. In sex chromosome disorders, there is a regular decrease in birthweight proportional to the number of sex (or possibly only X) chromosomes (Chen et al., 1971, 1972). The effect on birthweight of chromosome abnormalities seems to be mediated through cellular changes, probably through a prolongation or a shortening of specific phases in the cell cycle (Mitchinson, 1971).

A number of non-chromosome linked congenital anomalies have an effect on intrauterine growth, e.g. renal agenesis (Potter, 1965) and osteogenesis imperfecta congenita (Elias et al., 1978). Only two examples are given as it is beyond the scope of this review to describe all anomalies in which a deviation of the mean birthweight is found.

-Intrauterine infections

Intrauterine infections probably cause a slowing down of intrauterine growth. Concerning viral infection, this has been convincingly demonstrated only for rubella (Driscoll, 1967). Cytomegalovirus only causes growth retardation when there is congenital cytomegalic inclusion disease, and not, if there is symptomless excretion of the virus (Waterson, 1979). The influence of other viruses on fetal growth has not been proven (Waterson, 1979). Bacterial infections, mostly limited to the mother, have been held responsible for growth retardation. Studies comparing bacteriuric and non-bacteriuric women have failed to demonstrate this (Sweet, 1977).

-Multiple gestation

Birthweight in twin pregnancy is lower compared to birthweight in singleton pregnancy, although the total weight of a twin pregnancy exceeds that of a singleton pregnancy considerably (McKeown and Record, 1952; Van Bilderbeek, 1960; Gruenwald, 1970). Monochorial twins are lighter than bichorial. Gruenwald

(1970) explains this by the fact that monochorionic twinning represents a malformation arising within a single ovum (the choriopagus), while Bleeker (1979) suggests "that weight is less because monochorionic twins can make less effective use of their placenta due to the higher incidence of abnormal architecture than bichorionic twins".

Placenta.

-Umbilical cord

Whether or not the insertion of the umbilical cord influences birthweight is a point of discussion in literature. Adair and Thelander (1925) and Kloosterman (1969) observed a lower birthweight in cases of was a marginal or velamentous insertion of the cord contrary to Uyanwah-Akpom and Fox (1977). Bleeker (1979) confirmed Adair and Kloosterman's findings in twins, both mono- and bichorial.

A single artery, if not accompanied by other congenital anomalies, does not reduce birthweight (Longo, 1972). It is found in about 1% of mature singletons. In twins this percentage rises to 7%, and in spontaneous abortions and premature delivery, the frequency of a single artery is also higher (Kristofferson, 1969). Interesting is the observation of Ezaki (1972), who found a lower incidence of single arteries in a large series of induced abortions than at term. This might indicate an infection later in pregnancy, causing an endovasculitis with obliteration of one artery. In congenital rubella, this is a well-known (Driscoll, 1967), but this may also be induced by other viruses.

Macroscopic anomalies of the placenta.

-Small placenta

There is a rough correlation between placental weight and fetal weight, especially if the true blood-free weight of the placenta

is measured rather than the inaccurate, and thus uninformative, gross-weight (Kloosterman and Huydekoper, 1954; Garrow and Hawes, 1971). This does not mean that the fetus is small because the placenta is small. The reverse might even be true: namely, that the placenta, being a fetal organ, is small because the fetus, for whatever reason, is small. In infants of low birthweight, the placental fetal weight ratio is often normal or even slightly increased (Younosai and Haworth, 1969).

-Placenta circumvallata

In a placenta circumvallata totalis, birthweight is significantly lower. This condition is also accompanied by a higher incidence of fetal hypoxia and premature delivery (Fox, 1972).

-Hemangioma

A large hemangioma in the placenta causes a lower birthweight probably because of shunting, returning a part of the blood to the fetus unoxygenated (Fox, 1967[a]).

-Subamniotic hematoma

A subamniotic hematoma is caused by a rupture of an umbilical vene. Subamniotic hematomas are accompanied by a higher incidence of low birthweight, although the reason for this is not clear (De Sa, 1971).

-Infarction

Infarction of more than 10% of the placenta has a high incidence of low birthweight. This extended infarction is almost always accompanied by hypertension, or hypertensive complications, during pregnancy or by large retroplacental hematomas (Fox, 1967[b]; Wallenburg, 1969).

Besides infarctions due to a retroplacental bleeding, extended infarction arises from obstruction of multiple maternal arteriolae whereby pathology of the utero-placental vessels, as found in hypertensive complications of pregnancy, is almost always seen (Robertson, 1969; Wallenburg, 1973).

Low birthweight due to extended infarction is not only caused by a loss of villi due to the infarction, but probably also by a reduced utero-placental circulation. In cases where an extended perivillous fibrin deposition is found, decreasing sometimes the number of functioning villi by 20%, no fetal hazards are encountered (Fox, 1967[a]).

Microscopic anomalies of the placenta

Atherne (1966) showed that placentas from normal pregnancies have a mean villous surface area of about 11 square meters and a mean surface area of fetal vessels in the villi of about 12 square meters. In hypertensive pregnancies, this was, respectively, 7.4 m^2 and 10.2 m^2, and, in a normotensive pregnancy and a fetus below the 2.3 centile, this was 6.9 m^2 and 7.2 m^2. Thus, the exchange surface is less in hypertensive pregnancies and even lesser in normotensive pregnancy, when a small-for-date (SGA) fetus is born.

The syncytio-trophoblast has two functions: a synthetic and a transfer function. In the mature placenta, the villous syncytio-trophoblast is not morphologically homogenous. In many villi, thinned, anuclear areas of trophoblast are seen that directly overlie and appear to fuse with the wall of a dilated fetal capillary. These attenuated areas are called "vasculo-syncytial membranes" or "syncytio-capillary membranes". The membranous parts of the trophoblast are probably involved in the transfer function, while the non-membranous parts probably represent the synthetic function of the trophoblast.
A disturbance in the ripening of the syncytio-trophoblast may, because of a decrease in transfer function of the placenta, cause a slowing down of the fetal growth and finally cause fetal death. The cause of this disturbance in placental development is far from clear (Fox, 1975).

Maternal factors.

-Ethnic differences and maternal stature

Ethnic differences are well established. The mean birthweight for Americans is 3580 gram compared, for instance, with 2866 gram mean birthweight for Indians (Roberts, 1969). But even within ethnic groups, there are differences. The difference in mean birthweight between Neapolitans and Norwegians, both belonging to the European Caucasians, is 400 gram (Meredith, 1970). There is a positive correlation between maternal weight and height before pregnancy and birthweight. When comparing mothers with a height of 150 cm and 165 cm, the difference in birthweight is about 300 gram (Thomson, 1960). There is also a positive correlation between weight gain during pregnancy and birthweight. The difference in birthweight between women who did not gain weight and who gained 20 kg is about 300-400 gram (Thomson et al., 1968; Fedrich and Adelstein, 1978). Of course this relationship may be simply one of cause and effect. Small maternal weight gains and small babies may be features of an inherently poor pattern of reproduction. In women, with a prepregnancy underweight of more than 10%, a lower birthweight than in a control group was found even when the weight gain during pregnancy was equal (Edwards, 1979). This confirms Naeye's observation (1979) that optimal weight gain, defined as the weight gain whereby perinatal mortality is lowest, is dependent on prepregnancy weight. The optimal weight gain of women with normal weight is 10 kg, 13.5 kg for those with an underweight of less than 90% and 6.7 kg for women with an overweight of more than 135%.

-Age and parity

Primigravidae of 35 years and over have a higher chance of getting a SGA child than primigravidae of lesser age (British Perinatal Mortality Survey of 1958, Butler and Alberman, 1969). The reason for this is not clear. Possibly its cause is vascular, since arterial pressure rises with age in primigravidae (MacGillavry, 1961). Parity, or at least the

difference between the first and any subsequent pregnancy, has the most and best known positive effect. The cause is unknown. It has been suggested that the uterine vasculature does not adapt at the first pregnancy (Kloosterman, 1966; Thomson et al., 1968; Gruenwald, 1975).

-Uterus

Congenital uterine anomalies influence birthweight by an increase in incidence of premature delivery (Dunselman, 1959; Green and Harris, 1976; Heinonen et al., 1982). Whether the incidence of the SGA fetus is also increased is not clear, although one can imagine that if a placenta is inserted partially on a septum, the utero-placental circulation might be compromised.

In uteri removed at hysterectomy, the vasculature was visualized radiologically and a correlation between abnormal vascular anatomy, such as two ascending arteries on one or both sides, and birthweight was found. The frequency of a birthweight of less than 2500 gram in the group with abnormal vasculature was twice that of the group with a normal vasculature (Burchell, 1978). The "improved" uterine circulation, resulting in higher birthweights in multipara, will be discussed.

-Other maternal diseases

Most diseases that influence birthweight in a negative way have, as a common point of application, the decreased placental perfusion. Hypertension, whether or not due to pregnancy itself, can diminish the maternal flow in the intervillous space, making the supply line to the fetus insufficient (Gruenwald, 1966; Butler and Alberman, 1969). This is, however, only true for hypertension with proteinuria. Birthweight is not lowered in women with uncomplicated hypertension (Baird et al., 1957; Page and Christianson, 1979). For women with congenital or acquired heart disease, SGA babies are described (Cannel and Vernon, 1963), but this could only be confirmed for women with a tetralogy of Fallot, whether or not corrected (Davis, 1967).

Women with a history of more than one SGA fetus had a significantly lower circulating blood volume than a control group (Croall, 1978). In kidney disease, the prognosis is dependent on the creatinine clearance before pregnancy and the development of toxemia during pregnancy. The latter, especially, decreases birthweight of children born to women with kidney disease (Werko et al., 1956). Also, in a disease such as systemic lupus erythematosus, prognosis is dependent on the involvement of the kidney in this autoimmune process and the development or existence of hypertension during pregnancy (Zulwan et al., 1980).

Anemia results probably in lower birthweight of the fetus (WHO, 1970). In severe anemia, hypertrophy of the placenta is seen (Beischer et al., 1970), as in women with severe heart disease (Clavero and Botella Llusia, 1963). The villous space is enlarged probably to compensate for the decreased oxygen content of the blood or the decreased circulation. Anemia, however, is difficult to define in pregnancy (see section 1.3.11).

Chronic illnesses of the mother, e.g. bronchitis, asthma bronchialis, tuberculosis and malaria, influence birthweight in a negative way (Roshovski et al., 1964; Jeliffe, 1966). Whether this is due to a decrease in birthweight or due to a shortening in length of gestation is not clear. In asthmatic patients, no growth retardation could be demonstrated (Gordon et al., 1970).

-Maternal glucose level.

Newborns of diabetic mothers are heavier than those of non-diabetic mothers (Pederson, 1954). This has been attributed to the maternal hyperglycemia which entails an increased supply and fetal content of glucose. Because of the concommitant high fetal glucose levels, more insulin is produced by the fetus and growth is promoted. A larger than average birthweight is also found for women with a gestational diabetes (Coelingh Bennink, 1980).

Maternal hypoglycemia or "hypoglycemic glucose tolerance tests" are significantly more associated with SGA infants (Abell and Beischer, 1976; Khouzami et al., 1981; Sokol et al., 1982).

Maternal (non-diabetic) hyperglycemia was not found to be related to high birthweight (> 90th centile) (Abell and Beischer, 1976), although in a more recent evaluation of the same material, Oats et al. (1980) found a significantly higher percentage of hyperglycemic mothers in the group of newborns with a birthweight above 4540 g or the 99th centile. However, 77% of the women producing these large babies had a normal glucose tolerance test. This indicates that hyperglycemia is not necessarily the cause of fetal overgrowth. Another remarkable observation of this study was that the incidence of diabetes or gestational diabetes among women of this high birthweight group was not increased.

So, low maternal glucose levels seem to be related to low birthweight of the newborn and there are even suggestions that dextrose given to pregnant women intravenously might favor neonatal outcome in intrauterine growth retardation (Abell et al., 1976). High maternal glucose levels are related to fetal overgrowth in pathological conditions such as diabetes or gestational diabetes but non-pathological hyperglycemia is only correlated to extremely high birthweight. It is most likely that fetal insulin production is a factor involved in this fetal overgrowth but its role remains to be clarified.

-Smoking

Smoking decreases birthweight significantly, the difference between smokers and non-smokers being about 150-350 gram (Butler and Alberman, 1969; Naeye, 1978). It is not clear how this effect is mediated. The possibility that smoking depresses fetal growth mainly by depression of energy intake, as reflected by lower maternal weight gain (Rush, 1974), is contradicted by Meyer (1978) and Hajari et al. (1978), who found no differences in weight gain during pregnancy in smokers and non-smokers. Electron microscopic investigation of the placenta in heavy smokers points to a decreased placental perfusion (Asmussen, 1980). For a review about pregnancy and smoking, see Pirani (1978).

-Alcohol

Alcohol consumption during pregnancy may result in a congenitally abnormal fetus. The fetal alcohol syndrome is now known as a distinct entity (Jones et al., 1973). Alcohol also lowers birthweight (Little, 1977). This slowing down of growth starts, probably, in the first half of pregnancy since it results in a proportional SGA fetus (Hinckers, 1978).

-Heroin and Methadone

Heroin addicts give birth to children with a low weight for gestational age. Although it is difficult to exclude other factors in this group, there seems to be enough evidence that heroin alone can impair growth (De Lange, 1979). Methadone seems to influence birthweight to a lesser extent than heroin, but SGA fetuses occur in about 30% of cases (Newman et al., 1975).

-Environmental factors

It is extremely difficult to separate environmental factors from maternal ones, except in extreme environmental circumstances such as high altitude. At high altitude birthweight was found to be less than in a comparable population living at sea level (McClung, 1979), although Cotton et al. (1980) could not confirm this. The most important environmental factor, however, is the socio-economic status of the mother. This is an ill-defined conglomerate, consisting of many factors: education, housing, sanitation, psychic factors influencing people's attitudes and priorities with regard to attaining their status in life, health and medical care and last, but not least, nutrition. All over the world, there is a difference in perinatal mortality and birthweight in the different social classes (Gruenwald, 1966; Butler and Alberman, 1969; Tafari et al., 1980). When rats were fed a restricted diet for several generations, more than one generation was needed for the offspring to attain normal size again (Chow, 1968).

In humans, it is impossible to measure effects of nutrition during generations. Gruenwald et al. (1967) studied the

influence of changed socio-economic circumstances on birthweight in Japan during a period of twenty years (1947-1967). After an initial decrease due to the war deprivation, birthweight rose in this period above pre-war levels without an increase in duration of pregnancy. They also found that the difference in the rate of fetal growth correlated with stature at 6 years of age: children born in the years 1957-1958 were heavier than those born in 1945-1946.

-Duration of pregnancy

The most important factor that determines birthweight is the duration of pregnancy. All "intrauterine" growth charts show this (Lubchenco et al., 1963; Thomson et al., 1968; Kloosterman, 1970). The fetus seems to grow till the end of pregnancy. However, birthweight is the end result of intra-uterine growth and the intra-uterine growth charts are composed of cross-sectional data. Data on birthweight before 36 completed weeks of pregnancy are, however, derived from pregnancies considered as abnormal. One can only guess about real intra-uterine growth before this time. Echoscopy might be useful to establish the intra-uterine growth pattern in the near future.

The placenta seems to grow, according to these cross-sectional data, up to the end of pregnancy as well. However, the only conclusion about these data would be that as pregnancy continues, fetus and placenta become heavier.

Near the end of pregnancy, the growth rate slackens (see Figure 1.1.4.I). This faltering of growth just before birth is unique in the sense that no animal growth studies to date show a comparable period of growth faltering at the end of intra-uterine life (Campbell, 1976). The reason must be found in the maternal environment, because soon after birth the child increases growth rate and regains the size predicted by its original growth pattern.

There are a number of theories about the cause of this slowing of the growth rate.

Gruenwald (1966): Members of a human population with similar adult stature follow the same straight portion of the growth curve up to 37 weeks of gestation, i.e. as long as growth support exceeds the metabolic needs for realizing the growth potential. One of the factors causing the faltering of growth after 37 weeks is socio-economic. It is a shortage of the maternal supply line of the nutritional needs and not a placental insufficiency, because in perivillous fibrin deposition, 20% of villi might be afunctional without fetal compromise (Fox, 1967). A significant growth retardation in the Rhesus monkey could be produced only when 30-50% of the placental mass had been lost (Hill, 1974).

Kloosterman (1965): the placenta stops growing in the last weeks of pregnancy, but the fetus continues to grow until a certain ratio between placental and fetal weight is attained; then the fetus is born. At the end of pregnancy, the placenta is the limiting factor for fetal growth. Bleker et al. (1977) produced some echoscopic evidence that the placenta stops growing before the end of pregnancy. Limitation of fetal growth by the maternal nutritional capacity seems unlikely because in multiple pregnancy, the mother demonstrates a nutritional capacity that is never reached in singletons.

Briend (1979, 1980): the raising of the trunk in the upright position of the human body provokes a forward projection of the sacrum and the lower lumbar vertebrae (Bieniarz et al., 1966). This decreases the available space for the pregnant uterus and interferes with the normal maternal hemodynamics. Aorta and vena cava inferior can be compressed at the L_4-L_5 level. Aortic compression results in a fall in arterial pressure distal to the lumbar lordosis and decreases uterine blood flow. Compression of the vena cava provokes a diminuation of the blood volume and a drop in cardiac output. When standing in the upright position of the human body, a similar compounding effect is provoked by change in hydrostatic pressure in the venous system due to gravity. These hemodynamic effects imply that the cardiovascular system of pregnant women is not perfectly adapted to the upright position and, during evolution, its suboptimal efficiency may

have become a limiting factor for fetal growth. It is a nutritional effect due to an insufficient circulation and not due to nutrition itself. Children, when breast-fed, grow perfectly well in spite of their nutritional needs being much greater than during intrauterine growth. The last hypothesis may explain:

a. Differences in birthweight in social classes. Tafari (1980) and Briend (1980) observed significant differences in birthweight in women who performed physical hard work and women who did not.
b. The difference in birthweight between primigravid and multi parous women. In the latter there will be more space available for the pregnant uterus.
c. The faltering of growth in twins that starts already round the 32th week of pregnancy (Bleker et al., 1979).

It can be concluded that many factors may influence intra-uterine growth and finally birthweight. When birthweight, or variables that are possibly related to birthweight, are studied, the study population should be well defined, and, within the possibilities of the study, all variables, that are known to be related to birthweight, should be noted carefully.

1.5. General considerations about the effect of nutrition on the course and outcome of pregnancy (summary of Chapter 1).

Numerous studies relating nutrition, vitamins and minerals to course and outcome of pregnancy have been performed. These studies have been inspired by observations that changes in habitual food pattern and in vitamin status parameters are related to pathology in pregnancy. A marked reduction in food intake during pregnancy results in a decrease in birthweight. This observation was, however, made in dramatic circumstances (World War II) (Smith, 1947; Antonov, 1947; Sindram, 1953). Another confirmation of this relationship was found in animal experiments in which severe food restriction and an artificially induced vitamin deficiency were able to produce severely malnourished and sometimes congenitally affected offspring (Winick, 1970; Roeder and Chow, 1972). In the literature of the first half of this century, success was described in reducing pathology of pregnancy by supplying extra food, vitamins or minerals to pregnant women. In the 1950's, a number of extensive and well-organized studies concerning this relationship were done: the Vanderbilt study (Darby et al., 1953; McGanity et al., 1954), the Aberdeen study (Thomson, 1958, 1959[a], 1959[b]) and the Amsterdam study (Van de Rijst, 1962). In hardly any of these studies, a relationship between nutrition, vitamin intake or vitamin blood levels and pathology of pregnancy could be found. The positive relationship between energy intake and birthweight was "overruled" by the relationship between midpregnancy weight and birthweight (Thomson, 1959[a]). Only a (too) high energy intake was found to be related to toxemia (Thomson, 1959[a]).

Still the debate went on as Rush et al. (1976) explained the clinical observation relating birthweight to weight gain during pregnancy as a nutritional effect.

Well-organized intervention studies were thought to solve this problem, but only a few conclusions could be drawn from these studies (section 1.2.6). In one, the Guatamalan study, it seems proven that energy supplementation increased birthweight and reduced the percentage of low birthweight. However, the

effect is marginal. Another problem in these studies is that as long as one is not informed about the influence of supplemented food on the physical activity of the women concerned, one is never sure it is a real effect of energy intake or an effect caused by altered physical activity (Tafari et al., 1980).

Summarizing on macronutrients during pregnancy; the fetus seems well-protected against changes in maternal food intake. Probably, only a severe decrease reduces birthweight. The fetus itself may furnish protection against such events with the aid of its placenta. Early in pregnancy, an energy bank in the form of stored fat is formed. There are indications derived from animal experiments that, in a similar way, protein storage exists, making the fetus even less dependent on the protein intake of the mother in the last part of pregnancy. How the feto-placental unit modulates the maternal metabolism is not clear. Estrogens, progesterone and human placental lactogen are likely to be involved in this process, but other feto-placental hormones may be involved as well.

From observational and other types of studies (see section 1.2.5), it can be concluded that the nutritional status before pregnancy, expressed as the prepregnant weight, is related to birthweight and duration of pregnancy. Too many calories are related to toxemia, but, due to the difficult and different definitions of toxemia, this relationship may even be doubted. A relationship between nutrition and lactation in industrialized countries is doubtful. The relationship of nutrition in general to congenital anomalies, except for the "nutrient" alcohol has also never been demonstrated. The possible relationship between congenital anomalies and some micronutrients is discussed below.

Focusing now from the macronutrients to the micronutrients, no relationship between the course and outcome of pregnancy and the intake and/or blood levels of vitamins and minerals could be demonstrated in the observational studies of the 1950's. The same conclusions can be drawn from studies in which vitamin and mineral supplements were given (Hemminski and Starfield, 1978). The only causal relationship between a specific vitamin

deficiency and a complication more frequently seen during pregnancy is the folacin deficiency as a cause of megaloblastic anemia, at least in some parts of the world (see section 1.3.8). There may be a causal relationship between relative vitamin deficiencies and the occurence of central nervous system defects since peri-conceptional vitamin supplementation seems to decrease the recurrence in a high risk group (Smithells, 1980; Laurance, 1981).

However, it is evident (Bergner and Susser, 1970) that many studies which demonstrate a relationship between a certain vitamin deficiency and certain pathological conditions of pregnancy were based on only a few observations or on poorly designed studies. Extrapolation of observations from animal experiments, done under extreme conditions, to the human situation, must be interpreted with great caution (Committee on Maternal Nutrition, FNB/NRC, 1970).

From the strong fixation on the pathological conditions of pregnancy and due to the negative findings, the idea has developed that changes in, for example, vitamin status parameters during pregnancy are caused by changes (adjustments) in the maternal physiology. A number of studies have been published in which the course of one or more vitamin status parameters during an uncomplicated pregnancy has been described (Heller et al., 1973, 1974; Baker et al., 1975; Kübler and Moch, 1975; Vir et al., 1980, 1981). These studies almost always regard cross-sectional observations or are based on a certain moment during pregnancy, such as delivery. Generally a decreasing concentration of vitamin blood levels is observed during pregnancy pointing to maternal depletion. The causes which, among others, may be responsible for the hypovitaminemia of pregnancy are the increase in circulating plasma (blood) volume, the increase in renal plasma flow and glomerular filtration, possibly causing an increased excretion, and changes in hormonal balance which may cause an increased vitamin turnover and altered tissue retention (see section 1.1.1 and 1.1.3). Except for these physiological changes in maternal metabolism, fetal vitamin accumulation together with a

suboptimal maternal vitamin intake may be involved (Metcoff, 1976).

Only a few studies have been performed, with the purpose of explaining the alterations of the vitamin status during pregnancy. The studies of Hall et al. (1976) and Fleming (1972) indicate that hemodilution and urinary excretion may be important determinants of folacin plasma concentrations during pregnancy. Alteration of vitamin status parameters in women using oral contraceptives indicate that hormonal changes during pregnancy may play an important role as well (see section 1.1.3). Environmental factors, such as smoking and drinking habits, may also influence vitamin needs and vitamin metabolism. Beaton (1979) has postulated that a better approach to evaluate nutrient needs during pregnancy might be to study the alterations in nutrient stores between the entry and exit from the reproductive cycle.

Knowledge of the manner and speed in which the normalization of nutritional status parameters to their non-pregnant values occurs in the postpartum period might provide a better insight into the "net" nutrient cost of pregnancy. However, only a few studies have been performed on changes in vitamin status parameters during pregnancy and the postpartum period, and, most of them cover only the first 6 weeks after delivery.

In some (recent) studies, a significant relationship between vitamin status parameters and the clinical parameter birthweight has been demonstrated while others could not confirm these observations (Kübler and Moch, 1975; Rolschau et al., 1979; Gandy and Jacobson, 1977[a]). Such discrepancies may be due to differences between preconceptional vitamin stores in the population studied. Birthweight is, however, influenced by a large number of exo- and endogenous factors (see section 1.1.5), and the complexity introduced by all these factors and the possible interactions between these factors make it extremely difficult to evaluate the role of nutrition and that of vitamins, during pregnancy in particular, and may also explain the apparent discrepancies.

CHAPTER 2.

Patients, materials and methods.

2.1. Patient selection.

Pregnant women, who fulfilled the criteria described below while visiting the antenatal clinic of the University Hospital of Utrecht, were asked to participate in this study, and, admitted only after informed consent was obtained.

Selection criteria were:

- Caucasian race
- Duration of pregnancy less than 14 weeks
- Regular menstrual period (between 24-32 days) and known date of the last menstrual period
- Hormonal contraception stopped three or more months before conceiving and at least one spontaneous menstrual period after stopping this form of contraception
- Uncomplicated obstetrical history with the exception of spontaneous abortion; however, no history of a habitual abortion.

Assessment of general health was based on the patient's history and a routine physical examination. The obstetrical history was obtained from hospital files, and additional information was asked if a delivery had taken place elsewhere. The duration of pregnancy was checked by echoscopy. If a deviation of 10 days or more between the duration of pregnancy, according to the last menstrual period and the duration of pregnancy determined by the crown-rump length, according to Robinson's curve (Robinson and Fleming, 1975), was repeatedly found, the patient was rejected from the study.

Only women having had a normal pregnancy were evaluated. The criteria to define normal pregnancy were:

- No signs of toxemia. Toxemia was defined as a diastolic blood pressure repeatedly exceeding 90 mmHg and/or edema on hands and face and/or albuminuria of more than 0.5 g/24 hrs.
- No admission to the hospital during pregnancy
- No long-term medication except for iron. Iron was only therapeutically given if the Hb was below 6.8 mmol/l
- No dietary restriction or dietary advice except the one to use a limited amount of salt
- Spontaneous labor after 36 and before 41 completed weeks (259-294 days)
- Vaginal delivery of a singleton infant
- Apgar score of the child more than 7 after 5 minutes
- No major congenital abnormalities
- Blood loss less than 1000 cc and no blood transfusion
- Uncomplicated puerperium.

The birth(per)centile was calculated and according to this birth centile 3 groups were formed. Dutch birth centiles were composed from a large number of birthweights (n = 80,000) by Kloosterman (1969, 1970). The birth centile can be calculated when birthweight, sex and duration of pregnancy are known. Birth centile is a better indicator of intrauterine growth than birthweight as the latter parameter is strongly dependent on the duration of pregnancy and, although less, on the sex of the newborn.

- The S-Reference group: birth centile between the 10th and 90th centile
- The < P10 group: birth centile at or below the 10th centile
- The > P90 group: birth centile at or above the 90th centile.

The reasons for dividing the already normal study population according to birth centile, are the following:

- To make the S-Reference group as normal as possible. Even after a "normal" pregnancy, minor pathology is probably more frequently encountered in women whose children were below the

10th or above the 90th centile

- To compare data from the < P10 and > P90 group with those of the S-Reference group.

2.2. Procedures during and after pregnancy.

In Table 2.2.I is shown when women and their children were seen during and after pregnancy, and which investigations and measurements were made at what time.

TABLE 2.2.I. Procedures during and after pregnancy.

		DURING PREGNANCY			DELIVERY		POSTPARTUM		
	Admission to the study	16 weeks	28 weeks	34 weeks	Mother	Child	6 days	6 weeks	6 months
Blood collection	-	+	+	+	+	+	+	+	+
Blood volume	-	+	+	+	-	-	+	+	+
Dietary history	-	+	-	+	-	-	-	-	+

In the study population the following anthropometric measurements were done:

- Maternal and paternal height in centimeters, using a Geigy-Braun measuring scale.
- Maternal weight in kilograms, taken at each prenatal visit, 6 days, 6 weeks and 6 months postpartum, using a Volke and Halke weighing scale (type SECA).
- Maximal weight gain - the highest measured weight during pregnancy minus the prepregnant weight.
- Weight during delivery - the last recorded weight on the antenatal record before delivery.
- The child was weighed within one hour after birth, using a Berkel weighing scale, type Piccolo. Weight recorded in grammes.
- Length of the child in centimeters, was taken as described by Roord and Ramaekers (1978).

Besides the anthropometric measurements described, the following parameters were noted:

On admission to the study:

- Woman's age.
- Parity: all previous children born after a gestation of 16 weeks or more.
- Number of abortions.
- Conception month, based on last menstrual period and menstrual interval.
- Smoking habits.
- Prepregnant weight, based on patients information.

During pregnancy:

- Use of iron tablets.
  The use of iron tablets was catagorized as follows: no tablets at all, only during pregnancy (more than 4 weeks), only after pregnancy and during and after pregnancy. The use of tablets was not checked.
- Smoking habits.
  Smoking was noted as no-smoking, smoking between 1 and 10 cigarettes a day and more than 10 cigarettes a day.

During delivery:

- Placental weight in grams, after removing cord, membranes and blood, using a Krups weighing scale.
- Placental index: $\frac{\text{the placental weight in grams}}{\text{the weight of the child in grams}}$
- Blood loss in ml.
- Ponderal-index: $\frac{\text{the weight of the child in grams}}{(\text{length of the child in cm})^3} \times 100$
- Birth centile: according to the Kloosterman curve.

2.3. Dietary survey.

Every woman participating in the study, was interviewed in the 16th and 34th week of pregnancy as well as 6 months postpartum. Habitual food intake was assessed by a dietary history method, covering retrospectively the amounts and frequency of foods

onsumed in the previous 4 months. A differentiation was made between weekend and working days. During the interview food models and cubic measures were used. To increase the validity of the data obtained a cross-check was done at the end of each interview (Van Staveren et al., 1981). The interviews were performed by three trained dieticians during the regular visits of the women to the antenatal clinic.

All food intake data were encoded according to the Uniform Food Encoding (UFE) system in the Netherlands (Hautvast, 1975). In this system each food has a code number of 4 figures. In the UFE system the number of foods has been considerably extended compared with the Netherlands Food Table and covers almost all frequently consumed foods in the country. Encoding of the dietary data was done by the interviewing dietician. All encoded dietary intakes were finally transferred to a punch card and analysed by computer.

Vitamin B12 and D contents of food products not included in the Netherlands Food Table used for the calculation of nutrient intakes, were taken from McCance and Widdowson (1978). A control program to detect mistakes concerning code numbers and extreme or improbable quantities of food products was included in the computer program. When irregularities were detected, the original intake data were checked and verified by the dietician.

2.4. Blood collection and blood volume measurement.

During the antenatal- and postnatal period, the participants came to the clinic at 9 a.m. after an overnight fast, and blood was drawn from an antecubital vein using the venoject system. Women were laying in a lateral or semilateral position. Before the vena puncture they had been resting in this position for at least 10 minutes. After inserting and taping of the needle, the cuff was released for at least 30 seconds to minimize stases. Forty cc of blood were removed, 15 cc into tubes containing EDTA. After removing this blood, 20 cc of a 1.25% solution of

Evans Blue in 5% glucose was injected through the same needle, which was flushed with a few cc of 5% glucose after injecting the dye. The women remained in the lateral or semilateral position. Fifteen minutes after administration of the dye, 5 cc blood was removed from the antecubital vein on the other side using, again, the venoject system. The same procedure as mentioned before was repeated: 30 seconds after releasing the cuff, blood was drawn.
The blood was taken to the Department of Clinical Biochemistry of the Department of Obstetrics and Gynecology of the University of Utrecht (Drs.G.P.J.Alsbach); 5 cc of EDTA-blood was used for measuring hemoglobin, hematocrit, and erythrocytes. These measurements were done within an hour after venapuncture with the exception of blood that was taken during delivery. During working hours (8 a.m. till 5 p.m.), this was done immediately. After 5 p.m. this EDTA-blood was stored at 4°C and measured the next day. However, centrifugation (see below), was always done before storage.

20 cc blood was clotted for one hour at room temperature and centrifuged (10 minutes/3000 rpm), and plasma was frozen in several aliquots. The remaining 5 cc of clotted blood was used for blood volume measurements (see below). The 10 cc EDTA-blood was stored at 4°C.

Twice a week this frozen plasma and EDTA-blood were collected and brought to the Department of Clinical Biochemistry, CIVO Institute of Toxicology and Nutrition TNO (Dr.W.H.P.Schreurs). After arrival an aliquot of EDTA-blood was frozen directly at minus 20°C for total riboflavin analysis, and another aliquot was diluted 1:20 with a freshly prepared 0.2% Na-ascorbate solution and frozen at minus 20°C for folacin determination in the erythrocyte. The rest of this EDTA-blood was centrifuged, and plasma was frozen in different aliquots at minus 20°C. The remaining cells were washed three times with normal saline (0.9%), and the leucocytes were removed. After washing, the erythrocyte suspension was diluted 1:1 with a 0.5% Sterox-SE (Baker Chemicals, Deventer, The Netherlands) solution and frozen at minus 20°C.

The enzyme stimulation tests were all done within 3 weeks after arrival of the sample at the institute. The steroid hormones, 25-OH-Vitamin D and pyridoxal-5'-phosphate (PLP) determinations were done at the same time after all samples had been collected (maximal storage time 24 months). The other determinations were all done within one to 6 months after arrival of the sample at the laboratory and, until that time, stored at minus 20°C. According to literature and our own research in this area, these storage conditions are acceptable.

Blood volume measurement

The Evans Blue method was used for measuring plasma volume. Sterile ampoules containing 25 mg/20 cc Evans Blue (Merck Company, no. 3169) were prepared by the Pharmacy of the University Hospital of Utrecht. The Evans Blue used in this study is not the same dye available now under the same number, used only for microscopy, but was the special Evans Blue made until 1978 by the Merck Company for blood volume measurements. At 16 weeks gestation, 0.01 cc Evans Blue, taken from the ampule used, was added to 1.5 cc serum. It is important that the reading of the standard should be close to that of the dyed sample from the pregnant woman since the relationship between dye concentration and optical density is not strictly linear. At 28 and 34 weeks, the prepared standard was, therefore, diluted to parallel the more or less expected dilution in pregnancy (Mollison, 1956). At 28 and 34 weeks, 0.01 cc Evans Blue was added to, respectively, 1.9 cc and 2.1 cc serum. Serum of blood taken 15 minutes after the dye injection was measured in the same way.

Since blood was taken while the subject was in a fasting state, serum can be used without problems and it is not necessary to use plasma (Mollison, 1956; Hytten and Paintin, 1963). Injection of the dye was done according to Hytten and Paintin (1963) with the exception that women were lying in the lateral position. Care was taken that all dye was injected or that losses were measured. In principal, it seems desirable to take a series of blood samples, for example at 15, 25 and 35

minutes after injection of the dye. In practice, it is doubtful whether this extra trouble is repaid. Often, the three points do not fall precisely on a straight line so that dye concentration at zero-time cannot be precisely measured, although differences are less than 3% (Mollison, 1956). In three non-pregnant volunteers we sampled at 10, 15 and 20 minutes and the observed differences in calculated plasma volume were less than 5%. In almost all studies, a single sample taken at 10 or 15 minutes is the procedure used.

Plasma volume was calculated according to the following formula:

$$20 \times \frac{\text{cc standard}}{0.01} \times \text{extinction standard serum} \Big/ \left(\text{extinction "15 minutes" serum} \times \frac{100}{97.5} \right)$$

The extinction was measured at 620 nm, using a spectrophotometer type VARIAN-TECHTRON MOD. 635. After determination of plasma volume, blood volume was calculated by using the following formula: Plasma volume $\times \frac{100}{100-Ht \times 0.91}$

In this formula the venous hematocrit is corrected for the whole body hematocrit. No correction has been made for the "trapped" plasma in the red cell volume. This is not considered within the study since changes in red cell and plasma volume, but not their absolute values, are considered important (Chesley, 1972).

The anthropometric measurements, blood collection and blood volume measurements were done by a research nurse especially assigned to the project. Measurements and blood collection during and directly after delivery were done by the midwife in charge of the delivery ward.

2.5. Blood determination.

2.5.1. Hematological parameters.

Hemoglobin was measured using the hemoglobin cyanide method (NEN 2407, Zijlstra and Van Kampen, 1962).
Hematocrit was measured using a Hettich centrifuge (3.000 g at 13.000 rpm for 5 minutes).
Erythrocytes were counted in an electronic cell counter type ANALYS-34.
All tests were done in duplicate.

2.5.2. Vitamin status parameters.

Retinol (vitamin A)
After saponification in 1N ethanolic KOH (20 minutes at 60°C), 100 µl serum was extracted with 1 ml xylene and then separated by high pressure liquid chromatography (column, 250 x 4.6 mm ID stainless steel filled with Polygosil 60-5 (Macherey-Nagel/GmbH & Co, Düren, FRG); Eluent, n-hexane: methylene chloride: isopropanol (90:10:1.2, v/v) . Retinol content was measured fluorometrically (extinction, 433 nm; emission, 470 nm) (Fankel, 1978).

25-Hydroxy-Vitamin D (25-OHD)
25-OHD serum content was measured using a modified competitive protein binding (CPB) assay, according to Edelstein et al. (1974), without column purification of the extract. 200 µl serum was extracted with dichloromethane:methanol:water (2.5:2.5:1.5, v/v). Recoveries were between 90-95%. Diluted rat serum (1:15,000) was used as the binder after precipitation of the β-lipoprotein fraction with Heparin-$MnCl_2$ (Belsey et al., 1971).

Riboflavin (vitamin B2)
Total riboflavin content in whole blood was determined fluorometrically (extinction, 436 nm; emission, 518 nm), according to Baker and Frank (1975) after extraction with trichloroacetic acid.

Pyridoxal-5'-phosphate (vitamin B6)
Plasma pyridoxal-5'-phosphate (PLP) content was determined by the radioenzymatic method of Chabner and Livingstone (1970). Tyrosine decarboxylase apoenzyme was purchased from Sigma Chem. Company (St.Louis, Missouri, USA) and was used without further purification.

Folacin
Serum folacin content was measured, using a CPB-assay with a folate binding milk protein based on the method originally described by Dunn and Foster (1973). Reagents were used from the Quanta-Count Folate radioassay kit (Bio-Rad. Laboratories, Richmond, California, USA) (5-Methyl-tetrahydrofolate was used as the standard). Red cell folacin was measured using the same procedure after 1:20 dilution of a whole blood aliquot with a 0.2% sodium ascorbate solution, containing 4% bovine serum albumin. Whole blood lysates were stored at minus 20°C until CPB-analysis. Red cell folacin content was calculated as follows:

$$\text{Red cell folacin} = \frac{\text{whole blood folacin content (nmol/l)}}{\text{hematocrit}} \times 100$$

Vitamin B12
Vitamin B12 serum content was measured using a CPB-assay with Intrinsic Factor (IF) covalently coupled to Sephadex as the binder, based upon the method originally described by Wide and Killander (1971). Reagents were used from the Phadebas B12-Test kit (Pharmacia Diagnostics; A.B., Sweden). More recently published studies from Kolhouse et al. (1978) have indicated that most commercially available IF-preparations are contaminated with the

so-called R-proteins, resulting in relatively higher values. B12-content measured with R-protein binders, or contaminated IF-binders, is commonly referred to as "total" vitamin B12, while B12-content measured with purified IF-binders is referred to as "true" vitamin B12. Between "total" and "true" vitamin B12 contents, a highly significant correlation has been observed (Kubasik et al., 1980).

ETK stimulation test
The transketolase (E.C. 2.2.1.1) enzyme activity (ETKA) in hemolysates of washed erythrocytes and the in vitro stimulation with thiamine diphosphate (TDP) were measured according to the kinetic method described by Smeets et al. (1971) with ribose-5'-phosphate as the substrate.

EGR stimulation test
The glutathion reductase (E.C. 1.6.4.2) enzyme activity in hemolytases of washed erythrocytes and the in vitro stimulation with flavine adenine-dinucleotide (FAD) were measured according to Tillotson et al. (1972) with oxidized glutathion (GSSG) as the substrate but with a higher NADPH-concentration (168 µMol) as described by Bayuoumi et al., 1976).

EGOT stimulation test
The glutamate-oxaloacetate transaminase (E.C. 2.6.1.1) enzyme activity in hemolysates of washed erythrocytes and the in vitro stimulation with pyridoxal-5'-phosphate (PLP) were measured according to Stanulovic et al. (1967), using reagents from the GOT-test kit (Test Combination GOT, UV-method, Cat. No. 191337, Boehringer Mannheim Diagnostics, Germany).

2.5.3. Routine clinical chemistry methods.

Protein
Serum total protein content was measured by a colorimetric method with biuret-reagent, originally described by Gorter and De Graaf (1955). Sera were calibrated against Wellcome control serum (batch K 5916, Wellcome Reagents Limited, London, England).

Albumin
Serum albumin content was measured by a colorimetric method with bromocresol green, according to Doumas et al. (1971). Sera were calibrated against Wellcome control serum (batch K 5916).

Alkaline phosphatase (AP) (E.C. 3.1.3.1)
AP activity in serum was measured colorimetrically at 25°C using reagents and protocol from Boehringer Mannheim Diagnostics (Germany) (Alkaline Phosphatase optimized colorimetric method, Cat. No. 123854).

L-γ-Glutamyltransferase (γ-GT) (E.C. 2.3.2.1)
γ-GT activity in serum was measured colorimetrically at 25°C using reagents and protocol from Boehringer Mannheim Diagnostics (Germany) (γ-GT new colorimetric method, Cat. No. 125954).

Alanine aminotransferase (GPT) (E.C. 2.6.1.2)
GPT activity in serum was measured colorimetrically at 25°C using reagents and protocol from Boehringer Mannheim Diagnostics (Germany) (GPT optimized UV-test, Cat. No. 124567).

Cholesterol
Serum cholesterol content was measured using a direct colorimetric method with the Liebermann-Burchard reagent, according to Huang et al. (1961). The procedure was standardized according to NEN-protocol 2415.

Triglycerides
Serum triglyceride content was measured with an enzymatic method using reagents and protocol from Boehringer Mannheim Diagnostics (Germany) (Biochemica Test Combination Triglycerides, UV-method; Cat. No. 124966).

Urea
Serum urea content was measured by an enzymatic method based upon the method originally described by Fawcet and Scott (1960), using the reagent kit from Boehringer Mannheim Diagnostics (Germany) (Biochemica Test Combination Ureum, Cat. No. 124788).

Creatinine
Serum creatinine content was measured by a direct colorimetric method with Jaffé reagent based upon the method originally described by Bartels and Böhmer (1971) using the reagent kit from Boehringer Mannheim Diagnostics (Germany) (Biochemica Test Combination Creatinine, Cat. No. 124192).

Uric acid
Serum urate content was measured enzymatically using reagents and protocol from Boehringer Mannheim Diagnostics (Germany) (Urica-quant, Cat. No. 124761). This method was originally described by Kageyama (1971).

Iron and iron-binding capacity
Iron and its binding capacity in serum were measured by a colorimetric method with ferrozine according to Führ (1965). Percentage saturation was calculated by dividing serum iron content (SI) by the total serum iron-binding capacity (TIBC) and multiplying by 100 ($\frac{SI}{TIBC}$ x 100 = percent saturation).

Transferrin
Transferrin content in serum was measured by the single radial immuno-diffusion technique (Mancini et al., 1965) using the commercially available M-Partigen plates (Behring Werke, Marburg, Germany). Sera were calibrated against human standard serum (Batch 1003 G, Behring Werke).

Ferritin
Serum ferritin content was measured by a radioimmunometric method based upon the method originally described by Miles et al. (1974), using the Ferritin - Irma kit (Cat. No. 17-Fer-100-1) from Nordic Lab OY (Oulu, Finland). The antiserum provided in the kit was raised against human spleen ferritin, which was also used as the standard. Radioimmunometric determination of ferritin is preferred above radioimmunoassay, especially for determination at the lower concentration of ferritin levels (Lipschitz et al., 1981).

2.5.4. Hormonal analysis.

Estradiol (E2)
Plasma E_2 content was measured by direct radioimmunoassay (Oosterveen, 1981). The antiserum was raised against 6-keto-Estradiol coupled to bovine serum albumin via the 6-O-(carboxymethyl-) oxime (Makor Chem. Ltd., Jerusalem, Israel). Endogenous estradiol binding to serum proteins (sex hormone binding globulin (SHBG) was blocked by the addition of excess testosterone (10 ng/tube/5 µl plasma) (Jurjens et al., 1975).

Estriol (E3)
Total plasma estriol content was measured by direct radioimmunoassay using the estriol (total) II RIA kit (Code IM 1040) from the Radiochemical Centre, Amersham, U.K.).

Cortisol
Serum cortisol content was measured by direct radioimmunoassay using the

Cortisol
Serum cortisol content was measured by direct radioimmunoassay using the Cortisol RIA premix kit (Cat. No. KCO D2) from Diagnostic Products Corporation (Los Angeles, California, USA).

Progesterone
Plasma progesterone content was measured by radioimmunoassay after extraction of the plasma with 20 volumes n-hexane. The antiserum used was raised against 11α-hydroxy-progesterone hemisuccinata-BSA (Makor Chem., Jerusalem, Israel), using the procedure of Baumiger et al. (1974).

Human Placental Lactogen (PHL)
Serum HPL content was measured by radioimmunoassay using the HPL-RIA kit from Nordic Lab. (Cat. No. 19-HPL-100-1, Oulu, Finland).

Prolactin
Serum prolactin content was measured by a radioimmunoassay using the Radormon Prolactin kit from Kabi-Diagnostica (Stockholm, Sweden). The standard provided in the kit was calibrated against the MRC 71/222 standard preparation (1 ng of the standard provided in the kit = 1 ng NIH Reference preparation VLS no. 3 = 25 μU).

Thyroxine (T4)
Serum T_4 content was measured by radioimmunoassay using the T_4 RIA (PEG) Diagnostic Kit from Abbott Laboratories (Los Angeles, California, USA).

T3 Resin uptake (T3-U)
To assess latent binding capacity of thyroxine binding proteins, mainly thyroxine binding globulin (TBG), the T_3-Resin Uptake test was performed (a.o. Clark and Horne, 1965). The T_3-Uptake Assay kit (Cat. No. KTU D1) from Diagnostic Products Corporation (Los Angeles, California, USA) was used.

Free thyroxine index (FTI)
The FTI was calculated as follows: $FTI = \frac{T3\text{-}U(\%)}{100} \times$ serum T_4 concentration (nmol/l).

2.6. Quality control.

From every woman participating in the study, blood samples were collected between the 16th week of pregnancy and 6 months postpartum. The project involved 116 women, whose blood samples were collected and analysed between May 1978 and June 1980. As already indicated (section 2.4) most samples were analysed within 6 months after they were collected. Enzyme stimulation tests were performed within 3 weeks, while steroid hormones, PLP and 25-OHD were measured only after all samples had been collected. To assess the inter-assay variation during the whole experimental period, a strict system of internal quality control was introduced. In all series of measurements, quality control sera and pool sera were included at different levels. One batch of control or pool serum was used throughout the whole experimental period as much as possible. In those cases where it was not possible to use the same batch of control or pool serum throughout the whole period, e.g. in case of the enzyme stimulation tests, the different batches overlapped amply.

The inter-assay variation coefficient (CV) calculated for the various assays are summarized in Tables 2.6.I, 2.6.II and 2.6.III. In Table 2.6.IV the reference values and cut-off points for some vitamin status parameters in non-pregnant adults used at the Institute CIVO-Toxicology and Nutrition-TNO, are given. These reference ranges were based upon measurements in populations of apparently healthy blood donors.

Also indicated in this table are the mean values ($\pm$ SD) for some non-pregnant (female) groups, obtained in previous studies performed at the Institute.

TABLE 2.6.I Mean value (± SD) and the inter-assay variation coefficient (CV) of the quality control samples measured during the whole project period I : Vitamin status parameters.
S = serum B = whole blood Pl = plasma.
P = Pool serum/blood
NMS = Control serum (Nuclear Medical Systems Inc, Newport Beach, California, USA).

Parameter	Mean value ± SD of pool or control serum (blood)	N	CV (%)	Control serum
Retinol (S)	1.00 ± 0.18 µmol/l	11	18	P
25-hydroxy-vitamin D (S)	33 ± 5 nmol/l	24	15	P_1
	93 ± 10 nmol/l	24	11	P_2
	173 ± 22 nmol/l	24	13	P_3
ETK Activity	10.7 ± 0.9 U/L	61	8	P
αETK	1.09 ± 0.05	61	5	P
EGR Activity	104 ± 7 U/L	39	7	P
αEGR	1.04 ± 0.04	39	4	P
EGOT Activity	69 ± 4 U/L	33	6	P
αEGOT	1.81 ± 0.10	33	5.5	P
Riboflavin (B)	0.34 ± 0.02 µmol/l	45	6	P
Pyridoxal phosphate (PLP)	6.4 ± 1.1 nmol/l	23	16	P_1
	22.6 ± 1.5 nmol/l	23	7	P_2
	54.3 ± 4.1 nmol/l	19	8	P_3
Folacin (S)	6 ± 0.6 nmol/l	12	10	P_1, NMS
	11 ± 0.9 nmol/l	12	8	P_2, NMS
	22 ± 1.8 nmol/l	12	8	P_3, NMS
Vitamin B_{12} (S)	270 ± 27 pmol/l	12	10	P_1, NMS
	550 ± 47 pmol/l	12	9	P_2, NMS

TABLE 2.6.II Mean value (± SD) and the inter-assay variation coefficient (CV) of the quality control samples measured during the whole project period. II : Hormonal parameters.
S = serum P = poolserum

Parameter	Mean value ± SD of pool serum				N	CV (%)	Control serum
Estradiol (S)	10	±	1.5	nmol/l	22	15	P_1
	26	±	3.0	nmol/l	22	12	P_2
	77	±	9.0	nmol/l	22	12	P_3
Estriol (S)	35	±	1	nmol/l	8	3.5	P_1
	377	±	10	nmol/l	8	3	P_2
	1132	±	46	nmol/l	8	4	P_3
Cortisol (S)	0.08	±	0.01	µmol/l	11	12	P_1
	0.55	±	0.05	µmol/l	11	10	P_2
	1.66	±	0.14	µmol/l	11	8	P_3
Progesterone (S)	151	±	19	nmol/l	13	12	P_1
	649	±	70	nmol/l	13	11	P_2
	1149	±	151	nmol/l	13	13	P_3
Prolactin (S)	6.4	±	1.1	ng/ml	14	18	P_1
	28.2	±	4.4	ng/ml	14	15	P_2
	64	±	9	ng/ml	10	14	P_3
HPL (S)	2.9	±	0.3	ng/ml	14	11	P_1
	8.7	±	1.1	ng/ml	14	12	P_2
T_4 (S)	83	±	5	nmol/l	14	6	P_1
	165	±	10	nmol/l	14	6	P_2
T_3-uptake (S)	29.3	±	1.6	%	22	6	P_1
	35.6	±	2.0	%	22	6	P_2

TABLE 2.6.III Mean value (± SD) and the inter-assay variation coefficient (CV) of the quality control samples measured during the whole project period. III : Clinical chemical parameters.
S = serum P = poolserum

Parameter	Mean value ± SD of control or pool serum			N	CV (%)	Control serum
Iron (S)	18.0	± 1.3	µmol/l	18	7	Monitrol 147 A[1]
Total Iron Binding Capacity (TIBC)	65	± 4	µmol/l	36	6	Monitrol 147 A[1]
Ferritin (S)	13	± 3	ng/ml	29	21	P_1
	30	± 5	ng/ml	29	16	P_2
	354	± 40	ng/ml	29	12	P_3
Transferrin (S)	204	± 4	g/l	19	2	K-7003[2]
Total protein (S)	54.5	± 0.6	g/l	14	1	Precinorm 724[3]
Albumin (S)	41.3	± 0.5	g/l	9	1	Monitrol 147 A[1]
Cholesterol (S)	5.7	± 0.3	mmol/l	24	2	P
Triglycerides (S)	1.78	± 0.07	mmol/l	22	4	P
Creatinine (S)	157	± 9	µmol/l	11	6	Monitrol 153 A[1]
Urea (S)	8.44	± 0.24	mmol/l	5	3	Precinorm 835[3]
Uric acid (S)	315	± 20	mmol/l	11	7	Precinorm 835[3]
Alk. phosphatase (S)	197	± 13	U/L	13	7	Precinorm 835[3]
SGPT (S)	15.6	± 1.1	U/L	14	7	Monitrol 147 A[1]
γ-GT (S)	44	± 2	U/L	12	4.5	Precinorm 724[3]

1) Monitrol 147 A and 153 A, Dade R, Düdingen, Switzerland
2) Behring Werke, Marburg, Germany
3) Precinorm 724 and 853, Boehringer Mannheim Diagnostics, Germany

TABLE 2.6.IV Reference values and cut-off points for some vitamin status parameters in non-pregnant adults (Institute CIVO-Toxicology and Nutrition-TNO).

	Non-pregnant reference range	Cut-off point	Mean value (± SD) observed for an apparently healthy adult (non-pregnant) reference group (blood donors/laboratory workers)	
			$\bar{x}$ ± SD	N
Retinol (S) (μmol/l)	1.2 - 2.6 > 0.7*	< 1.1 < 0.7*	1.84 ± 0.42 1.78 ± 0.38	135 (M/F) 21 (F)
25-hydroxy-vitamin D (S) (nmol/l)	30 - 100	< 20	62 ± 24 [1] 40 ± 22 [2] 95 ± 35 [2]	74 (M/F) 23 (F) 23 (F)
ETK activity (E) (U/mmol Hb)	9.2 - 13.2	-	11.5 ± 1.8	74 (M/F)
αETK (E)	1.00 - 1.20	> 1.25	1.10 ± 0.05	74 (M/F)
Vitamin B_2 (B) (μmol/l)	0.25 - 0.40	< 0.22	0.33 ± 0.04	74 (M/F)
EGR activity (E) (U/mmol Hb)	68 - 131	-	98 ± 13	74 (M/F)
αEGR (E)	1.00 - 1.30	> 1.30	1.15 ± 0.11	74 (M/F)
Pyridoxal phosphate (P) (nmol/l)	15 - 90	< 15	48 ± 20 40 ± 19	89 (M/F) 24 (F)
EGOT activity (E) (U/mmol Hb)	53 - 95	-	69 ± 16	74 (M/F)
αEGOT (E)	1.65 - 2.10	> 2.20	1.85 ± 0.20	74 (M/F)
Folacin (S) (nmol/l)	4.4 - 22	< 3.6	8.9 ± 2.9 9.0 ± 3.5	74 (M/F) 80 (F)
Vitamin B_{12} (S)	220 - 750	< 180	398 ± 108	74 (M/F)

Abbreviations: B = whole blood; S = serum; E = erythrocytes; P = plasma
M = males; F = females; 1) samples collected in March/April
2) samples collected in July/August
* ICNND and NAS/NRC guidelines (Sauberlich et al., 1974)

TABLE 2.6.V Reference values of the hematological, biochemical and hormonal parameters assessed for non-pregnant adults.

	Non-pregnant reference values	Source
Hematology		
Hb mmol/l	7.4 - 9.6	1)
Ht L/l	0.36 - 0.46	1)
Erythrocytes 10^{12}/l	3.7 - 5.0	1)
MCV fl	85 - 105	1)
MCH amol/l x 10^3	1.7 - 2.2	1)
MCHC mmol/l	19 - 23	1)
Blood volume L (mean value)	3.8	2)
Plasma volume L (mean value)	2.5	2)
Red cell volume L (mean value)	1.36	2)
Clinical chemistry		
Total protein g/l	62 - 78	3)
Albumin g/l	38 - 52	3)
Cholesterol mmol/l	4.0 - 7.3	3)
Triglyceriden mmol/l	0.85 - 2.0	3)
Creatinine serum µmol/l	44 - 120	3)
Urea serum mmol/l	3.0 - 7.3	3)
Uric acid serum nmol/l	< 360	3)
Alkaline phosphatase U/l	50 - 135	3)
SGPT U/l	< 18	3)
γGT U/l	< 40	3)
Serum iron µmol/l	14 - 32	3)
TIBC µmol/l	35 - 78	3)
Transferrin g/l	2 - 4	3)
Percentage saturation %	0.25 - 0.60	3)
Ferritin ng/ml	20 - 200	3)
Hormones		
Estradiol 17β nmol/l	< 2	1)
Progesterone nmol/l	< 64	1)
Prolactin ng/ml	< 20	1)
Cortisol µmol/l	< 0.65	1)
Thyroxine nmol/l	60 - 170	3)
T_3-uptake %	23 - 33	3)
FTI	15 - 56	3)

1) Cokcl, AZU

2) Wissenschaftliche tabellen, Geigy, 1976

3) Range stated by Kit manufacturer (section 2.5)

2.7. Statistical methods (Ir.J.T.N.M.Thissen, statistician, IWIS-TNO).

Correlations

The calculated correlation coefficients are Pearson correlation coefficients except otherwise stated. With all coefficients, graphs have been made (not printed in this report) to look at the scatter of the points to ensure a right interpretation.

Change of variables in time

The difference between the means of a variable at two different points of measurement has been tested with Student's t-test on paired observations.

Change of dietary intake variables in time

The differences between the means of the dietary intake variables for the three different periods have been tested with a randomized block analysis of variance where the blocks are women and the treatment is period. In the case the P-value was less than 0.05, the l.s.d. (least significant difference) was calculated at a two-sided level of 0.05.

Influence of one factor on some variables

To investigate the influence of one factor, e.g. smoking, on some variable e.g. birth centile, analysis of variance has been carried out. In case the P value was less than 0.05, the l.s.d. (least significant difference) was calculated at a two-sided P level of 0.05.

Influence of more than one factor on some variables

The designs with more than one factor were all inorthogonal. So, inorthogonal analyses of variance have been carried out. The main effect of each of the factors was adjusted for the main effects of the other factors; the two factor interactions were adjusted for all main effects and the other two factor interactions (if present) and so on.

Regression of a variable on another variable adjusted for a factor

Three regressions have been carried out. First the single factor was put in the regression model. Then the second regression was carried out with the factor and the variable in the model assuming the same linear relation between the two variables for each of the levels of the factor. Thereafter, the second model was extended with the interaction factor level, giving the third model. This model assumes a different linear relation between the two variables for each of the levels of the factor.

The differences in the residual sums of squares of the successive models have been used to perform F-tests at a P level of 0.05.

Multiple regression (stepwise and subset selection)

To explain the variation in some dependent variables, multiple regressions have been carried out. The regression model contained both factors and variables as independent variables. The following assumptions have been made:

- the relationships between the dependent variable and the independent ones are linear
- these relationships are independent of the levels of the factors
- there are no interactions between factors.

First, a great number of independent variables had been chosen. With this total set of independent variables, the percentage of variance accounted for has been calculated.

The following question to be answered was: which subsets of the independent variables could give an almost equally percentage variance accounted for. To obtain a first idea, stepwise multiple regression has been carried out on the total set of variables. This procedure has the drawback of getting only one "best" subset. Moreover, the program in GENSTAT accepted only a subject if data for all variables were completely present, so the number of available subjects was limited in most cases.

After that, the total set of independent variables has been reduced. On this new set a subset selection procedure in regression analysis has been carried out with a branch and bound algorithm Screen (1981) from G.M.Furnival and R.W.Wilson (1974). This procedure selects for every value of k (the number of independent variables in the regression model) the best p (number given by the user) subsets. Best means the greatest R^2 (i.e. the square of the multiple correlation coefficient).

2.8. Account for selected parameters.

Maternal weight, prepregnant weight, weight gain during pregnancy, paternal height and smoking habits were selected because a relationship exists between the birth(weight) centile of the newborn and these parameters (section 1.4.1 and 1.4.3). The birth centile of the newborn - a parameter that can be calculated when birthweight, sex of the newborn and the duration of pregnancy are known - was selected since it represents intra-uterine growth better than birthweight due to the great dependence of the last on the duration of pregnancy (section 1.4.3).

The Ponderal-index was chosen because it differentiates better than the birth centile between proportional and dysproportional intra-uterine growth (section 1.4).

Blood, plasma and red cell volume measurements were performed since: 1) a strong relationship was demonstrated between plasma volume during pregnancy and birthweight of the newborn (section 1.1.1 and 1.4.3) 2) levels of biochemical parameters may be influenced by the hemodilution occurring in pregnancy (section 1.3).

For evaluation of the (human) vitamin status a number of tests are available (Sauberlich et al., 1976). These tests can be roughly divided into two categories: 1) direct tests in which the concentration of some vitamin or related metabolite is measured in blood or urine, 2) indirect or functional tests in which the availability of the active vitamin at the cellular

level is measured, like the enzyme stimulation tests and the tryptophan loading test.

The pros and cons of the various parameters for assessment of the vitamin status were discussed in section 1.3. The parameters measured in this study, summarized in section 2.5 (Table 2.6.IV), were selected both on available data about validity and accuracy, as well as on more practical criteria. Direct measurement of blood thiamin and vitamin B6 were abandoned because of the problems in the standardization of the microbiological methods, which were the only available methods for these vitamins at the time of the study.

Vitamin E, K, niacin, pantothenic acid and biotin status parameters were not measured in this study because these vitamins are unknown to be associated with clinical deficiency symptoms in humans, and because the parameters available for evaluation of the vitamin status for these vitamins are not as fully validated in all cases (see section 1.3.10).

Another criterium to exclude vitamins from this study was that no reliable or practical method for determination was available, like for vitamin K. Vitamin C was left out because the expected problems in sample handling and transport, and in sample storage due to the known instability of this vitamin.

For evaluation of the iron status a number of tests are available. We selected the most commonly used parameters, i.e. serum iron, percentage saturation, total iron binding capacity and serum transferrin. Serum ferritin was selected as well since it can be used as a parameter representing the iron stores (section 1.3.11).

Hormonal and routine clinical chemical parameters measured in this study were selected because of one of the following considerations: 1) general evaluation of the (nutritional) health status, 2) assessment of organ function, i.e. liver and kidney function tests, also to exclude pathology and 3) to serve as independent variables in the multiple regression analyses to explain for the variance in the fall of some vitamin blood or serum levels during pregnancy, as well as the variance in birth centiles (sections 6.6 and 8.9, respectively).

CHAPTER 3.

The study population.

In the period of May 1978 to May 1979, women visiting the prenatal clinic of the Department of Obstetrics of the University Hospital of Utrecht were asked to participate in the study. One hundred-sixteen women fulfilling the intake selection criteria were prepared to join the study. During the study seven dropped out: two after an abortion and five because of recurring problems with the vena puncture.
The last six months postpartum control was in May 1980.

After completing the study, 23 women did not fulfill the "normal" pregnancy and "normal" puerperium criteria (see section 2.1.1). The reasons for rejecting these women from the study were (numbers of subjects are given in parentheses):
- premature delivery (6)
- gestational diabetes (4)
- blood transfusion postpartum (5)
- toxemia (3)
- caesarean section (2)
- admission into the hospital during pregnancy for other reasons (3).

Eighty-six women fulfilled all criteria. One joined the study twice. She was pregnant within six months after her first child and was readmitted when she requested to join the study again. Only the data of her second pregnancy were used, so 85 cases were finally evaluated.

The birth centile of the children in 70 cases was between 10th and 90th centile of the Kloosterman curve (Kloosterman, 1969, 1970). This group is considered as the S-Reference group (n = 70). For 10 cases, the birth centile was on or below the 10th centile of the Kloosterman curve, and this group is considered as the "smaller than P10 group" (n = 10). The birth centile was on or above the 90th centile of the Kloosterman curve in 5 cases, the "larger than P90 group" (n = 5).

In Table 3.1, the following data for the three groups are given: age, height, prepregnant weight, maximal weight gain, duration of pregnancy, blood loss during delivery and partner's height. Not unexpectedly, there is a tendency for a larger mean height, prepregnant weight and weight gain as the birth centile increases. In Figure 3.1 and in Table 3.2, the changes in weight during and after pregnancy are described. The pattern of weight changes for the S-Reference and < P10 group are almost similar. The maximal weight gain expressed as a percentage of the prepregnant weight for the three groups is, respectively, 18.5% (S-Reference group), 17.3% (<P10 group) and 22% (>P90 group).

In Table 3.3, data concerning gravidity, parity, smoking habits, iron medication, breast-feeding and contraception are given. Forty-five of the 85 subjects smoked, a rather high percentage. In the < P10 group, all women smoked and seven out of ten smoked more than 10 cigarettes a day. These data on smoking habits were collected by the research nurse of the project, who worked outside the prenatal clinic and was not involved in the prenatal care; she kept her records apart from the prenatal records. There is a remarkable difference in the information on smoking habits when comparing her records with the prenatal records: the number of cigarettes in the prenatal record was always less than in the project records.

Only 7 out of the 85 subjects were given iron tablets during pregnancy, mostly because the Hb fell below 6.8 mmol/l. In 3 cases, the woman asked for iron supplement. Fifteen women were given iron tablets postpartum. Fifty-five percent of the total group did not breast-feed at all, a situation that has

fortunately changed today. Only 16% were still breast-feeding after 6 weeks.

Seventy percent of the women started oral contraception, almost all within 14 days after delivery.

In Table 3.4, data concerning the child and placenta are given: sex, weight, length, birth centile and Ponderal index of the child, and weight and index of the placenta (placental weight divided by birthweight). It is remarkable that the mean birth centile of the total group is 42.2. One might have expected in this group of healthy women and uneventful pregnancies, a mean birth centile at least on, and probably above, the 50th centile. This will be further discussed in chapter 8.

In Table 3.5, the conception months are given for the different groups. The S-Reference groups is equally divided over the summer and winter periods. There may be a tendency for conception of the < P10 group in the winter months, but numbers are too small for any conclusion.

CHAPTER 4.

Hematological, biochemical and hormonal parameters in the population studied.

-Introduction

In this chapter the aforementioned parameters will be described and shortly discussed. These parameters have been measured mainly because there was proof or there were indications that they are involved in the mechanisms inducing changes in vitamin blood levels or are involved in the mechanisms that determine the birth centile of the newborn (section 2.8). The literature about the changes of these parameters and about the possible mechanisms inducing these changes during pregnancy was reviewed in section 1.1.2. It was thought to be outside the scope of this study to give an extensive description and explanation of the observed changes of all parameters measured, and most parameters have been compared with the generally accepted values found in literature.

In the figures belonging to the description of the parameters described in this chapter, the P10, P50 and P90 values for the S-Reference group are given. For comparison the median of the group with a birth centile on or below the 10th centile has been given as well. Findings from the small group with a birth centile on or above the 90th birthweight centile are not presented. More detailed data of the S-Reference group are found in the tables underneath the figures: numbers, means, standard deviations and ranges. For some parameters, it has been tested whether the changes during and after pregnancy differ significantly, using the Student's t-test (section 2.6). $P < 0.05$ was considered to be significant.

Detailed data of the < P10 group and > P90 group are shown in separate tables, which will be referred to in the text.

In the figures, the 10th and 90th centile points for the different moments of measurements have been connected by interrupted lines and the 50th centile points by an uninterrupted line. This may give rise to optical illusions as it may suggest a linear increase or decrease of a certain parameter from the moment the measurement has been performed. This has not been our intention. The lines have been drawn only to make it easier to compare the different points at the different moments. As has been described in Chapter 2, the moment of drawing maternal blood during delivery was not standardized, and sometimes it was possible only shortly after delivery of the baby. This has definitely influenced the partus values of hormones produced by the feto-placental unit, especially when they have a short half-life, e.g. human placental lactogen. The hormonal values obtained during delivery are only described and are not used in the statistical analyses in the following chapters.

-Hematology

In Tables 4.1, 4.3 and 4.5, the changes in blood, plasma and red cell volume are described for the S-Reference group and are illustrated in Figures 4.1, 4.2 and 4.3. A more detailed description of the data of the < P10 and > P90 group is given in Table 4.13.

The mean increase in plasma volume is 37%, which corresponds with the figures found in literature (Chesley, 1972). Red cell volume increased by only 15%, which is rather low, probably due to the lack of routine iron supplementation. The absolute increase is 220 ml; this corresponds well with figures given by Hytten and Leitch (1971) for non-iron supplemented gravidae (section 1.1.2). In Tables 4.2, 4.4 and 4.6, the significance of differences of the blood, plasma and red cell measurements are given. As can be seen from these data, there is a significant increase of blood and plasma volume before the 16th week of pregnancy. This contrasts with the increase in red cell volume

that starts in the second trimester. Blood volume values postpartum have returned to the non-pregnant state about 6 weeks postpartum since no significant differences between the 6th week and 6th month values could be found. However, there is a small, but significant difference in plasma volume values between 6 weeks postpartum and 6 months postpartum. Red cell volume is not found to be significantly different between these two moments. The median of the < P10 group is found to be below the 50th centile of the S-Reference group during pregnancy, but approaches the 50th centile in the postpartum period. This suggests a smaller increase (particularly early in pregnancy) in this group; however, due to the small number of subjects, no conclusions may be drawn.

Changes in hemoglobin, hematocrit and erythrocytes are illustrated in Figures 4.4, 4.5 and 4.6. Data of the S-Reference group are given in Tables 4.7, 4.9 and 4.11. The significance of changes during and after pregnancy are given in Tables 4.8, 4.10 and 4.12. The findings are consistent with the observations described in the literature (Hytten and Lind, 1973). Already early in pregnancy, a decrease of all these parameters is found probably due to the increase in plasma volume. The lowest values are found at 28 and 34 weeks. These values were not significantly different from each other. For all three parameters, the values found during delivery were significantly above the 34 weeks values.

Six weeks postpartum, the non-pregnant situation has not yet been reached as 6 month postpartum values are all significantly higher.

When comparing the median of the < P10 group with the median of the S-Reference group, a remarkable early increase in hemoglobin and hematocrit values is observed during pregnancy. This harmonizes with the observation of Mau (1977), who observed a high percentage of dysmature children in women with high hemoglobin levels in the third trimester.

In Figures 4.7 to 4.9 and Tables 4.14 to 4.20, findings are presented for the Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin

Concentration (MCHC) of the erythrocytes. During pregnancy, no consistent, significant changes in all these three parameters could be observed, which is in accordance with the observations described in literature (see section 1.3.11). This indicates that the erythrocyte itself does not change during pregnancy.

In the first 6 weeks after delivery, there is a decrease for all three parameters, the 6 weeks postpartum mean value being lower than all mean values measured during pregnancy. Six months postpartum, all values are found within the non-pregnant reference range. The median values of the < P10 group are all found to be around the median of the S-Reference group. Detailed data concerning the < P10 and > P90 are described in Table 4.20.

-Total protein and albumin

Changes in total protein and albumin serum concentrations are illustrated in Figures 4.10 - 4.11 and described in Tables 4.21 - 4.24 and 4.29. Total serum protein decreases during pregnancy by 5 g/l: a maximal decrease of about 7.5%. This is less than the 10 g/l generally described in literature (Hytten and Lind, 1973). However, this 10 g/l is an overall mean, and in the studies cited, rather large differences in mean decrease can be observed. In the postpartum period, there is a slow increase, and highest non-pregnant values are measured 6 months postpartum, the difference between 6 weeks postpartum and 6 months postpartum being significant (Table 4.22).

Serum albumin shows a similar pattern, although the decrease (about 18%) is more pronounced than that of total protein: the concentration falls quickly, reaching the lowest point at 28 weeks, whereafter no significant changes are observed during pregnancy. After delivery, there is again an increase with its highest value measured at 6 months postpartum. The difference between 6 weeks and 6 months postpartum is significant.

Except for the rather low values of protein and albumin of the <P10 group at the 16 weeks point, no different trend is observed when comparing median values of this group with the S-Reference group.

Data concerning the < P10 group and > P90 group are given in Table 4.29.

-Cholesterol and triglycerides

Serum cholesterol and triglycerides observations are given in Figures 4.12 - 4.13 and Tables 4.25 - 4.29.

The mean increase in cholesterol level during pregnancy is 35%, a percentage in accordance with percentages given in literature (section 1.1.2). The mean increase in triglyceride level in this group is 140%. This increase is slightly below the percentages given in literature (section 1.1.2). The pattern of increase is the same. The main decrease postpartum occurs in the first 6 weeks, but there is a small, significant fall in the period thereafter (see Tables 4.26 and 4.28).

Median values of the <P10 group are all found around the median of the S-Reference group.

Data for the < P10 and > P90 group are given in Table 4.29.

Hormonal parameters

The possible mechanisms which induce the enormous rise in the hormones described have been reviewed in section 1.1.2, together with their respective functions during pregnancy.

-Estrogens

Data concerning estradiol 17-β and estriol serum levels are presented in Figures 4.14 and 4.15 and Tables 4.30 and 4.38. These data are in accordance with data given by Klopper (1980). The median of the < P10 group during pregnancy is not different from the median of the S-Reference group. It is slightly below the median of the latter only during delivery.

-Progesterone

Findings on progesterone serum levels are given in Figure 4.15 and Tables 4.31 and 4.38. Mean values at the measurement points are above those given by Klopper, but progesterone values during pregnancy vary considerably from one author to another (Klopper,

1980[a]). For the median of the < P10 group, a similar picture as in the estrogen curves is found.

-Prolactin

Prolactin findings are given in Figure 4.16 and Tables 4.32 and 4.38. These data are in accordance with the findings on prolactin during pregnancy published by Rigg et al. (1977). The median of the < P10 group is well within the range of the S-Reference group.

-Human Placental Lactogen (HPL)

Data concerning HPL are given in Figure 4.17 and Tables 4.33 and 4.38. These data agree with findings in literature (Klopper, 1980[a]). The median of the < P10 group is found to be in the lower range of the S-Reference group in the last part of pregnancy, but still well above the 10th centile of the reference group.

-Thyroid parameters

Thyroxine, T3-uptake and Free Thyroxin Index (FTI) are illustrated in Figures 4.18, 4.19 and 4.20 and are presented in Tables 3.34, 4.35 and 4.36. In agreement with findings reported in literature (section 1.1.2), early in pregnancy there is already a sharp increase in total T4 and the T3-uptake is depressed. The FTI during pregnancy is slightly below the 6 weeks postpartum value.

Median values of the < P10 group for all the parameters are found to be around the median of the S-Reference group.

-Cortisol

Cortisol findings are illustrated in Figure 4.21 and described in Tables 4.37 and 4.38. The data are in accordance with data found in literature (section 1.1.2). There is a gradual rise during pregnancy, and values double during pregnancy when compared to non-pregnant values. Median values of the < P10 group are not different from those of the S-Reference group.

Data concerning the < P10 and > P90 group of the above

mentioned hormones are described in Tables 4.38 and 4.39.

To check whether the healthy pregnant women we studied had a normal kidney and liver function, we determined creatinine, urea, uric acid, alanine-amino transferase (SGPT) and gamma-glutamyl transferase (γGT) serum levels during and after pregnancy. Our findings are in the normal range and are described in Tables 4.40 and 4.41 and illustrated in Figures 4.22 and 4.23.

Summarizing: all parameters measured are generally in agreement with observations in the literature. As mentioned in the introduction of this chapter, these parameters are merely measured to be used as variables which might contribute to the explanation of the observed changes in vitamin status or the variation in birth centile (Chapter 6 and 8).

When a parameter is found to decrease or increase during pregnancy, it should be realised that this means a decrease or increase per ml or liter blood, plasma or serum. As was shown in Figures 4.1, 4.2 and 4.3, there are impressive rises in blood, plasma and red cell volumes. A certain parameter may decrease by about 10% during pregnancy, while in fact the total intravascular circulating amount may increase. However, this does not necessarily mean that the factor plasma expansion is a (partial) cause of an observed decrease.

The values for most parameters of women from the < P10 group are found well within the range of the S-Reference group. The only striking differences are observed in some of the hematological parameters; different patterns of plasma volume, hemoglobin and hematocrit changes are observed during pregnancy. However, this group consisted only of 10 women; a group too small to allow any conclusions.

CHAPTER 5.

Dietary intake during pregnancy.

5.1. General description.

The habitual daily energy and nutrient intake was estimated from dietary histories obtained in the 16th (I) and 34th (II) week of pregnancy as well as 6 months postpartum (III). Vitamin and mineral supplementation was excluded, except ascorbic acid containing sirops. From 62 women in the S-Reference group complete data were available for all periods (I-III). Data for periods I and II were available from 5 women, and one woman had data only for period I.

In the <P10 birthweight group complete data were available from all women. The results of the dietary survey for the S-Reference group are presented in Tables 5.1 to 5.5 and can be summarized as follows:

-Energy and macronutrients

Mean energy intake in the early months of pregnancy (I) was found to be about 160 kcal (0.65 MJ) higher compared with that calculated at 34 weeks of pregnancy (II) and 230 kcal (0.95 MJ) higher than at 6 months postpartum (III) (Table 5.1). These differences are significant. Total fat and protein, but not carbohydrates, contributed significantly to the higher energy intake at I when compared with II.

The relative contributions of the macronutrients to the total energy intake are given in Table 5.2.

The contribution of total protein remains rather constant, around 14% of the total energy intake, although there is a small, but significant difference between II and III. About 70% of the protein consumed is of animal origin. This percentage is about the same in all three periods.

The contribution of fats decreases during pregnancy, increasing to higher values postpartum. Differences are significant between all periods. The contribution of saturated, mono- and poly-unsaturated fats to the total fat intake shows a rather consistent pattern with a relatively high contribution from saturated and mono-unsaturated fats in all periods.

The contribution of alcohol to the total energy intake, being about 2.5% in the "non-pregnant state" (III), is neglectable during pregnancy.

The relatively lower contribution of fats and alcohol to the total energy intake during pregnancy is paralleled by a significant increase in carbohydrate consumption both in absolute amounts and as energy percentages. The extra energy from carbohydrates during pregnancy seems to be mainly derived from the mono- and disaccharides and only to a lesser extent from polysaccharides as indicated in Table 5.2.

-Micronutrients

Mean intake of micronutrients, given in Table 5.1, is significantly higher in the early months of pregnancy (I) compared with the mean intake calculated 6 months postpartum (III). At 34 weeks (II) micronutrients can be roughly divided into two groups: one group of nutrients in which the intake is still above that at 6 months postpartum (calcium, thiamin, riboflavin, vitamin B6, B12 and C) and a group in which the intake has lowered and is not significantly different from that at 6 months postpartum (iron, niacin, retinol and vitamin D).

However, the variation in micronutrient intake within the group is considerable at all moments, variation coefficients ranging between 25 and 40%. Distribution around the mean intake approaches a Gaussian distribution for most of the nutrients except for vitamin D where the distribution is strongly skewed

to the right.

In Table 5.3, the calculated nutrient densities are summarized. Nutrient densities of calcium, riboflavin, vitamin B6, B12 and C are higher during pregnancy (I,II) when compared with those postpartum (III). The intake of the other micronutrients increases proportionally with energy intake.

Changes in dietary behavior may also be derived from changes in nutrient and energy intake within persons. Therefore, we calculated rank correlations between intakes at the three different periods for a number of nutrients as shown in Table 5.4. The significant, mutual rank correlations indicate that the usual dietary pattern and the relative position of the subjects in terms of intake of a given nutrient remained substantially unchanged during pregnancy (I,II).

The more remarkable change of correlation coeficients between period I and III was obtained for energy, protein, fat, calcium, (heme)-iron, thiamin and riboflavin. Nutrients which also showed the most remarkable changes in nutrient density. Considering rank correlations for proteins and iron intake, it is worth noting that the substantially lower correlation coefficients between period I and III compared with those obtained between period I and II, are mainly limited to animal protein and heme-iron, suggesting a significant change in the consumption of meat and meat products. Similar for calcium and riboflavin respectively, suggesting a significant change in cheese and milk consumption.

-Changes in the consumption of food products

Changes in nutrient intake are, of course, derived from changes in amounts of food products consumed. Such a relationship is relatively simple and clear for those nutrients confined to only one food product or to a small group of food products, but is rather complex for nutrients where a number of food products contributes to the daily intake.

Table 5.5a, summarizes the mean contribution of various groups of food products to the total amount of energy consumed at the different periods.

During pregnancy the consumption of dairy products (except cheese), brown bread and fruit tends to increase while the relative contribution of meat, meat products and sweets tends to decrease.

The mean percentual contribution of food products or groups of food products to the intake of some macro- and micronutrients are summarized in Table 5.5b.

Although the contributions of animal and vegetable proteins to the total energy intake remained rather constant in the three respective surveys (Table 5.2), there is some difference in the main contributing food products, especially for the intake of animal products which shows a trend for an increased contribution to animal protein from dairy products parallel with a lower contribution from cheese and meat products in the course of pregnancy when compared to the postpartum period.

Fat intake shows a trend similar to that of animal protein, an increased contribution from dairy products together with a lower contribution from cheese and meat when compared with the postpartum situation. Particularly the decrease in the consumption of fat meat products and cheese is responsible for the decreasing fat intake during pregnancy, whereas the consumption of dairy products contributes more to the fat intake. The higher carbohydrate intake during pregnancy is mainly caused by an increased consumption of dairy products, which also accounts for the higher calcium and riboflavin intake.

Changes in relative contributions of food products are more complex in the case of thiamin and vitamin B6. The relative contribution from meat is lower during pregnancy than postpartum for both vitamins, while the contribution of dairy products, fruit and bread (full grain) is higher and probably accounts for the higher absolute intake of both thiamin and vitamin B6. The relative higher fruit consumption found for pregnant women also accounts for the higher absolute vitamin C intake. The relative decrease in consumed meat and cheese, especially in the second half of pregnancy, probably accounts for the lower intake of retinol (and vitamin D?) compared to the estimated intake at 16

weeks (I).

Differences in the contribution to heme and non-heme iron parallel those for animal and vegetable protein.

-Limitations of the method

Although the dietary history is considered to be a valid method in tracing food habits and food consumption, the data presented here should be interpreted only carefully and with great caution. Bias may be introduced by the fact that three dieticians were involved in this study.

Inaccuracy is introduced by using Food tables to calculate nutrient intake from estimated consumption amounts and by the lack of knowledge about nutrient absorption, nutrient interrelations etc. (see Chapter 1, section 2.3).

The changes in food patterns observed, may indicate trends but no more than that, and are not to be interpreted at an individual level.

5.2. Energy and nutrient intake according to parity, smoking behavior, season and pregnancy outcome.

-Diet and parity

Mean daily energy and nutrient intake from women of the S-Reference group are compared according to parity and are shown in Table 5.6.

A significantly higher intake for nulliparae compared to multiparae (parity > 1) was observed for carbohydrates and vitamin C. Differences were significant in all periods. Also the mean energy intake was higher for the nulliparae at all periods, but differences never reached significance.

Mean alcohol intake was higher for the multiparae, but variation in alcohol consumption is considerable.

-Diet and smoking behavior

Mean daily energy and nutient intakes are compared for women in

the S-Reference group according to smoking behavior (Table 5.7). Women who smoked also consumed significantly more alcohol. For the other nutrients, no significant differences could be observed between the two groups. A significantly higher intake for fat and protein intake in period I compared to period II was found for the non-smokers, but not for the smokers. Energy intake was not significantly different for the two groups during and after pregnancy.

-Diet and season

Mean daily energy and nutrient intakes are compared for women in the S-Reference group who were interviewed in the summer period (April-September) with those in the winter period (October-March). Between both periods, no significant differences in nutrient and energy intakes could be demonstrated for any of the nutrients, as shown in Table 5.8. Only for thiamin, a significantly higher intake in the summer period was found at the 16th week survey, but not for the 34th week and 6 months postpartum survey. We have no explanation for this finding which may result from mere chance.

-Diet and pregnancy outcome

Mean daily energy and nutrient intakes for the S-Reference group and for the group of women delivering babies with birthweights below the 10 th centile (<P10 group) are shown in Figure 5.1. Significance of difference was not tested here because the < P10 group consisted of only 10 women. From Figure 5.1, no consistent trend for differences in mean nutrient intakes is apparent.

The possible relationship between nutrition and birthweight and weight gain in pregnancy is discussed in more detail in Chapter 8.

5.3. Nutrient intake relative to recommended daily allowances.

Nutrient needs and recommended daily allowances (RDA) during pregnancy were discussed in Chapter 1, section 2.2. The

allowances recommended by the Netherlands Nutrition Council, the WHO, the British DHSS and the Food and Nutrition Board of the NAS/NRC (USA) are summarized in Table 1.2.2.I.

The energy and nutrient intakes from our S-Reference group are calculated in percentages of the allowances of the Netherlands Nutrition Council (Figure 5.2).

The mean intake data obtained during pregnancy were compared to the recommendations for the second and third trimester of pregnancy, the 6 months postpartum data to those for moderately active non-lactating women aged 20-35. The findings were not adjusted for maternal weight or body size.

As can be concluded from Figure 5.2 nutrition during pregnancy and 6 months postpartum for our S-Reference group can be considered adequate or even abundant with respect to the intakes of energy and some nutrients, i.e. protein, calcium, retinol, thiamin, riboflavin and vitamin C. Iron is the only nutrient that does not meet the recommendations during pregnancy. The contribution of fats to the total energy intake was around 40%, from which only 5 to 5.5% was poly-unsaturated (Table 5.2). Thus the fat consumption is relatively high and does not provide probably enough poly-unsaturated fatty acids.

The diet from our S-Reference group also provides relatively high amounts of mono- and disaccharides, about 25% of the total daily energy intake.

When we compare our data from the dietary surveys of the S-Reference group to American standards, which also include recommendations for vitamins B6, B12 and D, we obtaine virtually the same picture.

The nutrient intakes, as percentages of NAS/NRC (USA) allowances, are represented in Figure 5.3. Again iron as well as vitamin B6 and vitamin D intake are far below the recommended allowances, both during pregnancy and 6 months postpartum.

As indicated in Table 5.9 none of the women in our S-Reference group meets the NAS/NRC allowances for vitamin B6, vitamin D and iron during pregnancy, and only a few do so at 6 months postpartum. For the other nutrients, estimated intakes are adequate.

5.4. General discussion.

The habitual food intake in our S-Reference group was assessed by a dietary history method. As could be expected, mean energy and nutrient intakes, calculated from these dietary histories, were generally satisfactory in all periods, with exception of iron, vitamins B6 and D. For reasons mentioned in section 1.2.4 interpretation of dietary survey data and comparison of these data with similar data published in literature is subject to many pitfalls. Comparison of our data, given in Tables 5.1 to 5.9 with data reported for comparable groups (some of these studies were summarized in Table 1.2.4.I) shows a considerable agreement with most of the studies. A small, but significant increase in daily energy intake of pregnant women in their first months of pregnancy, followed by a decrease, of about 100-200 kcal (0.4-0.8 MJ) during the last months of pregnancy is a similar trend in most studies, including ours (Darby et al., 1953; Lunell, 1969; Beal, 1971; Papoz et al., 1980).

Remarkable higher intakes for healthy Dutch gravidae were reported in the older studies by Van der Rijst (1962) and Den Hartog et al. (1953). However, another method of food consumption survey was used. Whether this difference with the data reported in this study reflects indeed a higher energy intake of pregnant women about 20-30 years ago (due to a higher energy expenditure?) or just reflects a methodological effect is open for speculation.

Concerning changes in food consumption pattern, we observed an increase in the consumption of dairy products paralleled by a decrease in meat consumption. Also, the consumption of fruits tended to be higher during pregnancy. These changes may account for the observed changes in nutrient intake, i.e. for calcium, riboflavin and vitamin C. On the whole the dietary pattern concerning nutrient intake seems rather consistent during and after pregnancy with only minor changes. Changes in the consumption of foods are more evident for dairy and meat products, but are not convincing. Similar trends in food consumption pattern were described by Lunell (1969), Beal (1971)

and Papoz et al. (1980). We did not observe lower variation coefficients for calculated nutrient intakes within the group as described by Beal (1971).

No significant effects of smoking or parity on energy and nutrient intakes in our group of pregnant women could be demonstrated except for a significantly higher carbohydrate and vitamin C intake in nulliparae compared with multiparae (Table 5.6) and a significantly higher alcohol consumption in women who smoked (Table 5.7).

A higher energy intake in nulliparae compared with multiparae was reported by Lunell (1969) and Van der Rijst (1962). In some other studies, the intake of one or more of the micronutrients is reported to be somewhat higher in nulliparae, e.g. calcium (Morse et al., 1975).

An association between smoking and energetic consumption during pregnancy has been suggested (Philipps and Johnson, 1977; Picone et al., 1980, cited in Wack and Rodin (1982); Haworth et al., 1980). Smitthels et al. (1977) studying maternal nutrition in early pregnancy observed no effect of smoking on energy intake; nor did we.

A seasonal variation in the intake of energy, protein, calcium, iron and some B-vitamins was reported by Darby et al. (1953) and Van der Rijst (1962). The fact that we could not observe such a season dependent nutrient intake may be related to the relative higher availability nowadays of most food products throughout the year compared with the situation 20-30 years ago.

Comparison of our nutrient intake data with recommended daily allowances (Figures 5.2 - 5.3 and Table 5.9) showed that mean intakes meet or even exceed the recommendations of the Netherlands Nutrition Council for most nutrients, indicating an adequate or even abundant nutrient supply in relation to nutrient requirements with the exception probably of iron. Comparing our data with American standards (NAS/NRC), which involve recommendations for most of the micronutrients, indicate that the iron, vitamin B6 and D intake might be marginal (Table 5.9). The NAS/NRC standards are established at levels considered

to be adequate for the needs of practically all healthy persons (approximately 2 SD above the median requirement for nutrients and at the median level for energy requirement). Intakes below the RDA carry increasing risk for undernutrition among some individuals. The great majority of individuals, however, require less than the RDA (section 1.2.2).

Endogenous vitamin D synthesis significantly contributes to daily vitamin D body needs (section 1.3.2), so the actual supply of vitamin D might have been adequate in this group of women, assuming a "normal" solar exposure. A final "proof" whether vitamin D supply (both exogenous and endogenous) was indeed adequate in our group may come from (biochemical) laboratory findings (Chapter 6). The same is true of course for the other nutrients, including vitamin B6 and iron. Iron and vitamin B6 intake below recommended allowances has been reported in many studies covering comparable groups of pregnant women, i.e. women living in industrialized societies with a generally abundant supply of food (section 1.2.4).

Unfortunately we were not able to calculate folacin intakes from our dietary histories, but it is not unlikely that folacin intake in our group was also relatively low, as observed in several comparable studies (Elsborg and Rosenquist, 1979; Papoz et al., 1980).

The higher recommendations for pregnant women compared with those for non-pregnant women are generally related to the second and third trimester. It is, therefore, remarkable that in many of the studies on nutrient intake of pregnant women, including this study, the highest figures are observed in the first months of pregnancy with a significant decrease in habitual food consumption during late pregnancy.

As indicated in section 1.2.1, energy needs are spread fairly evenly over most of the whole pregnancy period, and even the total metabolized energy needed between the 10th and 30th week is higher than in the last 10 weeks of pregnancy. Maybe this possibly hormone-induced physiological adaptation of pregnancy is responsible for an increased appetite in early pregnancy just to provide an energy (and nutrient?) bank for use

in late pregnancy and lactation.

An uncertain factor in this study is the effect of vomiting in early pregnancy on the "true" nutrient intake. Thomson (1958) reported a "surge of appetite" near the end of the first trimester sometimes concurrent with the time nausea and vomiting disappeared. This surge may represent some catch-up appetite.

Conclusion Chapter 5:

1. The habitual daily energy and nutrient intake of our S-Reference group was estimated using a dietary history method at week 16 and 34 of pregnancy and at 6 months postpartum. In comparison with recommendations from the Netherlands Nutrition Council mean intake was generally satisfactory, except probably for iron. Compared with American standards (Food and Nutrition Board, NAS/NRC) the mean iron, vitamin B6 and D intakes were below recommendations.
2. Mean energy intake estimated at week 16 was significantly higher than that at week 34 or at 6 months postpartum. The relative contribution of fats to the total energy intake decreased during pregnancy whereas that of carbohydrates was higher during pregnancy compared with 6 months postpartum.
3. Minor changes were observed in the food consumption pattern. The consumption of dairy products and fruits tended to be slightly higher during pregnancy, that of meat and meat products slightly lower.
4. Mean micronutrient intake was significantly higher at week 16 compared with that at 6 months postpartum. At week 34 the estimated intake of calcium, thiamin, riboflavin, vitamins B6, B12 and C was still significantly higher than at 6 months postpartum. Between the iron, retinol, niacin and vitamin D intake at week 34 and that at 6 months postpartum respectively, differences were not longer significant.
5. Parity, maternal smoking and season did not affect maternal dietary intake, except for a significantly higher carbohydrate and vitamin C intake in nulliparae compared with multiparae and a significantly higher alcohol consumption in women who smoked.

CHAPTER 6.

Maternal and fetal (cord blood) vitamin status.

6.1. Changing values of some vitamin status parameters in blood during pregnancy and postpartum among healthy females (S-Reference group).

To describe the changes of the vitamin status parameters during and after pregnancy, the 10th, 50th and 90th centile were calculated for vitamins A, D, B6, B12, thiamin, riboflavin and folacin, respectively, and are illustrated in Figures 6.1.1 - 6.1.13. Measurements were made only at selected times during and after pregnancy. Blood samples were taken in the 16th, 28th and 34th week of pregnancy, at parturition and 6 days, 6 weeks and 6 months postpartum, respectively. In the figures also the 10th, 50th and 90th centile of the measurements in cord blood are illustrated as well as the ranges for the respective parameters obtained from non-pregnant healthy adults as a reference for mean values and cut-off points (see Table 2.6.IV).

Tables 6.1.1 - 6.1.25 (odd numbers only) represent the mean values, standard deviations, minimum and maximum values obtained for the S-Reference group in this study.

Differences between the measurements at the different times were tested for statistical significance, using Student's t-test (see section 2.7). The significance matrices obtained are presented in Tables 6.1.2 - 6.1.26 (even numbers only). Differences were considered significant when $p < 0.05$.

To become a global idea about the ratio of the inter- and intra-individual variability, linear correlation coefficients between the same parameters at different time points, were calculated (Table 6.1.27).

The occurrence of abnormal values in percentages for each of the vitamin status parameters at the various stages of pregnancy and in the postpartum period, using the interpretation criteria for non-pregnant adults (Table 2.6.IV) are presented in Table 6.1.28.

The findings for the respective vitamins follow in the subsequent paragraphs.

-Vitamin A

During pregnancy the serum retinol level falls. Compared with the non-pregnant reference range, retinol levels were considerably lower already at the 16th week of pregnancy (Figure 6.1.1). Differences between the 16th and 28th week mean values and between those of the 16th and 34th week were statistically significant, unlike the differences between the 28th and 34th week mean values (Table 6.1.2). This indicates that the major decrease might have occurred in the first half of pregnancy.

At parturition, extremely low retinol levels were found for most, but not for all women, resulting in a strongly skewed distribution around the mean value. Whether this phenomenon represents a substantial decrease in serum retinol content during the last weeks of pregnancy or is only representative for the parturient state cannot be concluded from these data.

At the 6th day postpartum (pp), the mean serum retinol level was again above the 1 µmol/l, which is at least two times higher than the mean values at parturition and is also higher than the mean values determined at the 28th and 34th week of pregnancy.

A significant increase in serum retinol level was observed between the 6th day and 6th month pp. Although the mean value at 6 months pp is still lower when compared with that obtained for non-pregnant females, the observed range is compatible with the (non-pregnant) reference range.

As will be discussed in more detail in section 6.7, lactation and oral contraceptive use affect serum retinol levels in the postpartum period and may account for the relatively low levels at 6 months pp.

During pregnancy up to 90% of the women had serum retinol

levels below 1.1 µmol/l, 6 months pp, this percentage was about 40% (Table 6.1.28).

When compared with international standards, like the reference standards from the Interdepartmental Committee on Nutrition for National Development (ICNND) (Sauberlich et al., 1976), the occurrence of abnormal values is much lower. According to these interpretation criteria, levels between 0.35 and 0.70 µmol/l (10-20 µg%) should be considered low, while values below 0.30 µmol/l are considered deficient, i.e. are usually associated with low liver reserves of vitamin A and an increased prevalence of clinical signs of deficiency. The lowest value observed in our group 6 months pp was 0.4 µmol/l, still above the 0.30 µmol/l level, while 4 women in the S-Reference group fell into the "low" category.

Between measurements at the various times, low, but significant linear correlation coefficients were obtained (Table 6.1.27). Correlation coefficients became lower when more time elapsed between measurements.

Between the levels measured at parturition and at 6 days pp, a significant correlation was also obtained ($r = 0.45$; $p = < 0.001$), although the absolute levels were considerably different.

From the 4 women of the S-Reference group with a serum retinol level at 6 months pp below 0.7 µmol/l, 3 demonstrated a low (<0.7 µmol/l) level at the 16th week of pregnancy.

One woman from the S-Reference group became pregnant within 6 months after delivery of her first baby and entered the study for the second time. The time course in serum retinol content showed a remarkable similarity during the two consecutive pregnancies as indicated below:

	1th pregnancy	2nd pregnancy*
16 wk	0.9 µmol/l	1.0 µmol/l
34 wk	0.7 µmol/l	0.6 µmol/l
partus	0.6 µmol/l	0.5 µmol/l
6wpp	1.1 µmol/l	1.4 µmol/l
Cord	0.6 µmol/l	0.8 µmol/l

* data from the first pregnancy were not used for the calculations of the S-Reference group.

-Vitamin D

Mean serum 25-hydroxy-vitamin D (25-OHD) content remained rather constant during and after pregnancy (Figure 6.1.2).

At all times, 25-OHD levels were compatible with the non-pregnant reference range. The highest values were measured in the 6 months pp samples. The mean value at 6 months pp was significantly different from mean values at all other measurement points (Table 6.1.4).

Between measurements at the various stages of pregnancy and in the postpartum period, highly significant correlations were obtained. However, the individual serum 25-OHD levels are subject to seasonal variation (see section 1.3.2). In Table 6.1.I mean values are shown that were obtained after allowance was made for these seasonal effects.

TABLE 6.1.I Mean value and standard deviation of serum 25-OHD levels of women from the S-Reference group, during and after pregnancy, according to time of sampling.

	Serum 25-OHD level (nmol/l)				
	wk 16	wk 28	wk 34	P	6mpp
women sampled in March/April (N = 9 - 13)	40 ± 13	43 ± 17	38 ± 17	32 ± 9	54 ± 20
women sampled in July/August (N = 10 - 14)	74 ± 27	81 ± 24	71 ± 23	70 ± 19	91 ± 21

As illustrated above the mean values measured during pregnancy are always lower than at 6 months postpartum. This trend seems more pronounced in winter (early spring). The subject of seasonal variation in vitamin status parameters, including vitamin D, is further discussed in section 6.5.

Throughout all stages during and after pregnancy the occurrence of 25-OHD serum levels in the marginal or deficient (non-pregnant) range was negligible, i.e. below 5% (Table 6.1.28).

-Thiamin

Mean basal, i.e. unstimulated, erythrocyte transketolase (ETK) activity showed a gradual fall during pregnancy (Figure 6.1.3).

At the end of pregnancy, a mean basal ETK activity of 7.5

U/mmol Hb is found, while for non-pregnant controls the mean value was 11.5 U/mmol Hb (see Table 2.6.IV). Between mean values measured at the 16th, 28th and 34th week of pregnancy, significant differences were obtained but not between the 34th week and partus values (Table 6.1.6).

In the postpartum period, there was a gradual recovery to non-pregnant (reference) values. Both between the 6th day and 6th week pp and between the 6th week and 6th month pp, a significant increase was observed. The variation within the group remained rather constant (between 20-30% at all stages). Between measurements at the various stages in pregnancy and postpartum period, significant correlations were obtained, except for the values measured at parturition (Table 6.1.27).

The changes in basal ETK activity were not paralleled by similar changes in the ETK stimulation ratio. As illustrated in Figure 6.1.4, the mean ETK ratio showed only a minor change. Differences between measurements never reached significance except the 6 weeks pp mean value which was slightly, but significantly, lower than any other mean value measured during or after pregnancy. Between measurements, no significant relationship could be demonstrated for the ETK stimulation ratio (Table 6.1.27). Although the mean ETK stimulation ratio remained rather constant during pregnancy, the occurrence of high values (> 1.25) increased up to 25% at the end of pregnancy. At 6 months pp, this percentage is again at the same level as in the 16th week of pregnancy, i.e. around 10% (Table 6.1.28).

TABLE 6.1.II Linear correlation (Pearson) between paired observations of ETK activity and stimulation ratio from women of the S-Reference group.

	16th wk	34th wk	P	6mpp
ETK activity vs. αETK	-0.46*	-0.40*	-0.57*	-0.35*

* $p \leq 0.001$

From the 7 women in the S-Reference group who showed stimulation ratios > 1.25 at 6 months pp, only 3 were also within the marginal/deficient range at the end of pregnancy. Between basal ETK activities and -stimulation ratios (αETK), a

significant negative correlation was obtained as illustrated in Table 6.1.II.

The fall in basal ETK activity without a proportional change in stimulation ratio suggests a decrease in transketolase apoenzyme content in the erythrocyte during pregnancy.

-Riboflavin

For assessment of the riboflavin status during pregnancy both whole blood riboflavin content and the Erythrocyte Glutathion Reductase (EGR) stimulation test were used as the parameters.

Whole blood riboflavin content hardly changed during the first period of pregnancy and after pregnancy and remained within the non-pregnant reference range as illustrated in Figure 6.1.5. At the end of pregnancy, there was even a slight increase of riboflavin between the 28th and 34th week. Also, between the 34th week mean value and that at parturition differences were significant (Table 6.1.10).

In the postpartum period, whole blood riboflavin content fell again within the first 6 weeks after delivery. The mean value at 6 weeks and 6 months pp is very similar with that established for a non-pregnant reference group (Table 2.6.IV), i.e. 0.32 $\pm$ 0.04 and 0.33 $\pm$ 0.04 µmol/l, respectively.

At any stage of pregnancy or in the postpartum period low values (< 0.22 µmol/l, the lower cut-off point) were observed for women in the S-Reference group. Significant linear correlation coefficients were obtained between measurements (Table 6.1.27).

The EGR stimulation test: Basal, or unstimulated, EGR activity showed a small, but significant, fall in the first period of pregnancy (Figure 6.1.6). The mean level measured in the sample taken at parturition is, however, significantly higher than that at 34 weeks. In the postpartum period there is a further increase. At 6 months pp a mean activity of 99 $\pm$ 22 U/mmol Hb was obtained which is significantly higher compared with that at parturition or that at the 16th week of pregnancy (Figure 6.1.6, Tables 6.1.11 and 6.1.12), but compares very well with the mean

value of 98 ± 13 U/mmol Hb obtained for a non-pregnant reference group. The variation within the group remained rather constant throughout the whole period with a coefficient of variation around 20%. Between measurements, highly significant correlation coefficients were obtained (Table 6.1.27).
The EGR stimulation ratio (EGR) showed a consistent pattern (inversely) proportional with the changes found for the EGR activity (Figure 6.1.7). The changes occurring in the first period of pregnancy were small, and differences never reached significance except between the 34th week and partus values. In the postpartum period, mean stimulation ratios remained rather constant and were slightly lower compared with values measured during pregnancy (Tables 6.1.13 - 6.1.14). The mean value at 6 months pp (1.12 ± 0.14) compares very well with 1.15 ± 0.11 obtained for a non-pregnant reference group (Table 2.6.IV). The occurrence of stimulation ratios > 1.30 increased to 25% at the end of pregnancy, but was < 5% at 6 months pp (Table 6.1.28). From the 3 women of the S-Reference group with stimulation ratios > 1.30 at 6 months pp, 1 demonstrated abnormal values at all other stages of the study, one also at the 16th and 28th week of pregnancy, while another had "normal" values at all previous examinations. Between measurements, significant correlations were obtained for the S-Reference group (Table 6.1.27).

Between the respective parameters EGR activity, stimulation ratio and whole blood riboflavin content a significant relationship was observed (Table 6.1.III).

TABLE 6.1.III Linear correlation (Pearson) between paired observations of some parameters of the riboflavin status from women of the S-Reference group. (Bl=blood).

	16 wk	34 wk	6mpp
EGR activity vs. αEGR	-0.66**	-0.76**	-0.56**
αEGR vs. riboflavin (Bl)	-0.55**	-0.58**	-0.30*
EGR activity vs. B_2 (Bl)	0.60**	0.73**	0.53**

**$p \leq 0.001$ * $0.001 < p \leq 0.01$

The erythrocyte apoenzyme content, calculated as the product of basal activity and stimulation ratio, showed no consistent trend during nor after pregnancy.

Relationship between riboflavin status and hematological parameters

In literature a relationship has been suggested between riboflavin status, hemoglobin level and iron status (see section 1.3.5). We calculated linear (Pearson) correlation coefficients between these parameters, but could not confirm such a relationship as is indicated in Table 6.1.IV.

TABLE 6.1.IV Linear correlation (Pearson) between paired observations of some heamatogical and riboflavin status parameters from women of the S-Reference group.

	αEGR			whole blood riboflavin		
	16 wk	34 wk	6mpp	16 wk	34 wk	6mpp
Hb	NS	-0.22*	NS	0.39**	0.34*	0.23*
ht	NS	-0.26*	NS	0.40**	0.41**	0.23*
serum Iron	NS	NS	NS	NS	NS	NS
% saturation	NS	NS	NS	NS	NS	NS
Ferritin	NS	NS	-0.29*	NS	NS	NS

NS: not significant ** $p \leq 0.001$ * $0.001 < p \leq 0.01$

-Vitamin B6

Plasma pyridoxal-5'-phosphate (PLP) content falls to very low levels during pregnancy when compared with levels obtained for non-pregnant females (Figure 6.1.8). The decrease is significant until the 34th week, but between the 34th week and the partus values, no significant difference was observed (Table 6.1.16). Although preconceptional PLP levels were unknown, the major decrease in plasma PLP levels seemed to have occurred in early pregnancy, i.e. before the 16th week. In the postpartum period, mean PLP levels increased significantly from 8 nmol/l at 6 days pp to 24 nmol/l at 6 months pp (Table 6.1.15). For a non-pregnant female reference group, a mean value of 40 ± 19

nmol/l was established (Table 2.6.IV).
At 6 months pp, about 25% of the women showed PLP plasma levels still below 15 nmol/l, the lower limit of the acceptable range established for non-pregnant adults (Table 6.1.28). At the 16th week of pregnancy the percentage of women showing the lower PLP plasma was already 55% and increased up to 100% at the end of pregnancy. The variation within the group is considerably with variation coefficients ranging between 20 and 60%. The distribution around the mean value is strongly skewed to the right, especially at 6 months pp.

Between measurements at the various stages of pregnancy and in the postpartum period significant correlations were obtained (Table 6.1.27). From the 15 women of the S-Reference group with plasma PLP levels at 6 months pp below 15 nmol/l, 10 had already values below that level at all previous examinations.

The erythrocyte Glutamate-Oxaloacetate Transaminase (EGOT) stimulation test: In contrast with the significant fall in plasma PLP content in the course of pregnancy, EGOT activity and stimulation ratio showed less dramatic changes. Basal, i.e. unstimulated, EGOT activity remained almost constant during and after pregnancy (Figure 6.1.9). The highest mean value (68 $\pm$ 14 U/mmol Hb) was observed 6 months pp and compares very well with the mean value established for the non-pregnant reference group (69 $\pm$ 14 U/mmol Hb). Differences between the 6 months pp value and that at any other stage during and after pregnancy were significant (Table 6.1.18). The variation within the group showed some increase when pregnancy progressed, variation coefficients ranging between 20-30%. Between measurements significant linear correlation coefficients were obtained (Table 6.1.27).

The EGOT stimulation ratio (αEGOT) showed a consistent pattern proportional to the course of basal EGOT activity, with only minor changes during and after pregnancy (Figure 6.1.10).

The highest ratios were observed 6 days pp, the lowest at 6 months pp (2.13 $\pm$ 0.37 and 1.87 $\pm$ 0.18, respectively). The

latter range is fully compatible with the range established for non-pregnant adults (1.85 ± 0.20). The variation within the group increased as pregnancy progressed. The occurrence of αEGOT values > 2.20, indicative for B6-deficiency according to the range obtained for non-pregnant adults (Table 2.6.IV), increases from about 7.5% to 25% at the end of pregnancy and even to 32% at 6 days pp (Table 6.1.28). At 6 months pp only one woman of the S-Reference group showed an αEGOT > 2.20. At previous examinations this woman fell within the acceptable range (< 2.20) except at 6 weeks pp when αEGOT was also elevated (2.47). Between measurements at various times significant linear correlations were obtained, but correlation coefficients between the postpartum measurements seemed to be low compared with those during pregnancy (Table 6.1.27). Also, between measurements during and after pregnancy, correlations were low or not even significant. Between the respective vitamin B6 status parameters measured in this study, linear correlation coefficients were calculated at the various stages of pregnancy and in the postpartum period. Those obtained at 16 and 34 weeks, as well as at 6 months pp are summarized in Table 6.1.V.

TABLE 6.1.V Linear correlation (Pearson) between paired observations of some vitamin B_6 status parameters from women of the S-Reference group.

	16 wk	34 wk	6mpp
EGOT activity vs. αEGOT	-0.75**	-0.80**	-0.50**
PLP vs. EGOT activity	0.26*	0.52**	0.55**
PLP vs. αEGOT	-0.30*	-0.38*	-0.30*

**$p \leq 0.001$ * $0.001 < p \leq 0.01$

The relative stability of EGOT activities and stimulation ratios suggest no significant change in erythrocyte apoenzyme content during and after pregnancy.

-Vitamin B12

Serum vitamin B12 content steadily falls in the course of pregnancy (Figure 6.1.11). Between the 16th and 28th and also

between the 28th and 34th week differences were significant, but not between the 34th week and the values measured at parturition (Table 6.1.22). The mean fall in serum B12 content is about 70 pmol/l. Within the first weeks postpartum, vitamin B12 serum content increased to the non-pregnant level. Mean level at 6 weeks pp was 448 ± 127 pmol/l which agrees well with the mean value of 398 ± 108 pmol/l obtained for non-pregnant adults (Table 2.6.IV). Between the 6th week and 6th month pp, no significant further increase was observed (Table 6.1.21).

Vitamin B12 levels below 180 pmol/l, suggesting marginal or deficient vitamin B12 body stores, were not observed during this study. Between measurements at the various stages during and after pregnancy, significant linear correlation coefficients were obtained (Table 6.1.27).

-Folacin

Folacin status in the course of pregnancy was assessed by determination of both serum and red cell folacin content.

Serum folacin content showed a significant fall during pregnancy (Figure 6.1.12). Between the 16th and 28th and between the 28th and 34th week values differences were significant, but not so between the 34th week and partus values (Table 6.1.24). Postpartum, there was a slight, but significant rise in the first 6 weeks, but surprisingly, serum folacin levels remained low between the 6th week and 6th month pp.

The mean value at 6 months pp (4.4 ± 2.4 nmol/l) is considerably low when compared with the mean value of 9.0 ± 3.5 nmol/l, established for a non-pregnant female reference group (Table 2.6.IV). The occurrence of low serum levels, i.e. below the lower limit of the acceptable range established for non-pregnant adults, increased from 10 to 55% between the 16th week of pregnancy and parturition and was still 45% at 6 months pp (Table 6.1.28).

Between measurements, low, but significant, correlations were obtained, but linear correlation coefficients were lower when more time had elapsed between measurements (Table 6.1.27).

Red cell folacin content is considered a better parameter of body folacin stores than serum folacin content (see section 1.3.8). Although the radioassay for red cell folacin has not yet been as completely evaluated as for serum folacin, we measured red cell folacin content using the same assay as for serum folacin (see section 2.2.6). Red cell folacin levels showed a broad variance at all stages during and after pregnany. The distribution of values around the mean was strongly skewed to the high values. Red cell folacin content tended to decrease when pregnancy progressed (Figure 6.1.13). The levels measured at the 16th week were significantly higher than at any other time during pregnancy (Table 6.1.26). A slight, but insignificant rise was observed postpartum. The considerable variance may have prevented the demonstration of significant differences in the postpartum period.

When our data for red cell folacin levels are compared with reference values published in literature and based upon similar methodology as in this study, or with the reference range specified by the Kit-manufacturer (Bio-Rad Laboratories), i.e. 260-1400 nmol/l, it appears that our findings constitute low, but acceptable mean values during and after pregnancy. Assuming a level of 220 nmol/l to be the lower limit of the acceptable range for non-pregnant adults, about 35% of the women in the S-Reference group had low folacin stores at the end of pregnancy. At 6 months pp this percentage is still 20% (Table 6.1.28).

No correlations were obtained between the 16th and 28th week measurements and those at the other stages of pregnancy and postpartum period (Table 6.1.27).

TABLE 6.1.VI Linear correlations (Pearson) between paired observations of serum and red cell folacin content from women of the S-Reference group.

	16 wk	34 wk	P	6mpp
serum vs. red cell folacin	0.26*	0.50**	0.55**	NS

NS: not significant ** $0.001 \leq p < 0.01$ * $0.01 < p \leq 0.05$

Between serum and red cell folacin content weak, but significant linear correlation coefficients were obtained except at 6 months pp (Table 6.1.VI).

Relationship between folacin status and red cell indices

Folacin deficiency becomes clinically manifest by a macrocytic anemia (see section 1.3.8) characterized by a decreeased hemoglobin level and hematocrit (ht) and an increase in Mean Cell Volume (MCV) and Mean Red Cell Hemoglobin content (MCH) but normal Mean Cell Hemoglobin Concentration (MCHC), i.e. a normochromic, macrocytic anemia (when not complicated by iron deficiency). No significant relationship between serum folacin content and any of the red cell indices and hemoglobin content could be demonstrated neither during nor after pregnancy.

Discussion 6.1

Functional parameters of the vitamin status, such as the enzyme stimulation tests and parameters reflecting tissue stores (liver or blood cell content), are generally considered to reflect long-term nutrient status, while serum or plasma levels may be affected by recent dietary intake.

Pregnancy is a relatively short-term event. As has been reported by many workers in this field, some vitamin status parameters show a considerable change in the course of pregnancy, especially vitamin blood levels (section 1.3). This is confirmed in our study which indicates that blood vitamin levels fall already early in pregnancy. Many older studies looked for possible relationships between this hypovitaminemia of pregnancy and the incidence of specific pathology. This focus on pathology was probably based upon the similarity in changes of the vitamin status parameters in the course of pregnancy and those observed during vitamin depletion in the non-pregnant situation.

The changes in biochemical indices of the vitamin status were, therefore, considered a threat during pregnancy. Many

studies on vitamin requirement in pregnancy have been reported, including supplementation studies to establish the dose needed to keep vitamin status parameters during pregnancy within ranges considered acceptable for non-pregnant (female) adults. The idea that changes in most biochemical parameters during normal pregnancy represent physiological adaptations or adjustment has gained acceptance. Starting point in this study was also that changes in vitamin status during normal uncomplicated pregnancy should be considered as normal physiology and can be used to establish reference values, one of the goals of this study.

Another objective was to answer the question "why do parameters change", i.e. what are the main determinants for the falling blood levels. This subject will be discussed in section 6.6.

A discussion will follow about the changes in vitamin status parameters observed in our study and we will compare our data with similar studies on populations of pregnant women living in industrialized countries with a rather unlimited food supply.

Vitamin A: The mean serum retinol levels measured in our study at the various stages of pregnancy are generally lower than those reported in comparable studies, as indicated below:

Authors	1th Tr. (µmol/l)	2nd Tr. (µmol/l	3rd Tr. (µmol/l)	Non pregnants (µmol/l)
Darby (1953, USA)	1.2	1.2	1.1	0.9
Gal and Parkinson (1974, England)	1.1	1.4	1.5	1.4
Kübler/Moch (1975, Germany)	-	1.9	1.8	1.7
Baker et al. (1975, USA)	-	-	0.8	1.2
This study (Netherlands)	1.0	0.8	0.8(34wk) 0.4(Partus)	1.8

As pointed out in section 1.3.1, and illustrated above, the observed trend in serum retinol content during pregnancy is rather inconsistent. Some workers find an increase, others no significant change or some decrease as in our study. Methodological differences might account for these differences between studies (different response of retinolesters?). We used

a specific fluorometric assay after HPLC purification of the serum extract, while in all other studies the classical colorimetric method with the Carr and Price reagent was used for determination of the total serum retinol content, including retinylesters. Remarkably, somewhat higher serum retinol levels were found in non-pregnant subjects using the HPLC-method (the P5 value for a non-pregnant reference population changed from 0.8 to 1.1 μmol/l). From our data it seems that during pregnancy lower serum retinol levels are found compared to the colorimetric assay. We have no explanation for this discrepancy. When we compare our data with the internationally accepted criteria based on the colorimetric method, the incidence of "low" values is considerably lower, and less than 5% at 6 months postpartum.

Gal and Parkinson (1974) performed a similar longitudinal study as we did and found a rather irregular pattern: a decrease in early pregnancy followed by an increase in mid-pregnancy (they sampled in each month of pregnancy). They also observed a sharp decrease in serum retinol content before and during labor, followed by a rise after delivery, but such extremely low retinol levels at parturition as observed in our study have not been reported before. The values measured in the samples taken at parturition may, however, represent an "artifact" because time of sampling and handling of the blood samples was the least standardized at parturition. During parturition complex hemodynamic changes occur and retinol levels measured in these samples may not be representative for the serum retinol content at the end of pregnancy. Another explanation may come from the results of the earlier studies reported by Hillman and Rosner (1958). They observed a decrease in serum retinol levels induced by forced exercise. More recent studies from Morita and Nakano (1982) confirm the relationship between acute and chronic stress and vitamin A metabolism. It is tempting to speculate about a possible relationship between the low serum retinol levels at parturition and maternal stress during parturition. As discussed in section 1.3.1, no direct relationship has been established,

between serum and liver retinol content, except in extreme situations.

Postpartum, a significant increase was observed towards the non-pregnant reference range, especially in the first days after delivery. At 6 months pp, mean serum retinol content was still lower compared with a non-pregnant female reference group (Table 2.6.IV). A postpartum rise in serum retinol content was also described by Darby et al. (1953), Morse et al. (1975) and Gal and Parkinson (1974). As already mentioned, only a slight decrease, if any, in serum retinol levels during pregnancy was observed in these studies and the postpartum rise was, in some cases, even above non-pregnant values.

Darby (1953) suggested an association between the postpartum changes in serum retinol content and the changes in serum lipid composition.

As will be discussed in section 6.7 serum retinol levels in the postpartum period are affected by lactation and use of oral contraceptives. These factors might explain, at least partly, the lower mean retinol level at 6 months pp when compared with a non-pregnant reference group.

Vitamin D: Mean serum 25-OHD content showed a remarkable constancy during and after pregnancy (Figure 6.1.2).

Serum 25-OHD content is, however, season dependent (see section 1.3.2). Longitudinal studies on changes in serum 25-OHD content are, therefore, difficult to interpret because of the bias introduced by the fact that pregnancy covers at least three seasons. This "problem" is circumvented in cross-sectional studies in which all blood samples are taken in the same period. Comparison of our results with some studies in which no adjustment was made for seasonal effects, is shown below:

Authors	10-20 wk (nmol/l)	28-32 wk (nmol/l)	33-40 wk (nmol/l)	N
Dent/Gupta (1975, England)	51 ± 12	43 ± 8	38 ± 6	14
Reiter et al. (1979, USA)	46 ± 16	50 ± 21	41 ± 16	25
This study (Netherlands)	50 ± 21	53 ± 26	53 ± 26	70

The study described by Reiter et al. (1979) had a cross-sectional design while Dent and Gupta's and our study were longitudinal. Both the Dent/Gupta study as well as Reiter's study seem to indicate a decreasing trend in serum 25-OHD level in the course of pregnancy. Because these data were not adjusted for seasonal effects, such trends may result from a different distribution of the samples taken over the respective seasons. The same trend in serum 25-OHD level is seen after allowance was made for seasonal variation, i.e. lower pregnancy values compared with the 6 months pp values (Table 6.1.I). Also when compared with the mean values obtained in winter and summer for a group of healthy non-pregnant females, values measured at the end of pregnancy are lower, i.e. 32 $\pm$ 9 nmol/l versus 40 $\pm$ 22 nmol/l (late winter) and 70 $\pm$ 19 nmol/l versus 95 $\pm$ 35 nmol/l (summer). Lower serum 25-OHD levels at the end of pregnancy were reported by Turton et al. (1977) and Weisman et al. (1979). Hillman and Haddad (1975) observed these lower 25-OHD levels during pregnancy only during the winter period (section 1.3.2).

The vitamin D binding globulin (DBG) concentration increases during pregnancy and this does not explain the slightly lower 25-OHD values during pregnancy. May be, pregnant women spend less hours outside than non-pregnant women or deliberately expose their skin less to the sun. Dietary intake is, at least for vitamin D, from minor importance as endogenous synthesis is by far the main source for vitamin D (see section 1.3.2).

Thiamin: Comparison of results with the transketolase stimulation test as parameter of the thiamin status in pregnancy with other studies, shows in general a similar picture of higher percentage of elevated stimulation ratios (αETK).

The mean αETK values ($\pm$ SD) reported by Heller et al. (1975), Vir et al. (1980) and those found in our study are summarized below:

Authors	13 - 18 wk	25 - 30 wk	31 - 36 wk	N
Heller (1975, Germany	1.13 ± 0.10(28)	1.13 ± 0.11(30)	1.13 ± 0.10(27)	54-171
Vir (1980, England)	-	1.16 ± 0.16(28)	1.16 ± 0.13(38)	60/48
This study (Netherlands)	1.14 ± 0.10(10)	1.14 ± 0.10(18)	1.17 ± 0.14(25)	70

The data reported by Heller et al. were established in a cross-sectional study, while Vir's and our study had a longitudinal design. The incidence of abnormal values is indicated between parenthesis. The values are compared with cut-off points established for non-pregnant controls. In the case of αETK this cut-off point represents the upper limit of the accepted non-pregnant range, i.e. 1.23 (Heller), 1.20 (Vir) and 1.25 (this study), respectively. The incidence of abnormal values remains rather constant in the course of pregnancy, according to Heller et al., while in our study, some increase was noted as pregnancy proceeds. Interpretation of these data in terms of increased risk is doubtful and should be done carefully.

What we already described in our previous pilot-study (Van den Berg et al., 1978), confirmed by Dirige et al. (1979) and demonstrated again in this study, is that basal ETK activities decrease in the course of pregnancy (see section 1.3.4). Because mean αETK values remain on a constant level and mean erythrocyte hemoglobin levels do not change significantly, the fall in basal ETK activity indicates a fall in cellular apoenzyme content during pregnancy.

A change in apoenzyme content has been reported both in case of chronic vitamin deficiency as well as during vitamin supplementation, probably induced by a change in cellular coenzyme content (Greengard and Gordon, 1963), or as a result from hormonal effects (Rose, 1978) (see also section 1.3.6). Compared with the other erythrocyte enzymes used for stimulation tests (EGR and EGOT) the ETK apoenzyme is the least stable, both *in vivo* and *in vitro* (Bayoumi et al., 1976; Thurnham, 1981). No

association between low apoenzyme levels and an increased incidence of high αETK levels was observed by Dirige et al. (1978). Contrary to their findings we found a slight, but significant negative correlation between basal ETK activities and stimulation ratios (Table 6.1.II). So lower basal activities are associated with a higher incidence of increased stimulation ratios. Such an association is present during and after pregnancy. The low, but significant, correlation between measurements indicates that the decrease in basal ETK activity occurred for all women.

In the postpartum period, ETK activities are gradually restored to non-pregnant values. At 6 months pp the mean value was 10.8 ± 2.2 U/mmol Hb, compatible with the mean value of 11.5 ± 1.8 U/mmol Hb established for a non-pregnant reference group (Table 2.6.IV).

ETK stimulation ratios showed an irregular pattern in individuals. Between measurements, no significant relationship could be observed (Table 6.1.27), both during and after pregnancy. Similar observations were reported by Vir et al. (1980) (see section 1.3.4).

Riboflavin: Mean whole blood total riboflavin content showed only minor changes in the course of pregnancy and remained within the reference range established for non-pregnant controls (Figure 6.1.5). Relatively few data on longitudinal changes in riboflavin blood levels have been published; most studies are directed towards the situation at the end of pregnancy. Some of the mean values reported at parturition are summarized below:

Authors	Period	Mean value (± SD) (µmol/l)	N
Clarke (1971, USA)	Partus	0.55 ± 0.08	20
Baker (1975, USA)	Partus	0.55	133
Decker (1975, Germany)	(2nd Tr.)	0.31 ± 0.05	57
	(3rd Tr.)	0.39 ± 0.09	57
Knobloch (1979, Tsech.Sl.)	Partus	0.37 ± 0.04	11
This study (Netherlands)	(16th wk)	0.30 ± 0.04	70
	Partus	0.35 ± 0.05	66

The differences obtained between absolute mean values may be accounted for by different riboflavin intake, or supplementation but also by methodological differences.

From our data, a slight increase in whole blood riboflavin content during pregnancy is apparent. At parturition the highest mean value was obtained. Considering the increase in blood volume and number of erythrocytes as pregnancy progresses, riboflavin content per erythrocyte (mean cell riboflavin content, MCRC) tended to increase (Table 6.1.VII).

TABLE 6.1.VII Mean cell riboflavin content during and after pregnancy (S-Reference group).

Period	MCRC (µmol/l)
16 wk	0.78
34 wk	0.83
Partus	0.89
6mpp	0.76

Glutathion reductase (EGR)stimulation test: Compared with the few reported studies on changes in blood riboflavin content, more information from the literature is available regarding changes in the EGR stimulation ratio (αEGR) during pregnancy.

Longitudinal studies were reported by Decker et al. (1975), Vir et al. (1981) and Bates et al. (1982). Cross-sectional data were obtained by Heller et al. (1974) (see section 1.3.5). These reported data are summarized below and compared with the results of this study:

Authors	Period	% abnormal (cut-off point)	Mean value (± SD)	N
Heller et al. (1974,	7-12 wk	7	1.15 ± 0.13	(49)
Germany)	25-30 wk	26 (>1.20)	1.17 ± 0.15	(90)
	37-47 wk	33	1.23 ± 0.19	(156)
Decker et al. (1975,	2nd Tr.	37 (>1.20)	1.19 ± 0.21	(57)
Switzerland)	3rd Tr.		1.17 ± 0.23	(57)

Vir et al. (1981, England)	2nd Tr.	22		1.13 ± 0.19	(60)
	3rd Tr.	21	(>1.20)	1.10 ± 0.19	(48)
	3 dpp	30		1.13 ± 0.23	(27)
Bates et al. (1981, England)	32 wk	20	(>1.30)	1.19 ± 0.08	(28)
	lactation			1.19 ± 0.11	(31)
This study (Netherlands)	16 wk	15		1.16 ± 0.15	(70)
	34 wk	25	(>1.30)	1.18 ± 0.21	(70)
	Partus	15		1.14 ± 0.22	(66)
	6mpp	5		1.12 ± 0.14	(59)

As can be concluded from this table, there is in general a good agreement between these studies. We found slightly higher mean stimulation ratios when pregnancy proceeds and an increasing occurrence of values above the non-pregnant cut-off point (i.e. the upper limit of the accepted range for non-pregnant subjects). Basal EGR activity showed a proportional decrease during pregnancy parallel with the slight increase in αEGR, suggesting no significant change in EGR apoenzyme content. This was also reported by Dirige et al. (1978).

A modulating effect of the iron status upon the riboflavin status has been reported by Decker et al. (1975) and Ramachandran and Iyer (1974). We found no evidence for such a relationship (Table 6.1.IV). The significant correlation obtained between αEGR and hemoglobin content, as well as between blood riboflavin and hemoglobin content, may reflect the fact that both hemoglobin and riboflavin are related with the number of circulating erythrocytes rather than a causal relationship.

The slight increase in mean EGR stimulation ratio and the higher occurrence of abnormal values seems contradictory to the observed increase in cellular riboflavin content. However, between basal EGR activity, stimulation ratio and cellular riboflavin content, significant correlations were obtained. A possible explanation for the contradictory findings may be that the increase in cellular total riboflavin content is not paralleled by a proportional increase in cellular FAD content.

Both riboflavin derived coenzymes, FAD and FMN, are present

in the red cell. FAD is the coenzyme involved in the glutathion reductase reaction, while FMN is an inhibitor of the EGR enzyme. Compared with the Transketolase stimulation test, the αEGR values showed a more consistent pattern, and between measurements, significant correlations were obtained (Table 6.1.27).

Vitamin B6: The low PLP values measured in early pregnancy are a most remarkable finding in this study and in this respect our results seem to deviate from reported data as indicated below:

Authors	3rd month (nmol/l)	6th month (nmol/l)	9th month (nmol/l)	N
Hamfelt and Tuvemo (1972, Sweden)	23 ± 13	11 ± 10	5 ± 5	19
Reinken and Dapunt (1975, Germany)	41 ± 11	18 ± 6	9 ± 3	16
Shane and Contractor (1975, England)	-	-	19 ± 4.5	10
Lumeng et al. (1976, USA[1)])	70 ± 24	± 40 ± 10	14 ± 3	10
This study (Netherlands)	15 ± 5	9 ± 4	8 ± 3	69

[1)] Women receiving 2.5 mg of pyridoxine supplementation.

In most studies the main decrease is observed in the second trimester, even when pyridoxine is supplemented (Hamfelt and Tuvemo, 1972; Lumeng et al., 1976; see also section 1.3.6).

A low mean PLP level at the end of pregnancy with a high incidence of abnormal values (below the lower limit of the acceptable range obtained for non-pregnant controls) is a common finding in all studies (see section 1.3.6). The relatively higher values reported by Shane and Contractor (1975) may reflect methodological differences because thay used a fluorometric assay, while in all other studies summarized, including this study, a radioenzymatic method was used for

determination of plasma PLP content.

When compared with our non-pregnant reference group (Table 2.6.IV) the mean PLP plasma level at the 16th week of pregnancy is already considerably lower, suggesting a significant fall early in pregnancy.

A quite different time trend was observed with the EGOT stimulation test. Mean EGOT stimulation ratio (αEGOT) remained at a rather constant level throughout pregnancy, although at a significantly higher level when compared with the mean level at 6 months pp (Tables 6.1.19 and 6.1.20). The incidence of abnormal values, indicative for vitamin B6 deficiency, increased from 5 to 25% at the end of pregnancy. Similar data for changes in αEGOT in the course of pregnancy have been reported by Heller et al. (1973), Hamfelt and Tuvemo (1972) and Livingstone et al. (1978) (section 1.3.6).

Similar to Lumeng (Lumeng et al., 1976) we might have also concluded from our data that the EGOT stimulation test is less sensitive than plasma PLP level to detect marginal of deficient vitamin B6 status, we feel that interpretation of our data in terms of sensisitivy and specificity may be misleading. Changes in the maternal metabolic environment undoubtedly affect vitamin status parameters and may result in a different time trend for the respective parameters. As will be discussed in more detail in section 6.6 and Chapter 9 our results may be explained by a shift from plasma PLP into the tissues.

At 6 months pp when maternal physiology may be supposed to have been restored to the non-pregnant state, plasma PLP levels are still low when compared with the non-pregnant reference range (Table 2.6.IV). Twenty-five percent of the measured PLP levels 6 months pp are still below 15 nmol/l, the lower limit of the acceptable range, while the mean value is about half the mean value established for a non-pregnant reference group. αEGOT values were perfectly normal at 6 months pp. The still lower plasma PLP levels postpartum may indicate maternal vitamin depletion during pregnancy with preferential repletion of tissue stores in the postpartum period. Before statements about the

"pregnancy cost" for vitamin B6 are justified, based upon sequential measures of biochemical indices both during and after pregnancy, other interfering factors should be considered first.

Changes in dietary intake, lactation and oral contraceptive therapy in the postpartum period may be such factors which affect vitamin B6 status. This will be discussed in more detail in section 6.7.

Vitamin B12: Literature is quite unanimous about the changes in vitamin B12 serum content during pregnancy: a steady fall of about 75-100 pmol/l in the course of pregnancy, followed by a rapid recovery to non-pregnant values in the postpartum period (section 1.3.7). A similar pattern was obtained in our study as indicated below where our data are compared with reported values:

Authors	Type of assay	1th Tr.	2nd Tr.	3rd Tr.
		mean value (pmol/l)		
Metz et al. (1965, South Africa)	MB[1)]	315	270	230
Hansen et al. (1964, Sweden)	MB	225	190	140
Green et al. (1975, South Africa)	MB	200	195	140
	RID[2)]	450	440	340
This study (Netherlands)	RID	390	350	320

[1)] MB: microbiological assay [2)] RID: radioisotope assay

As illustrated above, radioisotopic methods generally show higher vitamin B12 levels when compared with microbiological assays. Using both methods, a highly significant correlation can be obtained for pregnant women (Green et al., 1975); Kalemagham and Krishnaswamy, 1977). Green et al. (1975) observed that differences between these methods were not consistent during pregnancy. Discrepancies were greatest in early pregnancy and decreased when duration of pregnancy increased. This observation was based upon a cross-sectional study. Their total group consisted of 111 women. Only small groups of different women were compared at the various stages of pregnancy, so

interpretation in terms of different time trends between both methods seems doubtful. The extent of such an effect may also depend on the type of binder used in the radioisotopic assay as the discrepancies obtained between radioisotopic and microbiological assays are caused exclusively by the R-proteins (see section 2.6). Green et al. used a pure R-type binder (diluted chicken serum) in their assay, while in our study an Intrinsic Factor (IF) preparation was used although it was contaminated with R-proteins.

The time course in serum vitamin B12 content during pregnancy seems different from the other water soluble vitamins. Serum PLP and folacin fall in early pregnancy and remain on a rather constant level in the second half of pregnancy, while the main decrease in vitamin B12 serum levels occurs in the second and third trimester (Figure 6.1.11).

Although a relationship has been established between serum vitamin B12 concentration and muscle stores in late pregnancy (Edelstein and Metz, 1969), it seems unlikely that the fall in serum content represents depletion of maternal stores.

The rapid postpartum recovery and the failure of vitamin B12 supplementation to prevent the fall in serum levels (Metz, 1965) suggest that plasma dilution and the complex changes in transcobalamin levels (Fernandez-Costa et al., 1982) may account for the changes observed. As already indicated the possible explanation(s) and interpretation of changes in vitamin status parameters will be discussed in section 6.6.

Folacin: Comparison of this study with other reported studies on folacin status in pregnancy (see section 1.3.8) is hampered by similar difficulties as in case of vitamin B12, i.e. differences introduced by different methodology in serum and red cell folacin measurement. In most reported studies, especially the earlier ones, results are based upon determination of folacin content by a microbiological assay (with L.Casei as test organism) while we used a radioassay (see section 2.6.1). Although good correlation has been obtained between both assays levels measured by radioassay (RID) are generally lower compared

with the microbiological assay (MB). Also systematic differences may occur between radioassay methods, depending on purity of the folate binding protein (FBP) used, pH of the assay, standard used, etc. Folate radioassay has the advantage of better reproducibility, is less time consuming and can be better standardized compared with microbiological assay. The discrepancies observed between both methods have not yet fully been explained.

Some data reported on changes in serum folacin levels during pregnancy are summarized below (see also section 1.3.8).

Authors	Type of assay	1th Tr.	2nd Tr.	3rd Tr.	N	Non-pregnant
		approximate mean value (nmol/l)				
Chanarin (1968, England)	MB	13.5	10	10	101	10-40
Hamfelt and Tuvemo (1972, Sweden)	MB	14	12	8	19	-
Martinez and Roe (1977, USA)	MB	14[1)]	9[1)]	6[1)]	58	-
		9[2)]	9[2)]	6[2)]		-
Rolschau et al. (1979, Denmark)	RID	-	14	10	40	-
Ek and Magnus (1981, Norway)	MB	15	9	8	48	13
This study (Netherlands)	RID	6.5	5	4	69	9

MB: microbiological assay
RID: radioisotope assay
[1)] no preconceptional OC-use
[2)] preconceptional OC-use

In all studies, healthy, well-nourished women with uncomplicated pregnancies were involved. There is a general tendency for serum folate levels to fall during pregnancy. Our data suggest a considerable fall early in pregnancy; our 16th week mean value is lower than that established for a non-pregnant female reference group, while in the third trimester no significant further decrease appears. A similar sequence was reported by Chanarin (1968), although our absolute mean values are considerably lower, probably because of

methodological differences.

Ek and Magnus (1981), however, found in their longitudinal study evidence for an increase in serum folate levels above non-pregnancy values in early pregnancy, i.e. before the 10th week. It should be noted that their observations in early pregnancy are based upon measurements in only 5-11 women, while this number increased up to 50 at the following stages of pregnancy.

Using our data, a rise in serum folate in early pregnancy cannot be excluded since we first sampled at the 16th week of pregnancy. In the Ek and Magnus study, a sharp decrease between the 10th and 16th week was observed and the mean value at the 16th week was also below their non-pregnant reference range.

An interesting observation which may explain this discrepancy was made by Martinez and Roe (1979), who found considerable lower serum folate levels in early pregnancy in women who had used oral contraceptives for at least 3 months before conception compared with women who did not.

Similar effects of long-term use of oral contraceptives on vitamin status in pregnancy were reported by Roepke and Kirksey (1979) in the case of vitamin B6.

We also measured red cell folacin levels in the course of pregnancy and observed a rather similar trend as for serum folacin content (Figure 6.1.13).

Besides methodological differences in folacin assay with respect to type of assay, i.e. microbiological versus radioassay, determination of red cell folacin content is even more complicated because erythrocyte folates are mainly present as polyglutamates which should be hydrolysed to the corresponding monoglutamate forms before assay. Because folates are extremely instable, strict standardization of extraction and assay procedures are essential to avoid artifacts (see section 1.2.4). Red cell folacin is, however, thought to reflect overall folacin body stores better than serum folate (see section 1.3.8).

Some data on changes in red cell folacin levels during pregnancy are summarized below.

Authors	Type of assay	1th Tr.	2nd Tr.	3rd Tr.	Non-pregnant
		aproximate mean value (nmol/l)			
Chanarin (1968, England)	MB	350	300	260	360
Hamfelt and Tuvemo (1972, Sweden)	MB	300	350	330	-
Martinez and Roe (1977, USA)	MB	550[1)]	440[1)]	390[1)]	-
Rolschau et al. (1979, Denmark)	RID	-	300	400	-
Ek and Magnus (1981, Norway)	MB	800	1000	850	700
This study (Netherlands)	RID	560	490	450	(-)

MB: microbiological method
RID: radioisotope assay
1) no preconceptional OC-use
2) preconceptional OC-use

Reported levels of red cell folacin content at the various stages of pregnancy are quite variable. As already mentioned this variability may be due to methodological differences, although other factors like local nutritional conditions, folacin supplementation, etc. may also be involved.

We found the fall in red cell folacin parallel to that of serum folacin levels with a similar time course as described by Chanarin, although absolute levels were different. Ek and Magnus, however, found an increase up to the second trimester, followed by a decrease in the last weeks of pregnancy, but at term, mean red cell folacin content was still above non-pregnant mean values. Relatively higher red cell folate levels at term were also reported by Rolschau et al. (1979) and Avery and Ledger (1975).

The fall in red cell folacin content, although less dramatic than for serum folacin with the majority of values remaining within the acceptable range, is rather unexpected and suggests depletion of maternal folacin stores in pregnancy, as was

already observed by Kitay (1969), who stated: "It is doubtful whether a pregnant woman with normal stores and on adequate intake, can avoid at least subclinical deficiency".

Even more unexpected is the lack of recovery to non-pregnant values in the postpartum period. At 6 months postpartum, 45% of the women in our S-Reference group still showed serum folacin levels indicative for a marginal of deficient folacin status. Twenty percent of this group showed deficient, or marginal red cell folacin levels. It should be noted that low folacin levels were not associated with subclinical or hematological abnormalities at any stage of pregnancy or in the postpartum period.

Concerning postpartum changes in serum and red cell folacin the literature is rather limited and concerns, in nearly all instances, the first 6 weeks postpartum. Only Temperley (1968) reported also lowered serum levels 3 months postpartum. Willoughby and Jewell (1968) observed the lowest red cell values in a group of unsupplemented, pregnant women at 6 weeks postpartum, but did not provide data extending that period.

Contrary to the observations from Leck (1977), we found a significant relationship between measurements at the 16th week of pregnancy and again one year later at 6 months postpartum for serum folacin but not for red cell folacin (Table 6.1.27).

Conclusions 6.1

1. Vitamin blood or serum levels of retinol, PLP, vitamin B12 and folacin fall during pregnancy. The mean values measured for these parameters at the 16th week of pregnancy were already below the ranges obtained with non-pregnant females. Between the 16th week and the time of parturition levels decreased with about 10 (vitamin B12) to 50 (retinol, PLP, folacin) percent. Serum 25-OHD levels remained rather constant in this period, while whole blood riboflavin content even slightly increased at the end of pregnancy.
   Between measurements of the same parameter at the various stages of pregnancy, a significant correlation was obtained for all vitamins.

2. The changes in vitamin blood or serum levels were observed for all women and showed a rather parallel time course. The main decrease seemed to have occurred in the first half of pregnancy, except for serum vitamin B12, where the main decrease occurred in the second and third trimester.
3. With the enzyme stimulation tests for assessment of the thiamin, riboflavin and vitamin B6 status, less obvious changes during pregnancy were observed. However, basal ETK activity was significantly lower during pregnancy (about 20%).
4. The occurrence of values, below or above the non-pregnant reference range increased during pregnancy for nearly all parameters. The highest incidences were found for serum retinol (70%), folacin (55%) and plasma PLP (100%). For serum vitamin B12 and 25-OHD as well as for whole blood riboflavin the occurrence of values outside the non-pregnant reference range remained below 5% throughout pregnancy.
5. In the postpartum period most parameters of the vitamin status changed, within the first 6 weeks, to values within the non-pregnant range, except serum folacin and plasma PLP. At 6 months postpartum low levels for serum folacin and plasma PLP were observed in, respectively, 45 and 25% of women from the S-Reference group. With the enzyme stimulation tests, especially for the basal ETK activity and EGOT stimulation ratio, a more gradual recovery was observed up to 6 months postpartum.

6.2. Fetal-maternal relationship; vitamin status parameters in cord blood relative to maternal vitamin status.

Apart from genetic factors, adequate growth in intrauterine life is depending on an adequate supply of oxygen and nutrients. To provide an optimal environment for the developing fetus while maintaining maternal health (i.e. body stores), physiological adjustments in maternal organisms occur (see section 1.1). As a result, nutrients are repartitioned between the fetal and

maternal compartments, a process controlled both by homeostatic and homeorhetic regulation mechanisms (Bauman and Currie, 1980).

The placenta is the vital link between both compartments and can not simply to be regarded as a passing-hatch between mother and fetus. It plays a crucial, but still poorly understood role affecting both maternal and fetal metabolism.

Although the fetal compartment may still be regarded as a "black box", measurement of vitamin levels and other vitamin status parameters in cord blood may provide a better insight into placental function. An important question in this study was about the relationship between the fetal and maternal vitamin status and the relevance for fetal growth and development. We measured, therefore, some biochemical indices of the vitamin status both in maternal and in cord blood.

-Findings

Mean value, standard deviation, range and the 10th, 50th and 90th centiles of the values measured in the cord blood of babies born to mothers belonging to the S-Reference group were presented in Tables 6.1.1 - 26 and Figures 6.1.1 - 13.

Linear correlation coefficients obtained between maternal and cord blood values are presented in Table 6.2.1.

In this table the ratios between mean values in cord and maternal blood (C/M ratio) are also given.

The C/M ratio is > 1 for all water soluble vitamins (at least those measured in this study) and < 1 for serum 25-OHD content and serum retinol. For the latter vitamin, a C/M ratio of 1.1 is obtained by taking the maternal values at parturition. However, using the maternal values measured in the 34th week of pregnancy or at 6 days pp, which may be more representative for maternal retinol status at the end of pregnancy (see section 6.1), C/M ratios of 0.71 and 0.52 are obtained, respectively. Stimulation ratios for the red cell enzymes (ETK, EGR and EGOT) in cord blood were significantly lower than maternal values (C/M ratio < 1) as might be expected from the higher cord blood levels for the water soluble vitamins. The basal enzyme

activities were 1.5 - 2 times higher in cord blood compared with maternal values at term. Between maternal and cord αETK values, no significant correlation was obtained, while for αEGOT correlation was low and only slightly significant. The highest C/M ratios were obtained for PLP and folacin indicating extensive transfer across the placenta.

Between measurements of different indices from the same vitamin status in cord blood, such as riboflavin, vitamin B6 and folacin, as well as between paired enzyme activities and stimulation ratios, significant correlations were obtained except between serum and red cell folacin contents and between plasma PLP contents and EGOT stimulation ratios, as shown in Table 6.2.I.

TABLE 6.2.I Linear correlation (Pearson) between paired observations of some vitamin status parameters in cord blood. NS: not significant.

Vitamin	Correlation	r	Significance
Thiamin	ETKA-αETK	-0.22	$p < 0.05$
Riboflavin	EGRA-αEGR	-0.56	$p < 0.001$
	EGRA-WBRC[1)]	0.28	$p < 0.05$
	αEGR-WBRC[1)]	-0.33	$p < 0.01$
Vitamin B_6	EGOT-αEGOT	-0.66	$p < 0.001$
	EGOT-PLP (plasma)	0.39	$p < 0.001$
	αEGOT-PLP (plasma)	-0.19	NS
Folacin	Serum-red cell content	0.12	NS

1) whole blood riboflavin content

-Relationship between vitamin status parameters in maternal and cord blood according to birthweight

Mean value, standard deviation and median value of the measurements at the various stages of pregnancy and in the postpartum period for women in the < P10 group (see section 2.1) are summarized in Table 6.2.2. Median values obtained for the < P10 group were also indicated in Figures 6.1.1 - 13.

For the > P90 group consisting of 5 women, only the median values are shown in Table 6.2.3. Comparing the mean and median values obtained at the various stages of pregnancy and

postpartum between the three groups, i.e. the < P10, > P90 and the S-Reference group, no consistent difference can be observed except for vitamin B6 status parameters, especially for the EGOT stimulation test. At all stages during pregnancy the basal EGOT activity was significantly lower, αEGOT ratios significantly higher, for women from the < P10 birthweight group compared with those from the S-Reference group. The possible relationship between maternal vitamin status parameters and birthweight was further assessed by regression analyses. These data are presented and discussed in section 8.5.

As illustrated in Table 6.2.II only slight differences, if any, are found between the C/M ratios obtained for the S-Reference group and the < P10 group, respectively.

TABLE 6.2.II Ratio between mean values for some vitamin status parameters measured in cord blood and in maternal blood, taken at parturition, for the S-Reference group and the < P10 birthweight group.

Parameters	S-Reference group	< P10 group
Retinol 34th week	0.70	0.60
Retinol partus	1.10	1.40
Retinol 6 days pp	0.52	0.50
25-OHD	0.68	0.72
ETK activity	1.98	1.44
αETK	0.92	0.91
Riboflavin	1.29	1.14
EGR activity	1.50	1.50
αEGR	0.87	0.91
PLP	6.0	4.8
EGOT activity	1.97	2.4
αEGOT	0.69	0.67
Vitamin B_{12}	1.73	1.89
Folacin (serum)	4.0	4.1
Folacin (erythrocytes)	2.7	2.2

-Discussion and conclusions 6.2

As illustrated in Table 6.2.1, vitamin content in cord blood is invariably higher than in maternal blood, at least for the water soluble vitamins, indicating active transfer from the maternal

into the fetal compartment. The C/M ratios found in this study generally agree with reported ratios (see section 1.3), although a broad variance is apparent from the literature. For plasma PLP ratios between 2-10 have been reported (Hamfelt and Tuvemo, 1972; Brophy and Siiteri, 1975; Lumeng et al., 1976; Reinken and Dapunt, 1978 and Ejderhamm and Hamfelt, 1980). From the B6-supplementation studies described by Lumeng et al. (1976) and Hamfelt and Tuvemo (1972), no clear relationship between supplementation dose during pregnancy and the resulting C/M ratio is apparent.

In the case of 25-OHD serum levels, however, higher C/M ratios were reported when maternal 25-OHD levels were elevated, suggesting a regulating role for the placenta (Paunier et al., 1978). This cannot be concluded from our data which show a linear relationship between maternal and cord blood values. Linear relationships were also reported by Birbeck and Scott (1980).

The difference in C/M ratios obtained between vitamin status parameters illustrates that different mechanisms may account for transfer of vitamins across the placenta. These mechanisms are complex and have not yet been elucidated for all vitamins (Hill and Longo, 1980). Differences in protein binding between the maternal and fetal circulation (for retinol, 25-OHD, vitamin B12 and folacin), metabolic conversion and different transplacental transfer between vitamins and metabolites (riboflavin, folacin?), placental vitamin metabolism (vitamin B6), as well as carrier-mediated, energy dependent placental transfer (vitamin C) were all found to be involved and may account for maternal-fetal transfer (see section 1.3). The highest C/M ratios were found for serum folacin and plasma PLP. As discussed in the previous section (6.1) especially for these vitamins some depletion of maternal stores was apparent during pregnancy. The high C/M ratio may indicate the existence of specific and active mechanisms for both vitamins to provide the fetus with its requirements.

For those vitamins present in both plasma and erythrocytes, i.e. vitamin B6, riboflavin and folacin, it seems that C/M

ratios for whole blood or red cell vitamin content are generally lower than those for serum or plasma levels. The C/M ratio obtained for whole blood riboflavin content (section 1.3) may even be accounted for by the difference in red cell number between maternal and cord blood because the mean cell riboflavin content (MCRC) is not significantly different, i.e. 0.89 and 0.88 µmol/l respectively. Similar observations were reported by Clarke (1971) and Knobloch et al. (1979). The higher basal ETK, EGR and EGOT activities in cord blood are thought to reflect the higher level of immature cells in the fetal circulation.

The basal enzyme activity of a red cell population is influenced by mean cell age, younger cells having the highest enzyme activity (Thurnham, 1981). Mean stimulation ratios for ETK, EGR and EGOT are significantly lower compared with maternal values at term, but the differences are most marked in case of EGOT, probably reflecting higher intracellular PLP levels.

Between maternal and cord blood values, a significant correlation was obtained for all parameters except for αETK. Visual inspection of the plots of maternal versus cord values showed a linear relationship in all cases. The significant linear correlations between cord and maternal levels indicate that maternal vitamin status is an important determinant of cord blood levels.

Whether cord blood values are reliable indicators of "true"fetal vitamin status is doubtful. However, it was beyond the scope of our study to follow changes in vitamin blood levels in early life, but reported data suggest that considerable changes may occur for serum and red cell folacin levels (Hibbard and Kenna, 1975; Ek and Magnus, 1979), vitamin D (Hillman and Haddad, 1975), vitamin B6 (Ejderhamm and Hamfelt, 1980)and vitamins E and C (Vobecky et al., 1976). In spite of this, the C/M ratio is considered to be an important indicator for identifying aberrations in placental vitamin transport. Baker et al. (1977) observed significantly lower C/M ratios for folacin, vitamin B12 and pantothenate for small for gestational age (SGA) babies compared with normal weight, term infants. Between maternal blood levels for both groups, no significant

differences were obtained. Brophy and Siiteri (1975) reported significantly lower C/M ratios for plasma PLP in pre-eclamptic than in normal pregnancies.

The significance of maternal and fetal vitamin status with respect to fetal growth and development is another issue. A positive relationship between birthweight and maternal vitamin status has been reported a.o. for vitamin B6, thiamin and retinol (Kübler and Moch, 1975; Reinken and Dapunt, 1978; see also section 1.3). Roepke and Kirksey (1979) and Schuster et al. (1981) reported lower Apgar-scores of infants born to mothers with poor vitamin B6 status during pregnancy. Gandy and Jacobson (1977) described a higher incidence of SGA babies in mothers with low serum folacin levels compared with mothers with a higher serum folacin level. However, in many other studies and most supplementation studies (reviewed by Hemminki and Starfield, 1978), no significant effects could be demonstrated. In our study, evidence for only vitamin B6 was obtained for a relationship of fetal growth and maternal vitamin status which will be discussed in more detail in Chapter 8.

From the data presented in this section on fetal-maternal relationships of vitamin status parameters, it can be concluded that:

1. Between parameters of the vitamin status measured in cord and maternal blood, taken at parturition, a statistically significant linear relationship was found except for the ETK stimulation ratio (αETK). The levels of the water soluble vitamins in cord blood are generally higher, those for the fat soluble vitamins generally lower, than in maternal blood.
2. Mean maternal EGOT-activity was significantly lower, and αEGOT significantly higher during pregnancy, for women in the < P10 birthweight group compared with the S-Reference group. For the other parameters no significant relationship with birthweight was found. The ratios between mean cord and maternal values for the vitamin status parameters (the C/M ratio) were about the same in the S-Reference group and the

< P10 birthweight group. The highest ratios were obtained for the plasma PLP and serum folacin levels.

6.3. The effect of maternal smoking on maternal and cord blood vitamin status parameters.

Maternal smoking reduces fetal growth (see section 1.5 and Chapter 8). Whether this effect is mediated by a change in dietary intake is still a matter of debate (see Chapter 5). In this study no significant effect of smoking on nutrient intake could be demonstrated (Table 5.7). To assess the effect of maternal smoking on the biochemical indices of maternal and neonatal vitamin status, all parameters were calculated according to smoking habit and are shown in Table 6.3.1. No significant differences were found between smokers and non-smokers for serum retinol, 25-OHD, PLP, folacin and vitamin B12, as well as for basal ETK activity and stimulation ratio (αETK). However, the riboflavin and vitamin B6 status seemed both affected by maternal smoking. Cord blood total riboflavin levels were significantly lower among neonates born to mothers who smoked during pregnancy when compared with those of non-smoking mothers. Maternal riboflavin levels were not affected. As illustrated in Table 6.3.1, EGR activity and stimulation ratio (αEGR) in cord blood were not significantly different between babies born to smoking compared with those from non-smoking mothers. Also maternal EGR activity and stimulation ratio were not significantly different among both groups.

Although no differences could be demonstrated for the plasma levels of PLP between smoking and non-smoking mothers the results with the EGOT stimulation test were affected. EGOT activities were significantly lower, αEGOT ratios significantly higher during pregnancy of the smoking compared with the non-smoking mothers. After the puerperium differences between both groups disappeared. Cord blood levels were not significantly different for the vitamin B6 status parameters

between babies from smoking versus non-smoking mothers.

Data for the EGOT stimulation ratio (αEGOT) at all stages during pregnancy and postpartum for the smoking and non-smoking groups are summarized in Table 6.3.I.

TABLE 6.3.I EGOT stimulation in non-smokers (I, n=37), smokers (< 10 cigarettes/day, II, n=15) and smokers (> 10 cigarettes/day, III, n=18).

	Group I		Group II		Group III		Significance
	mean	SD	mean	SD	mean	SD	
16 weeks	1.94	0.15	1.99	0.10	2.05	0.15	I-III*
28 weeks	1.98	0.18	2.01	0.18	2.08	0.2	NS
34 weeks	1.92	0.26	2.18	0.29	2.12	0.24	I-II* I-III*
P	1.95	0.26	2.10	0.37	2.20	0.29	I-III*
6 days pp	2.04	0.32	2.21	0.49	2.26	0.35	I-III*
6 weeks pp	1.92	0.23	2.05	0.18	2.03	0.21	NS
6 months pp	1.85	0.17	1.87	0.24	1.9	0.16	NS
Cord	1.39	0.14	1.45	0.23	1.40	0.14	NS

* $p < 0.05$; NS: not significant

As shown in Table 6.3.I differences between groups I and III were significant at all stages during pregnancy except at week 28. Only at week 34 differences between groups II and III became statistically significant. A similar picture was obtained with the basal EGOT activities: significantly lower values for the smoking groups (I, II) during pregnancy. At 6 months postpartum the EGOT activity in group III was still significantly lower than in group I.

Discussion

The lower riboflavin levels in cord blood, but not in the blood of parturient women, suggest a reducing effect of smoking on transplacental transfer of riboflavin into the fetus rather than a dietary effect. However, cord blood EGR activity and stimulation ratio are not different between neonates from smoking and non-smoking mothers.

In the case of vitamin B6 the situation is reversed: only maternal values are affected, but not cord blood values. The

observation of lower EGOT activity and higher αEGOT ratio at the various stages of pregnancy indicate a consistent effect of smoking on vitamin B6 status. Plasma PLP levels were, however, not statistically different between smokers and non-smokers, but it should be noted that, for both groups, plasma PLP levels are in the deficient range for most of the women during pregnancy (section 6.1). Vitamin B6 intake was not statistically different between smoking and non-smoking gravidae (Table 5.7).

Effects of smoking on nutritional status of pregnant women were also reported by Kübler and Moch (1975) who described a "better" nutritional status with respect to retinol, thiamin and vitamin B6, in gravidae who did not smoke or consumed alcohol during pregnancy compared with those who did. Vir et al. (1981) reported significantly higher EGR stimulation ratios in blood samples taken 3 days pp for smoking than for with non-smoking women. Between the second and third trimester values, no significant differences between both groups were obtained.

McGarry and Andrews (1972) reported a small, but significant difference in serum vitamin B12 content between smoking and non-smoking gravidae. The effect was most evident in early pregnancy. The lower B12 levels in the serum of smokers were explained by the cyanide inactivation of B12 coenzymes in the liver. Such an effect cannot be concluded from our data. Crosby et al. (1972) described a negative relationship between serum carotene level and the number of cigarettes smoked. They also found lower amino acid serum levels in smokers compared with non-smokers.

Hall et al. (1976) reported a significant fall in the serum folacin levels in smoking, but not in non-smoking women during pregnancy. We also found lower serum folacin levels for smokers, differences were not significant, however. According to Hall et al. the fall in serum folacin levels observed for the smokers was due to a difference in plasma volume expansion between smoking and non-smoking women (see section 6.6).

In conclusion there is only a smoking effect with respect to the vitamin B6 status, an effect which is only present during pregnancy. The underlying mechanism of the effect of smoking on

vitamin metabolism is still unknown. As indicated in Chapter 5 a dietary intake mediated effect seems unlikely. In pregnancy, placental circulation may be affected, but why only cord blood riboflavin content is affected and not the other vitamin levels is difficult to explain. In our study there is an association between smoking and alcohol consumption. Alcohol may also interfere with the vitamin metabolism. However, this is only true in more extreme situations (alcoholism), but in our group alcohol consumption was very low. In a review of the literature, Wack and Rodin (1982) concluded that little reliable information was available at the moment about the effects of smoking on nutrient metabolism.

6.4. Vitamin status and parity.

In Figure 6.4.1 mean values and standard deviations of some biochemical indices of the vitamin status, measured in this study, are presented for women from the S-Reference group, according to parity. Therefore, the S-Reference group was split into two groups:

1. One group of nulliparae (n = 39)
2. One group of multiparae (parity > 1, n = 31).

As can be seen in Figure 6.4.1 no consistent differences were apparent between both groups. For none of the parameters differences were statistically significant, neither during and after pregnancy nor in cord blood.

In Chapter 5 evidence was presented for a significantly higher vitamin C and carbohydrate intake from the diet in nulliparae compared with the multiparae. For the other nutrients and the energy consumption differences were not significant. Vitamin C blood levels were not measured, however, in this study. In some of the reported studies evidence was obtained that mothers with previous pregnancies had a slightly poorer vitamin status, in particular for vitamin B6 (Heller et al., 1973; Kübler and Moch, 1975). A remarkable observation was

reported by Reinken and Dapunt (1978) who found serum PLP levels increased at the onset of pregnancy as the number of pregnancies increased. As already noted their conclusions were based upon observations in a small group consisting of 16 women and only 6 women with parity ≥ 2. Generally, studies on vitamin status during pregnancy performed in western societies with healthy gravidae indicate no significant differences in vitamin status according to parity (Vir et al., 1980, 1981; see also section 1.3). A different situation may exist in developing countries. The incidence of megaloblastic anemia has been reported to increase, with increasing parity (see section 1.3.8). Succeeding pregnancies and subsequent lactation with a relatively short birth-interval, may gradually deplete maternal folacin stores. However, in healthy, well nourished, pregnant women parity seems not to be related to folacin status as found in this and other studies (Hall et al., 1976). On the other hand, our data for serum folacin and plasma PLP levels at 6 months postpartum, presented in section 6.1, are suggestive for maternal vitamin depletion during pregnancy while replenishment of maternal stores after delivery takes at least 6 months (section 6.7). As no significant differences in vitamin status parameters between nulli- and multigravidae could be demonstrated in this study the birth-interval in our population has obviously been long enough to replenish maternal stores.

6.5. Effect of seasonal variation in maternal and cord blood vitamin status parameters.

A season dependent trend in biochemical indices of the nutrient status may reflect a season dependent nutrient intake, due to a difference in availability of certain food products between seasons, like for fresh vegetables. Such trends have been reported in several studies, including studies with pregnant women (Gal and Parkinson, 1974; Kübler and Moch, 1975; Martinez and Roe, 1977).

To assess whether season dependent trends in biochemical

indices of the vitamin status could be demonstrated in this study, all measurements performed were grouped according to the time of the year the blood samples were collected. Measurements performed on blood samples taken from women in the S-Reference group at the respective stages of pregnancy and in the postpartum period were divided over 6 groups corresponding with two-months periods in which the samples were collected.

Mean value, standard deviation and median values were calculated and the P50 values are shown in Table 6.5.1 for the 16th and 34th week, as well as the partus, 6 months pp and cord blood values.

As expected, the seasonal variation is most evident for serum 25-OHD levels (section 6.1). For some of the other parameters some seasonal variation seemed also to be apparent, especially for serum retinol and the EGOT stimulation ratio. Serum retinol levels were lowest in the period between March and June. In Figure 6.5.1 mean serum retinol levels are shown at the various stages of pregnancy according to the time of sampling. Variance analysis indicated a significant seasonal variation in maternal serum levels at all stages of pregnancy and in the postpartum period, but not in cord blood. Such a seasonal variation in serum retinol levels was also reported by Gal and Parkinson (1974) and Kübler and Moch (1975). Remarkably, we measured the lowest levels in spring and early summer while in in the other studies, the lowest levels were found in autumn and winter. In the postpartum period, the highest levels were obtained in the samples collected in November/December. So, although differences are significant the seasonal effect seems rather inconsistent. Such an inconsistency might result from the small size of the subgroups and the bias introduced by lactation and the use of hormonal contraceptives in the postpartum period, as will be discussed in section 6.7.

The slightly lower mean values of serum folacin measured in the summer period may also reflect interference with other factors. However, variance analysis revealed no significant variation for serum folacin.

The variation in EGOT stimulation ratios (αEGOT) was,

however, significantly explained by seasonal effects, also after allowance was made for maternal smoking (see section 6.3) (the same was true for smoking effects after allowance was made for seasonal variation). Higher stimulation ratios were found in the winter period, lower values in the summer. For the 6 months pp values as well as for cord blood values, no significant differences according to season could be demonstrated.

As already mentioned in section 6.1 seasonal variation is most evident for serum 25-OHD levels. This is further illustrated in Figure 6.5.2. A consistent seasonal trend is present at all stages of pregnancy and postpartum. The variance ratios obtained with the variance analysis are highly significant ($p < 0.0001$) and demonstrated again that changes in serum 25-OHD content primarily reflect the extent of solar exposure.

As demonstrated in Chapter 5 nurient intake for the S-Reference group was not significantly different between summer and winter. Biochemical indices of the nutrient status in general do not reflect a significant diet induced seasonal variation either, except for vitamin B6.

Conclusions section 6.3 - 6.5

1. Maternal smoking resulted in significantly lower riboflavin levels in cord blood and lower basal EGOT activity and increased EGOT stimulation ratios in maternal blood, compared with non-smokers. The effect of smoking on the EGOT stimulation test was only evident in the pregnancy period but could not be demonstrated in the samples taken postpartum.
2. Vitamin status parameters in maternal and cord blood were not related to parity.
3. Part of the variation in serum 25-OH-vitamin D, retinol level and the EGOT stimulation ratio at the various stages of pregnancy and in the postpartum period is explained by seasonal effects. Seasonal variation is most evident in case of serum 25-OH-vitamin D levels which are about two times higher in summer compared with winter and early spring. Serum

retinol levels were lowest in spring and early summer, while the mean EGOT stimulation ratio was significantly higher in winter than in summer. In the case of serum retinol, the effect was less consistent.
For the other vitamin status parameters no seasonal variation was apparent.

6.6. Determinants of the fall in vitamin blood levels during pregnancy.

When considering the changes in some of the vitamin status parameters during pregnancy as normal physiology in a group of healthy women, the question arises as to the underlying mechanism. Which factors are related to changes in vitamin status parameters during normal pregnancy? The factors commonly suggested to explain the "hypovitaminemia of pregnancy" are (Metcoff, 1978):

- the increase in circulating blood or plasma volume,
- inadequate dietary vitamin intake (i.e. below requirements),
- an increase in renal vitamin clearance,
- fetal sequestration,
- an increase in vitamin catabolism,
- an increase in maternal tissue retention.

Although all these factors have been suggested as possibly having a causal relationship with the changes in some of the vitamin status parameters during pregnancy, little experimental evidence has been presented. One of the objectives of this study was, therefore, to trace the determinants of the fall in vitamin blood or serum levels during normal human pregnancy.

The changes observed for some vitamin status parameters during pregnancy were described in section 6.1. Vitamin status parameters were in some cases affected by maternal smoking, seasonal variation or different parity, and, therefore, may account for some of the inter- and intra-individual variation, but are unlikely to explain the fall in vitamin serum level.

Some of the factors mentioned above were assessed and

quantified in this study, such as dietary intake and plasma dilution. Other factors, like fetal sequestration and maternal tissue retention, are more difficult to quantify in human studies. We will first discuss the relationship between the increase in circulating blood or plasma volume and the fall in some vitamin blood or plasma levels, as well as the relationship between dietary intake and the changes in some vitamin status parameters during pregnancy. Next, these and other possibly related variables will be used as independent variables in multiple regression analysis to explain the fall in some vitamin serum levels between the 16th and 34th week of pregnancy.

- Is plasma dilution a causative factor in the fall in vitamin blood(plasma) level during pregnancy?

Data about the changes in circulating blood and plasma volume were presented in Chapter 4. Based on these data, the total amounts of circulating vitamin were calculated for serum retinol, folacin and vitamin B12, plasma PLP and whole blood riboflavin. The results are presented in Table 6.6.1 and Figure 6.6.1. Differences between measurements were tested for statistical significance. The significance matrix is given in Table 6.6.2.

As shown in Table 6.6.1 and Figure 6.6.1 a different time trend is obtained for the respective vitamins. Serum retinol and vitamin B12 levels decrease somewhat proportionally with the increase in plasma volume, resulting in a rather constant amount of circulating vitamin both during and after pregnancy.

Plasma PLP and serum folacin level fall relatively more than could be accounted for by a volume effect only while the whole blood riboflavin content increases.

In the postpartum period, the total amount of circulating vitamin decreases in the case of riboflavin, increased significantly for plasma PLP, but remained constant for serum retinol, folacin and vitamin B12. Total folacin levels in the postpartum period were significantly lower than those measured at any stage of pregnancy (Table 6.6.2).

It should be emphasized that the increase in the total distribution volume during pregnancy is actually greater than that calculated by the increase in blood volume as both the extravascular and extracellular water volumes also increase during pregnancy (see section 1.1). Whether an inverse proportionality between a decrease in concentration and an increase in circulating volume necessarily reflects a causality, is questionable. This relationship will be further discussed with the multiple regression analysis.

- Are dietary factors related to the fall in vitamin blood(plasma) levels during pregnancy?

Data about the habitual intake of energy and nutrients for our S-Reference group, at the various stages of pregnancy were presented and discussed in Chapter 5. It was concluded that when nutrient intake was compared with recommended allowances the mean vitamin intake for our S-Reference group was adequate or even abundant with the possible exception for vitamin D and B6.

However, as discussed in section 6.1, vitamin status parameters, especially vitamin blood or serum levels, changed to values indicative for maternal vitamin depletion when compared with non-pregnant reference ranges.

To assess whether a relationship could be established between dietary and biochemical variables of the vitamin status, correlations were calculated between the estimated vitamin intakes obtained at the 16th and 34th week and corresponding biochemical parameters. The Pearson correlation coefficients are summarized in Table 6.6.3. Since dietary folacin intake could not be calculated from the dietary history data, we used total energy intake as an approximation of the folacin intake. For serum retinol, folacin and plasma PLP, the decrease in serum or plasma level between the 16th and 34th week was correlated with the estimated vitamin (energy) intake obtained both at the 16th and 34th week.

As shown in Table 6.6.3, correlation coefficients are generally low or insignificant. Between the 16th week values for

αETK, αEGR and plasma PLP and the respective vitamin intakes (M_I), a weak correlation was obtained. The fall in vitamin serum or plasma level was not related to a difference in vitamin intake, at least for serum retinol, plasma PLP and possibly serum folacin. The relative small number of data used in the calculations are explained by the fact that these correlations were calculated as part of the multiple regression analysis, described below, for which only the data from women with complete files for all of the variables were available (see section 2.7).

Another approach to assess a relationship between dietary and biochemical measurements of the nutrient status is to calculate sensitivity and specificity of dietary data for the identification of abnormal biochemical values.

Sensitivity can be defined as the percentage of women with substandard (abnormal) biochemical values, i.e. below a certain cut-off point, identified by a substandard dietary intake. Specificity is defined as the percentage of women with above-standard biochemical values identified by above-standard dietary intake.

We calculated the specificity and sensitivity of our dietary history data obtained at the 16th (M_I) and 34th week (M_{II}), respectively, using the corresponding biochemical data. We also used the combined dietary data (M_I and M_{II}) to calculate sensitivity and specificity for the 34th week values. The cut-off points used for the biochemical parameters were the same as those given in Table 2.6.IV. Dietary intake was related to the recommended daily allowances accepted by the Netherlands Nutrition Council (Table 1.2.2.I). For vitamin B6 a standard value of 1.2 mg/per day was chosen arbitrarily for the interpretation of intake data during pregnancy. The results obtained for retinol, thiamin, riboflavin and vitamin B6 are presented in Table 6.6.4. Although also dietary intake data were available for vitamin B12 and D, sensitivities and specificities were not calculated for these vitamins, because in both cases the occurrence of substandard biochemical values was insignificant, and, in the case of vitamin D, the estimated

intake was below recommendations in all, except one case. Mean vitamin B12 intake was adequate at all times.

As illustrated in Table 6.6.4, the sensitivity of the dietary data for the identification of abnormal biochemical values is generally higher with the 34th week data. However, this higher sensitivity is accompanied by a lower specificity. When the combined dietary data from the 16th and 34th week were used instead of using only the 34th week data, a slightly higher sensitivity was obtained while the specificity remained about the same, or was even higher. This was true for serum retinol and αEGOT values, but not for e.g. αETK values. Rather extreme values for sensitivity and specificity are obtained for whole blood riboflavin and plasma PLP, caused by the absence of abnormal values (whole blood riboflavin) or a 100% incidence of low values (plasma PLP). However, specificity and sensitivity are dependent on the standards used for the interpretation of dietary and biochemical data. Accepting the RDA as the standard value for classification of the dietary data will result in relatively too high sensitivity percentages, because requirements for most individuals are, by definition, lower than the estimated RDA for the group as a whole. On the other hand criteria for interpretation of biochemical data are also rather arbitrary, although they are in general more on the lower side of the reference range than the dietary standards, resulting in relatively higher specificity. The absolute percentages as presented in Table 6.6.4 should, therefore, be interpreted carefully.

Multiple regression analysis

Besides dietary and volume related factors other factors may also be associated with the change in vitamin serum level. A number of such possible causally related factors were summarized in the introduction of this section. It is tempting to speculate that some of these factors, like a pregnancy induced change in vitamin retention, vitamin catabolism or vitamin transport are

associated with the shift in hormonal balance. Generally the control of nutrient metabolism is exercized partly by hormones. During pregnancy, the amounts of hormones secreted, the rate of hormonal degradation and excretion differ at different stages of pregnancy resulting in a modulation of normal metabolism (Naismith, 1980). Some of the changes in the parameters of the vitamin status observed during pregnancy can be simulated by hormonal therapy as in oral contraceptive medication (see section 1.3).

To trace the determinants of the fall in vitamin blood levels during pregnancy, regression analysis was performed using the difference of some vitamin blood or plasma levels between the values at the 16th and 34th week as the dependent variable and dietary, hormonal and biochemical parameters as independent variables. Data from the total group were used in the analysis (S-Reference group, <P10 group, > P90 group). The independent variables available for selection in the multiple regression analysis are summarized in Table 6.6.5. These variables were considered to reflect the hormonal status, dietary vitamin intake, distribution volume and kidney function. After stepwise multiple regression analysis, the subset selection procedure was performed using GENSTAT (see section 2.7). The subset selection procedure was carried out with a reduced number of independent variables to increase the degrees of freedom. Only those independent variables which were most likely associated, were considered in the subset selection procedure, which was also based upon the results with the stepwise multiple regression analysis. In stepwise multiple regression only the "best set of variables" is selected while in the subset analysis also information is obtained about the second best set, etc. allowing a better insight in the consistency of the independent factors selected to be associated with the fall in vitamin blood level (section 2.7).

The regression analysis was performed for serum retinol, folacin and vitamin B12 and plasma PLP to account for the variance in the changes in vitamin serum levels. The results of these analyses can be summarized as follows:

Serum retinol Δ (wk 16 - wk 34)

Stepwise multiple regression analysis (N = 34)

Variance accounted for with all available variables (Table 6.6.5): R^2 = 90.8; F-ratio = 14.5; $p < 0.005$

Variables selected in stepwise regression:

	Variance explained (R^2)	F-ratio*	Significance*
1) Serum retinol level at wk 16	70.7	83.1	$p < 0.005$
2) Δ(wk 34 - wk 16) serum Alk.phosphatase	76.0	8.3	$p < 0.01$
3) Δ(wk 34 - wk 16) serum HPL	78.8	5.2	$p < 0.05$
4) Serum cortisol level at wk 34	82.6	7.5	$p < 0.01$
5) Δ (wk 34 - wk 16) serum cholesterol	83.3	2.2	NS

* F-ratio and p-value given the set of other variables already selected.

Subset selection analysis (N = 60)

-Regression with 1 variable:

	R^2	F-ratio**	Significance**
1) Serum retinol at wk 16	68.1	124	$p < 0.005$
2) Free Thyroxine Index (FTI) at wk 34	14.4	9.8	$p < 0.005$
3) Serum estradiol at wk 34	11.3	7.4	$p < 0.01$
4) Δ(wk 34 - wk 16) serum Alk.phosphatase	11.0	7.2	$p < 0.01$

** F-ratio and p-value given no variable already selected.

5) Serum prolactin at wk 34	8.1	5.1	$p < 0.05$

-Regression with 2 variables:

	R^2	F-ratio*	Significance*
Serum retinol level at wk 16			
with 1) Serum estradiol at wk 34	71.7	7.3	$p < 0.01$
or 2) Serum HPL at wk 34	70.7	5.0	$p < 0.01$
or 3) FTI at wk 34	70.1	3.8	NS
or 4) Serum cortisol at wk 34	70.0	3.5	NS

-Regression with 3 variables:

	R^2	F-ratio*	Significance*
Serum retinol level at wk 16 and serum estradiol at wk 34			
with 1) Serum cortisol at wk 34	74.1	5.2	$p < 0.05$
or 2) Serum HPL at wk 34	74.1	5.2	$p < 0.05$
or 3) Maternal weight gain (wk 34 - wk 16)	73.3	3.3	NS

-Regression with 4 variables:

	R^2	F-ratio*	Significance*
Serum retinol level at wk 16, serum estradiol and cortisol at wk 34			
with 1) Serum HPL at wk 34	76.4	5.2	$p < 0.05$
or 2) Weight gain (wk 34 - wk 16)	76.3	5.0	$p < 0.05$
or 3) FTI at wk 34	75.6	3.4	NS

-Regression with 5 variables:

	R^2	F-ratio*	Significance*
Serum retinol level at wk 16 and serum estradiol, cortisol and HPL level at wk 34			
with 1) Weight gain (wk 34 - wk 16)	78.4	5.3	$p < 0.05$
or 2) FTI at wk 34	78.1	4.5	$p < 0.05$

Extending the analysis with sets of more than 5 variables revealed no other factors with a significant contribution, so it can be concluded that the main factors associated with the fall in serum retinol level between the 16th and 34th week are:

Variable	Estimate	S.E.	F-ratio*	Significance*
Weight gain (wk 34-wk 16)	-0.0253	0.0112	5.1	$p < 0.05$
Serum cortisol (wk 34)	-0.3005	0.1141	6.9	$p < 0.025$
Serum estradiol (wk 34)	0.0039	0.0012	10.6	$p < 0.005$
Serum HPL (wk 34)	-0.0279	0.0121	5.2	$p < 0.025$
Serum retinol (wk 16)	0.8898	0.7322	147.7	$p < 0.005$

Intercept -0.2006; $R^2 = 78.3$

Plasma PLP Δ (wk 16 - wk 34)

Stepwise multiple regression analysis (N = 34)

Variance accounted for with all variables available (Table 6.6.5): R^2 = 59.7; F-ratio = 3.1; $p < 0.01$.

Variables selected in stepwise regression:

	R^2	F-ratio*	Significance*
1) Plasma PLP at wk 16	63.6	60.5	$p < 0.005$
2) Δ(wk 34 - wk 16) serum albumin	69.1	6.9	$p < 0.025$
3) Δ(wk 34 - wk 16) serum Alk.phosphatase	72.0	4.2	$p < 0.05$
4) Serum progesteron at wk 34	73.8	3.1	NS
5) Dietary vitamin B6 (M_{II})	75.2	2.6	NS

Subset selection analysis (N = 60):

-Regression with 1 variable:

	R^2	F-ratio**	Significance**
1) Plasma PLP at wk 16	66.3	114	$p < 0.005$
2) Δ(wk 34 - wk 16) plasma volume	4.6	2.8	NS

-Regression with 2 variables:

	R^2	F-ratio*	Significance*
Plasma PLP at wk 16			
with 1) Δ(wk 34 - wk 16) serum albumin	71.0	9.4	$p < 0.005$
or 2) Δ(wk 34 - wk 16) serum Alk.phosphatase	68.5	4.1	$p < 0.05$

-Regression with 3 variables:

	R^2	F-ratio*	Significance*
Plasma PLP at wk 16 and Δ(wk 34 - wk 16) serum albumin			
with 1) Δ(wk 34 - wk 16) plasma volume	73.3	4.7	$p < 0.05$
or 2) Relative increase plasma volume	73.0	4.1	$p < 0.05$

-Regression with 4 variables:

	R^2	F-ratio*	Significance*
Plasma PLP at wk 16, Δ(wk 34 - wk 16) serum albumin and plasma volume			
with 1) serum progesterone at wk 34	74.5	2.6	NS

No other variables could be selected which contributed significantly to the variance in the fall in plasma PLP between the 16th and 34th week. The regression coefficients of the main contributing factors are given below:

Variable	Estimate	S.E.	F-ratio*	Significance*
Δ(wk 34-wk 16) plasma volume	-1.871	0.836	4.7	$p < 0.05$
Δ(wk 34-wk 16) serum albumin	0.3058	0.0914	11.2	$p < 0.005$
Plasma PLP wk 16	0.6862	0.0579	140.2	$p < 0.005$

Intercept -5.769; $R^2 = 73.3$

Serum folacin Δ(wk 16 - wk 34)

Stepwise multiple regression analysis (N = 34)

Variance accounted for with all variables available: (Table 6.6.5)
R^2 = 16.2; F-ratio = 1.27; NS.

Variables selected in stepwise regression:

	R^2	F-ratio*	Significance*
1) Serum folacin level at wk 16	33.7	18.2	p < 0.005
2) Serum cortisol at wk 34	47.1	9.4	p < 0.005
3) Prepregnancy weight	51.3	3.7	NS
4) Serum estradiol at wk 34	52.6	1.8	NS

Subset selection analysis (N = 60)

-Regression with 1 variable:

	R^2	F-ratio**	Significance**
1) Serum folacin level at wk 16	43.1	44.0	p < 0.005
2) Serum cortisol at wk 34	30.0	25.0	p < 0.005
3) Δ (wk 34 - wk 16) serum cortisol	21.5	16.0	p < 0.005
4) Serum prolactin at wk 34	12.3	8.1	p < 0.005
5) Serum creatinine at wk 34	5.8	3.5	NS

-Regression with 2 variables:

	R^2	F-ratio*	Significance*
Serum folacin level at wk 16 with 1) Serum cortisol at wk 34	56.7	18.0	p < 0.005
or 2) Δ (wk 34 - wk 16) serum cortisol	52.2	11.0	p < 0.005

or 3) Serum prolactin at wk 34	48.0	5.2	$p < 0.025$
or 4) Serum creatinine at wk 34	45.0	1.9	NS

-Regression with 3 variables:

	R^2	F-ratio*	Significance*
Serum folacin level at wk 16 and serum cortisol at wk 34			
with 1) Serum prolactin at wk 34	59.9	4.4	$p < 0.05$
or 2) Serum progesterone at wk 34	58.7	2.7	NS
or 3) Δ(wk 34 - wk 16) serum cortisol	58.4	2.2	NS

No other factors could be selected which contributed significantly to the variance in the fall of serum folacin between the 16th and 34th week. The regression coefficients of the main contributing factors are given below:

Variable	Estimate	S.E.	F-ratio*	Significance*
Serum cortisol at wk 34	4.090	1.00	16.7	$p < 0.005$
Serum prolactin at wk 34	-0.0118	0.0056	4.4	$p < 0.05$
Serum folacin at wk 16	0.5314	0.0936	32.2	$p < 0.005$

Intercept -2.975; $R^2 = 59.9$

Serum vitamin B12 Δ(wk 16 - wk 34)

Stepwise multiple regression analysis (N = 34)

Variance accounted for with all variables available: R^2 = 27.7; F-ratio = 1.6; NS.

Variables selected in stepwise regression:

	R^2	F-ratio*	Significance*
1) Serum vitamin B12 at wk 16	44.5	28.0	$p < 0.005$
2) Δ(wk 34 - wk 16) serum prolactin	48.8	3.8	NS
3) Δ(wk 34 - wk 16) serum albumin	52.7	3.7	NS
4) Prepregnancy weight	55.3	2.8	NS
5) Serum progesterone at wk 34	55.7	1.3	NS

Subset selection analysis (N = 60)

-Regression with 1 variable:

	R^2	F-ratio**	Significance**
1) Serum vitamin B12 at wk 16	40.7	40.0	$p < 0.005$
2) Serum cortisol at wk 34	19.1	14.0	$p < 0.005$
3) Δ(wk 34 - wk 16) serum cortisol	13.7	9.0	$p < 0.005$
4) FTI at wk 34	5.0	3.0	NS

-Regression with 2 variables:

	R^2	F-ratio*	Significance*
Serum vitamin B12 at wk 16			
with 1) Serum cortisol at wk 34	46.6	6.0	$p < 0.025$
or 2) Serum progesterone at wk 34	44.5	3.9	NS

or 3)	Δ (wk 34 - wk 16) serum albumin	43.6	2.9	NS

-Regression with 3 variables:

		R^2	F-ratio*	Significance*
Serum vitamin B12 at wk 16 and serum cortisol at wk 34				
with 1)	Serum progesterone at wk 34	49.3	3.1	NS
or 2)	Δ (wk 34 - wk 16) serum albumin	49.1	2.8	NS

No other factors could be selected which contributed significantly to the variance in the fall of serum vitamin B12 level between the 16th and 34th week. The regression coefficients of the main contributing factors are given below:

Variable	Estimate	S.E.	F-ratio*	Significance*
Serum cortisol at wk 34	117.2	47.0	6.2	$p < 0.05$
Serum vitamin B_{12} at wk 16	0.552	0.102	29.1	$p < 0.005$

Intercept - 262.4; $R^2 = 46.6$

Discussion 6.6.

The plasma volume expansion during pregnancy is frequently mentioned in literature as a possibly important factor to account for the "hypovitaminemia of pregnancy" (Metcoff, 1978). The study reported by Hall et al. (1976) is considered as a "proof" of that theory. These authors measured serum folacin level and plasma volumes in a group of healthy primigravidae at various stages of pregnancy. From their observation that the total amount of circulating folacin in serum did not significantly change between the 12th and 38th week of pregnancy, they concluded that the fall in serum folacin was to be regarded as a dilution effect. This observation was made for a group of primigravid singleton, non-smoking women. However, for primigravid singleton smokers, the total amount of circulating serum folacin decreased signficantly. From their data, a mean decrease in serum folacin level between the 12th and 38th week with, respectively, 35 and 45% for non-smokers and smokers could be calculated. The plasma volume expanded in the same period with, respectively, 47 and 31% for non-smokers and smokers. The decrease in circulating folacin levels is, therefore, more manifest for the smokers, while the relative volume expansion was less for the smokers than for the non-smokers. Their conclusion seems to be not substantiated by their data.

In our study, serum folacin levels fell between the 16th and 34th week with 38 and 47%, respectively, in the non-smokers compared with the smokers, but the relative volume expansion was the same, i.e. 25 and 23%, respectively, for both groups. However, an inversed proportionality in effects, i.e. a mean decrease in serum concentration concomitant with a proportional increase in plasma volume, does not necessarily reflect a causal relationship. With the regression analysis only in case of plasma PLP and vitamin B12, volume related parameters were selected such as the change in plasma volume between the 16th and 34th week of pregnancy, as well as the decrease in serum albumin concentration. Whether this latter variable is indeed indicative for a dilution effect is questionable. The fall in

serum albumin level is relatively smaller than the increase in plasma volume, so the total serum albumin content increases during pregnancy (Figure 4.3 and 4.23). Albumin is the exclusive transport protein of PLP in plasma and a decrease in the serum albumin concentration may cause a fall in the plasma PLP level. However, the total binding capacity of serum albumin for PLP exceeds by far the total amount of circulating PLP (section 1.3.6).

The complex changes in some of the vitamin binding proteins in the circulation during pregnancy, such as found for the transcobalamins (section 1.3.7) and the folacin binding proteins (section 1.3.8), make it difficult to interpret the fall in vitamin serum level as a simple dilution effect.

The results obtained with the regression analyses are not very supportive for a causal relationship between plasma dilution and the hypovitaminemia of pregnancy. However, it can be argued that the low vitamin serum levels measured at the 16th week of pregnancy, were already affected by a dilution effect, i.e. a dilution effect before the 16th week. Although the increase in circulating plasma volume starts already early in pregnancy, there is still a considerable increase between the 16th and 34th week (Figure 4.3). So, although plasma dilution cannot be excluded as a causative factor associated with the falling vitamin serum levels, it is unlikely to be an important determinant of this phenomenon.

Dietary factors, i.e. an inadequate vitamin intake, are unlikely to be associated with the fall in vitamin blood level as observed for our S-Reference group (Tables 6.6.3 and 6.6.4). This finding was not quite unexpected as both measures have their intrinsic inaccuracies, especially dietary intake data, which are primarily intended for the judgement of groups and not for individuals (see section 1.2.3). Besides inaccuracies, which may have prevented the demonstration of stronger relationships, such a relationship should not be linear by definition (Kerr et al., 1982). The main conclusion to be drawn from our data is that the occurrence of substandard biochemical values at the end

of pregnancy is about the same for women with an estimated dietary intake above recommended allowances as for women with intakes below these recommendations.

Changes in vitamin metabolism were not assessed directly, but it was speculated that such changes are directed by changes in the hormonal balance. Therefore, hormonal variables were included as independent variables in the multiple regression analysis. In this analysis an association of hormonal factors with the fall in vitamin blood or serum level was found for serum folacin, retinol and vitamin B12. However, the increase in R^2, the coefficient of variation explained, was rather small after including these variables. An hormonal effect was most evident in the case of serum folacin where R^2 increased from 43% up to 62% after including serum cortisol, progesterone and prolactin in the analysis. For serum retinol and vitamin B12, R^2 increased with about only 8%.

Although these data are in favor of the idea that hormonal changes are involved with the fall in vitamin blood levels during pregnancy, they do not allow interpretation in terms of biochemical mechanism. However, our prime interest in the regression model was in the variables selected. The regression coefficients should not be taken as absolute, because colinearity and correlation among independent variables cannot be excluded. That not all the independent variables are really independent may be illustrated by the lower percentage of explained variance (R^2) obtained when all available variables were included, especially for serum folacin and vitamin B12. Another point is that serum hormone levels reflect the balance between production and excretion or metabolism of hormones, but are not necessarily related with the metabolic effects. Besides, the free, and not the total hormone level, measured in this study is thought to be physiologically active, while metabolic effects of hormones on vitamin metabolism may be modulated by synergism or antagonism among hormones.

The evidence of direct hormonal effects on vitamin metabolism are scarce and less well documented than hormone

induced effects on protein or energy metabolism. The older studies reported by Beher and Gaebler (1951) indicate that anabolic steroids lower vitamin excretion as well as lower the serum levels of retinol in normal rats. Evidence has also accumulated that corticosteroids and thyroid hormones have an effect on vitamin A metabolism (Morley et al., 1978). Corticosteroids induce a decrease in both serum and liver retinol content of rats (Clark and Coburn, 1955; Atukorala et al., 1981). A causative relationship between the shift in hormonal balance and the fall in vitamin blood or serum level during pregnancy is, therefore, not unlikely and deserves further study. As already mentioned before, the well documented effects of hormonal contraceptives on vitamin metabolism suggest such a causative relationship (see also section 1.3). Bamji et al. (1979) reported an increased half-life of the stable folacin pool in the liver, while that of the more labile pool was decreased after treating rats with oral contraceptive steroids.

Other factors possibly associated with the fall in vitamin blood or serum level, like an increased renal excretion or fetal accumulation, were not measured in this study. However, according to the literature reported, a significant increase in urinary excretion during pregnancy has been demonstrated, only in the case of folacin (Fleming, 1972). For the other vitamins, excretion of the intact vitamin, or related metabolites, is generally lowered during pregnancy (see section 1.3). So, with the possible exception of folacin, urinary excretion seems unlikely to be a causative factor of the "hypovitaminemia of pregnancy". The fact that the fall in vitamin levels occurs already in early pregnancy is also not in favor of fetal sequestration as an explaining variable, although the higher vitamin levels in cord blood (see section 6.2) illustrate the existence of active placental transport mechanisms.

Calculation of the total amounts of vitamins accumulating in the fetal compartment indicate that, at least for retinol, folacin and vitamin B12 these fetal stores are very small compared with maternal stores (assuming normal, adequate body stores), i.e. less than 2% (using data on fetal body composition

reported by Vaz-Pinto et al., 1975; Loria et al., 1977; Montreewasuat and Olson, 1979). However, the placenta itself may have a relatively high retentive capacity for vitamins as demonstrated for folacin (Landon and Hytten, 1975) and vitamin B6 (Klieger et al., 1969; Dempsey, 1978).

The observation that the fall in vitamin serum level between the 16th and 34th week of pregnancy was negatively correlated with the 16th week value for that vitamin is very interesting. This 16th week value gave by far the best explanation for the variance observed in the stepwise multiple regression and subset selection analysis. We speculate that this negative relationship observed for all the parameters involved in the analysis, should be interpreted as a resetting of the vitamin blood level. Such a resetting of maternal vitamin serum levels, already in early pregnancy, may serve a common purpose. Teleologically, these lower levels may protect maternal stores from excessive losses via the urine due to the increased glomerular filtration rate and excessive accumulation in the placenta or fetal compartment.

The most important "motive" for such a mechanism may, however, be to provide an extra vitamin store for the last months of pregnancy to assure an optimal fetal and postnatal development. A resetting of vitamin serum levels at a lower level can be brought about by an increased renal excretion or catabolism or by an increased tissue retention. As discussed above, an increase in renal excretion is not substantiated by reported experimental data, with the possible exception of folacin. An increased tissue retention seems a more likely mechanism. It would explain the observed, different time trends in plasma and red cell or indirect vitamin status parameters, like plasma PLP content and the EGOT stimulation ratio. We can only speculate about the biochemical basis of such a mechanism. An increased synthesis of tissue binding proteins, induction of coenzyme-dependent, apoenzymes, or a longer half-life of these proteins may all be involved. The selection of hormonal variables in the regression analysis may be supportive evidence for a hormone-mediated effect.

1. Total circulating amounts of serum retinol and vitamin B12 remained on a constant level during pregnancy, total blood riboflavin increased slightly, while total folacin and plasma PLP decreased. In the postpartum period, there was a significant increase in total plasma PLP, but not for total serum folacin.
2. Changes in some biochemical parameters of the vitamin status during pregnancy are not likely to be related with dietary vitamin intake. The sensitivity of dietary history data for the identification of abnormal biochemical values was higher for the data obtained at the 34th week of pregnancy than for the 16th week data. The higher sensitivity is accompanied by a lower specificity.
3. Multiple regression analysis of the difference between the 16th and 34th week value, using some of the vitamin serum or plasma levels as the dependent variable and dietary, hormonal, anthropometric and blood volume related variables as the independent variables, indicated that the initial 16th week value for the respective vitamin level was by far the main determinant of the fall in vitamin serum level, considering the other set of variables in the subset analysis. The explained variance amounted to between 40 to 70%.

   A small, but significant, increase in the explained variation (R^2), was obtained after including plasma volume related parameters in the regression model, in the case of plasma PLP.

   Hormonal variables were found to be associated in the case of serum retinol, folacin and vitamin B12. Such an hormonal effect was most evident for serum folacin, where R^2 increased from 43 up to 62% after including the serum cortisol, progesterone and prolactin levels in the analysis. It was postulated that the changes in serum or plasma vitamin levels, during normal pregnancy, represent a resetting of the vitamin homeostasis in blood.

6.7. Changes in some vitamin status parameters in the postpartum period for women of the S-Reference group: the vitamin cost of pregnancy.

If the changes in some of the vitamin status parameters, like the fall in blood or serum vitamin levels, during normal pregnancy should be considered as physiological adjustments and fetal and maternal vitamin requirements are adequatly covered by the habitual diet, a spontaneous reversal of these pregnancy induced changes in the postpartum period might be expected.

Longitudinal study of the changes in some vitamin status parameters, including the postpartum period, might provide a better insight in the "net vitamin cost" of pregnancy. Remarkably, this aspect has gained little attention to our knowledge.

Data about the vitamin status for our S-Reference group in the first 6 months postpartum, were presented in section 6.1 (Tables 6.1.1 until 6.1.25 and Figures 6.1.1 until 6.1.13). Most of the parameters showed a spontaneous, significant "recovery" towards the non-pregnant reference range.

At 6 weeks postpartum mean serum retinol, vitamin B12 and plasma PLP levels were again significantly higher compared with the mean values obtained in the 16th week of pregnancy. Between the 6th week and 6th month postpartum mean serum 25-OHD, plasma PLP, as well as the erythrocyte enzyme activities for ETK, EGR and EGOT, showed a further significant increase above the 6th week postpartum, and 16th week of pregnancy mean value. However, serum folacin levels remained low in the postpartum period. At 6 months postpartum the mean serum and red cell folacin level were still below the mean values measured at the 16th week of pregnancy. Comparison of the 6 months postpartum value with the reference ranges and cut-off points established for non-pregnant adults (Table 2.6.IV) indicated a relatively high occurrence of abnormal serum folacin values: 45% of the women demonstrated levels below the lower cut-off point (Table 6.1.28). For serum retinol and plasma PLP these percentages were 40 and 25, respectively. However, using the internationally accepted ICNND

criteria for interpretation of serum retinol levels, the percentage of abnormal values at 6 months postpartum fell below 10%, a percentage comparable with the other parameters measured.

Before discussing these findings, the effects of some "interacting" factors should be considered. Breast-feeding exerts an extra stress on maternal nutrient stores (WHO, 1965; 1974), so lactation may affect the changes in some vitamin status parameters in the first months postpartum.

Hormonal contraception may also affect vitamin metabolism, as has been demonstrated for retinol, folacin and vitamin B6 (see section 1.3).

The effect of both factors, as well as that of dietary intake, on the postpartum values of some vitamin status parameters measured for our S-Reference group is discussed below.

-Effect of lactation on the maternal vitamin status in the postpartum period

Only 12 women from the S-Reference group practised breast-feeding for more than 6 weeks; 16 women breast-fed between 2 and 6 weeks and 37 women did not breast-feed their baby or did so for less than 5 days.

The mean values and standard deviations for the maternal vitamin status parameters studied at 6 weeks and 6 months postpartum, according to the length of the lactation period, are presented in Table 6.7.1.

At 6 weeks postpartum, serum retinol levels were about the same for all three groups. At 6 months postpartum, however, women breast-feeding their children more than 6 weeks demonstrated lower values compared with the other groups where serum retinol levels had increased to values of around 1.35 µmol/l.

Mean serum folacin content was lowest for the group of women breast-feeding for more than 6 weeks, both at 6 weeks and 6 months postpartum. For the other parameters, no consistent

differences in time and between groups were apparent, although mean serum vitamin B12 levels were highest for women breast-feeding their babies for at least 2 weeks. The lower mean serum 25-OHD level at the 6th week postpartum obtained for the lactating group (> 6 weeks) could be accounted for by seasonal effects. By chance, nearly all 6th week postpartum samples for the lactating group were found to be collected between November and May. At 6 months postpartum no consistent differences were found between both groups.

-Effect of hormonal contraceptives on the maternal vitamin status in the postpartum period

After pregnancy most of the women in our S-Reference group started using oral contraceptives within the first month postpartum.

At 6 months postpartum, 53 women from the S-Reference group used oral contraceptives (OC), 47 used a combination pill, i.e. containing both estrogens and progestagens, 6 used progestagens only, 2 used an intrauterine device (IUD) while 15 women used other or no contraceptives.

In Figure 6.7.1, mean values and standard deviations are presented for the parameters of the vitamin status measured at 6 months postpartum for the group using the combined OC-type as well for the non OC-users. Differences were only statistically significant for serum retinol, folacin and vitamin B12 ($p < 0.05$).

-Interaction between the lactational and hormonal contraceptive effect on maternal vitamin status in the postpartum period

Some of the vitamin status parameters, like serum retinol, folacin and vitamin B12 levels were affected both by lactation and the use of hormonal contraceptives. The higher serum folacin levels found in the group of OC-users when compared with the group of non-users is rather unexpected and seems contradictory with other reported studies (see section 1.3.8). This apparent contradiction can be accounted for by the fact that women who used no oral contraceptives nearly all breast-fed their babies

for at least 6 weeks, while women who bottle-fed their baby started using the pill from the second week after delivery.

A further breakdown of the postpartum serum retinol, folacin and vitamin B12 values, according to lactation and oral contraceptive use, is presented in Table 6.7.I. The differences observed were tested by inorthogonal analysis of variance. The results with these analyses indicated that serum retinol levels in the postpartum period were primarily affected by oral contraceptive use, while serum folacin levels were primarily affected by lactation. The number of women involved in the analysis is small and results should be interpreted carefully.

TABLE 6.7.I The effect of lactation and oral contraception use on the serum retinol, folacin and vitamin B_{12} level at 6 weeks and 6 months postpartum (S-Reference group).

Parameter	Time	Length of lactational period	Postpartum + (N)	OC-use - (N)
Serum retinol (µmol/l)	6wpp	< 5 d	1.22 (26)	0.80 (4)
		> 6 w	1.15 (2)	0.89 (7)
	6mpp	< 5 d	1.40 (27)	0.85 (2)
		> 6 w	1.40 (2)	0.92 (6)
Serum folacin (nmol/l)	6wpp	< 5 d	4.2 (26)	4.1 (4)
		> 6 w	3.6 (2)	3.7 (7)
	6mpp	< 5 d	4.6 (28)	4.3 (3)
		> 6 w	3.1 (2)	3.2 (6)
Serum Vit. B_{12} (pmol/l)	6wpp	< 5 d	403 (26)	437 (4)
		> 6 w	408 (2)	444 (7)
	6mpp	< 5 d	401 (28)	392 (2)
		> 6 w	404 (1)	517 (5)

-Dietary vitamin intake and maternal vitamin status in the postpartum period

The relationship between the estimated dietary vitamin intake at 6 months postpartum and some corresponding biochemical indices of the maternal vitamin status was assessed in a similar way as was performed with the nutritional data obtained during pregnancy (section 6.6). Pearson correlation coefficients and data on specificity and sensitivity of the dietary history data are presented in Table 6.7.2. Low, but significant, correlations were found between dietary vitamin B6 intake and αEGOT values

and between retinol intake and serum retinol levels. However, the latter correlation coefficient was negative. Such a negative relationship seems very unlikely, and may result from the interaction with lactational and oral contraceptive effects. It illustrates also the pitfalls in the interpretation of simple linear product-moment correlations in relatively small populations. The sensitivity of dietary history data for the identification of abnormal biochemical values is low, while the specificity is high. Compared with the data presented in Table 6.6.4, the sensitivity is higher using the data obtained during pregnancy than with the postpartum data. However, the specificity is lower using the pregnancy data. As discussed in section 6.6, the percentages calculated are strongly dependent on the criteria used for classification of the data.

From tha data presented here, no strong relationship between dietary vitamin intake data and biochemical vitamin status parameters in the postpartum period can be concluded.

Discussion 6.7.

A number of studies have been published on maternal vitamin status during lactation. More recently, Thomas et al. (1980) reported evidence for an adequate nutritional status, measured at 6 months postpartum, for a small group of lactating women. Vitamin supplementation had no significant effect on the vitamin levels in breastmilk in their study with generally well-nourished women. In another study on lactating women of low socio-economic status, vitamin supplementation was necessary to maintain acceptable vitamin concentrations in milk, especially for vitamin B6 and folacin (Sneed et al., 1981).

In our study, the most significant effect of lactation on maternal vitamin status parameters was found for serum folacin levels, which showed a further decrease between the 6th week and 6th month postpartum, for women breast-feeding their babies for more than 6 weeks (Table 6.7.I). Although our S-Reference group, like the group studied by Thomas et al., may be considered well-nourished (Chapter 5), the differences between the studies

for some of the biochemical indices of the vitamin status (i.e. serum folacin) may result from the higher incidence of vitamin supplementation, including folacin, during pregnancy in some countries, such as the U.S., compared with the situation in the Netherlands (in our group, no vitamin supplements were taken during pregnancy).

The most pronounced effect of oral contraceptives on the maternal vitamin status in the postpartum period was observed for serum retinol (Figure 6.7.1).

The higher serum retinol levels found for OC-users, seems best explained by an estrogen-induced increase in the Retinol Binding Protein (RBP) concentration (see section 1.3.1). It is remarkable that the increase in serum retinol level between the 6th week and 6th month postpartum occurred only to OC-users, but not to non-users, irrespective of the length of the lactational period.

The lack of an effect of OC use on parameters of the vitamin B6 status may be a little surprising, because hormonal effects on vitamin B6 metabolism are well documented (see section 1.3.6). This effect is generally manifested by lower plasma PLP levels, whereas results with the EGOT or EGPT stimulation test are generally unaffected.

As has been noted before, plasma PLP levels are already marginal in the postpartum period (section 6.1), irrespective of contraceptive use for a considerable number of the women in the S-Reference group, suggesting a depletion of maternal vitamin B6 stores during pregnancy.

It should be noted that women started using oral contraceptives between the 2nd and 6th week postpartum, so most of them had used these pills no longer than 4-5 months before the blood sample was taken, which may be a relatively too short period to affect the vitamin B6 status.

Another aspect of the effects of oral contraceptives in relation to nutritional status during pregnancy was noted by Roepke and Kirksey (1979) and Martinez and Roe (1977), who studied the effect of long term OC use before conception on vitamin status parameters in the course of pregnancy. Both for

vitamin B6 (Roepke and Kirksey) and folacin (Martinez and Roe), a negative effect of long term OC use on preconceptional vitamin stores was indicated, resulting in lower blood levels during pregnancy. We had no data on the length of preconceptional OC use, so this aspect was not included in our study. All women involved in our study had stopped taking the pill at least 3 months before conception.

The capacity to "recover" spontaneously in the postpartum period was one of the criteria used in this study to evaluate the "vitamin cost of pregnancy". The only vitamin which did not fulfill this criterium was folacin: at 6 months postpartum mean serum and red cell levels were still below the mean value measured at the first time the women entered the study, i.e. at the 16th week of their pregnancy, while at this moment the mean value was already below that obtained with a non-pregnant female reference group. Compared with reference mean values also the mean serum retinol and plasma PLP level at 6 months postpartum were lower. So, at least for folacin it can be concluded from our data that depletion of maternal stores had occurred during pregnancy. In case of vitamin B6 such a conclusion seems not warrented from our data, although the relatively high occurrence of plasma PLP levels in the marginal range are very suggestive for maternal vitamin B6 depletion. The presence of low preconceptional stores, e.g. as a consequence of long term OC-use, cannot be excluded, however.

The lower mean serum retinol level at 6 months postpartum may result from the relatively high incidence of women not using oral contraceptives at that time. OC-use has an increasing effect on serum retinol levels (Figure 6.7.1, Table 6.7.I). A different percentage of OC-use between groups may, therefore, result in a different mean serum level. Unfortunately, we did not know the incidence of OC-use in our non-pregnant reference group, but it might explain the difference observed. Interpretation of serum retinol levels is complicated by the fact that, under normal conditions there is no direct relationship between serum levels and body (liver) stores (see sections 1.3.1 and 6.1). However, at all times postpartum, serum

retinol levels fell within the accepted normal range.

The slight, but significant, increase in mean EGOT activity, concomittant with a significant decrease in EGOT stimulation ratio, as well as the increase in mean red cell folacin level in the postpartum period, are indicative for a (preferential) replenishment of maternal tissue stores in the first months after delivery. The lower serum levels at 6 months postpartum may indicate that, in the case of folacin and vitamin B6, such replenishment takes at least 6 months. Such a situation of low serum or plasma levels, together with normal values for parameters of the same vitamin, assumed to reflect tissue stores, is representative for a so-called "biochemical deficiency", an early stage of vitamin depletion. This stage is not accompanied by clinical symptoms, but may represent an increased risk to develop complications associated with a marginal or deficient vitamin status, especially during situations of increased vitamin (nutrient) requirement. For the other parameters showing a spontaneous postpartum recovery to values within the non-pregnant reference range, it can be concluded that maternal stores, and dietary vitamin intake during and after pregnancy, were apparently adequate.

Conclusions 6.7.

1. The length of the lactational period was related to a further fall in serum folacin levels in the postpartum period. Both at 6 weeks and 6 months postpartum, mean serum folacin content was lowest in the group of lactating women (> 6 weeks) compared with mothers who breast-fed their babies for less than 5 days. Differences between both groups were also significant after correction for other intervening variables like oral contraceptive use. For the other parameters of the vitamin status, no significant effects of lactation could be demonstrated.
2. The use of hormonal contraceptives in the postpartum period resulted in significantly higher serum retinol levels compared with those of non-users. Minor effects were noted

for αETK, 25-OHD and vitamin B12 serum levels. These effects were not significant, however.

3. The changes in some of the vitamin status parameters in the postpartum period were probably not associated with the dietary vitamin intake in that period. Only between the dietary vitamin B6 intake and the EGOT ratios was a significant relationship obtained. However, the correlation coefficient was low.
4. From the significantly lower mean serum folacin level at 6 months postpartum compared with the mean level at the 16th week of pregnancy, a depletion of maternal folacin stores during pregnancy was concluded.
   The relatively high occurrence of plasma PLP levels in the marginal range is suggestive that also maternal vitamin B6 stores become depleted during pregnancy, although marginal preconceptional stores cannot be excluded.

CHAPTER 7.

Maternal and fetal (cord blood) iron status.

Introduction

In this chapter the changes in maternal iron status parameters during and after pregnancy are described and discussed with the emphasis on serum ferritin as the parameter representing the iron store (section 7.1). The remark made in the introduction of Chapter 4 can be applied to the figures of this chapter: namely that connecting the centiles at the different moments of measurement may give rise to optical illusions.

The relationship between maternal and cord blood iron status parameters, the influence of iron medication and the interrelationships between the iron status, red cell volume and hemoglobin level will be presented in sections 7.2, 7.3 and 7.4.

7.1. Changes in iron status parameters during and after pregnancy.

Data on serum iron, total iron binding capacity (TIBC), transferrin, percentage saturation and serum ferritin of women from the S-Reference group during and after pregnancy and of cord blood are presented in Tables 7.1.1, 7.1.3, 7.1.5, 7.1.7 and 7.1.9. The numbers, means, S.D.'s and ranges of measurements of these parameters are given.

The changes of these parameters during pregnancy and the cord blood values are illustrated in Figures 7.1.1, 7.1.2, 7.1.3, 7.1.4 and 7.1.5. In these figures, the 10th, 50th and 90th centiles of measurement of women from the S-Reference group and of cord blood are presented, as well as the median of the P10 group.

In Tables 7.1.2, 7.1.4, 7.1.6, 7.1.8 and 7.1.10, data on the significance of the observed differences are given.

More detailed information about the < P10 and > P90 groups can be obtained from Table 7.1.11.

Serum iron (Figure 7.1.1, Tables 7.1.1 and 7.1.2)
As can be expected from the literature (section 1.3.11), the mean serum iron level decreases significantly during pregnancy up to 34 weeks (between 16 and 34 weeks by 38%). No difference is found between 34 weeks and partus value. Six days postpartum, a slight decrease is seen, and thereafter, serum iron levels slowly increase, but values at 6 months postpartum are still significantly (19%) below the values obtained at 16 weeks. As these last values are probably already lower than the prepregnant values (hemodilution?), values obtained 6 months postpartum are definitely significantly below prepregnant values in this population.

Mean serum iron levels in cord blood are significantly higher than mean maternal serum levels at delivery. This indicates an active transport from the maternal to the fetal side.

Maternal values obtained during pregnancy and cord blood values are in accordance with observations in literature (see section 1.3.11).

The median value of the < P10 group are all found to be above the median value of the S-Reference group, probably caused by a lower hemodilution. In section 4.1, it was shown that the increase in plasma volume of women from the < P10 group shows a different pattern.

TIBC and Transferrin (Figures 7.1.2 and 7.1.3, Tables 7.1.3-6) The β-globulin transferrin, on which the iron binding capacity depends, increases considerably (about 50%) during pregnancy, probably under the influence of estrogens. The TIBC increases accordingly (see section 1.3.11).

For transferrin, values obtained 6 months postpartum are slightly, but significantly elevated (9%) as compared to values at 16 weeks of pregnancy. Although these values are in the normal non-pregnant range, a possible explanation might be that a number of women at 6 months postpartum were using oral contraceptives, which induce a slight increase in transferrin values (Jacobi et al., 1969).

TIBC increases during pregnancy with a same percentage as transferrin levels. Postpartum, a rapid decrease is seen, but values at 6 months postpartum are still significantly higher than at 16 weeks.

Transferrin values in cord blood are 50% below maternal values at partum. It is most likely that the fetus produces this protein itself because β-globulins probably do not cross the placenta (see also section 7.2).

The median transferrin value of the < P10 group is always below the median of the S-Reference group; a similar picture is seen with the TIBC. Although the < P10 group is too small for definite conclusions, these results suggest that a small feto-placental unit induces or is accompanied by lower transferrin values than a larger feto-placental unit.

Percentage saturation (Figure 7.1.4, Tables 7.1.7 and 7.1.8) Due to the increases in transferrin and the decrease in serum iron, the percentage saturation decrease during pregnancy.

$$\text{Percentage saturation} = \frac{100 \times \text{serum iron}}{\text{TIBC}}$$

As might have been expected from the previous observations, 6 months postpartum, significantly lower values are found as compared to the 16 weeks point. It is obvious from the description of serum iron and transferrin that the values of the percentage saturation in cord blood are significantly higher

than the maternal values at partum. The median value for the <P10 group is, as expected, to be found above median values of the S-Reference group, but within its range.

Summarizing: this overall picture suggests iron depletion during pregnancy. One might argue that values during pregnancy may be blurred by other changes such as hemodilution and hormonal changes, but if so, one might expect that soon after delivery and definitely 6 months after delivery, values are found in the non-pregnant range. Instead, we find lower serum iron values and percentage saturation, and higher values for TIBC than expected. These parameters must be considered with caution, due to the fact that serum iron fluctuates widely and is affected by recent ingestion of iron, and transferrin may be influenced by factors not related to iron metabolism. These parameters are not a reliable indication of iron stores. Serum ferritin, which is supposed to reflect the iron stores better, might answer this question (see also section 1.3.11).

Serum ferritin (Figure 7.1.5, Tables 7.1.9 and 7.1.10)
Serum ferritin decreases during pregnancy, reaching its lowest values in the third trimester (section 1.3.11). We find a similar pattern. The decrease between the 16th and 34th week of pregnancy is about 80%. At 6 days postpartum, a significant increase of 50% as compared to the partus levels is seen, which is followed by a significant decrease of 20% at the 6 week postpartum point by a further non-significant change up to 6 months postpartum. So, the mean serum ferritin value at 6 months postpartum is still 60% below the mean serum ferritin value obtained at the 16th week of pregnancy.

Our observations are in agreement with those of Van Eyk et al. (1978) and Puolakka et al. (1979), who found that postpartum serum ferritin levels increased, but that levels at 3 and 6 months postpartum, respectively, were below those of the first trimester. These and our findings suggest that pregnancy partly empties maternal iron stores and that refilling of the stores

may take considerable time since at 6 months postpartum, still lower levels are found compared to the first trimester. How long it may take to replenish the stores is unknown and is probably dependent on several factors e.g. dietary intake of iron, menstrual blood loss, choice of contraceptives. In our population with a estimated mean intake of 13 mg of iron per day (section 5.1), more than 6 months after delivery are required to replenish maternal iron stores. If an event requiring extra iron happens during that time, an iron deficiency anemia is likely to develop. We can illustrate this with a woman who, after becoming pregnant five months after the first pregnancy, was followed through her second pregnancy as well. Her data for serum ferritin, serum iron and hemoglobin are presented in Table 7.1.I. She received no iron tablets until the 28th week of her second pregnancy. As can be seen from Table 7.1.I, 6 weeks after her first delivery she had low serum ferritin (10 ng/ml) and serum iron levels (5 µmol/l). In the 16th week of her second pregnancy, almost the same levels were found and at 28 weeks a low Hb (5.2 mmol/l), microcytosis and reduced MCHC were present. Iron medication quickly resolved this iron deficiency anemia.

TABLE 7.1.I. Serum ferritin, serum iron and hemoglobin levels in two successive pregnancies. Iron tablets were prescribed in the 28th week of the second pregnancy.
F.P: First Pregnancy.
S.P: Second Pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Ferritin	F.P.	45	20	11	22	13	10	-	324
	S.P.	11	6	34	25	61	47	49	725
Serum iron	F.P.	21	26	5	4	7	5	-	25
	S.P.	5	4	19	13	7	20	19	37
Hemoglobin	F.P.	6.6	6.3	6.2	6.1	6.1	7.2	-	10.9
	S.P.	6.2	5.2	6.6	8.0	8.7	8.6	8.6	11.3

As to the < P10 group, we find that the median value of serum ferritin of this group is mostly slightly above the median of the S-Reference group (Figure 7.1.5).

Although before the introduction of the serum ferritin assay, no consensus could be found in literature as to whether a pregnancy depleted the maternal iron stores (see section 1.3.11), it appears from our and other studies on serum ferritin that pregnancy - at least partly - depletes the maternal iron stores, and that with a mean daily estimated intake of 13 mg, it takes a long time (more than 6 months) before this loss has been replenished. Undoubtedly the question will rise again if iron suppletion should be advised and when, during pregnancy or postpartum. We will leave this question and discuss this at the end of the chapter.

In Tables 7.1.12 to 7.1.16, the correlation coefficient of the different iron parameters between the moments during and after pregnancy are described.

A general trend is clearly apparent: the correlation between findings at successive points is better than between findings when the time elapses. This can be compared with the correlation coefficients of the vitamin status parameters (see section 6.1). Another observation is that the correlation between the values at 16 weeks and at 6 months postpartum are better than the correlations obtained between various findings at any other moment during pregnancy and the 6 months postpartum point. This is observed for serum iron, percentage saturation and serum ferritin and illustrated in Table 7.1.II.

TABLE 7.1.II. Pearson correlation coefficients between the 6 month postpartum value and values during pregnancy of serum iron, percentage saturation and serum ferritin of women from the S-Reference group. P = partus.

	Serum iron 6mpp	% Saturation 6mpp	Ferritin 6mpp
16 wk	0.31	0.38	0.44
28 wk	0.17	0.29	0.34
34 wk	0.07	0.11	0.18
P	0.08	0.16	0.17

Two explanations for this phenomenon may be possible. First, values measured during pregnancy, especially late in pregnancy,

are influenced by other parameters and may, for instance in serum ferritin, not really represent iron storage at that time. Second, it may be caused by differences in diet, absorption or other factors, i.e. women with higher values at 16 weeks have again higher values at 6 months postpartum, because their diet and or absorption is more adequate or menstrual blood loss is less, etc. In the case of serum ferritin, the second explanation is more attractive as no correlation (r = -0.007) is found between the 16 weeks and 6 weeks postpartum point (Table 7.1.15). For serum iron, the first explanation seems more likely because the correlation between 16 weeks and 6 weeks postpartum (r = 0.26, Table 7.1.12) and 6 months postpartum (r = 0.31) are almost the same.

TABLE 7.1.III Pearson correlation coefficients between blood loss and serum iron, percentage saturation, serum ferritin and red cell volume in the postpartum period of women from the S-Reference group.

	6dpp r	6wpp r	6mpp r
Serum iron	-0.25	-0.24	-0.22
% Saturation	-0.26*	-0.24	0.27*
Ferritin	-0.25	-0.21	-0.13
Red cell volume	-0.14	-0.02	-

* $p < 0.05$

In Table 7.1.III, the question was evaluated as to whether blood loss during delivery was related to the red cell volume as well as to iron parameters. As can be seen from this table, the red cell volume at 6 days and 6 weeks postpartum is not related to the blood loss. As for serum iron, a minimal negative trend is apparent up to 6 months postpartum, although significance is not reached. A similar trend was found for the percentage saturation. As to serum ferritin, this trend is seen only at 6 days postpartum and 6 weeks postpartum, but has disappeared at 6 months postpartum.

If there is a relationship at all, blood loss has only a minimal influence on the values of serum iron, percentage

saturation and ferritin in the postpartum period in a group of women losing less than 1000 ml of blood during delivery.

7.2. The relationship between maternal and fetal iron status.

In Tables 7.1.12 to 7.1.16, the correlation coefficients between maternal iron parameters at different moments during and after pregnancy and mixed cord blood are described. For serum iron and percentage saturation (Table 7.1.12 and 7.1.14), only a weak significant correlation was found between 6 days postpartum values and values in the cord blood (0.24 and 0.23, respectively). At all other points, especially at 34 weeks and at delivery, no correlation was observed.

As to serum ferritin (Table 7.1.15), no significant correlation between maternal and cord blood values could be detected.

Concerning TIBC and transferrin (Table 7.1.13 and 7.1.16), weak, but significant correlations with the cord blood values were found at and around delivery (34 weeks, delivery and 6 days postpartum). This suggests that the same mechanism, probably estrogens which induce the increase of transferrin in the mother, is related to the values of this β-globuline in the fetus as maternal transferrin does not cross the placenta. Summarizing, no relationships between maternal and fetal cord serum iron, percentage saturation and serum ferritin values were observed.

This confirms the observations of most authors (section 1.3.11: fetal-maternal relationship). Only Kaneshige (1980) found a correlation between maternal and cord ferritin levels during parturition. Fenton (1977) observed significant lower cord ferritin values when maternal values at parturition were below 12 ng/ml. We tested whether we could confirm this observation. In our S-Reference group, 12 women had serum ferritin values below 12 ng/ml. The mean and S.D. were 9.5 $\pm$ 1.7 ng/ml in this group compared to 21 $\pm$ 13 ng/ml for the

whole S-Reference group. Cord blood values in the first group were 305 ± 210 ng/ml compared to 342 ± 210 ng/ml for the S-Reference group, so for maternal values below 12 ng/ml, we could not find significantly lower cord values.

Nine babies had cord blood values below 150 ng/ml (mean 89 ± 48 ng/ml) and the corresponding maternal values were 18.6 ± 10.3 ng/ml; values not significantly different from the total group. Again this confirms the observation that no relationship is apparent between maternal and cord serum ferritin levels.

However, it should be kept in mind that it is questionable whether serum ferritin cord levels represent fetal iron stores. First, because the baby's serum ferritin levels increase during the first 24 hours after birth, remain constant for a week, followed by a slow decrease (Rios et al., 1975). So, values obtained 24 hours after delivery are probably more representative. Second, because it may be doubted whether extrapolation of the observation in adults that 1 ng/ml ferritin corresponds with 8 mg depot iron (Walters et al., 1973) is allowed for estimating the fetal reserve. The term fetus contains about 280 mg of iron (Table 1.4.II), and this corresponds in no way to the amount of fetal iron storage when adult data are used. So, we conclude that maternal serum ferritin levels are not related to cord serum levels, but it remains unclear whether this implies that maternal iron stores are not related to fetal iron stores.

7.3. Iron medication and iron status.

Iron tablets were not routinely prescribed, but were only advised when the hemoglobin level (Hb) was found to be below 6.8 mmol/l during pregnancy or below 7.0 mmol/l in the puerperium or when "clinical signs of anemia" were present. However, sometimes for unknown reasons, iron was not prescribed when the Hb was below the aforementioned levels. In the S-Reference group, 50 women received no iron at all to our knowledge (group 0). Seven received iron tablets during pregnancy, always in the second

half (group 1) and 10 received iron tablets after pregnancy (group 2). Three women used iron tablets both during and after pregnancy. In most cases, 200 mg ferrofumarate was given 3 times daily. In Table 7.3.1, the results obtained in the three groups are described for red cell volume, hemoglobin, serum iron, percentage saturation, serum ferritin and transferrin; the three persons who used iron both during and after pregnancy have been left out because of the small number. Although numbers in group 1 and 2 are also rather small, some differences are apparent.

Differences between group 0 and group 1: At 16 weeks we find a significantly lower Hb, serum iron and percentage saturation in group 1 than in group 0, whereas red cell volume, serum ferritin and transferrin are not different. During the second half of pregnancy, Hb remains significantly lower in contrast to serum iron levels and percentage saturation for which a difference can no longer be found. The decrease in these last two parameters seen in group 0 during pregnancy is almost absent in group 1, probably because of the iron medication. However, serum ferritin shows the same decrease in both groups during pregnancy. Postpartum, no differences between group 0 and 1 can be observed.

Differences between group 0 and group 2: At 16 and 28 weeks, no differences are found between the two groups. At the 34th week, differences appear. Red cell volume, serum iron and percentage saturation are all significantly lower in group 2, and these parameters remain lower up to the 6th day postpartum. Serum ferritin and transferrin are not different. The Hb becomes significantly different only at the 6th day postpartum.

At 6 weeks postpartum, only the red cell volume is still significantly lower in group 2. All differences have disappeared at 6 months postpartum.

Interpretation of these data is rather difficult, especially because the groups which received iron are rather small and the intake of iron tablets was not controlled. However, some trends can be observed. If serum ferritin levels during pregnancy reflect the maternal iron stores, the iron reserve at 16 weeks

was in all groups more or less the same. Group 1, however, had a lower serum iron, lower percentage saturation and lower Hb and for the latter reason received iron tablets. A possible explanation might be that the increase in plasma volume in this group was larger than in group 0. This was tested, and indeed, plasma volume in group 1 was found to be significantly higher as compared to group 0 (3.345 $\pm$ 0.354 for group I, 2.894 $\pm$ 0.329 for group 0). So, the main reason group 1 received iron medication during pregnancy was because a larger hemodilution occurred.

In group 2, iron tablets were not prescribed during pregnancy because Hb apparently remained above the limit of 6.8 mmol/l. In the last part of pregnancy, there are possibly signs that iron stores are more depleted than in group 0; serum iron and percentage saturation are significantly lower at 34 weeks and at partum. Serum ferritin levels are also lower, but the difference is not significant. Postpartum, the same situation exists, but also the hemoglobin level has decreased and iron is prescribed. The difference between group 2 and group 0 might be that women of group 2 started pregnancy with lower - although no significance could be proved - iron stores than group 0. Another reason for the lower Hb in the early puerperium in group 2 might be that blood loss during delivery was larger than in group 0. This was tested and it was slightly, but not significantly higher (448 $\pm$ 216 ml in group 2 compared to 365 $\pm$ 210 ml in group 0). So blood loss is probably not the main factor to explain the differences in the puerperium.

The fact that at 6 months postpartum significant differences between the three groups are no longer found remains remarkable. However, all groups show serum iron, percentage saturation and particularly serum ferritin levels below the values obtained at the 16th week of pregnancy. As iron was not supplemented routinely in this study, we are not able to say whether iron supplementation during pregnancy will prevent the depletion of iron stores. As stated in section 1.3.11, there is no consensus about this subject since Van Eyk et al. (1978) found no difference in serum ferritin levels 3 months postpartum between

supplemented and in unsupplemented pregnant women in contrast to Puolakka (1979), who found significantly higher levels in supplemented women at 6 months postpartum.

7.4. Relationship between iron status, red cell volume and hemoglobin content.

In section 1.3.11, we have described that in epidemiological studies, the incidence of iron deficiency (anemia) among groups of a population reported in literature largely depends on the parameter studied and the cut-off points used. During pregnancy, this situation becomes even more complicated due to the physiological changes: hemodilution and increase of carrier proteins.

In Table 7.4.I, we calculated the correlation coefficients between the red cell volume, Hb, serum iron, serum ferritin and transferrin.

The correlation coefficients between red cell volume and hemoglobin level decreases during pregnancy because of the larger increase of plasma volume. No correlation between red cell volume, serum iron and serum ferritin is observed.

The hemoglobin level during pregnancy is not correlated to either serum iron or serum ferritin levels. Only at partum and in the puerperium are weak correlation seen, but they have disappeared at 6 months postpartum.

Serum iron levels during early pregnancy are not correlated to serum ferritin levels, but at 28, 34 weeks, during delivery and in the puerperium, weak, but significant correlations are found. At 6 months postpartum this correlation has disappeared. This probably indicates that when iron stores are well filled, no correlation exists between circulating iron and stored iron. Only when iron stores become depleted a correlation becomes apparent. The moment (6 months postpartum) the stores are - even modestly - replenished, the correlation disappears.

TABLE 7.4.I Linear correlation coefficients between red cell volume, hemoglobin, serum iron, serum ferritin and serum transferrin of women from the S-Reference group at different time points.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
RED CELL VOLUME								
Hemoglobin	0.50***	0.36**	0.24*	-	0.57***	0.36**	0.42***	-
Serum iron	0.08	0.01	-0.11	-	0.19	0.11	0.02	-
Ferritin (Log)	-0.04	-0.11	-0.20	-	-0.15	0.15	0.16	-
Transferrin	0.07	-0.12	0.02	-	-0.13	-0.08	-0.08	-
HEMOGLOBIN								
Serum iron	0.10	0.09	0.12	0.39**	0.39**	0.11	0.22	-0.09
Ferritin (Log)	0.01	-0.19	0.14	0.10	0.24	0.34**	0.0002	0.51***
Transferrin	0.10	-0.03	0.004	-0.003	-0.28*	-0.42***	0.13	0.25*
SERUM IRON								
Ferritin (Log)	0.15	0.39**	0.53***	0.30*	0.32*	0.47***	0.16	0.51***
Transferrin	0.10	-0.39**	-0.21	-0.12	-0.01	-0.15	-0.02	-0.11
FERRITIN (LOG)								
Transferrin	-0.29*	-0.42***	-0.15	-0.21	-0.17	-0.64***	-0.20	-0.56***

* $0.01 < p \leq 0.05$

** $0.001 < p \leq 0.01$

*** $p \leq 0.001$

If we assume that serum ferritin is a reliable indicator of maternal iron stores during pregnancy, we find that the hemoglobin level is no reflection at all of the iron stores, and in the second and third trimester, serum iron only weakly reflects iron stores. However, it may be doubted whether serum ferritin during pregnancy reflects iron stores as reliably as in the non-pregnant situation. It may be influenced by other changes during pregnancy, for instance, hemodilution. In the non-pregnant situation 6 months postpartum, both hemoglobin and serum iron have no correlation with the iron store.

To go any further into this matter is beyond the scope of this study, and the data presented in Table 7.4.I are of interest as an illustration that establishing iron deficiency (anemia) by means of hemoglobin, serum iron, percentage saturation and/or TIBC is doubtful, particularly during pregnancy.

Conclusions

The course of the iron status parameters among not routinely iron supplemented gravidae in this study is in agreement with observations described in literature. The maternal iron stores, as reflected by the serum ferritin level, are rather depleted 6 weeks postpartum or at least the stores are lower than in the 16th week of pregnancy among women who had a mean dietary iron intake of 13 mg iron per day during pregnancy. Even more remarkable is the fact that 6 months postpartum serum ferritin level had hardly increased and so the iron stores seem to have not yet been refilled. At that time it is apparent from our observations and other studies on serum ferritin during and after pregnancy, that a pregnancy costs the mother some iron from her iron store and that it takes quite a long time, at least more than 6 months, before the iron stores are refilled (section 7.1).

As to the relationship between maternal and fetal iron status, as measured in mixed cord blood, we did not observe any consistent relationship. Especially, maternal serum ferritin levels showed no relation with cord blood levels. It can be argued, however, whether ferritin cord blood levels are a reflection of the fetal iron reserve as serum ferritin levels increase during the first 24 hours after birth, and extrapolation of adult data would suggest an enormous fetal iron store which is not in agreement with data available from carcass analyses (section 7.2).

Concerning iron medication given, no definite conclusions about the different groups can be drawn. It seems that women who received iron tablets during pregnancy had a large increase in plasma volume, causing the lower hemoglobin level. Women who

were given iron medication postpartum were possibly the ones that started pregnancy with a lower iron store (section 7.3).

Regarding the interrelations of different parameters, we find that the hemoglobin level is not related to maternal iron stores (serum ferritin). Serum iron appears to be weakly related to iron stores, but only when stores are low (section 7.4).

This leaves the question of whether iron supplementation should be advised and when. The pros and cons of routine supplementation have been summarized in section 1.3.11, and there appears to be absolutely no consensus on this point in literature. As long as no severe iron deficiency anemia develops, no surplus of pathological conditions is observed during pregnancy. The maternal iron stores does not seem to be related to the fetal iron stores, but in our opinion, more data about fetal serum ferritin over a larger period should be available before a definite answer to this question can be given. It is, however, obvious that a pregnancy depletes the iron stores, which will take considerable time to refill. Whether iron supplementation during pregnancy guarantees a higher iron store postpartum remains controversial (section 7.3). To our knowledge, no study has been performed on routine iron supplementation postpartum and the effect on serum ferritin levels.

The only advice which, in our opinion, seems reasonable is that, if a woman becomes pregnant again within (half) a year, iron supplementation should be given from the 20th week of pregnancy onwards or, if a woman plans to become pregnant again within a year, iron tablets should be given postpartum after the first delivery to replenish iron stores.

CHAPTER 8.

The relationships between birth centile and the bodily, nutritional and biochemical parameters assessed and the percentage of variance in birth centile and in Ponderal-index which can be accounted for.

8.1. Introduction.

In this chapter we will describe which correlations have been found between the birth centile of the newborn and the bodily, nutritional and biochemical parameters assessed in this study. Linear regressions of the birth centile on these parameters were calculated at all moments during and after pregnancy. This was not done for all parameters mentioned in Chapter 2 since parameters that were merely used as a check on normal organ functions (i.e. creatinine, urea, uric acid, SGPT and γGT) were left out. Hormonal parameters except for human placental lactogen (HPL) were left out as well because no evidence was found indicating any differences between the mean values of the S-Reference group and the < P10 group (see section 4.4). The correlations are calculated for the total group, i.e. the S-Reference group (n = 70), the < P10 group (n = 10) and the > P90 group (n = 5). The linear regressions have only been calculated for the birth centile, not for the Ponderal-index.

After the individual linear regressions were calculated, a stepwise forward multiple regression analysis has been carried out on the parameters, for which a correlation with the birth centile was found, to explore which percentage of the variance in birth centile and in Ponderal-index can be explained (Table 8.9.1).

This stepwise forward multiple regression analysis was carried out merely to obtain a first idea about the importance of the variables and so to be able to reduce the number of variables. A subset selection procedure was performed with this reduced number of variables. This subset selection procedure has the advantage that the explained variance in birth centile and in Ponderal-index is calculated for all possible combinations. A more detailed description of the statistical models used is given in section 2.7.

8.2. Linear regressions of birth centile on general characteristics of mother, father and newborn.

The findings of the linear regressions of birth centile on the general characteristics of mother, father and newborn are presented in Table 8.2.I.

TABLE 8.2.I Linear regressions of birth centile on general characteristics of mother, partner, newborn and placenta.

		Number	Correlation coefficient	Significance
Mother	Age	85	0.19	$p < 0.05$
	Height	85	0.23	$p < 0.05$
	Prepregnant weight	85	0.28	$p < 0.01$
	Max. weight gain	85	0.29	$p < 0.01$
	Weight gain 34 w-P	83	0.19	$p < 0.05$
	Smoking	85	-0.38	$p < 0.01$
Partner	Height	84	0.05	-
Newborn	Weight	85	0.91	$p < 0.0001$
	Length	85	0.65	$p < 0.0001$
	Ponderal-index	85	0.65	$p < 0.0001$
Placenta	Weight	85	0.62	$p < 0.0001$
	Index	85	-0.002	-

As could be expected (section 1.5), correlations were found between birth centile and height and weight of the mother, maximal weight gain (even the weight gain in the last 6 weeks)

during pregnancy and smoking. The correlation between birth centile and age cannot easily be explained. Since we used birth centiles, there has already been a correction for parity. A possible explanation may be that smoking habits are different in the younger age group. This will be discussed in sections 8.3 and 8.9. No correlation could be demonstrated between birth centile and the height of the father.

A high correlation ($r = 0.91$) is found between birth centile and birthweight, indicative of the normality of the population studied. A lower correlation is seen between birth centile and length of the baby ($r = 0.65$), paralleled by a similar correlation between Ponderal-index and birth centile. The Ponderal-index ($100 \times \text{Weight}/\text{Length}^3$) is used as a parameter which gives an impression of the intra-uterine nutritional status. Intrauterine malnutrition, especially in the last part of pregnancy, is characterized by a dissociation between weight and length. The Ponderal-index can at the same time be regarded as an indirect measure of the amount of soft tissue mass, including fat stores. The Ponderal-index correlates well with the fat-fold thickness at birth (Roord and Raemaekers, 1978). A disadvantage of the Ponderal-index is that length^3 is used. Exact crown-heel measurement of a newborn baby is not easy. We found inter-observer variations in crown-heel measurement to be less than 4%. Measurements of a child with a "mean" length of 50 cm were between 49 and 51 cm. If we assume a birthweight of 3500 grams, the variance in Ponderal-index may be about 12%; inaccuracy for weighing has not been accounted for. It will probably be very difficult to obtain better results in a normal clinical setting. Results should be judged against this background.

The birth centile is positively related to placental weight ($r = 0.62$). No, not even an inverse, relationship between birth centile and placenta index is observed, and this suggests a rather constant relationship between birth centile and placental weight (section 1.4). Considering maternal parameters, smoking is the parameter which is found to be highest correlated ($r = -0.38$) with birth centile. This is illustrated in Figure 8.2.I

where the grey clouds of smoke are found mostly over the lower centile groups.

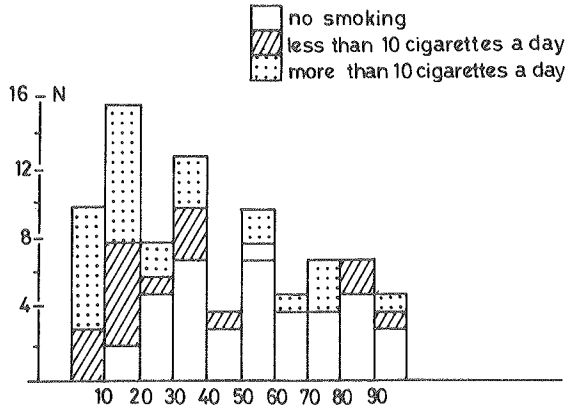


FIGURE 8.2.I Birth centiles and smoking in the population studied (n=85).

Since smoking seemed rather important, we decided to investigate whether smoking influenced other parameters as well, and the results will be described in the next section.

8.3. Smoking.

The S-Reference group (n = 70) was divided into three groups according to their smoking habits. Group I (n = 37) did not smoke, group II (n = 15) smoked less than 10 cigarettes a day and group III (n = 18) smoked more than 10 cigarettes a day during pregnancy. A one-way analysis of variance (section 2.7) was done on all parameters except kidney and liver function parameters. The results will not be described in detail, and only significant ($p < 0.05$) findings will be discussed.

Group I has been compared with group II and III. Differences between group II and III have not been tested because our main interest was to investigate possible differences between non-smokers and smokers, and not between light and heavy smokers.

Significant differences of the general characteristics for non-smokers and smokers are described in Table 8.3.I.

TABLE 8.3.I Differences in general parameters for non-smokers (group I, n=37) light smokers (group II, < 10 cigarettes/day, n=15) and heavy smokers (group III, > 10 cigarettes/day, n=18).

General Parameters	Group I mean (SD)	Group II mean (SD)	Group III mean (SD)	Significance of difference*
Birthweight	3534 (321)	3288 (374)	3266 (339)	I-II* I-III*
Birth centile	50.4 (22)	34.9 (24.5)	36.3 (24.9)	I-II* I-III*
Ponderal-index	2.9 (0.23)	2.8 (0.23)	2.7 (0.24)	I-II NS I-III*
Placenta-index	0.134 (0.026)	0.130 (0.022)	0.149 (0.015)	I-II NS I-III*

* $p < 0.05$

For all other general parameters (i.e. age, parity, maternal height, prepregnant weight, weight gain during pregnancy, height of the father, duration of pregnancy, length of the baby and placental weight), no significant differences were observed between the non-smoking group and the smokers. We did not find a significantly larger mean placental weight in smokers compared to non-smokers as is sometimes described in literature (Pirani, 1978). However, the mean placental index is significantly higher in group III compared to group I and II, suggesting some placental hypertrophy in group III since the mean birthweight in group II and III hardly differ.

Concerning blood volume and hematological parameters, no significant differences between the non-smokers and smokers could be detected. As an example, plasma volume values are given at 16 and 34 weeks during pregnancy and at 6 weeks postpartum (Table 8.3.II).

Hall et al. (1976) observed a significantly lower mean plasma volume in smokers compared to non-smokers; a difference observed at 38 weeks of pregnancy. As can be seen from our findings, we could not confirm this observation. However, the latest measurement in our study during pregnancy was at 34 weeks. If plasma volume values were lower in smokers at 38

weeks, this would mean a decrease in plasma volume in the smoking group since probably at 34 weeks, the maximal increase in plasma volume has occurred (see section 1.1). If a decrease of plasma volume would occur, this should be accompanied by an increase in hematocrit in the last part of pregnancy. In the above mentioned groups, no significant differences in hematocrit values could be observed. This makes it unlikely that plasma volume decreases significantly in the last 6 weeks of pregnancy among smokers and casts some doubt on Hall's observations.

TABLE 8.3.II Plasma volume during and after pregnancy in non-smokers (group I, n=37), light smokers (group II, < 10 cigarettes/day, n=15), and heavy smokers (group III, > 10 cigarettes/day, n=18).

Plasma vol./l at	Group I mean (SD)	Group II mean (SD)	Group III mean (SD)	Significance of difference
16 weeks	2.818 (0.340)	3.069 (0.464)	2.903 (0.364)	I-II NS I-III
34 weeks	3.530 (0.557)	3.601 (0.507)	3.566 (0.292)	I-II NS I-III
6 weeks pp	2.557 (0.357)	2.701 (0.479)	2.538 (0.384)	I-II NS I-III

Considering the iron status parameters (serum iron, total iron binding capacity, percentage saturation and serum ferritin), total protein, albumin, the hormonal parameters (thyroid function, estradiol 17-β, estriol, prolactin, progesteron, HPL and cortisol), no significant differences could be observed between non-smokers and smokers. The differences in vitamin status parameters between non-smokers and smokers are described and discussed in section 6.3.

Conclusion

When dividing the S-Reference group according to smoking habits (Group I non-smokers, Group II light smokers, Group III heavy smokers), we found as expected a significant lower birthweight and birth centile when comparing non-smokers with smokers. The length of the children in the three groups is not significantly different, although length is slightly lower in the heavy smoking group (group III). The Ponderal-index is only

significantly different when comparing group I and III; no difference is found between group I and II. Since the Ponderal-index correlates with the subcutaneous layer of fat (Roord and Raymaekers, 1978), this indicates a decrease in the subcutaneous layer of fat in children of heavy smokers. This contrasts the findings of d'Souza et al. (1981), who could not find a difference in subcutaneous fat measured by skin fold thickness in children of smokers and non-smokers. The differences we find in Ponderal-index are less pronounced than the differences in birthweight or birth centile and this suggests that "growth retardation" caused by smoking is more a "proportional growth retardation", whereby heavy smoking causes also a "dysproportional" growth retardation (a low Ponderal-index). This makes a nutritional component less likely to be a causative agent as is sometimes suggested (Rush, 1974) (section 5.5 and 8.4).

Placental weights are not different in the three groups and a significant difference is found only when comparing the placental indices of group I and III, suggesting some placental hypertrophy in group III (section 1.4).

The other parameters tested showed no differences. In contrast with the finding of Rush (1974), we found no difference in weight gain during pregnancy between the three groups. This is in agreement with studies published by Meyer (1978) and Hajari et al. (1979).

Lower blood and plasma volumes were not found in the smoking groups when compared to the non-smoking groups. This contrasts with the conclusion of Hall et al. (1976). However, their findings were inconsistent during pregnancy and, in our opinion, are more suggestive than actually proven.

Smoking and nutritional intake has already been mentioned in section 5.2 and will be further discussed in section 8.4.

Differences between non-smokers and smokers concerning their vitamin status are described in section 6.3.

8.4. Linear regressions of birth centile on the intakes of energy and macronutrients.

When investigating the relationship between birth centile (weight) and energy and macronutrients intake, one may correlate the birth centile with the total intake during pregnancy. However, when a dietary history is taken only twice during pregnancy and, as is shown in section 5.1, a significant difference is found between the two surveys concerning the intakes of energy and of macronutrients, it is not realistic to calculate total energy and macronutrient intake for the whole pregnancy from data obtained in these two surveys. Another point is that it is probably better to correlate the intake per kg of bodyweight. Whether this is realistic during pregnancy (when some 12.5 kg of bodyweight (see section 1.1.1) is rapidly accumulated) is questionable. In an attempt to avoid these pitfalls, we have calculated the correlations between energy- and macronutrient intake in three different ways:

a. The regressions of birth centile on the total daily intakes of energy and of macronutrients at the two moments during pregnancy when the dietary survey was performed (Table 8.4.I).

TABLE 8.4.I Linear regression of birth centile on the total daily intakes of energy and of macronutrients during the 16th and 34th week of pregnancy. Total group, n=85.

		Number	Correlation coefficient	Significance
Energy intake	16 w	82	-0.05	-
	34 w	82	-0.11	-
Fat intake	16 w	82	0.03	-
	34 w	82	-0.16	-
Protein intake	16 w	82	0.17	-
	34 w	82	0.11	-
Carbohydrate intake	16 w	82	-0.12	-
	34 w	82	-0.09	-

b. The regressions of birth centile on the intakes of energy and of macronutrients per kg of bodyweight at the time of the survey (Table 8.4.II).

TABLE 8.4.II Linear regression of birth centile on the intakes of energy and c macronutrients per kilogram of bodyweight at the 16th and 34th week of pregnancy. Total group, n=85.

		Number	Correlation coefficient	Significance
Energy intake per kg	16 w	81	-0.20	$p < 0.05$
	34 w	80	-0.22	$p < 0.05$
Fat intake per kg	16 w	81	-0.12	-
	34 w	80	-0.25	$p < 0.05$
Protein intake per kg	16 w	81	-0.03	-
	34 w	80	-0.009	-
Carbohydrate intake per kg	16 w	81	-0.23	$p < 0.05$
	34 w	80	-0.19	$p < 0.05$

c. The regressions of birth centile on the intakes of energy and of macronutrients per kg of prepregnant weight at the time of the survey (Table 8.4.III).

TABLE 8.4.III Linear regression of birth centile on the intakes of energy and of macronutrients during pregnancy calculated per kilogram of prepregnancy weight. Total group, n=85.

		Number	Correlation coefficient	Significance
Energy intake per kg	16 w	82	-0.17	-
	34 w	82	-0.21	$p < 0.05$
Fat intake per kg	16 w	82	-0.10	-
	34 w	82	-0.25	$p < 0.05$
Protein intake per kg	16 w	82	-0.007	-
	34 w	82	-0.02	-
Carbohydrate intake per kg	16 w	82	-0.21	$p < 0.05$
	34 w	82	-0.19	$p < 0.05$

As can be concluded from Table 8.4.I, no significant correlation can be observed between birth centile and the total intakes of energy and macronutrients.

Some correlations appear when correlating the intakes per kg of bodyweight with the birth centile at the time of the survey (Table 8.4.II).

There is a weak, but significant correlation between energy- and carbohydrate intake per kg of bodyweight at the 16 and 34 week survey and birthweight. The fat intake per kg of bodyweight

is significantly correlated only at 34 weeks. No correlation can be found between birth centile and protein intake per kg of bodyweight during pregnancy.

An almost identical picture emerges when replacing the kg of bodyweight at the time of the survey by the prepregnant weight (Table 8.4.III). The only difference being the energy intake per kg of prepregnant weight at 16 weeks which does not reach significance compared to the energy intake per kg of bodyweight at that time.

All correlations found have in common that they are all very weak and all negative, probably a disappointing finding for everyone who believes that alimentation during pregnancy, even in the western world, is correlated in a positive way with birthweight or birth centile.

It is disappointing as well for those who think that adding extra energy to the diet in pregnancy of women who smoke might overcome the decrease in birthweight of their children (Luke et al., 1981). The lack of a positive correlation between birth centile and energy- and macronutrient intake was not so unexpected, as most studies did not show a correlation either (Thomson, 1959[a]; Van der Rijst, 1962; Papoz et al., 1980).

The finding of negative correlations is more surprising. This may possibly be related to the observations that:

- Women who had a baby with a birth centile on or below the P10 consumed slightly more, although not significantly, energy and macronutrients than the S-Reference group (section 5.2 and Figure 5.1).
- Women who smoked delivered babies with a birth centile significantly below that of babies of women who did not smoke. The intakes of energy and of macronutrients in non-smokers and smokers did not show any significant difference, except for the consumption of more alcohol among smokers. There was even a tendency for smokers to consume more fat and carbohydrates at the 34 weeks survey than non-smokers (see section 5.2 and Table 5.7).

Considering these facts, the finding of negative correlations is less surprising. We have divided the total group

into smokers and non-smokers to see whether the negative correlation coefficients for the energy intake persisted in both groups or only in the smoking group. The results are summarized in Table 8.4.IV, and although no significance is reached, there is a tendency for higher negative r values in the smoking group, especially at 34 weeks survey. This suggests that the observation that the intake of energy, fat and carbohydrates have a negative correlation with the birth centile of the newborn may be caused by other variables. Whether this is true will be discussed in section 8.9.

TABLE 8.4.IV. Linear regressions of birth centile on total daily energy intake and energy intake per kilogram of prepregnancy weight for non-smokers and smokers.

		Number	Correlation coefficient	Significance
Non-smokers				
Energy intake	16 w	39	-0.01	-
	34 w	39	0.07	-
Energy intake per kg	16 w	39	-0.17	-
	34 w	39	-0.07	-
Smokers				
Energy intake	16 w	43	-0.05	-
	34 w	43	-0.23	-
Energy intake per kg	16 w	43	-0.12	-
	34 w	43	-0.25	-

We have also tested whether the intakes of energy, fat and carbohydrates are correlated with the (maximal) weight gain during pregnancy since there have been suggestions that weight gain during pregnancy is a nutritional effect. Considering that there is a possible correlation between weight gain during pregnancy and birthweight, therefore, birthweight would also be related to alimentation during pregnancy was assumed by Rush (1974) and Rush et al. (1976). We tested the total daily energy, fat and carbohydrate intake and the intakes per kilogram of prepregnant weight. The results are summarized in Table 8.4.V.

No significant correlation nor any persistent tendency could be observed between the intakes of energy, fat and carbohydrates

and the (maximal) weight gain. This contradicts Rush's assumption.

TABLE 8.4.V Linear regressions of maximal weight gain during pregnancy on total daily energy and macronutrient intakes and on energy and macronutrient intakes per kilogram of prepregnancy weight.

		Number	Correlation coefficient	Significance
Energy intake	16 w	82	0.06	-
	34 w	82	0.10	-
Energy intake per kg	16 w	82	-0.06	-
	34 w	82	-0.02	-
Fat intake	16 w	82	0.15	-
	34 w	82	0.004	-
Fat intake per kg	16 w	82	0.04	-
	34 w	82	-0.07	-
Carbohydrate intake	16 w	82	-0.09	-
	34 w	82	0.09	-
Carbohydrate intake per kg	16 w	82	-0.16	-
	34 w	82	-0.02	-

It is concluded that, according to the dietary histories performed at the 16th and 34th week of pregnancy, no positive correlation between the birth centile of the newborn and the intakes of energy and of macronutrients by the mother could be established. The negative correlations that were found between birth centile and the intakes of energy, fat and carbohydrates (when calculated per kg of bodyweight at the time of the survey or per kg of prepregnant weight) are probably due to the fact that women of the <P10 group and smoking women had the same food intake as was found in the S-Reference group, but had children with lower birth centiles. Whether the energy and macronutrient intake has a real negative influence on the birth centile is described in section 8.9.

Weight gain during pregnancy, although positively correlated with the birth centile (see section 8.1), was neither correlated with energy intake nor with the intake of fat and carbohydrates. This contradicts the assumption by some people that weight gain during pregnancy is a nutritional effect.

8.5. Linear regressions of birth centile on the vitamin status.

The linear regressions of the birth centile on some vitamin status parameters are described in Table 8.5.I. The results are given only if a relationship was found. No relationship at any moment could be demonstrated between birth centile and ETK activity or stimulation effect, vitamin B2 levels or EGR activity and stimulation effect, 25-hydroxy-vitamin D and vitamin B12.

TABLE 8.5.I Linear regressions of birth centile on vitamin status parameters during and after pregnancy. Total group, n=85.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Retinol	Number	84	84	84	79	74	79	71	71
	Correlation coefficient	-14	0.05	0.07	0.15	0.09	0.20	-0.18	0.28
	Significance						*		**
Folic acid serum	Number	80	83	82	77	72	77	74	71
	Correlation coefficient	-0.11	0.02	-0.02	-0.03	-0.11	-0.009	0.0003	0.03
	Significance								
Folic acid erythrocyte	Number	83	83	83	71	74	77	72	38
	Correlation coefficient	0.15	0.09	-0.009	0.05	0.09	0.17	0.26	0.09
	Significance							*	
PLP	Number	84	84	82	76	74	76	74	76
	Correlation coefficient	0.11	0.02	0.04	0.12	0.08	0.11	0.04	-0.005
	Significance								
EGOT-activity	Number	85	85	83	78	73	78	73	70
	Correlation coefficient	0.18	0.24	0.26	0.018	0.24	0.14	0.14	-0.02
	Significance	*	*	**		*			
EGOT-stimulation	Number	85	84	83	78	73	78	73	69
	Correlation coefficient	-0.24	-0.16	-0.24	-0.07	-0.25	-0.11	-0.05	-0.02
	Significance	*		*		*			

* $p < 0.05$
** $p < 0.01$

Only two studies described a relationship between either birthweight or dysmature children and maternal serum vitamin B1 levels and cord blood levels of vitamin B12, respectively (Kübler and Moch, 1975; Baker et al., 1977). We are unable to confirm these observations.

As to retinol serum levels, two significant correlations were found. The maternal serum level at 6 weeks postpartum and the level in the mixed cord serum correlated significantly with the birth centile. For the correlation at 6 weeks postpartum, it seems likely that this is induced by chance since, during pregnancy and at the other two moments after pregnancy, no other correlations are observed and no consistent trend is apparent. The correlation between birth centile and retinol cord blood level is difficult to explain. A positive correlation between retinol levels in the second and third trimester and birthweight was observed by Kübler and Moch (1975), but others were unable to confirm this observation (see section 1.3.1). Kübler and Moch did not mention a positive correlation between birthweight and retinol levels in cord blood. In our opinion, this observation should be considered just as it is: an observation that awaits further confirmation.

As to folacin serum levels and folacin content of the erythrocyte, we only found a correlation between birth centile and the folacin level in the erythrocyte at 6 months postpartum. Since no consistent trend of this relationship during and after pregnancy is apparent, we have no explanation for this finding at 6 months postpartum. Serum folacin levels do not show a relationship with the birth centile. There is still a controversy whether or not low maternal serum folacin levels are correlated with the birthweight of the newborn (see section 1.3.8). Even recently, Rolschau et al. (1979) observed a significantly higher birthweight when supplementing healthy Danish gravidae with 5 mg folacin from the 23rd week of pregnancy onwards. However, our results show no relationship between folacin levels and birth centile and this finding may cast some doubt as the "birthweight increasing" effect of folacin supplementation.

Concerning vitamin B6 serum levels (PLP) and cellular vitamin B6 parameters, EGOT activity and stimulation effect, we found no correlation between birth centile and PLP serum levels, but a rather consistent correlation during pregnancy and in early puerperium with the EGOT activity and stimulation effect.

Only during delivery no correlation was found, nor for the stimulation effect nor for the activity.

Some authors have described a relationship between birthweight and the vitamin B6 status, although others could not confirm this (see section 1.3.6). Our results seem to indicate a relationship as well. However, when we divided our S-Reference group into smokers and non-smokers, it was noticed that the EGOT activity was significantly lower in the smokers and the stimulation effect was significiantly higher (see section 6.3 and Table 6.3). We decided to test by means of regression analysis whether the relationship between the birth centile and the EGOT activity and stimulation effect was due to this smoking effect. As the highest correlation was reached at the 34 weeks point, this moment was used for analysis (Table 8.5.II).

TABLE 8.5.II Analysis of variance on birth centile with smoking and EGOT activity and stimulation effect.

	F-ratio	Significance
Smoking	6.54	$p < 0.005$
EGOT activity adjusted for smoking	0.87	NS
EGOT activity - smoking	1.64	NS
Smoking	6.45	$p < 0.005$
EGOT stimulation effect adjusted for smoking	0.95	NS
EGOT stimulation effect - smoking	0.90	NS

The percentage of variance in birth centile (the y variate) accounted for, is 11.7%, when only smoking is considered. Adding the EGOT activity, i.e. assuming the same linear relationship between birth centile and EGOT activity for non-smokers and smokers, the percentage of explained variance in birth centile remains the same (11.7%). Adding the interaction EGOT activity/smoking, i.e. assuming a different linear relationship between birth centile and EGOT activity for non-smokers and smokers, the percentage of variance increases slightly, but not significantly to 13%

When repeating this procedure for the EGOT stimulation effect, similar results were obtained.

The assumption that the observed relationship between birth centile and EGOT activity and stimulation effect was a smoking effect seems to be right. Therefore, women who smoke produce newborns with lower birth centiles and have lower vitamin B6 values at cellular level during pregnancy.

Summarizing: some correlations between the birth centile and maternal and cord blood vitamin levels are observed. Two weak correlations, the maternal retinol level at 6 months postpartum and the folacin level in the erythrocyte at 6 months postpartum are probably induced by chance since no such trend in the course of pregnancy or postpartum period was apparent. We have no explanation for the weak correlation between birth centile and retinol level in mixed cord blood and this observation needs further confirmation. The persistent correlation between the birth centile and the EGOT activity and stimulation effect during pregnancy and in early puerperium can almost completely be explained by smoking. However, it remains unclear why a significantly lower EGOT activity and higher stimulation effect is found during pregnancy among smokers and what the influence of these lower levels on the intrauterine growth of the fetus and placenta may be.

The overall conclusion about the linear regressions of the birth centile of the newborn on the maternal vitamin status during and after pregnancy and on the vitamin status in the mixed cord blood, is that no clear relationship could be demonstrated.

It may be argued that one should consider the overall food intake or general (biochemical) vitamin status before stating that no relationship exists between birth centile and alimentation during pregnancy or the maternal biochemical nutritional status. An overall low or even deficient intake of foods may exist, and this can be related to the birth centile while linear regressions of birth centile on single parameters do not show significant correlations.

To investigate this possibility, we calculated the mean birth centile of newborns of mothers with an overall low food intake (energy, protein and vitamins). Six or more parameters had to be below the recommended daily allowances of the NRC-NAS (Table 1.2.2.I) at all three nutrition surveys performed in this study. Seventeen women of the S-Reference group fulfilled this criterion. The mean birth centile in this group is 44.09 $\pm$ 25.29 compared to 43.05 $\pm$ 24.1 for the whole S-Reference group. So, no difference could be observed.

We also investigated whether the mean birth centile of newborns was different for women with biochemical signs of a multiple vitamin "deficiency" during pregnancy compared to the whole S-Reference group. Twelve women showed at all moments during pregnancy values for three or more blood vitamin status parameters below the non-pregnant reference range. The mean birth centile in this group is 36.07 $\pm$ 28.75 compared to 43.5 $\pm$ 24.1 for the whole S-Reference group: an unsignificant difference.

From these findings, it is concluded that there are no indications that women with an overall low food intake or with biochemical signs of a multiple vitamin "deficiency" during pregnancy produce newborns with a lower birth centile.

8.6. Linear regressions of birth centile on blood, plasma, red cell volume, hemoglobin (Hb) and hematocrit (Ht).

The results of the linear regressions of the birth centile on blood, plasma, red cell volume, Hb and Ht are described in Table 8.6.I.

During pregnancy and at 6 days postpartum, significant correlations are found between the birth centile and the blood, plasma and red cell volume. The correlations disappear at 6 weeks and 6 months postpartum, except for the still significant correlation of the birth centile with the total blood volume at 6 weeks postpartum. Our results are remarkably in agreement with the findings of Pirani et al. (1973), who found a correlation

coefficient of 0.43 between birthweight and plasma volume at 38 weeks of pregnancy, while the correlation coefficient we find at 34 weeks is 0.44. Even better correlations are described between birthweight and total plasma increase from 12 to 38 weeks (Pirani et al., 1973) or maximal plasma increase (Hytten and Paintin, 1963).

TABLE 8.6.I Linear regressions of birth centile on blood volume, plasma volume, red cell volume, hemoglobin and hematocrit. Total group, n=85.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Blood volume	Number	83	82	84	-	69	77	52	-
	Correlation coefficient	0.30	0.39	0.46	-	0.37	0.23	0.05	-
	Significance	xx	xxx	xxxx	-	xxx	x		
Plasma volume	Number	83	82	84	-	69	77	52	-
	Correlation coefficient	0.26	0.37	0.44	-	0.33	0.18	0.16	-
	Significance	xx	xxx	xxxx	-	xx			
Red cell volume	Number	83	82	84	-	69	77	52	-
	Correlation coefficient	0.22	0.30	0.36	-	0.28	0.16	-0.08	-
	Significance	x	xx	xxx	-	xx			
Hemoglobin	Number	84	85	84	74	74	79	74	41
	Correlation coefficient	-0.06	-0.14	-0.22	-0.18	-0.16	-0.20	0.16	0.07
	Significance			x			x		
Hematocrit	Number	84	85	84	75	74	79	74	41
	Correlation coefficient	-0.02	-0.16	-0.18	-0.10	-0.05	-0.08	-0.20	0.15
	Significance			x				x	

x : $p < 0.05$
xx : $p < 0.01$
xxx : $p < 0.001$
xxxx : $p < 0.0001$

We have tested whether we could confirm these findings. The correlations between birth centile and total plasma increase during pregnancy (16 to 34 weeks) and the maximal increase (the difference between the non-pregnant volume at 6 months postpartum and 34 weeks) were calculated and described in Table 8.6.II.

We do not find a better correlation between birth centile and total plasma increase during pregnancy as compared to the absolute plasma volumes at 28 and 34 weeks of pregnancy. This

may be due to the total plasma volume increase being calculated between 16 and 34 weeks in our study. As was described in section 4.1 and illustrated in Figure 4.1.2, a significant increase in plasma volume has already occurred at 16 weeks. However, even for the total increase of blood, and plasma volumes between 16 and 34 weeks, a highly significant correlation coefficient of 0.31 is reached.

TABLE 8.6.II Linear regressions of birth centile on absolute increase during pregnancy and decrease postpartum of blood, plasma and red cell volumes and on the decrease during pregnancy and increase postpartum of hemoglobin and hematocrit. Total group, n=85.

	Number	Correlation coefficient	Significance
Blood volume 16-34 weeks	82	-0.31	$p < 0.01$
Plasma volume 16-34 weeks	82	-0.31	$p < 0.01$
Red cell volume 16-34 weeks	82	-0.18	-
Hemoglobin 16-34 weeks	83	0.13	-
Hematocrit 16-34 weeks	83	0.12	-
Blood volume 34 w-6mpp	52	0.25	$p < 0.05$
Plasma volume 34 w-6mpp	52	0.27	$p < 0.05$
Red cell volume 34 w-6mpp	52	0.18	-
Hemoglobin 34 w-6mpp	73	-0.0004	-
Hematocrit 34 w-6mpp	73	0.09	-

We do not find better correlations when using the "maximal" plasma increase: the difference between the plasma volume at 34 weeks of pregnancy and at 6 months postpartum. The correlation coefficient even decreased ($r = 0.27$, Table 8.6.II). This is not in agreement with the observation of Hytten and Paintin (1963) that the maximal plasma volume increase correlates best with the

birthweight. The maximal plasma volume increase in Hytten's study was estimated for each individual, after correction for height and weight, from a curve constructed from measurements obtained for the whole group in an only partly longitudinal study. So, maximal plasma volume increases in their and our study are not completely comparable. Another possible explanation of this discrepancy may be that, during pregnancy, in our total group (n = 85) the percentage of non-smokers is 48% versus 52%. However, in the 52 measurements done at 6 months postpartum, this distribution has shifted even more to the smokers (42% non-smokers versus 58% smokers). Since no significant differences in plasma volume have been found between smokers and non-smokers, but a clear significant difference between birth centile of smokers and non-smokers, this slight over-representation of smokers at 6 months postpartum may reduce the correlation coefficient between maximal plasma volume increase and birth centile.

However, generally we can confirm the observations in the literature (see also section 1.5) that there is a correlation between birth centile (birthweight) and plasma volume during pregnancy, becoming more significant as pregnancy advances. Also, the red cell volume was found to be positively correlated with the birth centile although less significant than the plasma volume.

As both plasma volume and red cell volume are positively correlated with the birth centile, a strong correlation between the Hb or Ht and birth centile cannot be expected. As we see in Figure 8.6.1, a negative correlation is found only at 34 weeks for both. This confirms the observation that a high Hb during the third trimester is associated with the chance for the occurrence of the birth of a dysmature (<P10) child (Mau, 1977). No correlations are found with the decrease of the Hb and Ht during pregnancy (16-34 weeks) nor for the increase in the period 34 weeks to 6 months postpartum (Table 8.6.II). This may be explained by the significant decrease having already taken place before the 16th week (see Figure 4.1.4). In the period 34 weeks to 6 months postpartum, Hb and Ht are influenced by many

factors such as blood loss during delivery, iron content of the diet postpartum or iron medication postpartum.

The mechanisms which induce the increase in plasma- and red cell volume during pregnancy are still unclear (see section 1.1). There is a positive correlation between either total plasma volume or the increase in plasma volume and birth centile (weight). Whether a large plasma volume (increase) helps the fetus to grow larger or a large fetus (and placenta) induces a large plasma volume is still not resolved. Croall et al. (1978) found smaller non-pregnant plasma volumes in women who recurrently gave birth to small-for-date children when compared to controls. This suggests that plasma volume can restrict intrauterine development; on the other hand, it does not reflect the situation during pregnancy. We were not able to demonstrate a correlation between birth centile and plasma volume at 6 months postpartum. Another observation indicating that the feto-placental unit induces the increase in plasma volume is that in twin pregnancies significantly larger plasma volumes are measured than in singleton pregnancies (Hall et al., 1976).

8.7. Linear regressions of birth centile on iron status parameters.

The results concerning the relationship between birth centile and iron status parameters are described in Table 8.7.I.

Serum iron and the percentage saturation during pregnancy are negatively correlated with the birth centile. This may be explained by hemodilution. Because a positive correlation was observed between plasma volume and the increase in plasma volume and birth centile, a negative correlation between birth centile and serum iron or percentage saturation is not unexpected. The same phenomenon was observed when relating Hb and Ht to the birth centile (see section 8.5). However, it was not tested whether the increase in plasma volume really caused the fall of these parameters. Therefore, one has to be careful in assuming a causal relationship. If hemodilution is the main cause, one may

expect a higher correlation at 34 weeks than at 16 weeks. So, probably other mechanisms are involved as well.

TABLE 8.7.I Linear regressions of birth centile on iron status parameters during pregnancy and in cord blood. Total group, n=85.

		Number	Correlation coefficient	Significance
Serum iron	16 w	85	-0.21	x
	34 w	82	-0.18	x
	6mpp	73	-0.12	
	Cord	78	0.06	
Total iron binding capacity (TIBC)	16 w	85	0.13	
	34 w	84	0.12	
	6mpp	73	-0.005	
	Cord	78	0.23	x
% Saturation	16 w	85	-0.25	x
	34 w	83	-0.19	x
	6mpp	73	-0.14	
	Cord	78	-0.06	
Ferritin	16 w	85	0.04	
	34 w	84	-0.15	
	6mpp	74	-0.10	
	Cord	74	-0.02	
Transferrin	16 w	85	0.12	
	34 w	84	0.20	
	6mpp	75	0.03	
	Cord	79	0.22	

The positive correlations between birth centile and serum transferrin at 34 weeks and between birth centile and the total iron binding capacity (TIBC) and transferrin in cord blood are difficult to understand. It is assumed that the large increase in transport proteins is probably induced by estrogens. No correlation has ever been demonstrated between estrogen levels in the maternal or fetal compartment and birth centile (weight) (see section 1.1.3). To our knowledge, no one has ever tried to correlate the carrier protein increase during pregnancy with the increase in estrogen level. It may be that a larger feto-placental unit induces a larger increase in transport proteins both in the maternal and fetal compartment.

Serum ferritin concentrations, considered as a reflection of the maternal iron store, does not show any correlation with the

birth centile.

According to some studies, the iron content (Widdowson, 1951) and the serum ferritin level (Jansson and Holmberg, 1979) in the fetus increase with fetal weight, but in these studies fetal weight is meant to be the increase of fetal weight in the course of pregnancy (see section 1.3.11). No data are available in which serum ferritin levels in a group of children born at term are compared in relation to their birthweight. We could not observe any relationship between maternal serum ferritin level or ferritin level in cord blood and birth centile.

8.8. Linear regressions of birth centile on other biochemical and hormonal parameters studied.

Linear regressions of birth centile on protein, albumin, cholesterol and triglycerides during and after pregnancy and on human placental lactogen (HPL) during pregnancy are given in Table 8.8.I.

TABLE 8.8.I Linear regressions of birth centile on protein, albumen, cholesterol and triglycerides in serum during and after pregnancy and on human placental lactogen levels during pregnancy. Total group, n=85.

		Number	Correlation coefficient	Significance
Protein	16 w	84	0.09	-
	34 w	84	0.06	-
	6mpp	73	0.21	$p < 0.05$
Albumen	16 w	85	0.05	-
	34 w	84	0.11	-
	6mpp	74	0.15	-
Cholesterol	16 w	85	0.07	-
	34 w	84	0.03	-
	6mpp	75	-0.006	-
Triglycerides	16 w	85	0.02	-
	34 w	84	0.03	-
	6mpp	74	0.09	-
Human placental lactogen	16 w	85	-0.001	-
	34 w	84	0.04	-
	6mpp	84	0.09	-

As can be seen from Table 8.8.I, no correlations have been found between birth centile and other biochemical and hormonal parameters studied, except for a correlation between the maternal protein serum level at 6 months postpartum. We have no explanation for this weak correlation (r = 0.21). Thereby, the median values of the <P10 group are all found well within the range of the S-Reference group as is shown in Figure 4.10.

HPL is sometimes found to be positively related with placental weight (section 1.1.3) and placental weight is found to correlate significantly with the birth centile (Table 8.2.I). For this reason, linear regressions of birth centile on HPL were calculated. No correlation between these two variables could be demonstrated (Table 8.8.I).

8.9. The percentages of variance in birth centile and Ponderal-index that can be explained.

In the previous sections of this chapter, a correlation between birth centile and a number of parameters was demonstrated. Maternal age, height, prepregnant weight and maximal weight gain are positively related to the birth centile. Smoking is negatively correlated (section 8.2 and 8.3).

The intakes of energy, fat and carbohydrates per kg of bodyweight at 34 weeks of pregnancy is negatively correlated with the birth centile, although a real negative association is not likely (section 8.4).

The EGOT activity and stimulation effect, a vitamin B6 parameter at cellular level, is found to be correlated with the birth centile, although this relationship is probably confounded or induced by smoking (section 8.5).

Total blood, plasma and red cell volume are positively correlated with the birth centile as well as the increase of blood and plasma volume increase. Hemoglobin and hematocrit values at 34 weeks of pregnancy are negatively correlated with the birth centile, probably as a result of hemodilution (section 8.6). Serum iron and percentage saturation also showed weak

negative correlations with the birth centile at 16 and 34 weeks of pregnancy (section 8.7).

In this section, we will explore which percentage of the encountered variance in birth centile and Ponderal-index can be explained by these parameters. A positive or negative correlation with the birth centile was the main reason to select a parameter for further analysis, irrespective of whether its relationship could probably be explained by other variables.

Only two parameters, paternal height and human placental lactogen (HPL) values at 34 weeks, were added to the set of variables in the multiple regression analysis since these variables are said to be related either to birthweight or to placental weight (see section 1.4 and section 1.1.3). The placental weight - highly significantly related with the birth centile - was not included in the selected variables because this probably would raise the old question whether the child is large because the placenta is large or just the other way round (section 1.4).

To investigate which percentage of variance of the birth centile and Ponderal-index can be explained, regression analysis was carried out with birth centile and Ponderal-index as dependent variables and the aforementioned parameters as independent variables. These independent variables available for selection in the stepwise forward multiple regression analysis are summarized in Table 8.9.1. Because the statistical program accepts only "complete" cases, i.e. women for whom all data are available, the stepwise forward multiple regression was performed on 67 cases. It was used merely to obtain a first idea about the importance of the variables explaining the encountered variance. A subset selection regression procedure has been carried out with a selected number of variables. This procedure has the advantage that not only the "best subset" (as in the stepwise forward multiple regression analysis), but every combination (subset) of variables is presented. The statistical methods are described in more detail in section 2.7.

Birth centile

Stepwise multiple regression analysis (n = 67).
The variance accounted for with all available variables (Table 8.9.1) is 24.9% R^2 = 24.9 F-ratio 6.1 $p < 0.01$

The variables selected in stepwise regression are

	Percentage of variance accounted for	F-ratio*	Significance*
1) Smoking	17%	6.1	$p < 0.01$
2) Total plasma volume increase(34w-6wpp)	30.7%	6.1	< 0.01
3) Maternal age	35.8%	1.99	NS
4) Percentage of plasma volume increase	36.8%	1.03	NS
5) EGOT stimulation effect(34w)	36.85%	0.3	NS

* F-ratio and significance given the set of other variables selected.

Ponderal-index

Stepwise multiple regression analysis (n = 67).
The variance accounted for with all available variables (Table 8.9.1) is 1.2% R^2 = 1.2 F-ratio 1.03 N.S.

The variables selected in stepwise regression are

	Percentage of variance accounted for	F-ratio*	Significance*
1) Smoking	10.7%	3.72	$p < 0.05$
2) Plasma volume at 34w	15.5%	1.48	NS

3) Weight gain up to 34w	17.6%	2.08	NS
4) HPL levels at 34w	17.9%	1.44	NS
5) Paternal height	18.1%	0.63	NS

* F-ratio and significance given the set of other variables selected.

From this stepwise regression, it appears that 36.8% of the variance in birth centile and 18% of the variance in Ponderal-index can be accounted for by the selected variables. For the birth centile, only two variables, smoking and total plasma volume increase, contributed significantly. For the Ponderal-index, only one variable, smoking, contributed significantly to the explained variance.
From this it appears that the intake of energy, fat and carbohydrates, EGOT activity or stimulation effect, hemoglobin level, or serum iron do not contribute to the explanation of the variation in birth centile (and Ponderal-index), as had already cautiously been concluded in the previous section of this chapter.

It was decided to select the following parameters for the subset selection analysis:
- smoking
- plasma volume at 34 weeks
- maternal age
- maternal maximal weight gain
- maternal prepregnant weight
- maternal height.

Smoking and plasma volume were selected since both emerged as significantly contributing variables. The absolute plasma volume was chosen instead of the increase in plasma volume because a number of plasma volume measurements postpartum are missing and the subset regression model, like the stepwise regression model, accepts only "complete" cases.

Maternal age was chosen since it emerged, although not

significantly, as one of the independent variables in the multiple regression analysis. The last three variables were selected because, according to literature (section 1.4.3), they are related to birthweight and also because significant relationships were observed with the birth centile in this study (section 8.2). Maximal weight gain was selected instead of weight gain up to 34 weeks since it was considered to be a better parameter for comparison with data found in literature.

For the subset procedure 80 "complete" cases were available.

Birth centile

When the subset selection model with the above-mentioned variables is applied for explanation of the variation of the birth centile, the following results are obtained:

Regression with one variable:

	Percentage of variance accounted for	F-ratio*	Significance*
1) Plasma volume at 34w	22.2%	22.04	$p < 0.001$
2) Smoking	14.9%	9.51	< 0.01
3) Maximal weight gain	7.3%	6.5	< 0.05
4) Prepregnant weight	6.6%	5.8	< 0.05
5) Maternal age	4.5%	4.1	< 0.05
6) Maternal height	4.3%	3.99	< 0.05

* F-ratio and p-value given no variable already selected.

Regression with two variables:

		Percentage of variance accounted for	F-ratio*	Significance*
Plasma volume 34w				
with 1)	Smoking	32.4%	9.0	$p < 0.01$
2)	Maternal age	25.1%	4.2	< 0.05
3)	Maximal weight gain	23.0%	1.9	NS
4)	Maternal height	21.3%	0.15	NS
5)	Prepregnant weight	21.28%	0.10	NS

* F-ratio and p-value given the set of other variables already selected

		Percentage of variance accounted for	F-ratio*	Significance*
Smoking				
with 1)	Maximal weight gain	18.9%	5.0	$p < 0.05$
2)	Prepregnant weight	18.3%	4.5	< 0.05
3)	Maternal height	17.6%	4.05	< 0.05
4)	Maternal age	16.0%	2.7	NS

* F-ratio and p-value given the set of other variables already selected

Regression with three variables:

		Percentage of variance accounted for	F-ratio*	Significance*
Plasma volume 34w and smoking				
with 1)	Maternal age	34.4%	2.36	NS
2)	Maximal weight gain	33.3%	0.83	NS
3)	Prepregnant weight	32.5%	0.10	NS
4)	Maternal height	32%	0.08	NS

* F-ratio and p-value given the set of other variables already selected

From these data, it can be concluded that extending the analysis with more than two variables (plasma volume and smoking) revealed no other variables which contributed significantly in explaining the variation of the birth centile. This implies that the other variables are dependent on one of these two variables. From the combination with two variables, it appears that the significant relationship between birth centile and maximal weight gain, height and prepregnant weight as single parameters, disappears when these parameters are combined with plasma volume. When combining smoking and maternal age (subset of two variables), we find that maternal age no longer contributes significantly. Although between non-smokers and smokers, no significant difference in age could be observed (section 8.3), this finding indicates that the positive relationship between birth centile and age is probably caused by smoking less cigarettes.

Ponderal-index

When the subset selection model is applied for the explanation of the variation in Ponderal-index, the following results are obtained:

Regression with one variable:

	Percentage of variance accounted for	F-ratio*	Significance*
1) Smoking	10.7%	5.85	$p < 0.05$
2) Plasma volume at 34w	4.7%	4.06	< 0.05
3) Maximal weight gain	4.3%	3.65	NS
4) Maternal age	2.0%	1.7	NS
5) Prepregnant weight	0.42%	0.35	NS

* F-ratio and p-value given no other variables already selected

Regression with two variables:

	Percentage of variance accounted for	F-ratio*	Significance*
Smoking			
with 1) Plasma volume	14%	3.1	NS
2) Maximal weight gain	12.6%	1.75	NS
3) Maternal age	11.4%	0.60	NS
4) Prepregnant weight	11.2%	0.45	NS
5) Maternal height	11.0%	0.10	NS

* F-ratio and p-value given the set of other variables already selected

Smoking is the only variable that significantly explains some of the encountered variation of the Ponderal-index, the same conclusions as from the stepwise multiple regression analysis.

Summarizing: only two variables - smoking and (increase in) plasma volume - contributed significantly to the explained (about 35%) variation of the birth centile. The other variables available for selection appeared to be dependent variables, i.e. they did not contribute significantly. From the subset selection analysis, it is apparent that the relationship between birth centile and maternal prepregnant weight, height and weight gain during pregnancy disappeared when these variables are combined with plasma volume at 34 weeks. This indicates that plasma volume at 34 weeks is related to these variables. Possibly, weight gain during pregnancy and plasma volume (increase) are induced by the same mechanisms. Plasma volume at 34 weeks is also related to maternal height and prepregnant weight. This suggests a relationship between plasma volume at 34 weeks and prepregnant plasma volume. This may support Croall's (1978) observation that women who repeatedly gave birth to small-for-date children have lower non-pregnant plasma volumes than controls. So, (prepregnant) plasma volume may be a causal factor related to the birth centile. However, we are unable to confirm this finding since we did not observe a significant correlation between the birth centile and the non-pregnant plasma volume at 6 months postpartum (section 8.5). Therefore, a causal relationship between birth centile and prepregnant plasma volume remains doubtful. Whether the (increase in) plasma volume during pregnancy has a causal or coincidental correlation with the birth centile, i.e. "induces" or is induced by the feto-placental unit, is open to questions.

Smoking is the variable that definitely has a causal negative relationship with the birth centile. How smoking exactly affects the birth(weight) centile is not yet known (Pirani, 1978). Smoking is the only variable that contributes significantly to the explained variation of the Ponderal-index,

an index of proportional intrauterine growth of the mature fetus.

Since smoking is negatively correlated with the Ponderal-index, and the length of the newborn is not different between non-smokers and smokers, this indicates that smoking affects growth in weight more than growth in length, causing a "dysproportional or dystrophic" growth. The Ponderal-index is significantly lower among heavy smokers and this suggests a correlation between the number of cigarettes smoked and the decrease in Ponderal-index, an observation we could not confirm for the decrease in birth centile (section 8.3, Table 8.3.I).

We can conclude that the largest part of the variation in birth centile and Ponderal-index cannot be explained by the variables available for selection in the regression analyses. Other factors - definitely more important - must be involved. Undoubtedly nutrients play a role. It is not the quantity of maternal nutrient intake, but probably the quantity and quality of nutrients that reach the placenta and are transported to the fetus. This depends probably on maternal metabolic changes, utero-placental blood flow and placental transfer functions and capacity. On the other side of the cord, the fetus itself is involved in its growth by producing factors that induce or impede growth. These factors will probably give a further explanation of the large part of the variation in birth centile and Ponderal-index that can not be accounted for.

CHAPTER 9.

Summary, conclusions and recommendations.

In the introduction of this thesis the four objectives of this study were formulated:

1. To describe the changes of some vitamin and iron status parameters during normal pregnancy and in the period up to 6 months after delivery.
2. To investigate whether changes in vitamin level during normal pregnancy may be explained by physiological adjustments of pregnancy.
3. To investigate whether nutrition during pregnancy meets recommended daily allowances.
4. To investigate whether nutrition during normal pregnancy and biochemical parameters representing nutritional health during pregnancy are related to the birthweight centile of the newborn.

The study was performed among a group of healthy women who had a normal pregnancy and delivered vaginally a mature healthy baby. Measurements were performed at the 16th, 28th and 34th week of pregnancy, during delivery and at the 6th day, 6th week and 6th month after parturition. For a detailed description of the selection criteria, parameters measured and methods used, the reader is referred to Chapter 2.

Eighty-five women fulfilled all criteria. Seventy women delivered a baby with a birth centile between the 10th and 90th centile of the Dutch birthweight curve. This group is considered as the S(tudy)-Reference group. Ten women had a baby with a birth centile on or below the 10th centile and 5 had a baby on or above the 90th centile.

Based upon the literature reviewed in Chapter 1 and the results of our study presented in Chapters 3 to 8, the objectives can be answered as follows:

Objective 1: to describe the changes of some vitamin and iron status parameters during normal pregnancy and in the period up to 6 months after delivery.

Vitamin status parameters

In section 1.3 the extensive literature on the changes in maternal vitamin status during and after pregnancy was reviewed. Although a wide variety in reported data is apparent, it was concluded that many of the parameters of the vitamin status show a significant change in the course of pregnancy, especially vitamin blood levels and urinary vitamin excretion. These changes seem to occur even to healthy, well-nourished pregnant women. However, many of the available data are derived from cross-sectional studies considering especially the vitamin status at the end of pregnancy, whereas (longitudinal) data about the changes postpartum, beyond 6 weeks, are hardly found in literature.

In this connection we studied the course of some parameters of the vitamin A, D, B6, B12, thiamin, riboflavin and folacin status in a longitudinal design between the 16th week of pregnancy and 6 months after delivery. The findings for the S-Reference group are presented and discussed in section 6.1. As expected a decrease was found for serum retinol, vitamin B12 and folacin as well as for plasma PLP (Figure 6.1.1; 6.1.8; 6.1.11-13). For serum retinol, serum folacin and plasma PLP the 16th week values were already low compared with referene values of non-pregnant women, suggesting a fall already in early pregnancy. The mean serum 25-OH-vitamin D levels remained fairly constant during pregnancy although at all stages during pregnancy mean serum levels were significantly lower than that at 6 months postpartum (Figure 6.1.2). The whole blood riboflavin level also remained unchanged during the first months, but showed a significant increase at the end of pregnancy (Figure 6.1.5). The enzyme stimulation tests (ETK, EGR and EGOT) showed only minor changes. However, a significant fall in basal ETK activity was observed. Compared with reference ranges, established for non-pregnant (female) adults, the

occurrence of abnormal values increased during pregnancy for nearly all parameters, except for serum 25-OH-vitamin D, whole blood riboflavin and serum vitamin B12 (Table 6.1.28).

Between the maternal values for the vitamin status parameters at delivery and the corresponding cord blood values, a significant relationship was found, except for the ETK stimulation ratio (section 6.2).

Maternal smoking had a significant effect on the EGOT stimulation test in that higher αEGOT values were found for women who smoked during pregnancy compared with non-smokers. Such an effect could only be demonstrated during pregnancy. Also the whole blood riboflavin levels in cord blood were significantly lower for babies of smoking mothers. For the other parameters no significant effect of maternal smoking was found (section 6.3).

In section 6.4 the effect of different parity on maternal and cord blood vitamin status parameters was assessed, but differences according to parity could not be demonstrated during or after pregnancy.

Seasonal variation was observed for serum retinol, serum 25-OH-vitamin D and the EGOT stimulation ratio (section 6.5). This effect was most pronounced and consistent for serum 25-OH-vitamin D, the higher values being found in summer, the lower values in winter and early spring (Figure 6.5.2).

In the postpartum period, a spontaneous reversal of the pregnancy induced changes was found to occur within the first 6 months after delivery for most parameters except serum folacin levels. At 6 months postpartum the mean serum folacin level was still below that at the 16th week of pregnancy, and 45% of the women had levels still below the lower limit of the non-pregnant reference range. The mean serum retinol and plasma PLP levels at 6 months postpartum were also low compared with the non-pregnant reference mean values, but higher than those measured at any stage of pregnancy. Still 25% of the plasma PLP values were below the lower limit of the reference range (section 6.7).

Iron status parameters

From the literature review (section 1.3.11), it was concluded that there is no consensus whether the iron status during pregnancy is at risk. However, recent studies, in which serum ferritin was used as a parameter of iron stores, indicate that pregnancy depletes the maternal iron stores.

Concerning the relationship between the maternal and fetal iron status, most studies did not find a correlation. The fetal iron status at birth seems rather independent from the maternal iron status.

In this study we observed a significant decrease of serum iron, percentage saturation and serum ferritin during pregnancy (section 7.1). The total iron binding capacity increased significantly. This indicates a depletion of maternal iron stores, but these changes of iron status parameters may be caused by other factors during pregnancy.

After pregnancy, we found a slow increase of serum iron, percentage saturation and serum ferritin, but the mean values at 6 months postpartum were still significantly below the mean values obtained in the 16th week of pregnancy. Twenty percent of the S-Reference group had serum ferritin levels below the range of non-pregnant women (cut-off point 20 ng/ml) compared to 5% at the 16th week. The low values, in particular those observed postpartum, indicate that pregnancy and puerperium deplete the maternal iron stores.

We did not find a significant relationship between maternal and fetal iron status at birth. The fetal iron status parameters are significantly higher than maternal values, indicative of an active transport of iron from the maternal serum to the feto-placental unit (section 7.2).

Suggestions and conclusions Objective 1

1.1. The P10 and P90 values of the measurements for the vitamin status parameters among healthy pregnant women from this study can be used as a reference range for these parameters.

1.2. The increased occurrence of low serum folacin and plasma PLP levels at 6 months postpartum is suggestive for maternal folacin and vitamin B6 depletion during pregnancy of healthy women.

1.3. An uncomplicated pregnancy and puerperium depletes, at least partly, the iron store of healthy women consuming a mean daily iron intake of 13 mg (Chapter 5).

1.4. Replenishment of maternal stores takes at least more than 6 months for folacin, vitamin B6 and iron.

1.5. No relationship is found between the maternal and fetal iron status parameters at birth, in contrast to a maternal and fetal relationship observed for all vitamin status parameters measured in this study, except α-ETK.

Objective 2: To investigate whether changes in vitamin levels during normal pregnancy may be explained by physiological adjustments of pregnancy.

As was concluded from the literature review (section 1.3) there is still no consensus about the interpretation of the observed changes in vitamin status parameters in the course of pregnancy. The fact that no relationship between vitamin status parameters and the course and outcome of pregnancy could be demonstrated in most of observational and experimental studies supports the idea of a physiological adjustment. However, some studies suggest a probably non-optimal maternal vitamin status, interfering with an adequate fetal development (section 1.3 and 1.5). The direction of the changes during pregnancy, like the fall in vitamin blood levels, suggests a state of maternal vitamin depletion. As demonstrated in this and other studies, these changes occur in nearly all healthy pregnant women, even when vitamins are supplemented during pregnancy.

In this study, the extent and rate of the spontaneous reversal of the pregnancy-induced changes in some vitamin status parameters during the postpartum period was used as a criterium for "a physiological change" of such parameter during pregnancy. A spontaneous reversal after delivery towards values considered

adequate for the non-pregnant situation can be interpreted as an indication that the vitamin requirements imposed by pregnancy (i.e. the vitamin cost of pregnancy) were adequately covered by existing maternal stores and dietary supply. As described in section 6.1 and 6.7, this was true for nearly all parameters except for serum folacin and, to a lesser extent, for plasma PLP levels. Recovery of these two parameters probably takes more than 6 months after pregnancy.

Changes in vitamin status parameters during normal pregnancy are sometimes explained by the so-called physiological adjustments of pregnancy, like the increase in circulating blood volume, changes in organ function and hormonal balance (section 1.3). However, there is scarce experimental evidence about the determinants of the change in some vitamin status parameters.

To explore the determinants of the change in vitamin serum level between the 16th and 34th week of pregnancy, multiple regression analysis (section 2.7) was performed using dietary, bodily, hormonal and clinical-chemical parameters as independent variables (section 6.6). The variables available for selection in the multiple regression analysis are summarized in Table 6.6.5. The following results were obtained:

- for all vitamins involved in the analysis (retinol, folacin, vitamin B6 (PLP) and vitamin B12, a strong, inverse relationship between the fall in serum levels in the period from the 16th to the 34th week of pregnancy and the initial value at week 16 was observed. The variance accounted for by this variable was between 40 and 70%.
- volume related variables play only a minor role in case of vitamin B6 (plasma PLP), but there appears to be no relationship in the case of serum retinol and folacin.
- dietary variables (vitamin intake) were not associated with the change in vitamin blood levels.
- hormones correlated highly significant with changes in folacin level and were also associated with changes in serum retinol and vitamin B12 level.

Conclusions Objective 2

2.1. The changes in some vitamin status parameters during pregnancy should indeed be considered as a physiological adjustment of pregnancy.

2.2. The results from the regression analysis point to a hormone induced, resetting of vitamin blood (serum) level as part of a different regulation of maternal vitamin metabolism during pregnancy.

Objective 3: To investigate whether nutrition during pregnancy meets recommended daily allowances (RDA).

Data about the habitual dietary intake during pregnancy and at 6 months postpartum for our S-Reference group were presented and discussed in Chapter 5. During pregnancy minor changes in the food consumption pattern were noted. The consumption of dairy products tended to increase and that of meat products decreased. Mean energy intake estimated at the 16th week amounted 10 $\pm$ 1.9 MJ which was significantly higher than that at 34 weeks and at 6 months postpartum, i.e. 9.4 $\pm$ 2.2 and 9.1 $\pm$ 2.4, respectively. Mean micronutrient intakes were also significantly higher at the 16th week than at 6 months postpartum. Between the 34th week and 6 months postpartum, differences were still significant for calcium, thiamin, riboflavin, vitamin B12 and vitamin C. An effect of maternal smoking, parity or seasonal variation could not be demonstrated in any of the surveys, except for a significantly higher carbohydrate and vitamin C intake in nulliparae and a significantly higher alcohol consumption in women who smoked (section 5.2).

Comparison with recommendations from both the Netherlands Nutrition Council and the Food and Nutrition Board (FNB) of the NAS/NRC (see section 1.2) indicate that alimentation in this group was generally adequate (Table 5.9, Figures 5.2 and 5.3). However, the relative contribution of fat and of mono- and disaccharides to the total daily energy intake was rather high compared with these standards. Mean iron, vitamin B6 and D intake was below the American recommendations. Unfortunately, we

could not compute the daily folacin intake from our dietary data, but comparable studies indicate that dietary folacin intake during pregnancy is probably lower than recommended daily allowances (section 1.2.4).

Biochemical indices of the nutritional status during pregnancy and especially in the postpartum period measured in this study suggested a depletion of maternal iron, vitamin B6 and folacin stores during pregnancy (see Objectives 1 and 2). These biochemical findings may considered complementary and confirmative evidence for an inadequate iron, vitamin B6 and probably also folacin intake from the habitual diet. As discussed in section 6.6 and 6.7, no direct relationship between dietary and biochemical measures of the vitamin status during pregnancy could be established. Although inaccuracies in both variables may have prevented the demonstration of simple linear relationships, it also can be argued that the relatively high occurrence of abnormal values for these parameters at 6 months postpartum resulted from already marginal preconceptional stores for these nutrients due to an inadequate nutrient intake. Mean dietary vitamin D intake was also below recommendations. Although serum 25-OH-vitamin D levels were slightly lowered during pregnancy, values for all subjects were perfectly normal at 6 months postpartum. At no stage during and after pregnancy values were observed below the lower limit of the (non-pregnant) reference range. Vitamin D is an essential nutrient when UV-exposure of the skin is limited or even absent (see section 1.3.2). So it seems that endogenous vitamin D synthesis and dietary vitamin D supply were sufficient to maintain adequate maternal stores during and after pregnancy among the women in our S-Reference group.

Conclusions Objective 3

3.1. The estimated habitual intake of vitamin D, B6, iron and probably folacin were below recommended daily allowances.

3.2. Although no direct relationship between biochemical and dietary measures of the nutrient status could be established, the apparently marginal intake of iron and

vitamin B6 and probably of folacin seemed to be confirmed by the biochemical findings in the postpartum period. Endogenous vitamin D synthesis seemed sufficient for our S-Reference group to maintain an adequate vitamin D status throughout pregnancy.

Objective 4: To investigate whether nutrition during normal pregnancy and biochemical parameters representing nutritional health during pregnancy are related to the birthweight centile of the newborn.

From the literature review, no definite conclusion about this subject can be drawn (section 1.2). Fetal intrauterine growth seems to be largely, but not completely independent from maternal nutrition. On the other hand, nutrition is sometimes mentioned as a possible cause of unexplained dysmaturity, even in countries where no shortage of food exists. Moreover, maternal weight gain during pregnancy is positively correlated to birthweight of the newborn. This weight gain is assumed to be dependent on alimentation during pregnancy and so alimentation may influence birthweight.

In section 8.4, the linear regressions between birth centile of the newborn and estimated intakes of energy and macronutrients in the 16th and 34th week of pregnancy (n = 85) were described. Regressions were calculated for the total daily estimated intakes, the estimated intakes per kg of bodyweight at the time of the dietary survey and the estimated intakes per kg of prepregnant weight.

The following results were obtained:

- estimated total intakes of energy and of macronutrients during pregnancy were not related to the birth centile of the newborn (Table 8.4.I).
- estimated intakes per kg of bodyweight during pregnancy showed weak, but significant negative correlations with the birth centile for energy and carbohydrates at the 16th and 34th

week and for fat at the 34th week of pregnancy. Estimated protein intake per kg of bodyweight, was not correlated with the birth centile (Table 8.4.II).

- estimated intakes per kg of prepregnant weight showed an almost similar picture (Table 8.4.III).

This rather unexpected finding is probably caused by the fact that no differences were observed between the intakes of energy and of macronutrients of the S-Reference group (birth centile between the 10th and 90th centile) and of the < P10 group (birth centile below the 10th centile) and the fact that no differences in intake were observed between women who smoked and who did not smoke. Smoking (section 8.3) had a negative influence on the birth centile ($r = -0.38$). In a stepwise forward multiple regression analysis (section 8.9) the intakes of energy and of macronutrients were not selected as variables explaining the variance in birth centile or Ponderal-index.

It was also tested whether the estimated intakes of energy and of macronutrients were correlated with the maternal weight gain during pregnancy. No significant correlations could be demonstrated (Table 8.4.V).

Concerning the relationship between some maternal biochemical parameters of nutritional health during pregnancy and the birth centile, the following observations were made:

- vitamin status parameters during pregnancy were not correlated with the birth centile except for the EGOT activity and EGOT stimulation effect, a vitamin B6 parameter at cellular level. A consistent positive, significant correlation could be demonstrated between the EGOT activity and birth centile, while the EGOT stimulation effect showed almost similar negative correlations. EGOT activity was significantly higher and EGOT stimulation effect was significantly lower among women who smoked. When in addition to the variables EGOT activity and stimulation effect also the variable smoking was included in the correlation-eqution on the birth centile, the correlations of the B6 parameter with the birth centile disappeared; so these correlations are probably caused by smoking (section 8.5).

- weak, but significant, negative correlations were found between the birth centile and hemoglobin levels and hematocrit at 34 weeks (section 8.6).
- serum iron values at 16 and 34 weeks were also negatively correlated with birth centile (section 8.7). These correlations may probably partly be explained by the hemodilution of pregnancy.
- no correlations were observed between serum protein, albumin, cholesterol and triglyceride levels during pregnancy and birth centile (section 8.8).

In section 8.9, we described which percentage of the variance in birth centile and Ponderal-index of the newborn can be explained by parameters measured in this study, by means of a stepwise forward multiple regression analysis and, with selected parameters, a subset selection analysis. The parameters selected for these analyses are summarized in Table 8.1.

About 35% of the variance in birth centile could be accounted for. Only two variables contributed significantly: maternal plasma volume and smoking. The correlations between birth centile and maternal prepregnant weight, maternal height and weight gain during pregnancy disappeared when plasma volume was taken into account. This indicates that plasma volume is related to these variables. Weight gain and increase in plasma volume during pregnancy may possibly be induced by the same mechanisms. The relationship between plasma volume during pregnancy and maternal height and prepregnant weight suggests a relationship between plasma volume during pregnancy and prepregnant plasma volume. However, we did not observe a correlation between plasma volume at 6 months postpartum and birth centile, so whether (prepregnant) plasma volume is causally related to birthweight remains unclear. Whether (increase in) plasma volume during pregnancy has a causal or coincidental correlation with the birth centile, i.e. "induces" or is induced by the feto-placental unit, is also open to questions since the mechanisms inducing the increase in plasma volume are not known.

Smoking definitely has a causal negative relationship with

the birth centile. We did not observe a significant difference in birth centile between light and heavy smokers (section 8.3).

About 17% of the variation in Ponderal-index, an index of "proportional or dysproportional" intrauterine growth, could be accounted for. No significant difference in Ponderal-index was observed between non-smokers and light smokers, but only between non-smokers and heavy smokers (section 8.3). This seems to point to a relationship between the number of cigarettes smoked and dysproportional growth. Plasma volume did not contributed significantly to the explained variance of the Ponderal-index. From these observations it may cautiously be concluded that plasma volume is correlated with "proportional" growth, that smoking induces a "proportional growth retardation" (a lower birth centile) and that heavy smoking induces thereby also a "dysproportional growth" (a lower Ponderal-index).

Conclusions Objective 4:

4.1. No relationship could be demonstrated between the intakes of energy or of macronutrients and birth centile in this group of healthy women producing a healthy mature baby.

4.2. No relationship could be demonstrated between the intakes of energy or of macronutrients and maternal weight gain during pregnancy.

4.3. No relationship could be demonstrated between maternal biochemical parameters representing nutritional health and birth centile.

4.4. Maternal plasma volume and smoking are the variables that contributed significantly to the explained variance in birth centile. Smoking is the only variable with a clear causal influence on the birth centile.

4.5. The only variable which contributed significantly to the explained variance of the Ponderal-index was (heavy) smoking.

4.6. The largest part of the variance in birth centile (65%) and Ponderal-index (83%) of the newborn could not be explained by the parameters measured in this study.

General considerations from a teleological point of view

The fetus has long been considered to behave like a simple parasite. Because the mother is essential for the survival of the fetus, even for a long period after its birth, it was necessary for the human fetus to behave like an intelligent and modest parasite. This implies that, during the temporary symbiosis, maternal physiology should be changed for mutual interests. Probably by means of its hormones, the feto-placental unit models maternal physiology. Fetal and maternal interests do not always parallel and they sometimes had to compromise. For instance, the feto-placental unit probably induces the increase in plasma volume, thereby changing blood viscocity. Blood is more easily propelled and the utero-placental blood flow - like the blood flow through the kidney not privileged - is guaranteed better. This increased blood volume also protects the mother against the consequences of blood loss during delivery. Due to the concomitant increased renal plasma flow, however, more water soluble nutrients are excreted during pregnancy - the compromise?

The feto-placental unit probably also stimulates maternal appetite and an energy bank in the form of fat is laid down in the first 30 weeks of pregnancy. The relatively high fetal energy need in the last trimester is guaranteed and the mother is protected against the increased nutritional demands of her fetus. This mechanism is much more effective than following the recommendations of different nutrition councils who advise to increase food intake in the second half of pregnancy because of increased fetal needs. Each pregnant woman will tell you that she eats less in the last part of pregnancy because she feels already rather "full".

Naismith (1966, 1972, 1980) developed the concept of an anabolic and catabolic phase in the maternal organism during pregnancy. This concept was based upon experimental studies in pregnant rats, later extended to human studies, especially on fat and protein metabolism. The change-over from an anabolic towards a catabolic phase in maternal metabolism is brought

about by an altered hormonal environment due to the developing feto-placental unit. The anabolic phase would comprise the first months of pregnancy when fetal demands are relatively small. The anabolic phase is probably induced by the increased levels of progesterone, and during this period, protein and fat stores are deposited (Naismith and Fears, 1972). In the last months of pregnancy (i.e. the catabolic phase), these extra stores are mobilized again to support the increasing fetal needs. The increase in estrogen production by the feto-placental unit and probably human placental lactogen (HPL) may be responsible for the change from an anabolic towards a catabolic maternal metabolism.
This change in hormonal balance to support an optimal placental and, consequently, fetal development, directed by the feto-placental unit itself, can be considered as a typical homeorhetic control mechanism. The concept of an interaction of both homeostatic and homeorhetic regulation mechanisms in the partitioning of nutrients during pregnancy was first put forward by Bauman and Currie (1980). Homeorhesis, a term first introduced by Kennedy (1967), was defined as "the orchestrated change for the priorities of a physiological state, i.e. coordination of metabolism in various tissues to support a physiological state".

The changes observed in some vitamin status parameters during early pregnancy (the fall in vitamin blood levels) and, in the last trimester, the change in those parameters assumed to reflect body (tissue) stores are in favor of this concept. The results obtained from the regression analysis point in the same direction. The deposition of extra fat and protein stores is likely to be paralleled by an increased retention of fat-soluble vitamins and water soluble vitamins, respectively.

Animal experiments with rats have indicated that the amount of stored vitamin B6 (PLP) is associated with the size of the muscle protein compartment. This stored PLP becomes available only in a state of protein catabolism (Black et al., 1978). The reported reversal in the ratio between the 1.25-dihydroxy-vitamin D and 24.25-dihydroxy-vitamin D serum levels between the

maternal and fetal circulation (see section 1.3.2) may be another example of homeorhetic control.

The concept of an anabolic and catabolic stage in vitamin metabolism seems more attractive and realistic than the earlier concept of the fetus as a parasite of the maternal organism. The changes in maternal vitamin metabolism, like the resetting of vitamin blood levels, serve a mutual interest. This mechanism protects maternal stores from excessive losses via the urine as a result of the increased glomerular filtration rate, and it protects the fetus from excessive accumulation.

We stated that it was necessary for the human fetus to be an intelligent and modest parasite. It seems that the fetus has almost succeeded in reaching this goal. It is intelligent in a way that it creates stores of different nutrients during the first part of pregnancy. However, sometimes it still seems to behave like a simple parasite, as for instance, when iron or folacin are concerned. But mostly the fetus apart from being intelligent is also modest, reasonably and fairly sharing nutrients between itself and its mother. It can behave like this because, during pregnancy, the fetus with the aid of its placenta constantly resets maternal physiology for mutual interests.

Recommendations for further clinical and basic research

-Clinical

It can be concluded from our observations that, even after a normal pregnancy and puerperium, the iron, folacin and vitamin B6 stores are, more or less, depleted for a long time (more than 6 months) in a considerable part of the healthy population we studied. The question whether this depletion of stores should be treated and when is not easy to answer. One might argue: "It is a healthy group of women and they will probably refill their stores and this takes time". On the other hand one might argue: "Situations might occur within (half) a year postpartum in which it is preferable to have full stores of iron, folacin and vitamin B6, so why not try to prevent this by supplementation

through medication?" If one wants to supplement, the question is when? During pregnancy? This probably has the advantage that women are more motivated at this time, but it is not known whether this guarantees full stores postpartum.

A clinical study with or without supplementation during pregnancy or postpartum might answer these questions.

From a clinical point of view it would also be very interesting to repeat this study in parts of the world where alimentation during pregnancy is considered to be insufficient and to compare the results.

Another aspect which needs further research is what the effect will be of a marginal maternal iron, folacin and vitamin B6 at the end of pregnancy and in the first months postpartum on the contents of these and other constituents of breastmilk from lactating mothers.

-Basic research

From this study, it can be concluded that the birth centile of the newborn is not dependent on what a healthy Dutch mother eats during pregnancy. However, adequate intrauterine growth is depending on the availability of sufficient nutrients, so the key is the quantity and quality of nutrients that reach the placenta and are transported to the fetus. This is probably dependent on maternal metabolic changes, utero-placental blood flow and placenta transfer function and capacity. Research concerning intrauterine growth should be directed to that area. Other important determinants of intrauterine growth are factors which induce or impede growth; this will be the other direction for research to find an explanation of the variance in birth centile.

The control of maternal vitamin metabolism during pregnancy and the interaction of hormones and vitamins is another area for further research. Study of the changes in the partitioning of vitamins between the circulating blood and the tissues, as well as a study of vitamin excretion in relation to the changes in hormonal balance, especially in early pregnancy, may provide experimental evidence for the concept of an anabolic and catabolic stage of vitamin metabolism during (human) pregnancy.

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De doelstellingen van dit onderzoek waren:

1. het beschrijven van het verloop van vitaminen en ijzerstatus parameters tijdens een normale zwangerschap en tijdens de eerste 6 maanden na de bevalling;
2. te onderzoeken of veranderingen van de vitaminebloedspiegels tijdens de normale zwangerschap beschouwd kunnen worden als fysiologische aanpassingen in de zwangerschap;
3. te onderzoeken of de voeding tijdens de zwangerschap in overeenstemming is met de aanbevolen dagelijkse hoeveelheden;
4. te onderzoeken of voeding en biochemische parameters van de voedingstoestand tijdens de normale zwangerschap gerelateerd zijn aan de geboortegewichtspercentiel van het kind.

Dit onderzoek werd uitgevoerd bij een groep gezonde vrouwen die een normale zwangerschap doormaakten en spontaan bevielen van een voldragen gezond kind. Bloedmonsters werden afgenomen in de 16e, 28e en 34e zwangerschapsweek, tijdens de bevalling, op de 6e dag, in de 6e week en 6 maanden na de bevalling. Twee maal tijdens de zwangerschap, bij 16 en 34 weken, en 6 maanden postpartum werd een voedingsenquête afgenomen. Voor een beschrijving van de selectiecriteria, de gemeten parameters en de daarvoor gebruikte methoden, wordt verwezen naar hoofdstuk 2.

Vijfentachtig vrouwen voldeden aan de gestelde voorwaarden. Zeventig bevielen van een kind met een geboortegewicht tussen de 10e en 90e percentiel van de Nederlandse geboortegewichtscurve. Deze groep werd beschouwd als de S(tudie)-Referentie groep. Tien vrouwen bevielen van een kind met een geboortegewicht op of onder de 10e percentiel en vijf van een kind op of boven de 90e percentiel.

Gebaseerd op literatuurgegevens, waarvan een overzicht is gegeven in hoofdstuk 1 en de resultaten van het eigen onderzoek, die beschreven zijn in de hoofdstukken 3 tot en met 8, kunnen de doelstellingen als volgt worden beantwoord.

Doelstelling 1

In deel 1.3 werd een overzicht gegeven van de literatuur betreffende veranderingen in de vitaminestatus gedurende en na de zwangerschap. Hoewel de bevindingen nogal uiteenlopen werd geconcludeerd dat de meeste parameters van de vitaminestatus significant veranderen in de loop van de zwangerschap, vooral de vitaminebloedspiegels en de vitamine-excretie via de urine. Dergelijke veranderingen worden ook gevonden bij gezonde zwangeren met een ogenschijnlijk adequate voedselvoorziening. Veel van deze bevindingen zijn echter afkomstig van transversale studies, met de nadruk op de vitaminestatus aan het eind van de zwangerschap maar (longitudinale) gegevens over veranderingen na de bevalling ontbreken veelal, zeker over de periode na de zesde week postpartum. Er werd daarom een longitudinaal onderzoek uitgevoerd betreffende het verloop van een aantal parameters van de vitamine A, D, B6, B12, thiamine (B1), riboflavine (B2) en foliumzuurstatus tussen de 16e zwangerschapsweek en 6 maanden na de bevalling. De resultaten van de S-Referentiegroep zijn weergegeven en besproken in hoofdstuk 6 (deel 6.1). Zoals verwacht werd een daling gevonden voor het serum retinol (vitamine A), vitamine B12, foliumzuur en het plasma-PLP-(vitamine B6)-gehalte (Figuur 6.1.1; 6.1.8; 6.1.11-13). In geval van de serum retinol-, foliumzuur- en plasma-PLP-spiegel, waren de meeste gemeten waarden in de 16e zwangerschapsweek al laag ten opzichte van, voor niet zwangeren opgestelde, referentiewaarden. Dit is suggestief voor een daling reeds vroeg in de zwangerschap. De gemiddelde serum 25-OH-vitamine-D-spiegel bleef vrijwel onveranderd in de zwangerschap, echter de waarden waren steeds lager dan de waarden 6 maanden na de bevalling (Figuur 6.1.2). Het riboflavinegehalte in volbloed bleef constant, maar vertoonde een significante stijging aan het einde van de zwangerschap. Voor de enzymstimuleringstesten (de ETK (B1 statusparameter), EGR (B2 statusparameter) en EGOT (B6 statusparameter)) werden slechts kleine veranderingen waargenomen behalve een significant lagere basale ETK activiteit (Figuur 6.1.3).

Vergelijking van de gevonden waarden met referentiewaarden laat een stijging zien in het voorkomen van afwijkende waarden in het verloop van de zwangerschap voor vrijwel alle parameters, behalve het serum 25-OH-vitamine D, vitamine B12 en volbloed riboflavinegehalte (Tabel 6.1.28).

Tussen de vitaminestatus parameters bepaald in maternaal bloed, verzameld tijdens de bevalling, en in navelstrengbloed werd een significante relatie gevonden voor alle parameters, behalve voor de ETK stimuleringsratio (deel 6.2).

Het al of niet roken van de moeder had een significant effect op de resultaten met de EGOT stimuleringstest: hogere stimuleringsratio's (αEGOT) werden gevonden voor rooksters ten opzichte van niet-rooksters. Een dergelijk effect kon echter alleen in de zwangerschap worden aangetoond. Ook het volbloed riboflavinegehalte was significant lager in navelstrengbloed wanneer de moeder rookte, in vergelijking met dat wanneer de moeder niet rookte. Voor de andere parameters werd geen duidelijk rookeffect gevonden (deel 6.3).

Voor geen van de parameters werd een significant verschil tussen nulli- en multiparae aangetoond (deel 6.4).

De serum retinol, 25-OH-vitamine-D-spiegel en de EGOT stimuleringsratio vertoonden een seizoensafhankelijkheid (deel 6.5). Dit effect was het meest uitgesproken voor vitamine D, waarbij de hoogste waarden werden gevonden in de zomermaanden, de laagste in de winter en het vroege voorjaar (Figuur 6.5.2).

In de eerste 6 maanden na de bevalling vertoonden de meeste parameters een spontaan "herstel" van de door de zwangerschap geïnduceerde veranderingen, met uitzondering van het foliumzuurgehalte. Na de 6 maanden was het gemiddelde serum foliumzuurgehalte lager dan dat in de 16e zwangerschapsweek, terwijl in 45% van de gevallen een waarde beneden de ondergrens van het referentiegebied voor niet zwangeren werd gevonden. De gemiddelde plasma-PLP en serum retinol spiegels waren, 6 maanden na de bevalling, eveneens laag, echter hoger dan de gemiddelde spiegels gedurende enig tijdstip in de zwangerschap. In geval van het plasma-PLP-gehalte bedroeg het percentage afwijkende waarden nog steeds 25%.

IJzerstatusparameters.
Uit het literatuuroverzicht (deel 1.3.11) werd geconcludeerd dat er geen overeenstemming bestaat over de vraag of de maternale ijzerstatus tijdens de zwangerschap gevaar loopt. In recente studies, waarin serum ferritine als maat voor de ijzervoorraad werd gebruikt, vindt men echter aanwijzingen dat een zwangerschap de maternale ijzervoorraad vermindert. Uit de meeste studies blijkt geen verband tussen de ijzerstatus van moeder en kind, bepaald in navelstrengbloed bij de geboorte.

In dit onderzoek vonden wij een significante daling van het serumijzer, het ijzerverzadigingspercentage en het serum ferritine (deel 7.1). De totale ijzerbindingscapaciteit nam significant toe. Dit wijst op een vermindering van de maternale ijzervoorraad. Deze veranderingen van de ijzerstatus zouden echter ook door andere factoren, bijvoorbeeld mede door een verdunnningseffect als gevolg van het toegenomen plasmavolume, veroorzaakt kunnen worden. Na de zwangerschap namen wij een geringe stijging waar van het serum ijzergehalte, het verzadigingspercentage en het serum ferritine. De gemiddelde waarden 6 maanden na de bevalling waren echter significant lager dan de gemiddelde waarden tijdens de 16e zwangerschapsweek; voor serum ferritine en serumijzer respectievelijk 60 en 19%. Van de S-Referentie groep had 20% serum ferritinewaarden beneden de ondergrens (20 ng/ml) van de referentiewaarden voor niet zwangeren. Dit percentage was 5% in de 16e zwangerschapsweek. Deze lage waarden, in het bijzonder de waarden na de bevalling, duidden erop dat een zwangerschap en kraambed de maternale ijzervoorraad verminderen. Daarnaast duurt het meer dan 6 maanden voor de ijzervoorraad weer is aangevuld tot de hoeveelheid welke waarschijnlijk voor de zwangerschap aanwezig was bij gezonde vrouwen met een gemiddelde geschatte ijzeropname uit de voeding van 13 mg per dag.

Er werd geen verband gevonden tussen de ijzerstatus van de vrouw en van het kind bij de geboorte. De ijzerstatusparameters van het kind, gemeten in navelstrengbloed, waren significant hoger dan die van de moeder (deel 7.3).

Conclusies doelstelling 1:

1.1. de P10 en P90 waarden van de bepalingen van de vitamine-status parameters bij deze groep gezonde zwangeren kunnen dienen als referentiewaarden;

1.2. het vaker voorkomen van lage foliumzuur en vitamine B6 (PLP) serum (plasma) spiegels 6 maanden na de bevalling is suggestief voor een (gedeeltelijke) depletie van de maternale foliumzuur en (in mindere mate) de vitamine B6 voorraad gedurende de zwangerschap;

1.3. een normale zwangerschap en ongecompliceerd kraambed depleert, althans gedeeltelijk, bij gezonde vrouwen de ijzervoorraad indien de voeding gemiddeld 13 mg ijzer per dag bevat;

1.4. het aanvullen van de maternale ijzer, foliumzuur en vitamine-B6-voorraad tot waarden welke waarschijnlijk voor de zwangerschappen bestonden, duurt tenminste 6 maanden;

1.5. tussen de maternale ijzerstatusparameters, bepaald tijdens de bevalling en die van het kind, bepaald in navelstrengbloed, kon geen verband worden aangetoond. Een dergelijk verband bestond wel voor de vitaminestatus parameters, behalve voor de ETK stimuleringsratio (αETK).

Doelstelling 2.

Omtrent de interpretatie van de waargenomen veranderingen in vitaminestatus parameters, die zich in de loop van de zwangerschap voordoen, bestaat, zoals werd geconcludeerd in het literatuuroverzicht, geen overeenstemming (deel 1.3). Het ontbreken van een duidelijke relatie tussen de veranderingen in de biochemische parameters van de vitaminestatus enerzijds en het verloop en uitkomst van de zwangerschap anderzijds, zoals kan worden afgeleid uit de meeste beschrijvende en experimentele studies, ondersteunt het idee van een "fysiologische" aanpassing aan de zwangerschap. In andere onderzoeken echter, vindt men aanwijzingen dat een mogelijk niet optimale maternale vitaminestatus kan interfereren met de foetale ontwikkeling

(deel 1.3). De veranderingen van de meeste vitaminestatus parameters in de zwangerschap, zoals met name de daling van de meeste bloedspiegels, suggereert een depletie van de maternale voorraden. Dergelijke veranderingen doen zich echter evenwel ook voor bij gezonde, goed gevoede zwangeren, zelfs indien vitamines werden gesuppleerd. De mate waarin en de snelheid waarmee de vitaminestatus parameters na de bevalling spontaan "herstellen" van de door de zwangerschap geïnduceerde veranderingen, werden in dit onderzoek als criterium gebruikt om deze veranderingen te interpreteren als een al dan niet fysiologische aanpassing. Tevens kan hierdoor een indruk worden verkregen over de zogenaamde "vitamin cost of pregnancy". Zoals aangegeven in hoofdstuk 6 (deel 6.1 en 6.7) werd een dergelijk herstel voor de meeste parameters gevonden met uitzondering van het serum foliumzuur en in mindere mate het plasma PLP gehalte. Herstel van deze parameters na de zwangerschap kost kennelijk meer dan 6 maanden. De veranderingen in vitaminestatus parameters worden, zoals gezegd, soms toegeschreven aan aanpassingen in het "milieu interieur" van de zwangere, zoals de toename van het circulerend bloedvolume, veranderingen in orgaanfunctie en hormonale balans, etc. (deel 1.3). Over deze determinanten zijn echter weinig experimentele gegevens beschikbaar. Om een inzicht te verkrijgen in de determinanten van de daling tussen de 16e en 34e zwangerschapsweek voor een aantal vitamine serum (plasma) spiegels, werd multipele regressie-analyse uitgevoerd (zie deel 2.7) met een aantal voedings-, klinisch-chemische, hormonale en antropometrische parameters als onafhankelijke variabelen (deel 6.6). Deze onafhankelijke variabelen staan vermeld in Tabel 6.6.5. De resultaten kunnen als volgt worden samengevat:

- voor alle bij deze analyse betrokken vitaminen (A, B6 (PLP), B12 en foliumzuur) werd een zeer significant negatief verband gevonden tussen de daling in het serum of plasmagehalte tussen de 16e en 34e week en de initiële waarde in week 16. Na invoeren van deze beginwaarde werd tussen de 40 en 70% van de variantie verklaard;
- aan bloed- of plasmavolume gerelateerde parameters verklaarden slechts in geringe mate de optredende variantie ingeval van

het plasma-PLP-gehalte, terwijl voor serum retinol en foliumzuur een dergelijk verband niet kon worden aangetoond;
- de vitamine opname uit de voeding is niet geassocieerd met de veranderingen in de vitamine bloedspiegels gedurende de zwangerschap;
- hormonale variabelen vertoonden een sterke correlatie met de daling in het plasma foliumzuurgehalte. In mindere mate wordt een dergelijke associatie gevonden voor serum retinol en vitamine B12.

Conclusies vraagstelling 2:

2.1. veranderingen in vitaminestatus parameters gedurende een normale zwangerschap dienen inderdaad beschouwd te worden als een fysiologische aanpassing aan de zwangerschap;

2.2. de resultaten van de regressie-analyse duiden op een hormonaal geïnduceerde bijstelling van de vitamine bloed (serum) spiegel als onderdeel van een gewijzigde regulatie van het vitaminemetabolisme in de zwangerschap.

Doelstelling 3.

Gegevens over de gebruikelijke voedselconsumptie gedurende de zwangerschap en 6 maanden na de bevalling staan vermeld in hoofdstuk 5. In de loop van de zwangerschap blijkt zich een kleine verandering in het consumptiepatroon voor te doen: een wat hogere consumptie van melkproducten en fruit, gepaard aan een wat lager gebruik van vlees en vleesproducten. De gemiddelde energetische opname uit de voeding is in het begin van de zwangerschap significant hoger dan aan het eind en 6 maanden na de bevalling, respectievelijk 10,0 $\pm$ 1,9 MJ (16e week), 9,4 $\pm$ 2,2 MJ (34e week) en 9,1 $\pm$ 2,4 MJ (6 maanden postpartum). De opname van micronutriënten uit de voeding is eveneens hoger in de zwangerschap. Dit blijkt met name uit de geschatte opnames in de 16e week. Alleen voor calcium, thiamine (B1), riboflavine (B2), vitamine B12 en C zijn de verschillen tussen de 34e week en 6 maanden postpartum nog significant (Tabel 5.1).

Op geen van de onderzoeksmomenten konden significante verschillen in nutriëntenopname als gevolg van roken, pariteit of seizoensvariatie worden aangetoond met uitzondering van een significant hogere koolhydraat- en vitamine-C-opname voor nulliparae en alcoholconsumptie voor rooksters (deel 5.2).

Vergelijking van deze resultaten met aanbevelingen van de Nederlandse en Amerikaanse Voedingsraad (NAS/NRC) geeft aan dat het voedingspatroon van onze studiepopulatie over het geheel genomen als adequaat kan worden beschouwd (Tabel 5.9; Figuur 5.2 en 5.3). De relatieve bijdrage van vet en van mono- en disacchariden aan de totale energetische opname is echter wat aan de hoge kant. De gemiddelde ijzer-, vitamine B6- en D-opname liggen duidelijk onder de Amerikaanse normen. Helaas ontbreken gegevens over de foliumzuuropname, aangezien gegevens over het foliumzuurgehalte van voedingsmiddelen vooralsnog ontbreken in de gebruikte voedingsmiddelentabel (UCV Tabel) (deel 1.2.4 en 2.3). Het is echter niet onwaarschijnlijk dat ook de foliumzuur-opname uit de voeding laag was. Vergelijkbare studies duiden op een gemiddelde opname van $\pm$ 100 µg/dag, terwijl de WHO en de NAS/NRC hoeveelheden van 400 µg/dag aanbevelen voor zwangeren (deel 1.2.4). Op basis van biochemische parameters van de voedingstoestand werden in dit onderzoek aanwijzingen gevonden voor een (partiële) ijzer-, foliumzuur- en in mindere mate ook vitamine B6-depletie gedurende de zwangerschap (conclusies doelstelling 1 en 2). Deze biochemische bevindingen zouden als een aanvullend bewijs kunnen dienen voor een kennelijk ontoereikende ijzer-, vitamine B6- en mogelijk ook foliumzuur-voorziening uit de dageljkse voeding van zwangeren. Zoals aangegeven in deel 6.6. en 6.7 kon echter geen direct lineair verband worden aangetoond tussen de berekende nutriëntenopname uit de voeding en de biochemische parameters. Dit kan een gevolg zijn van intrinsieke onnauwkeurigheden in beide methoden, maar het voorkomen van abnormale waarden voor de betreffende parameters 6 maanden na de bevalling kan ook een gevolg zijn van reeds gedepleerde maternale voorraden voor de conceptie.
Ook voor vitamine D lag de gemiddelde opname onder de aanbevelingen. Hoewel echter gedurende de zwangerschap licht

verlaagde 25-OH-vitamine D serumspiegels werden gemeten, lagen de waarden zowel tijdens als na de zwangerschap voor alle proefpersonen steeds binnen het voor niet-zwangeren vastgestelde referentiegebied. Vitamine D is een essentiële nutriënt in situaties wanneer expositie van de huid aan zonlicht (UV-straling) onvoldoende of zelfs geheel afwezig is (deel 1.3.2). Uit deze gegevens blijkt dat voor onze onderzoeks-populatie de endogene vitamine D-productie en de vitamine D-opname uit de voeding kennelijk voldoende waren om de maternale voorraden tijdens en na de zwangerschap op peil te houden.

Conclusies doelstelling 3:

3.1. de geschatte opname van vitamine D, B6, ijzer en mogelijk ook foliumzuur is lager dan de aanbevolen dagelijkse hoeveelheden;

3.2. de marginale opname uit de voeding van ijzer, vitamine B6 en waarschijnlijk ook foliumzuur lijkt bevestigd te worden door de biochemische bevindingen met name in de tijd na de zwangerschap, alhoewel geen directe relatie kon worden aangetoond. De endogene vitamine D-productie bleek met het vitamine D opgenomen uit de voeding, in staat de vitamine D status tijdens en na de zwangerschap op peil te houden.

Doelstelling 4.

Uit het literatuuroverzicht kunnen geen duideljke conclusies worden getrokken (deel 1.2.5, 1.2.6 en 1.5). Enerzijds lijkt intra-uteriene groei grotendeels onafhankelijk te zijn van de maternale voeding, anderzijds wordt voeding regelmatig als mogelijke oorzaak genoemd van onverklaarde dysmaturiteit, zelfs in landen waar geen voedseltekort is. Ook wordt een verband verondersteld tussen de maternale gewichtsstijging en de energetische opname uit de voeding. Het positieve verband tussen gewichtsstijging en geboortegewicht wordt wel als bewijs aangevoerd dat voeding het geboortegewicht mede bepaalt.

In deel 8.2 is het verband beschreven tussen enkele algemene

parameters van de ouders en de geboortepercentiel van het kind. Aangezien roken van deze algemene parameters de hoogste correlatie had met de geboortepercentiel, is in deel 8.3 uitgewerkt in hoeverre roken de andere gemeten parameters beïnvloedt.

In deel 1.8.4 is het verband tussen de geboortepercentiel en de dagelijkse energetische opname en de opname aan vet, koolhydraten en eiwit beschreven. Het betreft de totale dagelijkse opname, de opname per kg lichaamsgewicht op het moment van het voedingsonderzoek en de opname per kg lichaamsgewicht voor de zwangerschap. Er werd een significant negatief verband waargenomen tussen de geboortepercentiel en de energetische opname en de opname van vet en koolhydraten per kg lichaamsgewicht tijdens en voor de zwangerschap (Tabellen 8.4.I-III). Deze onverwachte bevinding kan waarschijnlijk verklaard worden uit het feit dat er geen verschil in energetische opname en opname van vet en koolhydraten gevonden werd tussen de groep vrouwen met een kind tussen de 10e en 90e geboortepercentiel en de groep vrouwen met een kind beneden de 10e percentiel. Bovendien bleek er eveneens geen verschil in de geschatte opname van macronutriënten tussen niet-rooksters en rooksters, terwijl de gemiddelde geboortepercentiel in de rookgroep significant lager was (deel 5.2 en 8.3).

Ook kon geen verband aangetoond worden tussen de maternale gewichtsstijging tijdens de zwangerschap en de energetische opname van macronutriënten (Tabel 8.4.V).

Wat betreft het verband tussen de biochemische parameters van de voedingstoestand en de geboortepercentiel werden de volgende waarnemingen gedaan:

- vitaminestatus parameters, behalve die voor vitamine B6, bleken niet gerelateerd. De positieve correlatie tussen een vitamine B6-parameter (αEGOT) tijdens de zwangerschap en de geboortepercentiel bleek echter waarschijnlijk samen te hangen met roken;
- negatieve correlaties werden waargenomen met het hemoglobinegehalte en de hematocrit in de 34e zwangerschapsweek (deel 8.6);

- negatieve correlaties werden eveneens gevonden met de serum ijzerwaarden in de 16e en 34e week (deel 8.7). Deze negatieve correlaties zijn mogelijk gedeeltelijk te verklaren door de optredende hemodilutie tijdens de zwangerschap.

In deel 8.9 is beschreven welk percentage van de variantie in geboortepercentiel en Ponderal-index van het kind verklaard kon worden met de in dit onderzoek gemeten parameters. De statistische methoden staan beschreven in deel 2.7, de geselecteerde variabelen in Tabel 8.1. Ongeveer 35% van de variantie in geboortepercentiel kon worden verklaard. Slechts 2 variabelen, het maternale plasmavolume en roken, droegen significant bij. De correlaties tussen geboortepercentiel en het gewicht voor de zwangerschap, de lengte en gewichtsstijging tijdens de zwangerschap van de moeder (deel 8.2) verdwenen, wanneer het plasmavolume werd toegevoegd. Gewichtsstijging en toename van plasmavolume tijdens de zwangerschap worden mogelijk door dezelfde mechanismen geïnduceerd. Het verband tussen plasmavolume en lengte en gewicht voor de zwangerschap suggereert dat plasmavolume tijdens en na de zwangerschap met elkaar samenhangen. Wij konden echter geen verband aantonen tussen de geboortepercentiel en het plasmavolume 6 maanden na de zwangerschap. Het blijft de vraag of het plasmavolume en/of de toename van het plasmavolume, de groei en de grootte van de foeto-placentaire eenheid beïnvloedt of dat de foeto-placentaire eenheid de toename van het plasmavolume bepaalt. Roken heeft een duidelijk oorzakelijke negatieve invloed op de geboortepercentiel, waarbij echter geen significant verschil in geboortepercentiel werd waargenomen tussen matige en zware rooksters (deel 8.3). De Ponderal-index is een maat voor het verband tussen lengte en gewicht, met andere woorden, deze onderscheidt tussen gelijkmatige en ongelijkmatige (intra-uteriene) groei. Ongeveer 17% van de variantie in Ponderal-index kon worden verklaard. Slechts één variabele, roken, droeg hieraan significant bij en was negatief gecorreleerd. Er werd geen significant verschil in Ponderal-index gevonden tussen kinderen van niet-rooksters en matige rooksters, wèl tussen die van niet-rooksters en zware rooksters (deel 8.3). Dit lijkt te

wijzen op een verband tussen het aantal gerookte sigaretten en ongelijkmatige intra-uteriene groei. Uit deze waarnemingen kan voorzichtig worden geconcludeerd dat plasmavolume gecorreleerd is aan "gelijkmatige" groei, dat roken een "gelijkmatige groeivertraging" (lagere geboortepercentiel) veroorzaakt en dat veel roken daarnaast ook een "ongelijkmatige groei" (lagere Ponderal-index) induceert.

Conclusies doelstelling 4:

4.1. geen verband kon worden aangetoond tussen de geboorte-gewichtspercentiel en de energetische opname en de opname van macronutriënten uit de voeding bij gezonde vrouwen die van een gezond voldragen kind bevielen;

4.2. er kon geen verband worden aangetoond tussen de maternale gewichtsstijging tijdens de zwangerschap en de energetische opname en de opname van macronutriënten;

4.3. er werd geen duidelijk verband aangetoond tussen de geboortepercentiel en de biochemische parameters van de voedingstoestand van de vrouw;

4.4. het maternale plasmavolume en roken zijn de variabelen welke significant bijdroegen aan de variantie van de geboortepercentiel. Alleen roken heeft een duidelijk oorzakelijk verband;

4.5. de enige variabele welke significant bijdroeg aan de variantie van de Ponderal-index is (veel) roken;

4.6. het grootste deel van de variantie in geboortepercentiel (65%) en de Ponderal-index (83%) kon niet worden verklaard met de in dit onderzoek gemeten variabelen.

CURRICULUM VITAE

Henk van den Berg werd geboren op 28 juni 1947 te Ede (Gld).
Het eindexamen HBS-B werd in 1965 behaald aan het Christelijk Streeklyceum te Ede.
Hij studeerde scheikunde (S_2) aan de Vrije Universiteit in Amsterdam en legde in 1971 het doctoraal examen af (hoofdvak Biochemie, bijvakken Klinische Chemie en Analytische Chemie).
Hij was van 1972 tot 1975 als wetenschappelijk medewerker verbonden aan het Laboratorium voor Ontwikkelingsbiochemie (hoofd Dr.F.A.Hommes) van de afdeling Kindergeneeskunde van het Academisch Ziekenhuis te Groningen (hoofd Prof.Dr.J.H.P.Jonxis).
Sinds 1 februari 1975 is hij als biochemicus werkzaam op het CIVO-Instituut voor Toxicologie en Voeding-TNO te Zeist bij de afdeling Klinische Biochemie (hoofd Dr.W.H.P.Schreurs).
Hij is in 1971 getrouwd met Heleen Maat en vader van drie dochters: Corine, Leontine en Nijnke.

CURRICULUM VITAE

Hein Bruinse is op 19 april 1946 te Gorinchem geboren.
In 1964 werd het eindexamen gymnasium-β afgelegd aan het Stedelijk Gymnasium te Utrecht.
Van 1964 tot 1971 studeerde hij geneeskunde aan de Rijksuniversiteit te Utrecht. In mei 1971 werd het artsexamen afgelegd.
Als voorbereiding op uitzending naar de tropen bracht hij tien maanden door op de afdelingen chirurgie en gynaecologie van het ziekenhuis "De Lichtenberg" te Amersfoort en drie maanden op het Koninklijk Instituut voor de Tropen te Amsterdam. Van augustus 1972 tot oktober 1975 was hij als algemeen arts in dienst van de Keniaanse regering.
Hij begon zijn opleiding tot vrouwenarts in december 1975 in het R.K.-ziekenhuis te Sittard (hoofd Dr.A.M.C.M.Schellen) en vanaf mei 1977 in de kliniek voor Obstetrie en Gynaecologie van het Academisch Ziekenhuis te Utrecht (hoofd Prof.Dr.A.A.Haspels).
Sinds februari 1981 is hij als gynaecoloog op de afdeling Obstetrie van deze kliniek werkzaam.
Hij is gehuwd met Agnes Tigelaar en vader van een dochter, Sandra, en een zoon, Merijn.

TABLES AND FIGURES

CHAPTER 3

TABLE 3.1. General data concerning the mothers in the different groups. Mean, standard deviation and range are given.

		S-Reference group n = 70	P<10 group n = 10	P>90 group n = 5
Age (years)	$\bar{x}$	24.9	23.7	24.4
	SD	3.4	2.6	2.4
	Range	19-36	21-28	22-27
Height (cm)	$\bar{x}$	167	162	169
	SD	5.1	3.8	6.7
	Range	152-182	156-166	159-176
Prepregnant weight (kg)	$\bar{x}$	62.2	56	66.2
	SD	8.1	10.4	14.2
	Range	48-86	42.5-78.5	50-89
Partner's height (cm)	$\bar{x}$	177	175	180
	SD	7.1	8.5	10.7
	Range	164-194	164-186	166-195
Maximal weight gain (kg)	$\bar{x}$	11.5	9.7	14.5
	SD	3.2	4.8	4.1
	Range	4.2-19.6	4.3-19.5	8.5-19.4
Duration of pregnany (days)	$\bar{x}$	281	275	284
	SD	8	10	7
	Range	269-293	260-287	276-293
Blood loss during delivery (ml)	$\bar{x}$	388	288	360
	SD	212	135	152
	Range	100-1000	150-600	200-600

FIGURE 3.1. Weight before, during and after pregnancy of women from the S-Reference group (10th, 50th and 90th centile of measurements are shown). Open circles represent the median of the < P10 group.

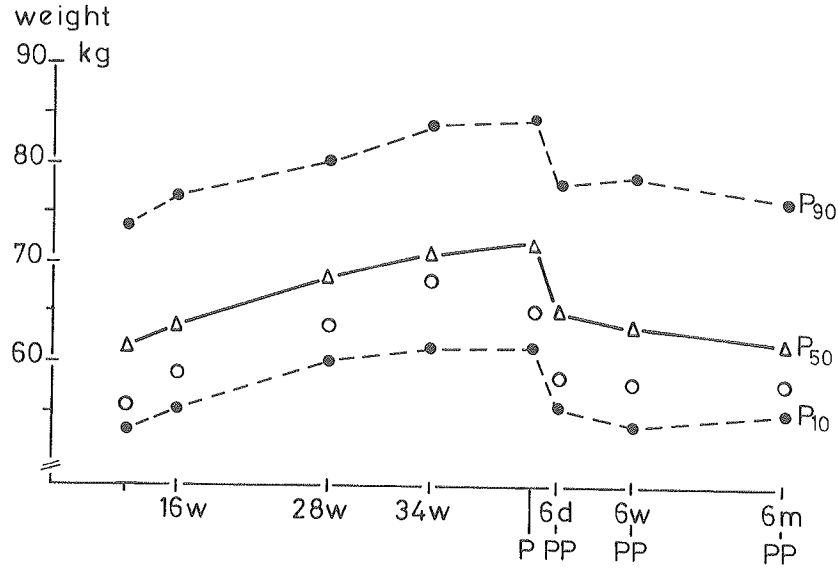


TABLE 3.2. Number, mean, S.D. and range of the weight from the S-Reference group before, during and after pregnancy. Number, mean and S.D. of the weight from the < P10 group and number and median of the weight from the > P90 group.

		0	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
Reference group	N	70	69	69	69	70	61	64	63
	$\bar{x}$	62.2	64.1	69.4	72.3	73.3	65.4	64.6	64
	SD	8.1	8.3	8.3	8.5	8.6	8.9	8.3	8.7
	Min.	48	48.9	53	55.1	55.1	47.7	47.3	47.4
	Max.	86	84	90.6	95.6	96.3	87.8	85.4	91.7
< P10 group	N	10	10	10	9	10	10	10	9
	$\bar{x}$	56	58.4	63	67.3	65.1	59.3	58.6	58.8
	SD	10.4	11.6	12.6	11.9	12.8	13.1	12.4	12.3
> P90 group	N	5	5	5	5	5	5	5	3
	Med.	65	69.1	74.8	77.6	81.8	71	68.4	66.1

TABLE 3.3. General data concerning the mothers in the different groups. Absolute numbers and percentages (in parentheses) are given.

		S-Reference group n = 70	P<10 group n = 10	P>90 grou n = 5
Gravidity	1	32 (45.7)	4 (40)	1 (20)
	2	29 (41.4)	5 (50)	3 (60)
	3	7 (10)	1 (10)	1 (20)
	4	2 (2.9)	0 -	0 -
Parity	0	39 (55.7)	5 (50)	4 (80)
	1	28 (40)	4 (40)	1 (20)
	2	2 (2.9)	1 (10)	0 -
	3	1 (1.4)	0 -	0 -
Smoking	nil.	37 (52.9)	0 -	3 (60)
	1-10 cig/day	15 (21.4)	3 (30)	1 (20)
	> 10 cig/day	18 (25.7)	7 (70)	1 (20)
Iron medication	nil.	50 (71.4)	9 (90)	3 (60)
	During pregnancy	7 (10)	1 (10)	0 -
	After pregnancy	10 (14.3)	0 -	2 (40)
	During and after pregnancy	3 (4.3)	0 -	0 -
Breast feeding	nil.	37 (52.9)	6 (60)	4 (80)
	2 weeks	5 (7.1)	2 (20)	0 -
	2-6 weeks	16 (22.8)	1 (10)	0 -
	> 6 weeks	12 (17.2)	1 (10)	1 (20)
Contraception postpartum	O.C.	47 (67.1)	7 (70)	4 (80)
	Depot provera	6 (8.6)	1 (10)	0 -
	I.U.D.	2 (2.9)	1 (10)	0 -
	None/other	15 (21.4)	1 (10)	1 (20)

TABLE 3.4. General data concerning the newborn and placenta in the different groups. Numbers, percentages (in parentheses), mean, standard deviation and range are given.

		S-Reference group n = 70	P<10 group n = 10	P>90 group n = 5
Sex	Male	29 (41.4)	3 (30)	3 (60)
	Female	41 (58.6)	7 (70)	2 (40)
Weight g	$\bar{x}$	3413	2647	4246
	SD	357	333	229
	Range	2800-4220	2030-3040	4030-4600
Weight g	Male	3484	-	-
	Female	3362	-	-
Length cm	$\bar{x}$	49.6	47.3	51.7
	SD	1.5	1.8	2.0
	Range	46-53	44-50	49.5-54
Kloosterman birth centile	$\bar{x}$	43.5	6.5	94.7
	SD	24.1	2.7	3.4
	Range	10.9-89.7	1.7-9.7	2.92-3.44
Ponderal index	$\bar{x}$	2.8	2.49	3.11
	SD	0.24	0.18	0.25
	Range	2.34-3.42	2.23-2.78	2.92-3.44
Placental weight g	$\bar{x}$	471	369	560
	SD	88	69	38
	Range	310-700	260-500	525-600
Placental index	$\bar{x}$	0.14	0.14	0.13
	SD	0.024	0.013	0.007
	Range	0.11-0.25	0.11-0.16	0.12-0.14

TABLE 3.5. Conception months of the different groups. The year is divided in summer and winter periods.

	SUMMER			WINTER			
	S-Reference group	< P10 group	> P90 group	S-Reference group	< P10 group	> P90 group	
April	6	0	1	5	3	1	October
May	7	0	1	8	0	0	November
June	4	2	0	5	1	0	December
July	6	0	1	4	1	1	January
August	6	0	0	8	1	0	February
September	7	1	0	4	1	0	March
Total	36	3	3	34	7	2	Total

TABLES AND FIGURES

CHAPTER 4

FIGURE 4.1 Total bloodvolume of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurement are shown). Open circles represent the median of the < P10 group. NP: mean non-pregnant reference value.

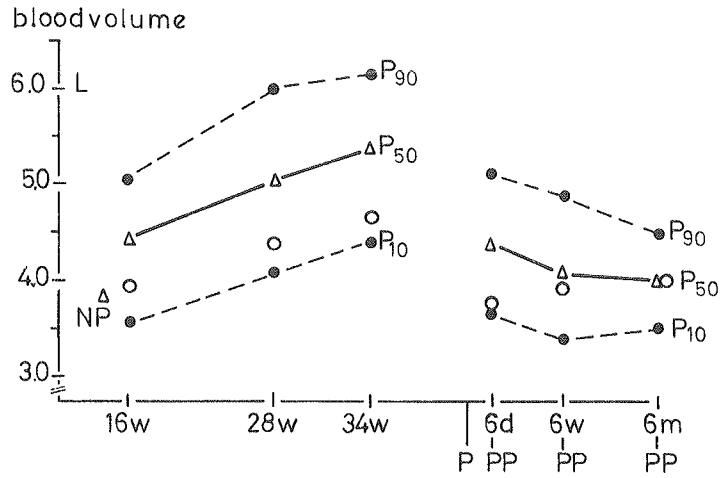


TABLE 4.1 Number, mean, SD and range of total bloodvolume measurements from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	69	68	70	-	55	63	44
$\bar{x}$	4.398	5.103	5.270	-	4.382	4.084	4.014
SD	0.548	0.678	0.710	-	0.651	0.513	0.491
Min.	3.050	3.810	3.770	-	2.410	3.080	2.870
Max.	5.810	6.710	7.030	-	6.070	5.190	5.450

TABLE 4.2 Significance of differences of bloodvolume measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
16 w	0	+	+	-	NS	+	+
28 w		0	+	-	ND	ND	ND
34 w			0	-	ND	ND	ND
P				0	-	-	-
6dpp					0	+	+
6wpp						0	NS

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

-: no measurement done

FIGURE 4.2 Total plasmavolume of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurement are shown). Open circles represent the median of the < P10 group. NP: mean non-pregnant reference value.

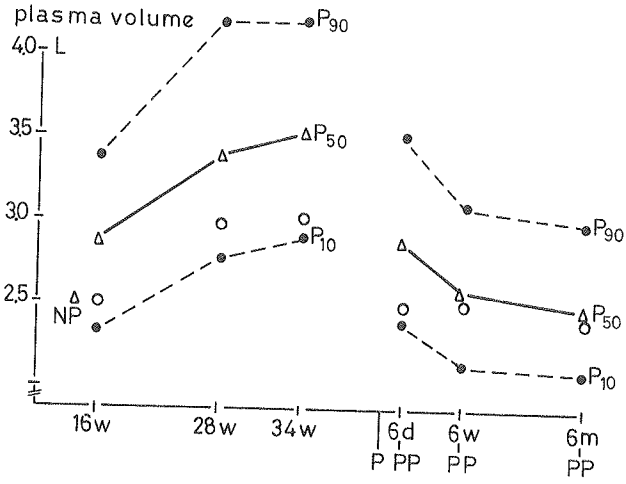


TABLE 4.3 Number, mean, SD and range of total plasmavolume measurements from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	69	68	70	-	55	63	44
$\bar{x}$	2.895	3.450	3.554	-	2.909	2.584	2.484
SD	0.383	0.485	0.486	-	0.471	0.380	0.315
Min.	2.080	2.430	2.470	-	1.510	1.800	1.600
Max.	3.970	4.400	4.690	-	4.190	3.920	3.200

TABLE 4.4 Significance of differences of plasmavolume measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
16 w	0	+	+	-	NS	+	+
28 w		0	+	-	ND	ND	ND
34 w			0	-	ND	ND	ND
P				0	-	-	-
6dpp					0	+	+
6wpp						0	+

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

-: no measurement done

FIGURE 4.3 Total red cell volume of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurement are shown). Open circles represent the median of the < P10 group. NP: mean non-pregnant reference value.

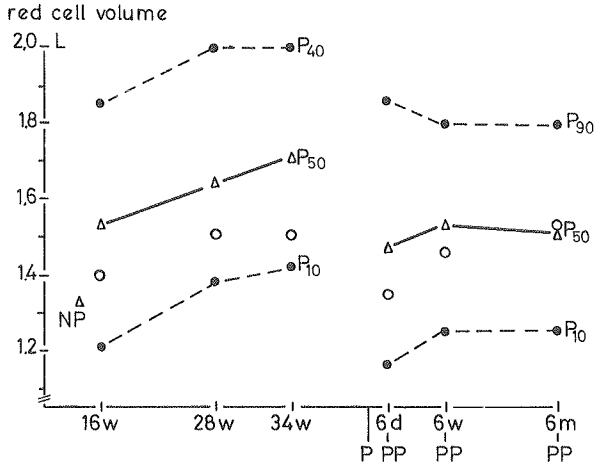


TABLE 4.5 Number, mean, SD and range of total red cell volume measurements from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	69	68	70	-	55	63	44
$\bar{x}$	1.521	1.670	1.750	-	1.494	1.527	1.533
SD	0.242	0.237	0.429	-	0.256	0.219	0.239
Min.	0.970	1.180	1.290	-	0.900	1.110	1.080
Max.	2.170	2.310	2.430	-	2.060	2.040	2.230

TABLE 4.6 Significance of differences of red cell volume measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
16 w	0	+	+	-	NS	NS	NS
28 w		0	+	-	ND	ND	ND
34 w			0	-	ND	ND	ND
P				0	-	-	-
6dpp					0	+	+
6wpp						0	NS

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

-: no measurement done

FIGURE 4.4 Hemoglobin levels of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference value.

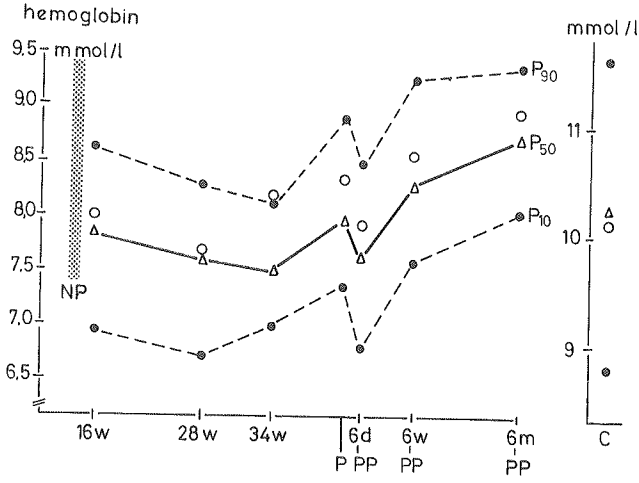


TABLE 4.7 Number, mean, SD and range of hemoglobin levels in maternal and in cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	61	60	64	62	33
$\bar{x}$	7.8	7.5	7.52	8.1	7.6	8.3	8.7	10.2
SD	0.7	0.64	0.5	0.6	0.7	0.54	0.6	0.9
Min.	6.2	5.2	6.2	6.8	5.6	7.5	6.8	8.0
Max.	9.2	8.9	8.8	9.5	9.1	9.6	10.5	12.0

TABLE 4.8 Significance of differences of hemoglobin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	NS	+	+	+	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $P < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.5 Hematocrit levels of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurement are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

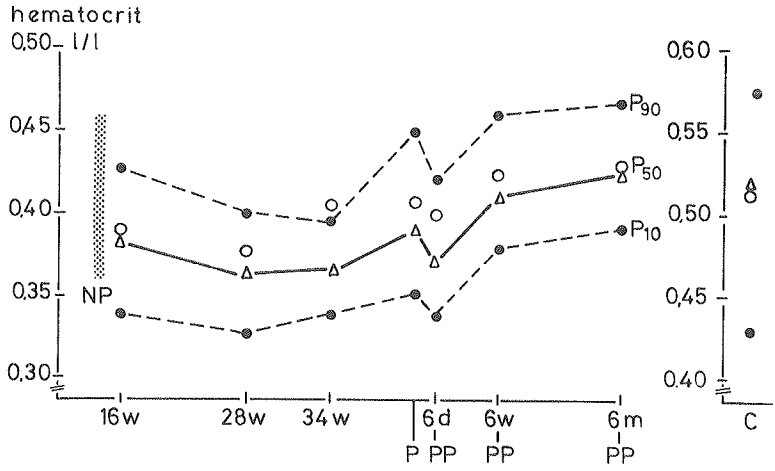


TABLE 4.9 Number, mean, SD and range of hematocrit measurements of maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	62	60	64	62	33
$\bar{x}$	0.38	0.36	0.367	0.395	0.373	0.412	0.424	0.51
SD	0.034	0.028	0.021	0.028	0.034	0.028	0.029	0.051
Min.	0.31	0.28	0.33	0.34	0.28	0.36	0.33	0.40
Max.	0.47	0.43	0.44	0.46	0.46	0.48	0.50	0.63

TABLE 4.10 Significance of differences of hematocrit measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	NS	NS	+	+	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $P < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.6 Number of erythrocytes of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference group.

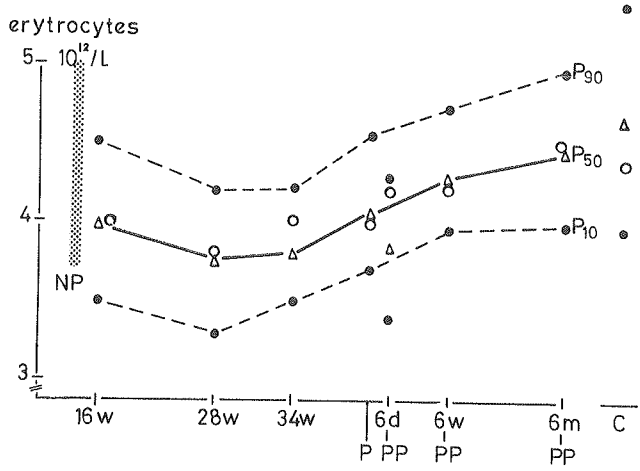


TABLE 4.11 Number, mean, SD and range of erythrocytes in maternal and in cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	62	60	64	62	33
$\bar{x}$	3.97	3.72	3.81	4.07	3.83	4.28	4.44	4.66
SD	0.34	0.34	0.31	0.35	0.43	0.30	0.39	0.53
Min.	3.2	2.9	3.0	3.5	2.6	3.5	3.6	3.9
Max.	4.8	4.8	4.9	5.1	5.5	4.9	5.8	5.9

TABLE 4.12 Significance of differences of erythrocytes measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	NS	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $P < 0.05$

NS: not significant

ND: no significance determined

TABLE 4.13. Number, mean and SD of blood-, plasma-, red cell volume, hemoglobin, hematocrit and erythrocyte measurements in maternal and cord blood from the < P10 group and number and median of these measurements in maternal and cord blood from the > P90 group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Blood volume	N	9	9	9	-	9	9	5	-
< P10	$\bar{x}$	4.009	4.541	4.681	-	3.941	3.928	3.990	-
	SD	0.659	0.607	0.604	-	0.634	0.454	0.697	-
	N	5	5	5	-	5	5	3	-
> P90	Med	4.640	6.070	6.220	-	4.940	4.300	3.780	-
Plasma volume	N	9	9	9	-	9	9	5	-
< P10	$\bar{x}$	2.591	3.032	3.040	-	2.542	2.448	2.378	-
	SD	0.350	0.452	0.412	-	0.384	0.235	0.291	-
	N	5	5	5	-	5	5	3	-
> P90	Med	3.080	3.970	4.420	-	3.190	2.720	2.440	-
Red cell vol.	N	9	9	9	-	9	9	5	-
< P10	$\bar{x}$	1.418	1.509	1.616	-	1.399	1.480	1.612	-
	SD	0.332	0.230	0.280	-	0.321	0.247	0.423	-
	N	5	5	5	-	5	5	3	-
> P90	Med	1.560	1.910	2.070	-	1.640	1.580	1.430	-
Hemoglobin	N	10	10	9	10	9	10	9	7
< P10	$\bar{x}$	7.9	7.7	8.1	8.3	8.0	8.4	8.8	10.1
	SD	0.48	0.71	0.69	0.50	1.03	0.66	0.97	1.04
	N	5	5	5	3	5	5	3	1
> P90	Med	7.9	7.5	7.4	7.7	7.1	8.2	8.6	-
Hematocrit	N	10	10	9	10	9	10	9	7
< P10	$\bar{x}$	0.38	0.37	0.39	0.40	0.39	0.42	0.43	0.50
	SD	0.023	0.031	0.032	0.024	0.047	0.026	0.037	0.071
	N	5	5	5	3	5	5	3	1
> P90	Med	0.38	0.36	0.37	0.38	0.35	0.41	0.40	-
Erythrocytes	N	10	10	9	9	9	10	9	7
< P10	$\bar{x}$	3.99	3.74	3.97	4.19	3.96	4.22	4.37	4.47
	SD	0.31	0.30	0.37	0.39	0.56	0.38	0.40	0.36
	N	5	5	5	3	5	5	3	1
> P90	Med	3.8	3.8	3.9	4.0	3.6	4.3	4.3	-

FIGURE 4.7 Mean Corpuscular Volume (MCV) of red cells of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

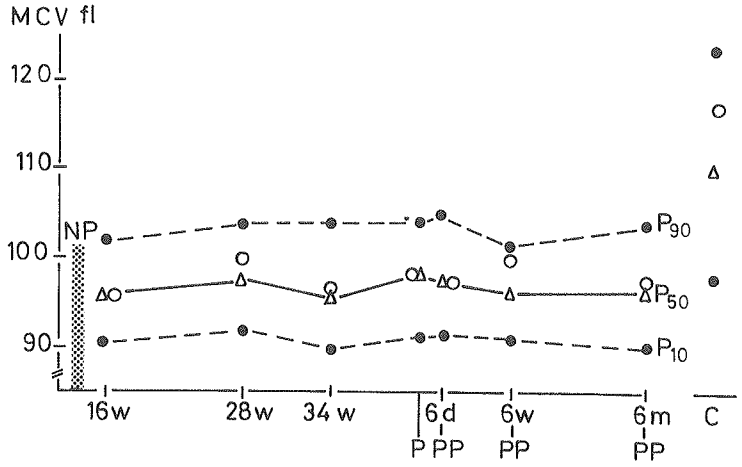


TABLE 4.14 Number, mean, SD and range of the MCV in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	61	60	64	62	33
$\bar{x}$	96.3	97.8	96.6	97.5	97.8	96.2	96.4	110
SD	4.2	5.0	5.4	5.0	5.6	4.4	5.1	9.7
Min.	89	83	81	86	75	84	86	91
Max.	111	109	110	112	113	108	110	128

TABLE 4.15 Significance of differences of MCV values from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	NS	NS	+	NS	NS	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	NS	+
6dpp					0	+	+	+
6wpp						0	NS	ND

+: significant $P < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.8 Mean Corpuscular Hemoglobin (MCH) of red cells of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

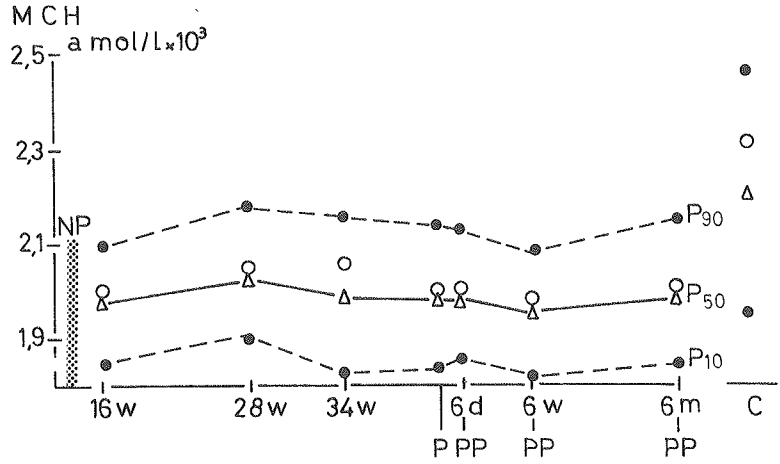


TABLE 4.16 Number, mean, SD and range of the MCH in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	61	60	64	62	33
$\bar{x}$	1.976	2.019	1.982	1.982	1.981	1.942	1.983	2.182
SD	0.085	0.114	0.126	0.103	0.123	0.107	0.110	0.199
Min.	1.789	1.576	1.597	1.707	1.491	1.667	1.700	1.741
Max.	2.206	2.273	2.300	2.220	2.323	2.225	2.268	2.550

TABLE 4.17 Significance of differences of MCH values from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	NS	NS	NS	+	NS	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	NS	+
6dpp					0	+	NS	+
6wpp						0	NS	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 4.9 Mean Corpuscular Hemoglobin Concentration (MCHC) of red cells of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

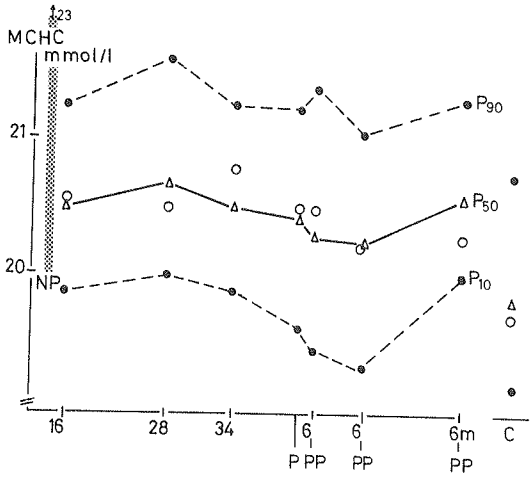


TABLE 4.18 Number, mean, SD and range of the MCHC in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	61	60	64	62	33
$\bar{x}$	20.5	20.7	20.5	20.4	20.3	20.2	20.6	19.8
SD	0.6	0.7	0.65	0.7	0.7	0.7	0.55	0.6
Min.	19.4	18.6	17.7	17.8	18.3	18.2	19.3	18.4
Max.	22.2	22.4	22.1	22.2	22.6	22.0	22.4	21.1

TABLE 4.19 Significance of differences of MCHC values from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	NS	+	+	NS	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	NS	+
6dpp					0	NS	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

TABLE 4.20 Number, mean and SD of MCV, MCH and MCHC measurements in maternal and cord blood from the < P10 group and number and median of these measurements in maternal and cord blood from the > P90 group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
	< P10 N	10	9	9	9	9	10	9	7
	$\bar{x}$	95.9	100.9	98.9	97.9	98.4	98.7	99	118.6
MCV fl	SD	3.4	5.1	1.9	5.8	5.0	5.6	5.4	10.5
	> P90 N	5	5	5	3	5	5	3	1
	MED	100	95	97	98	97	96	98	-
	< P10 N	10	10	9	9	9	10	9	7
	$\bar{x}$	1.981	2.070	2.082	2.003	2.018	1.993	2.023	2.29
MCH amol/lx10^3	SD	0.046	0.119	0.053	0.110	0.106	0.130	0.123	0.14
	> P90 N	5	5	5	3	5	5	3	1
	MED	2.025	1.946	1.975	1.927	1.935	1.907	2.000	-
	< P10 N	10	10	9	9	9	10	9	7
	$\bar{x}$	20.7	20.6	20.9	20.6	20.5	20.2	20.4	19.7
MCHC mmol/l	SD	0.6	0.5	0.5	0.6	0.4	0.7	0.8	1.3
	> P90 N	5	5	5	3	5	5	3	1
	MED	20.3	20.6	20.3	19.8	19.6	20	20.5	

FIGURE 4.10 Serum protein of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

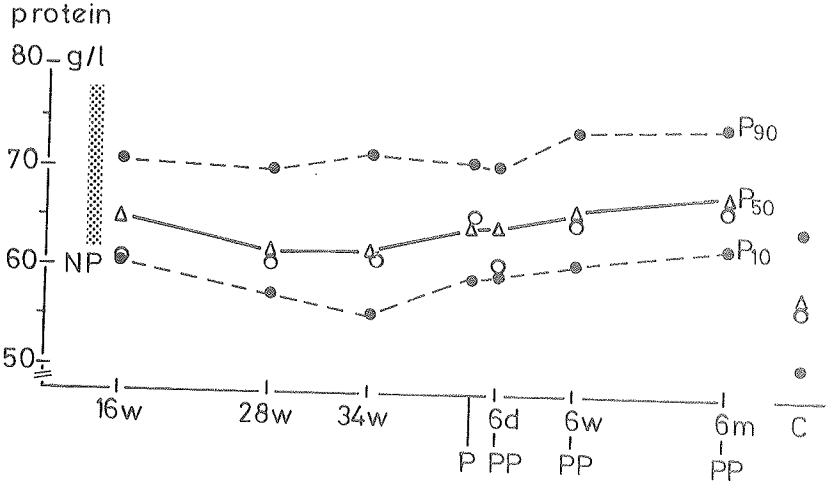


TABLE 4.21 Number, mean, SD and range of protein in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	61	64
$\bar{x}$	65	63	63	64	64	66	68	57
SD	4	6	7.5	6	5	5	4	5
Min.	54	51	52	54	50	58	60	45
Max.	77	87	88	86	83	79	77	67

TABLE 4.22 Significance of differences of serum protein from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	NS	NS	+	+	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.11 Serum albumin of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

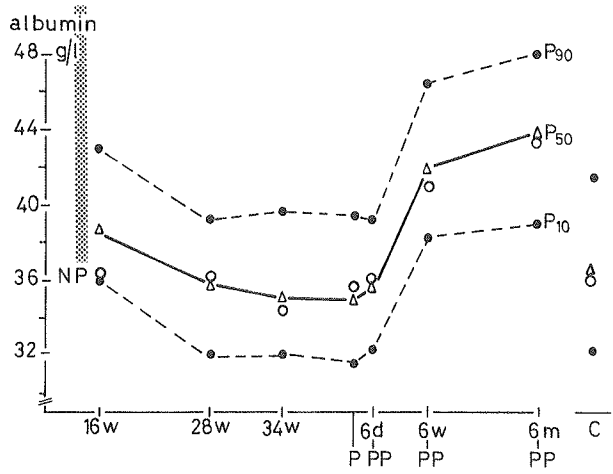


TABLE 4.23 Number, mean, SD and range of albumin in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	62	62
$\bar{x}$	38.5	35	35	35	35	42	43	36
SD	3	3	3	3	3	3	3	4
Min.	30	26	28	26	26	34	36	27
Max.	46	47	43	41	42	50	49	46

TABLE 4.24 Significance of differences of serum albumin from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.12 Serum cholesterol of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference value.

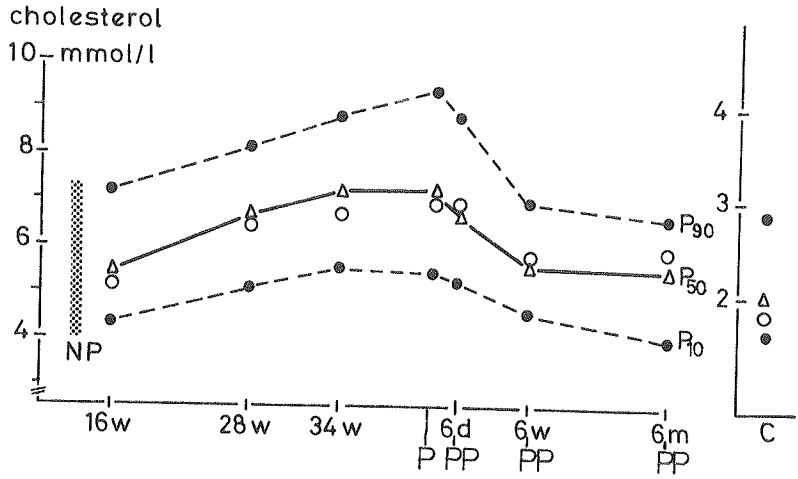


TABLE 4.25 Number, mean, SD and range of cholesterol in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	63	63
$\bar{x}$	5.6	6.7	7.2	7.25	6.9	5.7	5.4	2.1
SD	1.1	1.2	1.35	1.4	1.4	0.9	1.05	0.5
Min.	3.1	4.1	4.8	4.8	4.6	4.2	3.0	1.4
Max.	8.8	9.8	11.4	11.0	11.5	8.7	9.4	3.4

TABLE 4.26 Significance of differences of serum cholesterol from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	NS	NS	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 4.13 Serum triglycerides of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

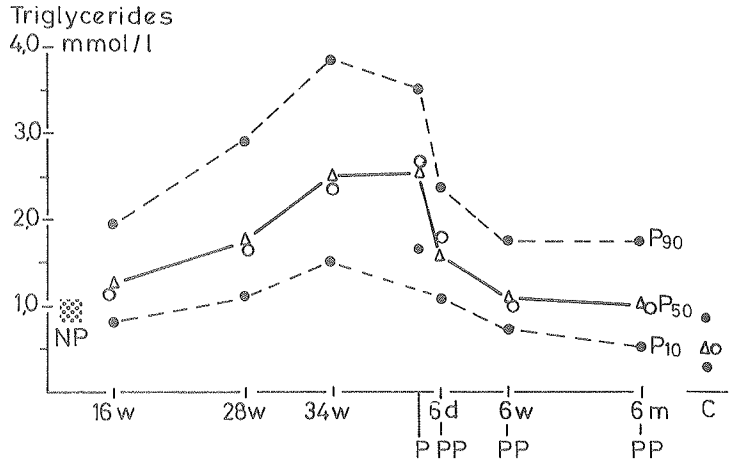


TABLE 4.27 Number, mean, SD and range of triglycerides in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	63	62	63
$\bar{x}$	1.28	1.91	2.54	2.66	1.70	1.24	1.12	0.55
SD	0.43	0.67	0.80	0.87	0.52	0.46	0.47	0.20
Min.	0.25	0.82	0.96	1.26	0.80	0.61	0.52	0.24
Max.	2.46	4.02	4.76	5.54	3.39	2.96	2.89	1.16

TABLE 4.28 Significance of differences of serum triglycerides from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	NS	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

TABLE 4.29 Number, mean and SD of protein, albumin, cholesterol and triglycerides values in maternal and cord serum from the < P10 group and number and median of these values in maternal and cord serum from the > P90 group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
< P10	N	9	10	9	10	9	10	9	10
	$\bar{x}$	63	64	63	69	63	67	66	59
Protein g/l	SD	6	5	7	8	6	2	1.5	7
> P90	N	5	5	5	4	5	5	3	4
	MED	65	60	66	62	61	65	72	58
< P10	N	10	10	9	10	9	9	9	9
	$\bar{x}$	38	35	35	37	35	41.5	42	36.4
Albumin g/l	SD	5	2	4	3	3	2.5	2.5	4
> P90	N	5	4	5	4	4	3	3	4
	MED	40	36	35	36	36	43	47	36
< P10	N	10	10	9	10	9	10	9	10
	$\bar{x}$	5.2	6.5	6.7	7.0	6.9	6.0	5.9	2.0
Cholesterol mmol/l	SD	0.7	1.0	1.1	1.1	1.5	1.0	1.3	0.6
> P90	N	5	4	5	4	5	5	3	4
	MED	4.7	5.7	6.2	6.7	5.6	5.1	5.2	2.3
< P10	N	10	10	9	10	9	10	9	10
	$\bar{x}$	1.25	1.81	2.64	2.62	1.91	1.24	1.13	0.69
Triglycerides mmol/l	SD	0.20	0.40	1.16	0.81	0.64	0.21	0.36	0.31
> P90	N	5	5	5	3	5	5	3	4
	MED	1.24	1.71	2.36	2.63	1.77	1.50	1.12	0.58

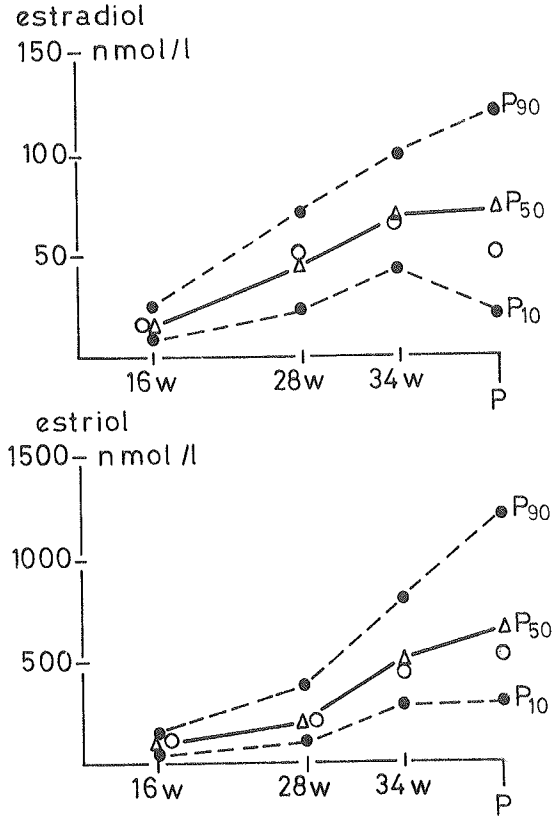


FIGURE 4.14. Serum estradiol 17β and estriol of women from the S-Reference group during pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

TABLE 4.30. Number, mean, SD and range of estradiol 17β and estriol in maternal serum from the S-Reference group during pregnancy.

	ESTRADIOL				ESTRIOL			
	16 w	28 w	34 w	P	16 w	28 w	34 w	P
N	69	70	68	67	67	70	67	65
$\bar{x}$	16	45	66	71	50.5	235	538	715
SD	8	16	24.5	34	30	90	239	344
Min	4	15	26	7	19	51	131	205
Max	43	102	140	149	162	490	1446	1446

FIGURE 4.15. Serum progesterone of women from the S-Reference group during pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

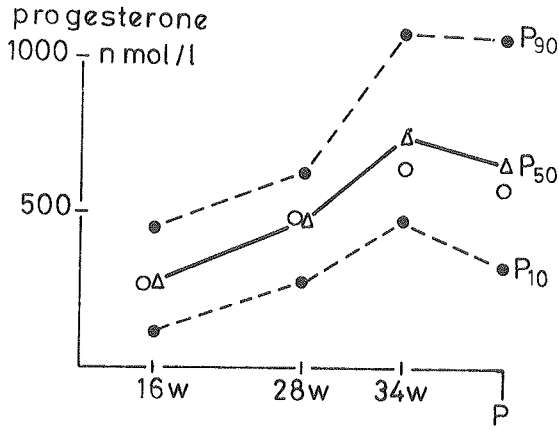


TABLE 4.31. Number, mean, SD and range of progesterone in maternal serum from the S-Reference group during pregnancy.

	16 w	28 w	34 w	P
N	67	68	66	64
$\bar{x}$	274	462	785	705
SD	118	166	263	320
Min	72	129	215	173
Max	593	1054	1686	1860

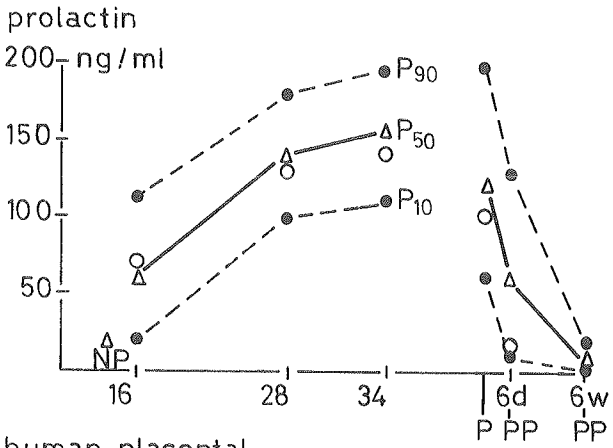


FIGURE 4.16. Serum prolacti
of women from the S-Referen
group during and after
pregnancy (10th, 50th and
90th centiles of measuremen
are shown). Open circles
represent the median of the
< P10 group.
NP: non-pregnant reference
value.

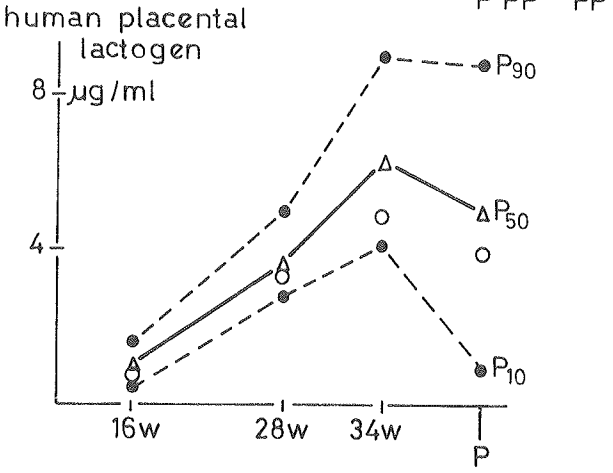


FIGURE 4.17. Serum values o
human placental lactogen
(HPL) of women from the
S-Reference group during
pregnancy (10th, 50th and
90th centiles of measuremen
are shown). Open circles
represent the median of the
< P10 group.

TABLE 4.32. Number, mean, SD and range of prolactin in maternal serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp
N	67	69	69	63	60	61
$\bar{x}$	69	140	153	127	66	14
SD	41	34.5	35	51	46	15
Min	18	38	48	26	5	1
Max	187	227	290	273	207	77

TABLE 4.33. Number, mean, SI
and range of HPL in materna
serum from the S-Reference
group during pregnancy.

16 w	28 w	34 w	P
70	70	70	65
1.1	3.8	6.6	5.1
0.5	1.0	1.85	2.9
0.5	2.1	3.8	0.6
3.5	6.7	12.8	14.0

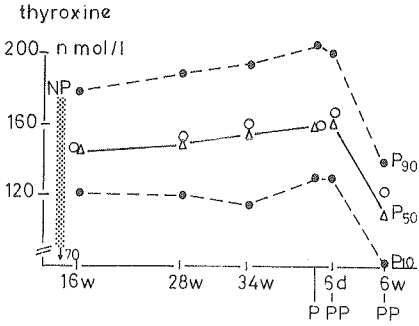


FIGURE 4.18. Serum thyroxine of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

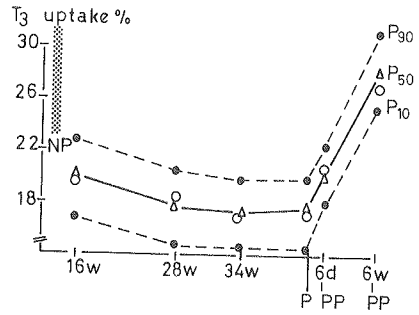


FIGURE 4.19. T3-uptake of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

TABLE 4.34. Number, mean, SD and range of thyroxine in maternal serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	70	70	70	65	60	64	-
$\bar{x}$	149	154	158	164	166	110	-
SD	23	26	30	30	28	20	-
Min	108	96	97	104	114	69.1	-
Max	222	225	243	245	259	148	-

TABLE 4.35. Number, mean, SD and range of the T3-uptake in maternal serum of the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	70	69	70	65	61	64	-
$\bar{x}$	20.1	17.7	17.4	17.9	19.8	28	-
SD	2.3	2.2	1.8	2.05	2.0	2.8	-
Min	15.6	13.4	13.6	13.3	16.1	21.9	-
Max	28.8	25.1	21.6	24.8	24.4	33.6	-

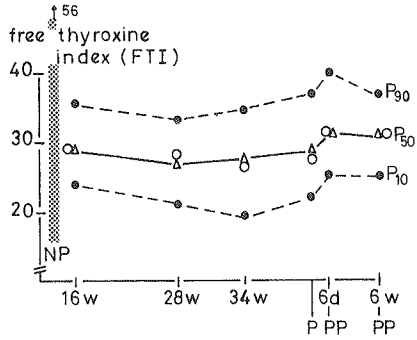


FIGURE 4.20. Free Thyroxine Index (FTI) of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

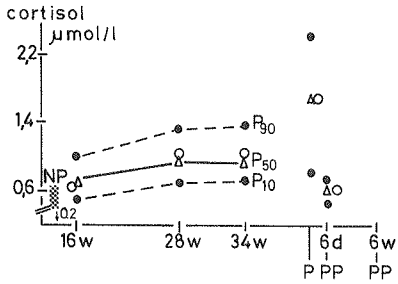


FIGURE 4.21. Serum cortisol of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

TABLE 4.36. Number, mean, SD and range of the FTI in maternal serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp
N	70	69	69	64	60	64
$\bar{x}$	29.7	27	27.2	29.4	32.7	30.6
SD	4.5	5.1	5.6	5.9	6.4	4.5
Min	20	17.2	16.1	18.6	21.4	20.7
Max	40	43.7	41.6	45.1	48	39.5

TABLE 4.37. Number, mean, SD and range of cortisol in maternal serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp
N	70	69	70	66	65	-	62
$\bar{x}$	0.74	0.96	1.00	1.66	0.59	-	0.75
SD	0.22	0.23	0.24	0.63	0.13	-	0.37
Min	0.39	0.52	0.56	0.71	0.40	-	0.16
Max	1.59	1.56	1.84	3.06	0.71	-	1.68

TABLE 4.38 Number, mean and SD of estradiol 17β, estriol, cortisol, prolactin, human placental lactogen and progesterone in maternal serum from the < P10 group and number and median of these hormones in maternal serum from the > P90 group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp
< P10	N	10	9	9	10	–	–	–
	$\bar{x}$	14	43	61	54	–	–	–
Estradiol nmol/l	SD	3	10	16	26	–	–	–
> P90	N	5	5	5	3	–	–	–
	MED	13	60	65	71	–	–	–
< P10	N	10	10	9	9	–	–	–
	$\bar{x}$	42	237	451	527	–	–	–
Estriol nmol/l	SD	11	86	150	207	–	–	–
> P90	N	5	5	4	3	–	–	–
	MED	70	288	588	902	–	–	–
< P10	N	10	10	9	10	9	–	9
	$\bar{x}$	0.73	1.05	1.09	1.79	0.63	–	0.87
Cortisol µmol/l	SD	0.25	0.27	0.29	0.67	0.21	–	0.18
> P90	N	4	3	4	2	–	–	2
	MED	0.64	1.01	1.12	1.83	–	–	0.68
< P10	N	8	10	9	10	9	10	–
	$\bar{x}$	80	132	148	117	38	9	–
Prolactin ng/ml	SD	54	46	47	62	31	5	–
> P90	N	4	4	5	2	5	5	–
	MED	48	132	130	139	40	7	–
< P10	N	10	10	9	10	–	–	–
	$\bar{x}$	1.1	3.8	5.4	4.4	–	–	–
HPL µg/ml	SD	0.8	1.5	1.9	1.4	–	–	–
> P90	N	5	4	5	2	–	–	–
	MED	1.1	3.3	6.1	4.1	–	–	–
< P10	N	9	10	9	9	–	–	–
	$\bar{x}$	247	448	687	618	–	–	–
Progesterone nmol/l	SD	112	174	251	329	–	–	–
> P90	N	5	5	4	3	–	–	–
	MED	198	507	714	630	–	–	–

TABLE 4.39 Number, mean and SD of thyroxine, T3-uptake and the free thyroxine index (F.T.I.) in maternal serum from the < P10 group and number and median of these parameters in maternal serum from the > P90 group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp
< P10	N	10	10	9	10	9	10	-
	$\bar{x}$	157	166	160	170	158	124	-
Thyroxine nmol/l	SD	36	36	23	34	31	25	-
> P90	N	4	4	5	2	5	5	-
	MED	152	167	183	178	170	120	-
< P10	N	10	10	9	10	9	10	-
	$\bar{x}$	19.4	17.6	16.8	17.4	20.2	26.5	-
T_3-uptake %	SD	1.8	1.7	1.6	1.4	2.1	4.2	-
> P90	N	4	4	5	2	5	5	-
	MED	17.9	16.6	16.6	17.1	20.5	24.8	-
< P10	N	10	10	9	10	9	10	-
	$\bar{x}$	30.3	29	27	39.8	32.1	32.1	-
F.T.I.	SD	7.0	5.5	4.6	7.3	7.1	3.9	-
> P90	N	4	4	5	3	5	5	-
	MED	26.9	29.9	26.5	30.0	32.7	30.4	-

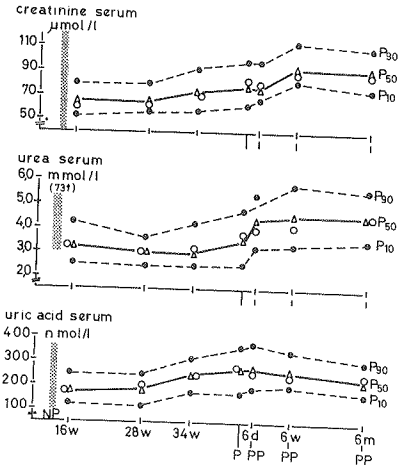


FIGURE 4.22. Serum creatinine, urea and uric acid of women from the S-Reference group during and after pregnancy (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

TABLE 4.40. Number, mean, SD and range of creatinine, urea and uric acid in maternal serum from the S-Reference group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp
Creatinine serum	N	70	70	69	64	60	64	63
	$\bar{x}$	66	67	74	78	80	95	92
	SD	11	10	14	15	13	13	13
	Min	31	33	41	37	41	67	64
	Max	102	96	111	114	109	130	128
Urea serum	N	64	69	68	67	60	64	62
	$\bar{x}$	3.2	3.0	3.1	3.6	4.5	4.6	4.6
	SD	0.7	0.7	0.8	0.9	0.8	1.0	1.05
	Min	1.6	1.2	1.5	1.7	2.6	2.7	1.6
	Max	5.0	5.4	6.4	6.2	7.2	7.0	7.5
Uric acid serum	N	58	66	67	64	60	64	63
	$\bar{x}$	182	192	242	275	282	262	235
	SD	43	50	55	67	67	54	53.5
	Min	98	96	104	150	138	179	147
	Max	266	365	366	432	457	407	328

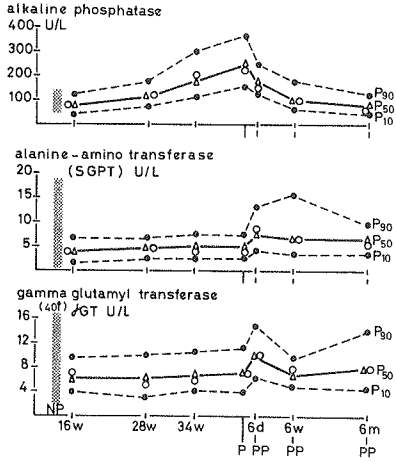


FIGURE 4.23. Serum alkaline phosphatase, serum alanine-amino transferase (SGPT) and serum gamma glutamyl (γGT) transferase of women from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group.

TABLE 4.41. Number, mean, SD and range of alkaline phosphatase, SGPT and γGT in maternal serum from the S-Reference group during and after pregnancy.

		16 w	28 w	34 w	P	6dpp	6wpp	6mpp
Alkaline phosphatase	N	70	70	70	67	60	64	62
	$\bar{x}$	81	120	202	267	194	115	84
	SD	26	41.5	64	86	58	40	30
	Min	30	47	81	117	89	51	30
	Max	158	248	341	610	436	237	202
Alanine amino transferase SGPT	N	69	69	69	64	59	61	62
	$\bar{x}$	1.85	4.2	4.75	4.45	7.4	7.85	6.7
	SD	2.2	1.6	1.7	1.7	3.6	4.9	2.15
	Min	1	1	1	1	2	2	2
	Max	12	9	10	9	19	21	12
γGT	N	70	70	69	66	60	63	62
	$\bar{x}$	6.2	6.1	7.0	7.3	9.6	6.8	8.7
	SD	2.15	2.50	3.0	3.3	3.9	2.0	4.15
	Min	1	2	3	1	5	2	3
	Max	13	13	16	19	22	12	23

TABLES AND FIGURES

CHAPTER 5

TABLE 5.1 Mean intake, standard deviation and 10th, 50th and 90th centiles of energy and nutrients in pregnant women (S-Reference group). I: at 16 weeks (n = 68); II: at 34 weeks (n = 67); III: 6 months postpartum (n = 62)

	Period	Mean	SD	CENTILES 10th	50th	90th	Statistical significance of difference [1]
	I	10.0	1.9	7.5	10.1	12.7	I-II
Energy (MJ)	II	9.4	2.2	6.5	9.7	11.8	I-III
	III	9.1	2.4	6.3	9.3	11.8	
	I	2405	450	1775	2400	3010	I-II
Energy (Kcal)	II	2245	520	1535	2325	2815	I-III
	III	2175	570	1505	2180	2800	
	I	83	18	61	82	105	I-II
Protein (g)	II	76	20	51	75	98	I-III
	III	76	16	53	75	98	
	I	106	24	-	105	-	I-II
Fat (g)	II	95	26	-	96	-	
	III	101	30	-	103	-	
	I	276	63	182	273	352	I-III
Carbohydrates (g)	II	267	72	181	267	347	II-III
	III	227	80	120	220	326	
	I	1.1	3.1	0.53	2.7	4.8	I-III
Alcohol (g)	II	1.8	4.8	0.58	2.9	8.8	II-III
	III	7.3	11.0	0.78	3.9	26.3	
	I	1308	479	745	1283	2046	I-III
Calcium (mg)	II	1295	546	728	1172	2030	II-III
	III	1097	417	603	1050	1636	
	I	13	3	10	13	17	I-II
Iron (mg)	II	12	3	8	11.5	16	I-III
	III	12	2.5	9	11	15	
	I	1.24	0.37	0.79	1.15	1.90	I-II
Retinol (mg)	II	1.13	0.36	0.74	1.07	1.64	I-III
	III	1.12	0.35	0.65	1.12	1.56	
	I	1.22	0.23	0.93	1.18	1.55	I-III
Thiamin (mg)	II	1.16	0.29	0.79	1.13	1.57	II-III
	III	1.07	0.25	0.80	0.99	1.40	
	I	1.96	0.57	1.31	1.89	2.71	I-III
Riboflavin (mg)	II	1.95	1.65	1.23	1.86	2.69	II-III
	III	1.69	0.51	1.07	1.64	2.29	

TABLE 5.1 continued

Nutrient	Period	Mean	SD	CENTILES 10th	50th	90th	Statistical significance of difference[1]
Vitamin B_6 (mg)	I	1.36	0.27	1.05	1.33	1.76	I-III
	II	1.32	0.38	0.87	1.31	1.86	II-III
	III	1.15	0.28	0.79	1.12	1.58	
Vitamin B_{12} (µg)	I	3.8	1.6	1.7	3.6	6.0	I-III
	II	3.7	1.5	2.1	3.5	5.6	II-III
	III	3.3	1.2	1.7	3.2	5.1	
Vitamin D (IU)	I	144	76	63	131	227	I-II
	II	115	61	53	109	177	I-III
	III	111	62	41	93	209	
Niacin (mg)	I	13	3	-	13	-	I-II
	II	12	3	-	12	-	I-III
	III	12	3	-	11	-	
Vitamin C (mg)	I	144	78	55	137	252	I-III
	II	121	76	34	112	224	II-III
	III	78	54	25	62	154	

1) Significances of $p < 0.05$ are indicated.

TABLE 5.2. Relative contribution of macronutrients to total energy intake of pregnant women (S-Reference group) at different stages of pregnancy and postpartum.
I: 16 weeks; II: 34 weeks; III: 6 months postpartum.

Nutrient	Period	Mean	SD	P50	Statistical significance of difference[4]
Protein[1]	I	14.0	2.5	13.8	II-III
	II	13.7	2.9	13.1	
	III	14.4	3.1	14.2	
Fat[2]	I	39.7	4.5	40.1	I-II
	II	38.1	5.4	39.1	I-III
	III	41.6	5.7	42.5	II-III
Carbohydrates[3]	I	45.8	5.4	45.4	I-III
	II	47.5	6.1	47.5	II-III
	III	41.4	6.9	41.5	
Alcohol	I	0.3	0.9	< 0.1	I-III
	II	0.5	1.2	< 0.1	II-III
	III	2.4	3.7	0.8	

1) Animal protein (En.%) Vegetable protein (En.%)

I	9.7	4.1
II	9.5	4.1
III	10.1	4.2

2) Saturated (En.%) Mono-unsaturated (En.%) Poly-unsaturated (En.%

I	17.9	14.9	5.5
II	17.3	14.3	5.2
III	18.7	16.0	5.5

3) Mono-disaccharides (En.%) Poly-saccharides (En.%)

I	25	21
II	27	20
III	21	20

4) Significance of difference between periods, indicated when $p < 0.05$.

TABLE 5.3 Nutrient density (mean value/1000 Kcal) of diets consumed by pregnant women (S-Reference group) at different stages of pregnancy and 6 months postpartum.

Nutrient	16 weeks	34 weeks	6 months pp
Calcium (mg/1000 Kcal)	544	577	504
Iron (total) (mg/1000 Kcal) xx	5.4	5.3	5.3
Retinol (mg/1000 Kcal)	0.52	0.50	0.51
Thiamin (mg/1000 Kcal)	0.51	0.53	0.51
Riboflavin (mg/1000 Kcal)	0.83	0.88	0.81
Vitamin B_6 (mg/1000 Kcal)	0.57	0.59	0.53
Vitamin B_{12} (µg/1000 Kcal)	1.58	1.64	1.53
Vitamin D (IU/1000 Kcal)	60	51	51
Niacin (mg/1000 Kcal)	5.6	5.3	5.5
Vitamin C (mg/1000 Kcal)	60	54	36
xx non-heme iron	3.9	3.9	3.7
heme iron	1.5	1.4	1.7

TABLE 5.4 Spearman rank correlation coefficients between energy and nutrient intake of pregnant women at the different stages of pregnancy and postpartum (S-Reference group).
I: 16 weeks; II: 34 weeks; III: 6 months postpartum.

Nutrient		I-II	I-III	II-III
Energy		0.58	0.41	0.64
Protein	Animal	0.52	0.26	0.60
	Vegetable	0.60	0.48	0.55
	Total	0.54	0.31	0.67
Fat	Saturated	0.58	0.48	0.64
	Mono-unsaturated	0.55	0.31	0.59
	Poly-unsaturated	0.54	0.33	0.50
	Total	0.57	0.42	0.67
Carbohydrates	Mono-disaccharides	0.52	0.55	0.38
	Poly-saccharides	0.59	0.50	0.45
	Total	0.60	0.50	0.42
Alcohol		0.81	0.63	0.61
Calcium		0.54	0.28	0.54
Iron	Heme	0.53	0.24	0.48
	Non-heme	0.51	0.51	0.51
	Total	0.48	0.38	0.48
Thiamin	mg/1000 Kcal	0.80	0.48	0.42
	Total	0.50	0.24	0.50
Riboflavin	mg/1000 Kcal	0.72	0.40	0.51
	Total	0.53	0.10	0.51
Vitamin B_6	mg/g protein	0.45	0.42	0.36
	Total	0.50	0.53	0.50
Ascorbic acid		0.33	0.33	0.53
Retinol		0.54	0.42	0.47

TABLE 5.5a. Mean relative contribution of groups of food products, expressed as energy percentage, to the diets consumed by pregnant women (S-Reference group) at different stages of pregnancy and postpartum.

Product	16 weeks	34 weeks	6 months pp
Potatoes	5.8	5.5	5.4
Bread (total)	13.5	13.7	12.8
Bread (white)	6.7	5.9	6.1
Bread (brown)	6.8	7.8	6.7
Fruits	6.7	7.3	4.2
Citrus fruits	3.1	3.5	1.7
Vegetables	2.1	2.3	2.2
Margarine/butter	8.4	8.4	7.9
Snacks	2.2	1.5	2.5
Sugar/cookies sweets	15.3	12.9	15.0
Fish	0.6	0.2	0.5
Meat (total)	13.3	12.6	15.4
Meat (lean)	1.5	1.6	1.6
Meat (mixed)	7.8	7.5	9.0
Meat (fat)	4.0	3.5	4.8
Dairy products	18.7	20.4	17.9
Cheese	5.3	4.5	5.6

TABLE 5.5b. Relative contribution of food products, or group of food products to the intake of macro- and micronutrients as calculated for the three respective surveys.

Nutrient	Food products	I 16 wk	II 34 wk	III 6 months pp
Protein (veget.)	Potatoes	11.2	10.9	10.3
	Bread	45.4	46.6	43.4
	Vegetables	17.6	19.3	19.0
Protein (animal)	Cheese	16.2	13.0	16.6
	Dairy products	27.6	35.7	24.0
	Meat products	38.1	38.1	42.9
Total fat	Cheese	9.3	8.4	9.7
	Margarine/butter	21.0	21.7	18.8
	Dairy products	11.1	13.8	9.8
	Meat products	23.9	23.3	26.8
Carbohydrates	Potatoes	9.7	9.0	9.8
	Bread	23.0	22.2	23.7
	Dairy products	11.5	13.5	11.1
	Sugar/sweets	11.8	10.8	14.0
Calcium	Cheese	25.0	19.4	28.2
	Dairy products	46.3	53.7	44.1
Iron - heme	Meat products	85.2	90.4	88.7
Iron - non-heme	Bread	23.4	22.6	23.7
	Vegetables	23.5	24.3	26.9
Retinol	Vegetables	34.7	39.1	39.4
	Cheese	11.2	9.9	12.4
	Margarine/butter	17.9	17.9	17.7
	Meat products	11.7	10.1	8.9
	Dairy products	6.9	7.6	6.3
Thiamin	Potatoes	9.4	8.9	9.0
	Bread	15.7	15.6	15.3
	Fruits	13.1	12.0	7.7
	Vegetables	9.3	10.0	10.2
	Dairy products	12.4	14.8	11.1
	Meat products	25.3	25.3	30.3
Riboflavin	Dairy products	42.4	48.9	39.7
	Meat products	18.1	15.1	18.3
Vitamin B_6	Potatoes	20.2	19.1	19.7
	Meat products	17.2	15.1	20.2
	Bread	12.8	13.3	13.1
	Fruits	13.4	16.0	8.6
	Vegetables	11.2	10.9	12.1
	Dairy products	12.7	14.9	12.2
Niacin	Meat products	35.2	35.4	38.4
	Bread	14.4	15.9	13.9
	Potatoes	13.8	14.2	13.0
Vitamin C	Fruits	69.8	71.2	62.6
	Vegetables	11.4	10.7	14.2
	Potatoes	10.6	11.5	15.9

TABLE 5.6. Mean energy and nutrient intake of pregnant women (S-Reference group) according to parity.
N = Nulliparae (n = 33)
M = Multiparae (n = 29), parity ⩾ 1

Nutrient	16 weeks		34 weeks		6 months pp	
	N	M	N	M	N	M
Energy (MJ)	10.3	9.7	9.7	9.1	9.5	8.5
Protein (g)	82	83	77	73	78	73
Fat (g)	105	106	95	96	102	100
Carbohydrates (g)	298*	253	286*	252	251*	200
Alcohol (g)	0.9	1.5	1.2	2.7	6.6	8.2
Calcium (mg)	1287	1365	1346	1200	1093	1102
Iron (mg)	13.1	13.1	12.4	11.8	12.0	11.2
Retinol (mg)	1.25	1.23	1.15	1.13	1.15	1.09
Thiamin (mg)	1.25	1.18	1.20	1.14	1.10	1.04
Riboflavin (mg)	1.95	2.00	2.00	1.85	1.76	1.62
Vitamin B_6 (mg)	1.40	1.33	1.36	1.32	1.14	1.17
Vitamin C	160*	123	139*	113	85*	70

* N > M; significant at 5% level

TABLE 5.7. Mean energy and nutrient intake of pregnant women (S-Reference group) according to smoking behavior.
NS = non-smoking (n = 33)
S = smoking (n = 29)

Nutrient	16 weeks		34 weeks	
	NS	S	NS	S
Energy (MJ)	9.9	10	9.0	9.8
Protein (g)	84	81	73	77
Fat (g)	106	106	91	103
Carbohydrates (g)	273	276	260	275
Alcohol (g)	0.4	2.4*	0.9	3.5*
Calcium (mg)	1294	1348	1168	1356
Iron (mg)	13.6	12.5	12.2	12.0
Retinol (mg)	1.18	1.29	1.13	1.14
Thiamin (mg)	1.23	1.19	1.15	1.20
Riboflavin (mg)	2.00	1.95	1.85	2.02
Vitamin B_6 (mg)	1.39	1.34	1.31	1.37
Vitamin C (mg)	139	142	120	133

* S > NS, significant at 5% level

TABLE 5.8. Mean energy and nutrient intake of pregnant women (S-Reference group) in summer and winter period.
S = summer (April-September)
W = winter (October-March)

Nutrient	16 weeks		34 weeks		6 months pp	
	S (n=28)	W (n=34)	S (n=34)	W (n=28)	S (n=30)	W (n=32)
Energy (MJ)	10.3	9.9	9.4	9.5	9.0	9.2
Protein (g)	84	82	75	76	74	77
Fat (g)	106	106	94	98	99	103
Carbohydrates (g)	287	268	273	268	227	227
Alcohol (g)	0.7	1.6	2.0	1.8	6.8	7.9
Calcium (mg)	1355	1297	1220	1349	1055	1137
Iron (mg)	13.7	12.7	12.4	11.7	11.7	11.5
Retinol (mg)	1.20	1.27	1.18	1.10	1.11	1.13
Thiamin (mg)	1.29*	1.16	1.18	1.16	1.06	1.08
Riboflavin (mg)	1.99	1.95	1.92	1.96	1.68	1.71
Vitamin B_6 (mg)	1.43	1.32	1.35	1.33	1.14	1.16
Vitamin C (mg)	157	130	136	116	73	83

* Intake in summer period significantly higher than in winter period ($p < 0.05$)

TABLE 5.9 Percentage of women in the S-Reference group with an estimated energy or nutrient intake below recommended daily allowances (RDA's given in Table 1.2.2.I). I: 16 weeks; II: 34 weeks; III: 6 months postpartum.

Nutrient	Period	Neth.Nutr. Council(1978)	WHO (1974)	NAS/NRC (1980)
Energy	I	27	62	42
	II	26	73	50
	III	33	52	35
Protein	I	15	0	35
	II	27	0	45
	III	12	0	0
Calcium	I	51	31	45
	II	62	34	55
	III	25	0	25
Iron	I	81	66	95
	II	81	72	100
	III	27	82	100
Retinol	I	22	7	30
	II	32	11	35
	III	22	17	20
Thiamin	I	37	0	88
	II	47	15	77
	III	10	20	50
Riboflavin	I	22	10	20
	II	30	7	27
	III	26	10	20
Vitamin B_6	I	-	100	100
	II	-	100	100
	III	-	100	100
Vitamin C	I	19	0	15
	II	30	12	22
	III	42	16	50
Vitamin B_{12}	I	-	35	60
	II	-	30	65
	III	-	15	45
Vitamin D	I	-	100	100
	II	-	100	100
	III	-	56	90

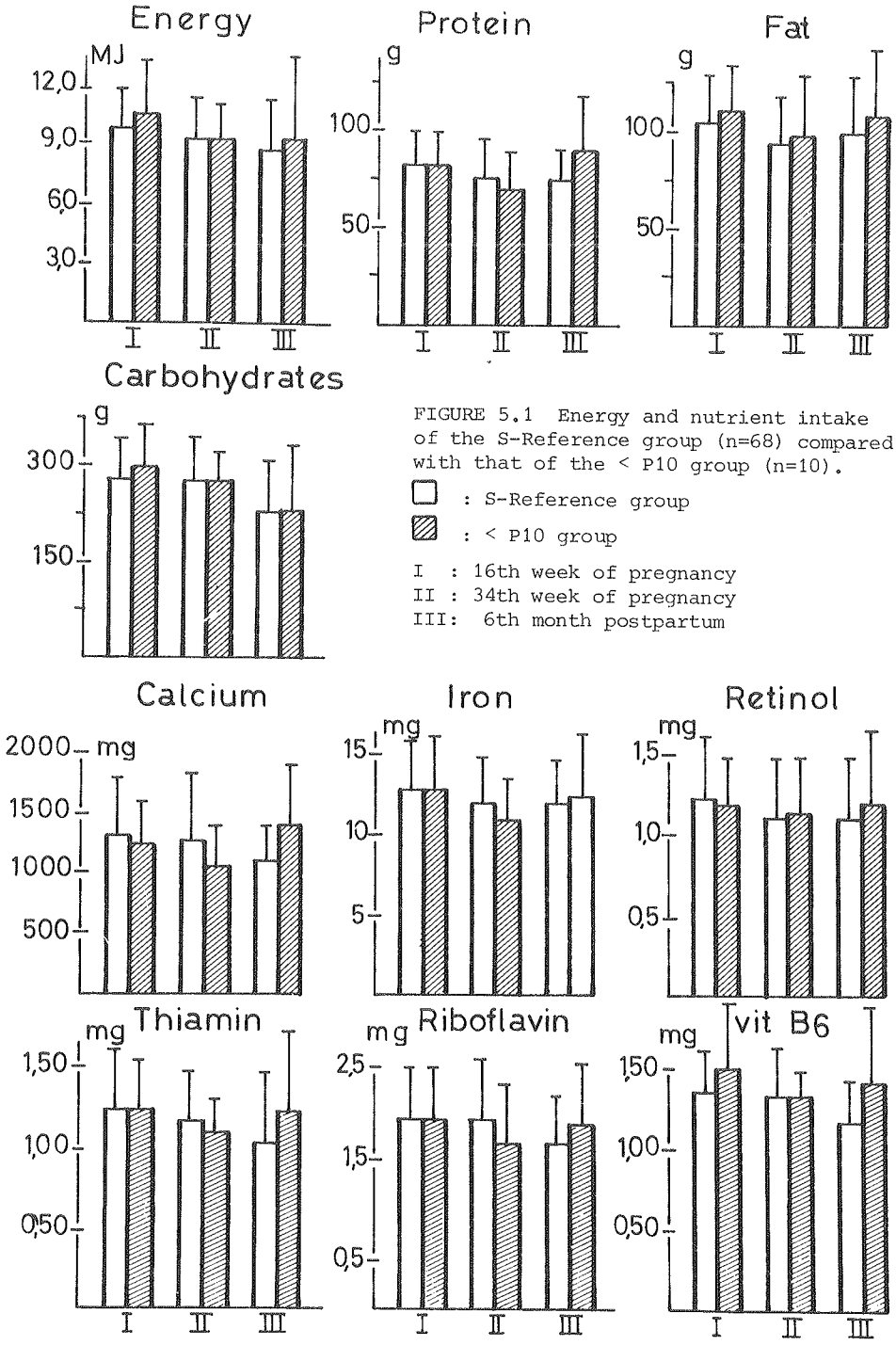


FIGURE 5.1 Energy and nutrient intake of the S-Reference group (n=68) compared with that of the < P10 group (n=10).

□ : S-Reference group

▨ : < P10 group

I : 16th week of pregnancy
II : 34th week of pregnancy
III: 6th month postpartum

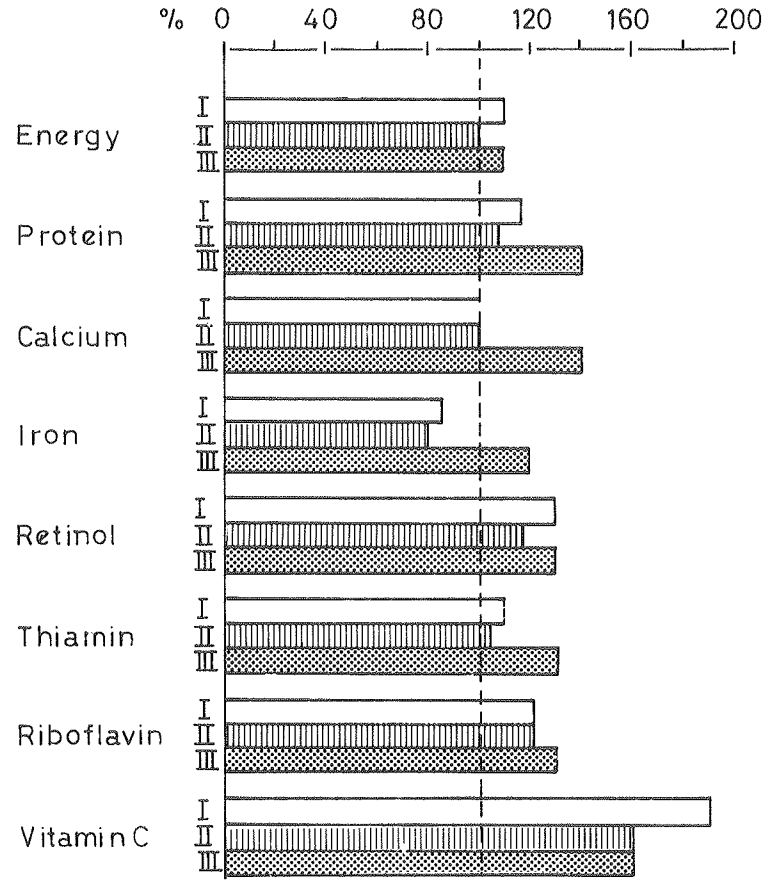


FIGURE 5.2. Energy and nutrient intakes relative to recommended daily allowances given by the Netherlands Nutrition Council. I: 16th week; II: 34th week; III: 6 months postpartum

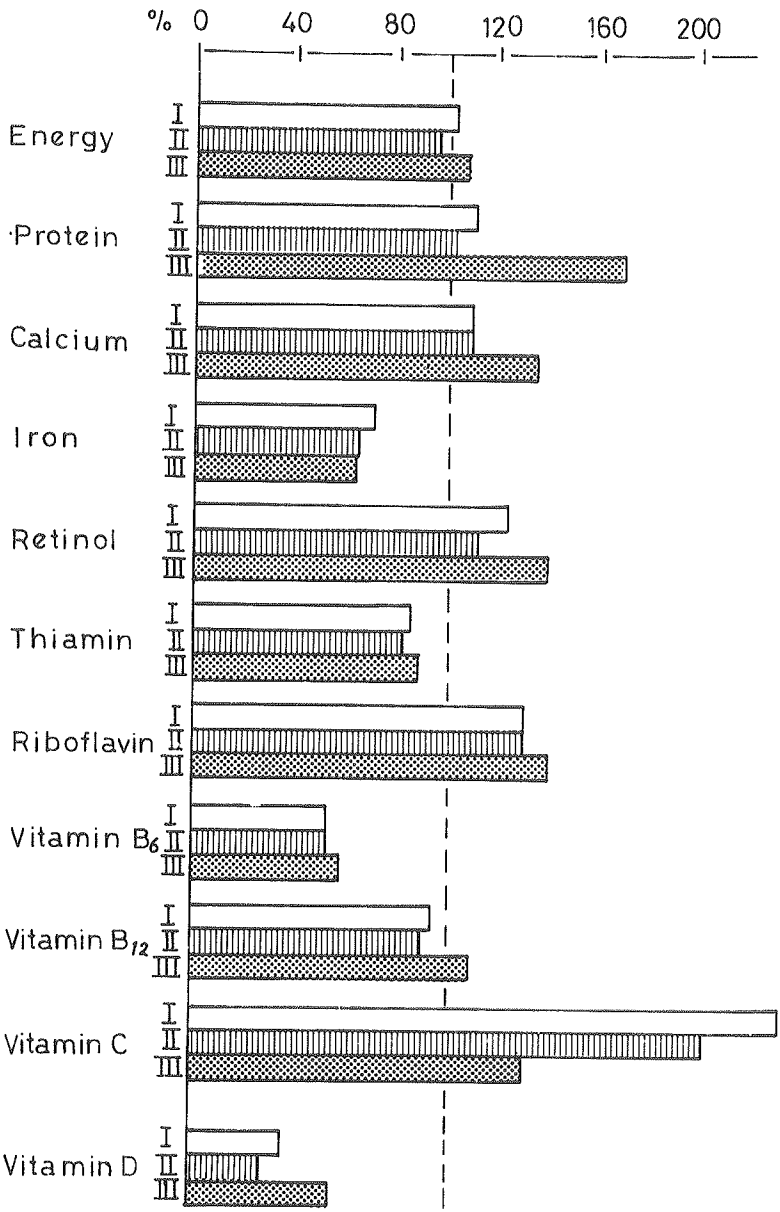


FIGURE 5.3. Energy and nutrient intakes relative to recommended daily allowances from the Food and Nutrition Board of the NAS/NRC (see Table 1.2.2.I)
I: 16th week; II: 34th week; III: 6 months postpartum

TABLES AND FIGURES

CHAPTER 6

FIGURE 6.1.1 Serum retinol levels of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

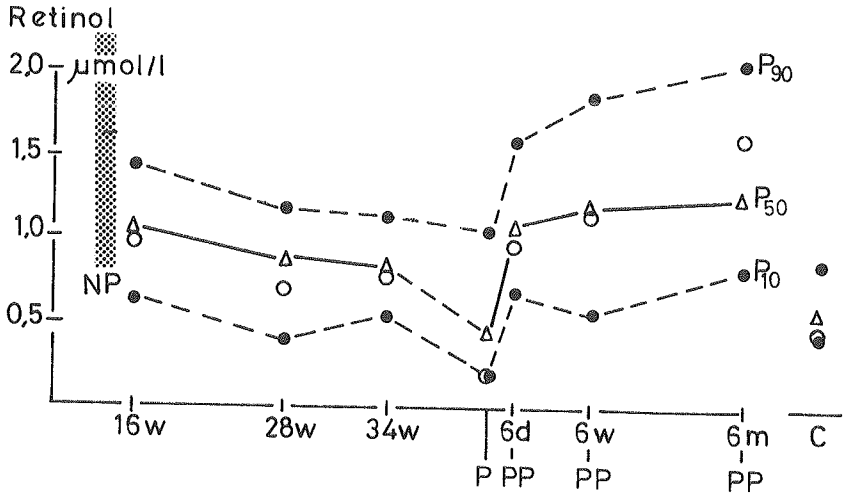


TABLE 6.1.1 Number, mean, SD and range of retinol values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	66	60	64	59	58
$\bar{x}$	1.01	0.81	0.78	0.48	1.06	1.18	1.30	0.55
SD	0.34	0.27	0.24	0.31	0.34	0.49	0.50	0.19
Min.	0.2	0.2	0.2	0.1	0.4	0.2	0.4	0.2
Max.	2.3	1.5	1.8	1.3	2.0	2.6	2.3	1.1

TABLE 6.1.2 Significance of differences in serum retinol measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	NS	+	+	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	NS
6dpp					0	NS	+	+
6wpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.2 Serum 25-OH-vitamin D levels of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

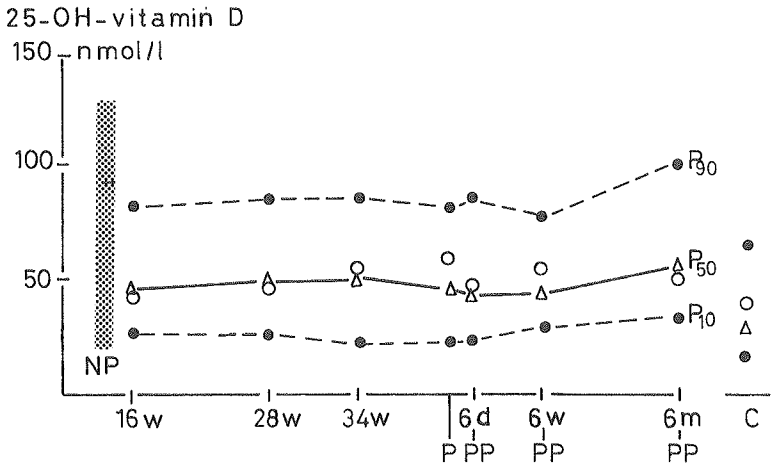


TABLE 6.1.3 Number, mean, SD and range of 25-OH-vitamin D values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	60	60
$\bar{x}$	50	54	53	50	49	50	62	34
SD	21	24	26	22	25	21	27	17
Min.	20	22	10	18	15	12	20	10
Max.	125	119	150	115	134	126	150	78

TABLE 6.1.4 Significance of differences in serum 25-OH-vitamin D measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	NS	NS	NS	+	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	+	+
6dpp					0	NS	+	+
6wpp						0	+	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 6.1.3 Erythrocyte transketolase (ETK)activities of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

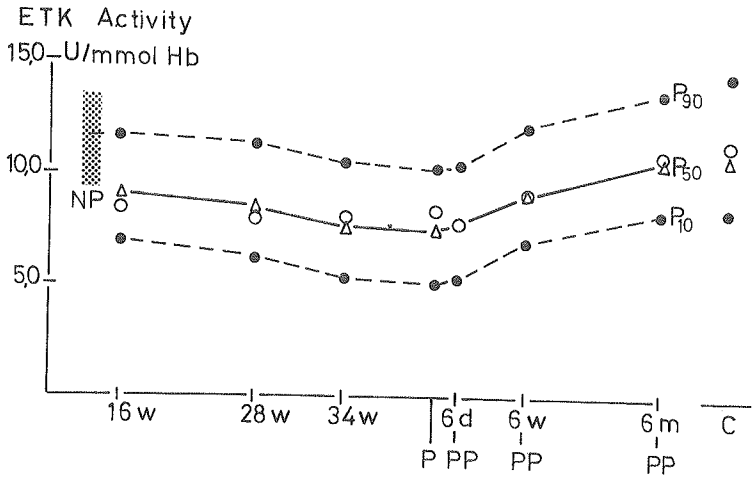


TABLE 6.1.5 Number, mean, SD and range of ETK activities in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	66	60	64	61	57
$\bar{x}$	9.1	8.5	7.8	7.5	7.8	9.3	10.8	11.1
SD	1.8	1.9	2.1	1.9	1.9	2.2	2.2	2.1
Min.	5.3	4.4	3.7	3.0	3.5	5.7	6.4	7.3
Max.	13.8	13.1	15.6	13.9	11.5	13.6	18.4	15.7

TABLE 6.1.6 Significance of differences in ETK activity measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	NS	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.4 ETK stimulation ratios of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

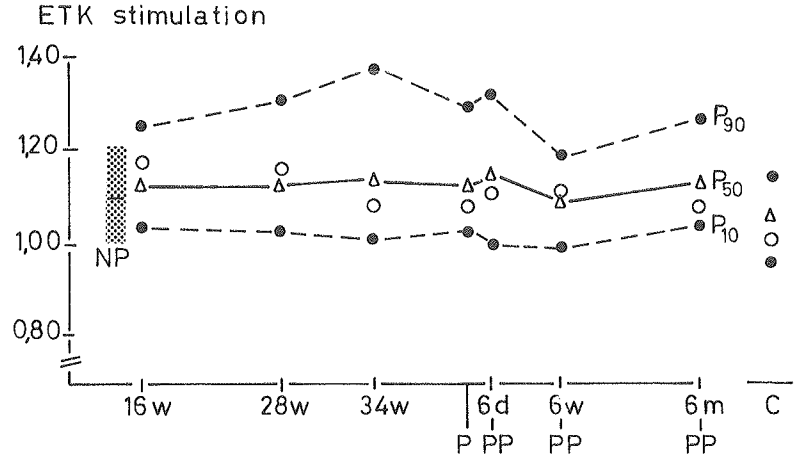


TABLE 6.1.7 Number, mean, SD and range of ETK stimulation ratios in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	64	60	64	60	57
$\bar{x}$	1.14	1.14	1.17	1.14	1.15	1.09	1.13	1.05
SD	0.10	0.10	0.14	0.12	0.12	0.08	0.08	0.07
Min.	0.99	0.97	0.89	0.93	0.86	0.94	1.02	0.78
Max.	1.50	1.46	1.70	1.47	1.53	1.28	1.32	1.19

TABLE 6.1.8 Significance of differences in ETK stimulation ratio measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	NS	NS	+	NS	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	NS	+
6dpp					0	+	NS	+
6wpp						0	+	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 6.1.5 Whole blood riboflavin levels during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

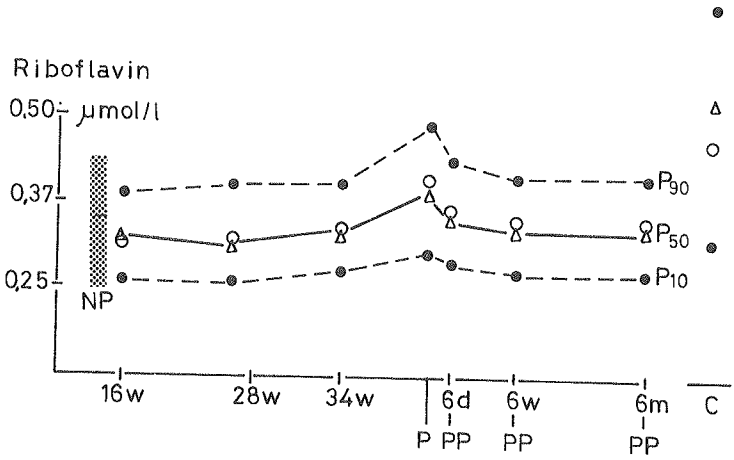


TABLE 6.1.9 Number, mean, SD and range of riboflavin values of maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6 dpp	6wpp	6mpp	Cord
N	70	69	69	66	60	62	62	62
$\bar{x}$	0.30	0.30	0.31	0.35	0.33	0.32	0.32	0.45
SD	0.04	0.04	0.03	0.05	0.04	0.05	0.04	0.10
Min.	0.22	0.23	0.23	0.22	0.23	0.24	0.22	0.22
Max.	0.42	0.43	0.39	0.49	0.44	0.47	0.43	0.62

TABLE 6.1.10 Significance of differences in whole blood riboflavin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6wpp						0	NS	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 6.1.6 Erythrocyte glutathion reductase (EGR)activities of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

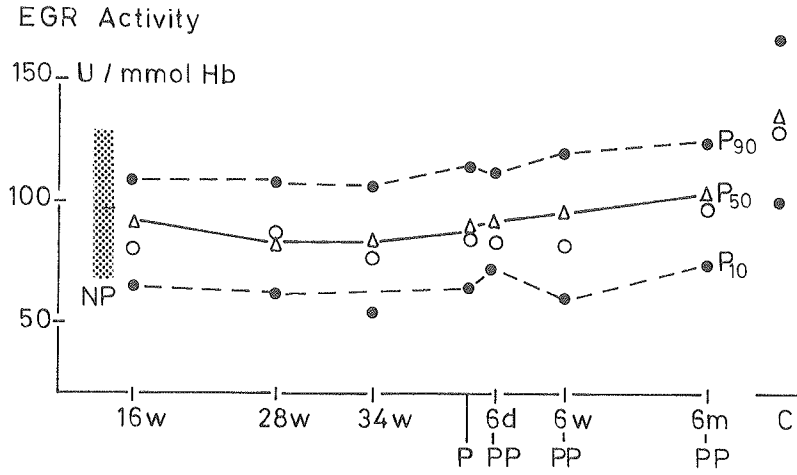


TABLE 6.1.11 Number, mean, SD and range of EGR activities in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	69	66	60	64	59	57
$\bar{x}$	88	84	81	89	91	93	99	134
SD	19	19	18	20	18	22	22	28
Min.	31	31	30	34	41	40	42	58
Max.	151	145	115	132	146	145	144	205

TABLE 6.1.12 Significance of differences in EGR activity measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	NS	NS	NS	+	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	NS	NS	+	+
6dpp					0	NS	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.7 EGR stimulation ratios of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

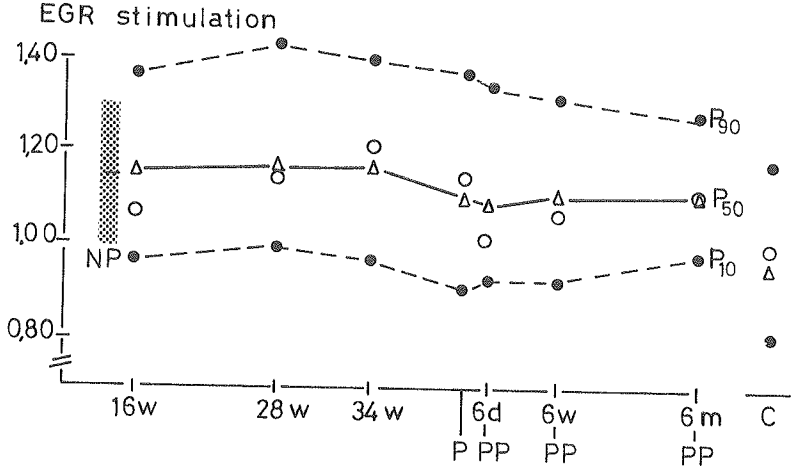


TABLE 6.1.13 Number, mean, SD and range of EGR stimulation ratios in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	66	60	63	59	57
$\bar{x}$	1.16	1.19	1.18	1.14	1.12	1.11	1.12	0.99
SD	0.15	0.18	0.21	0.22	0.18	0.15	0.14	0.17
Min.	0.82	0.90	0.88	0.73	0.87	0.87	0.86	0.73
Max.	1.62	2.05	2.27	2.11	1.90	1.76	1.78	1.76

TABLE 6.1.14 Significance of differences in EGR stimulation ratio measurements from the S-Reference group

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	NS	NS	NS	NS	ND
28 w		0	NS	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	NS	NS	NS	+
6dpp					0	NS	NS	+
6wpp						0	NS	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 6.1.8 Plasma pyridoxal phosphate (PLP) levels of women during and after pregnancy and of cord plasma from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP : non-pregnant reference group.

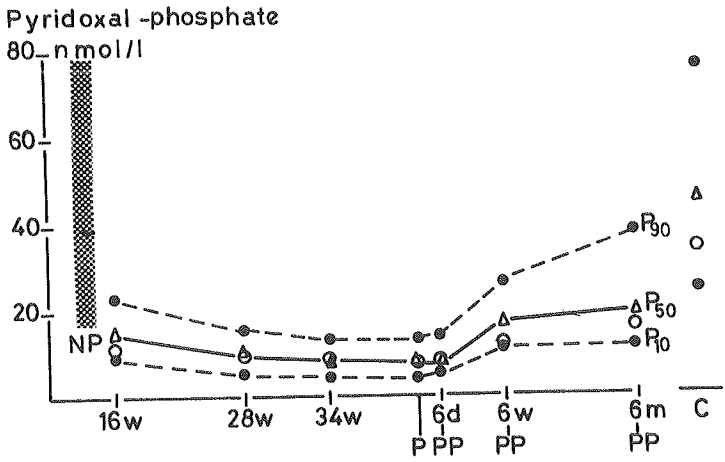


TABLE 6.1.15 Number, mean, SD and range of PLP values of maternal and cord plasma from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	69	68	64	60	61	62	63
$\bar{x}$	15	9	8	8	8	18	24	48
SD	5	4	3	3	3	6	15	20
Min.	7	4	3	3	4	7	9	5
Max.	30	25	22	17	16	40	112	103

TABLE 6.1.16 Significance of differences in plasma PLP measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.9 Erythrocyte glutamate-oxaloacetate transaminase (EGOT) activities of women during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

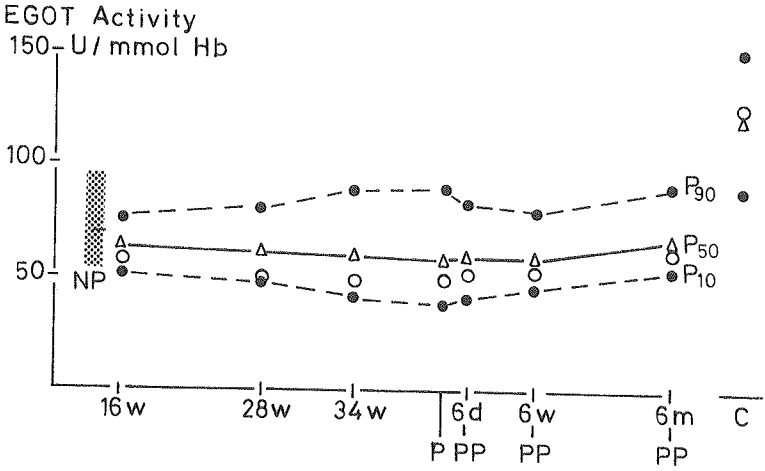


TABLE 6.1.17 Number, mean, SD and range of EGOT activities in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	66	59	63	61	57
$\bar{x}$	64	63	61	61	60	61	68	120
SD	11	12	17	18	17	15	14	24
Min.	46	41	35	29	25	30	45	55
Max.	105	95	106	113	104	101	110	164

TABLE 6.1.18 Significance of differences in EGOT activity measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	NS	+	NS	+	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	+	+
6dpp					0	NS	+	+
6wpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.10 EGOT stimulation ratios of women during and after pregnancy and in cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

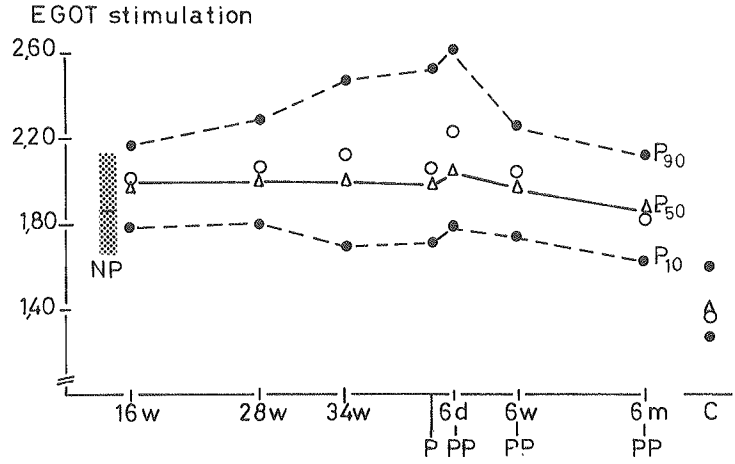


TABLE 6.1.19 Number, mean, SD and range of EGOT stimulation ratios in maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	69	70	66	59	63	61	56
$\bar{x}$	1.98	2.01	2.03	2.05	2.13	1.98	1.87	1.41
SD	0.15	0.18	0.28	0.31	0.37	0.22	0.18	0.17
Min.	1.61	1.63	1.42	1.41	1.27	1.50	1.29	1.04
Max.	2.27	2.50	2.61	2.86	3.64	2.69	2.30	2.19

TABLE 6.1.20 Significance of differences in EGOT stimulation ratio measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	NS	NS	+	+	NS	+	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	+	+
6dpp					0	+	+	+
6wpp						0	+	ND

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 6.1.11 Serum vitamin B_{12} levels of women during and after pregnancy and of cord serum from the S- Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

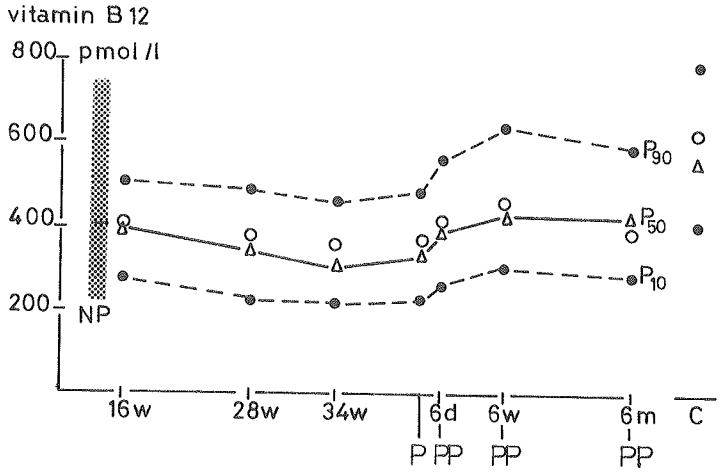


TABLE 6.1.21 Number, mean, SD and range of vitamin B_{12} values in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	69	70	70	65	61	64	56	61
$\bar{x}$	391	351	322	342	406	448	421	592
SD	97	108	92	101	120	127	114	169
Min.	220	187	136	184	209	221	215	336
Max.	736	664	579	624	900	800	691	1069

TABLE 6.1.22 Significance of differences in serum vitamin B_{12} measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	NS	+	+	ND
28 w		0	+	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	NS	+
6wpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.12 Serum folacin levels of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

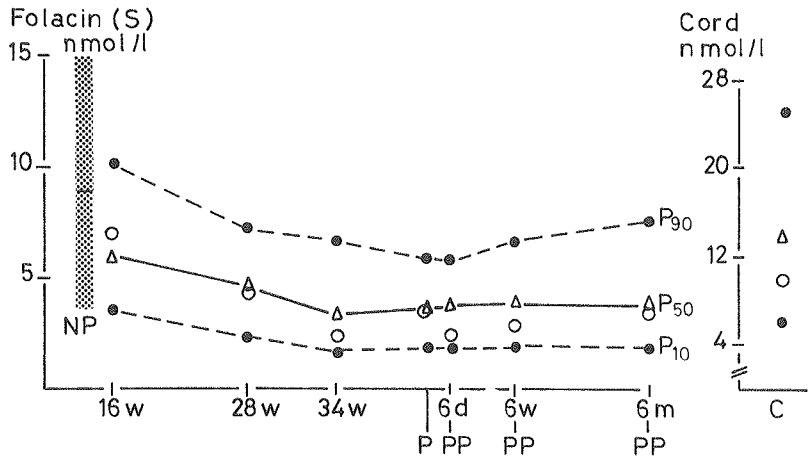


TABLE 6.1.23 Number, mean, SD and range of folacin values in maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	67	69	68	64	58	63	62	60
$\bar{x}$	6.5	4.8	3.8	3.8	3.8	4.1	4.4	14.9
SD	2.4	2.2	2.0	1.8	1.4	2.2	2.4	7.2
Min.	2.6	1.1	0.7	1.1	0.7	0.7	1.3	2.6
Max.	13.2	13.9	9.0	9.9	6.6	12.5	14.7	34.5

TABLE 6.1.24 Significance of differences in serum folacin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	NS	+
6dpp					0	+	+	+
6wpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 6.1.13 Erythrocyte folacin levels during and after pregnancy and of cord blood from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

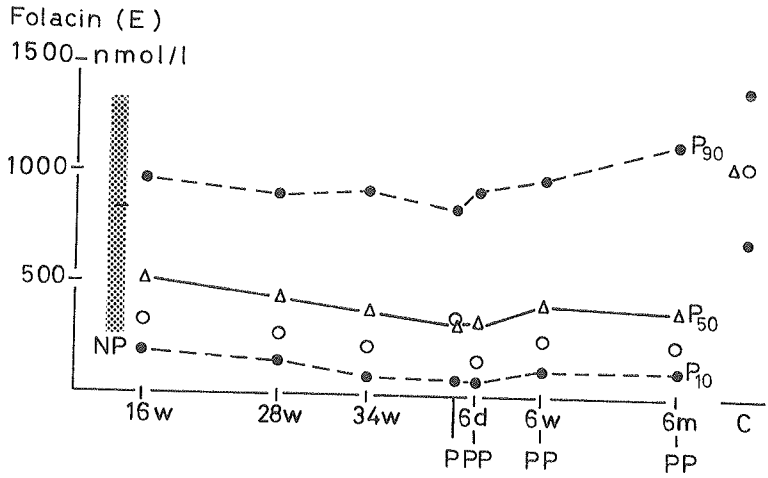


TABLE 6.1.25 Number, mean, SD and range of erythrocyte folacin values of maternal and cord blood from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	68	68	69	59	60	62	60	31
$\bar{x}$	560	485	451	393	465	502	516	1056
SD	310	281	335	312	458	415	395	270
Min.	145	127	58	20	61	48	38	256
Max.	1078	1457	1800	1470	2316	2725	1602	1430

TABLE 6.1.26 Significance of differences in erythrocyte folacin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	NS	NS	NS	ND
28 w		0	NS	NS	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	NS	NS	+
6dpp					0	NS	NS	+
6wpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

TABLE 6.1.27. Linear correlation coefficients between measurements for some vitamin status parameters at different stages of pregnancy and in the postpartum period for the S-Reference group.
1) $0.001 < p < 0.01$; 2) $0.001 < p$; NS = not significant; T_1: 16 w, T_2: 28 w, T_3: 34 w, T_4: partus, T_5: 6 d pp, T_6: 6 w pp, T_7: 6 m pp.

	Retinol	ETKA	αETK	Riboflavin	EGRA	αEGR	PLP	EGOT	αEGOT	Folacin(s)	Folacin(E)	Vit.B12
T_1-T_2	0.30[1)]	0.46[2)]	NS	0.74[2)]	0.73[2)]	0.58[2)]	0.69[2)]	0.45[2)]	0.45[2)]	0.36[1)]	0.28[1)]	0.51[2)]
-T_3	NS	0.31[1)]	NS	0.63[2)]	0.62[2)]	0.51[2)]	0.43[2)]	0.53[2)]	0.47[2)]	0.29[1)]	NS	0.51[2)]
-T_4	NS	NS	NS	0.45[2)]	0.62[2)]	0.43[2)]	0.29[1)]	0.49[2)]	0.54[2)]	0.31[1)]	NS	0.54[2)]
-T_5	NS	0.29[1)]	NS	0.71[2)]	0.62[2)]	0.44[2)]	0.50[2)]	0.55[2)]	0.35[2)]	0.34[1)]	NS	0.47[2)]
-T_6	NS	0.42[2)]	NS	0.76[2)]	0.62[2)]	0.30[2)]	0.57[2)]	0.42[2)]	NS	0.48[1)]	NS	0.40[2)]
-T_7	NS	0.49[2)]	NS	0.69[2)]	0.53[2)]	0.30[2)]	NS	0.48[2)]	0.27[1)]	0.38[1)]	NS	NS
T_2-T_3	0.31[1)]	0.53[2)]	0.25[1)]	0.63[2)]	0.77[2)]	0.77[2)]	0.50[2)]	0.60[2)]	0.48[2)]	NS	0.32[1)]	0.58[2)]
-T_4	0.29[1)]	NS	NS	0.39[2)]	0.73[2)]	0.72[2)]	NS	0.59[2)]	0.45[2)]	NS	NS	0.58[2)]
-T_5	NS	0.43[2)]	NS	0.62[2)]	0.73[2)]	0.74[2)]	0.52[2)]	0.53[2)]	0.36[2)]	NS	NS	0.49[2)]
-T_6	NS	0.37[2)]	NS	0.65[2)]	0.64[2)]	0.44[2)]	0.49[2)]	NS	NS	0.52[1)]	NS	0.36[2)]
-T_7	NS	0.51[2)]	NS	0.64[2)]	0.62[2)]	0.54[2)]	0.32[1)]	0.48[2)]	NS	0.53[1)]	NS	0.40[2)]
T_3-T_4	0.31[1)]	0.30[1)]	NS	0.59[2)]	0.76[2)]	0.80[2)]	NS	0.73[2)]	0.63[2)]	0.86[1)]	0.60[2)]	0.75[2)]
-T_5	0.28[1)]	0.57[2)]	0.30[1)]	0.57[2)]	0.68[2)]	0.75[2)]	0.36[2)]	0.79[2)]	0.55[2)]	0.71[1)]	0.42[2)]	0.49[2)]
-T_6	NS	0.36[2)]	NS	0.57[2)]	0.70[2)]	0.36[2)]	0.43[2)]	0.45[2)]	NS	0.35[1)]	0.45[2)]	0.44[2)]
-T_7	NS	0.50[2)]	NS	0.51[2)]	0.62[2)]	0.52[2)]	NS	0.54[2)]	NS	0.24[2)]	0.43[2)]	0.40[2)]
T_4-T_5	0.45[2)]	NS	0.27[1)]	0.54[2)]	0.80[2)]	0.80[2)]	0.31[1)]	0.76[2)]	0.63[2)]	0.75[1)]	0.50[2)]	0.67[2)]
-T_6	NS	NS	NS	0.48[2)]	0.67[2)]	0.37[2)]	0.35[2)]	0.46[2)]	0.40[2)]	0.34[2)]	0.29[1)]	0.57[2)]
-T_7	NS	NS	NS	0.29[2)]	0.62[2)]	0.40[2)]	NS	0.51[2)]	0.27[1)]	0.26[2)]	NS	0.52[2)]
T_5-T_6	0.29[1)]	0.57[2)]	NS	0.62[2)]	0.58[2)]	0.39[2)]	0.31[1)]	0.61[2)]	0.34[1)]	0.45[1)]	0.43[2)]	0.57[2)]
-T_7	NS	0.43[2)]	NS	0.58[2)]	0.40[2)]	0.44[2)]	NS	0.59[2)]	NS	0.26[2)]	0.38[2)]	0.42[2)]
T_6-T_7	0.28[1)]	0.36[2)]	NS	0.66[2)]	0.55[2)]	NS	0.39[2)]	0.51[2)]	0.30[2)]	0.51[2)]	0.31[1)]	0.48[2)]

TABLE 6.1.28 Occurrence of abnormal values in percentages for some vitamin status parameters in the S-Reference group when assessed according to cut-off points derived for non-pregnant controls.
(T_1 : 16 wk; T_3 : 34 wk; T_4 : Partus; T_7 : 6mpp).

Parameter	Cut-off point		T_1	T_3	T_4	T_7
Retinol	<1.1	µmol/l	60	90	95	40
	⩽0.7	µmol/l	10	25	70	5
25-OH-vitamin D	<20	nmol/l	5	10	5	<2.5
αETK	>1.25		10	25	25	10
Riboflavin	<0.22	µmol/l	<2.5	<2.5	<2.5	<2.5
αEGR	>1.30		15	25	15	5
Pyridoxal phosphate	<15	nmol/l	55	100	100	25
αEGOT	>2.20		7.5	17.5	25	<2.5
Vitamin B_{12}	<180	pmol/l	<2.5	<2.5	<2.5	<2.5
Folacin (S)	<3.6	nmol/l	10	45	55	45
Folacin (E)	<220	nmol/l	10	25	35	20

TABLE 6.2.1 Linear correlation coefficients between maternal and cord blood vitamin status parameters calculated for the S-Reference group.
1) $0.001 < p \leq 0.01$; 2) $0.0001 < p < 0.001$; 3) $p < 0.0001$
NS: not significant C: cord blood value
M: maternal blood value.

Parameter	Correlation (R)	C/M-ratio	N
Retinol (T_4)	$0.37^{1)}$	1.14	58
(T_5)	$0.38^{1)}$	0.52	49
25-OH-vitamin D	$0.91^{3)}$	0.68	60
ETK Activity	$0.53^{2)}$	1.48	56
αETK	NS	0.92	56
Riboflavin	$0.34^{1)}$	1.29	61
EGR Activity	$0.57^{3)}$	1.50	56
αEGR	$0.85^{3)}$	0.87	56
Pyridoxal phosphate	$0.56^{3)}$	6	62
EGOT Activity	$0.50^{2)}$	1.97	56
αEGOT	NS	0.69	55
Vitamin B_{12}	$0.36^{1)}$	1.73	59
Folacin (S)	$0.54^{3)}$	4	57
Folacin (E)	NS	2.7	29

TABLE 6.2.2 Mean value, median value (P50) and standard deviation (SD) for some vitamin status parameters at different stages during pregnancy and in the postpartum period for the < P10 group (N = 10)

Parameter		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Retinol (S) (µmol/l)	$\bar{x}$	0.98	0.94	0.78	0.34	0.92	1.09	1.52	0.47
	P50	1.00	0.70	0.80	0.20	1.00	1.15	1.60	0.45
	SD	0.26	0.38	0.25	0.25	0.38	0.58	0.41	0.13
25-OH-vitamin D (S) (nmol/)	$\bar{x}$	43	60	52	54	48	51	50	39
	P50	44	48	55	61	47	55	50	39
	SD	16	40	20	18	23	26	11	18
ETK Activity (U/mmol Hb)	$\bar{x}$	8.5	7.8	7.4	8.3	8.6	8.8	11.0	12
	P50	8.4	7.9	8.0	8.4	7.9	9.1	10.9	11.5
	SD	1.9	1.5	1.4	1.9	3.8	1.3	2.2	2.7
αETK	$\bar{x}$	1.23	1.15	1.18	1.12	1.15	1.12	1.12	1.02
	P50	1.18	1.16	1.08	1.08	1.11	1.11	1.08	1.01
	SD	0.15	0.08	0.19	0.12	0.16	0.06	0.07	0.06
Riboflavin (B) (µmol/l)	$\bar{x}$	0.32	0.32	0.33	0.38	0.34	0.34	0.33	0.43
	P50	0.31	0.31	0.32	0.38	0.34	0.34	0.33	0.42
	SD	0.04	0.04	0.05	0.06	0.02	0.04	0.04	0.07
EGR Activity (U/mmol Hb)	$\bar{x}$	83	80	80	86	89	87	105	129
	P50	81	82	77	87	84	82	98	130
	SD	9	14	17	14	16	18	15	24
αEGR	$\bar{x}$	1.11	1.13	1.17	1.12	1.07	1.13	1.10	1.02
	P50	1.07	1.15	1.21	1.14	1.02	1.07	1.11	1.00
	SD	0.13	0.14	0.18	0.10	0.13	0.17	0.13	0.18
Pyridoxal phosphate (P) (nmol/l)	$\bar{x}$	12	9	8	8	8	12	17	38
	P50	11	10	8	7	7.5	11	16	34
	SD	4	3	3	3.5	4	5	7.5	22
EGOT Activity (U/mmol Hb)	$\bar{x}$	56	53	49	51	49	52	64	121
	P50	57	49	49	49	52	54	61	125
	SD	9	10	8	12	11	11	9	23

TABLE 6.2.2 continued

Parameter		16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
αEGOT	$\bar{x}$	2.02	2.03	2.11	2.06	2.23	2.02	1.83	1.37
	P50	2.00	2.04	2.14	2.06	2.23	2.02	1.85	1.37
	SD	0.18	0.20	0.24	0.17	0.29	0.19	0.17	0.14
Vitamin B_{12} (S) (pmol/l)	$\bar{x}$	415	378	354	373	398	431	388	707
	P50	400	384	362	372	407	459	388	630
	SD	151	95	78	62	89	123	124	238
Folacin (S) (nmol/l)	$\bar{x}$	6.7	4.2	3.5	3.2	2.5	2.6	4.0	13.3
	P50	7.0	4.2	2.4	3.7	2.4	2.9	3.7	9.7
	SD	1.5	1.6	1.9	1.2	0.7	2.1	1.7	6.5
Folacin (E) (nmol/l)	$\bar{x}$	403	318	500	403	284	284	328	884
	P50	336	274	214	347	170	261	240	1053
	SD	209	179	436	278	260	174	202	378

TABLE 6.2.3 Median value for some vitamin status parameters at different stages during pregnancy and in the postpartum period for the > P90 group (N = 5).

Parameter	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Retinol (µmol/l)	1.10	1.05	0.70	0.20	1.00	1.40	1.50	0.80
25-OH-vitamin D (nmol/l)	36	54	50	38	34	36	68	20
ETK Activity (U/mmol Hb)	8.9	8.4	6.5	6.7	7.3	9.0	11.0	12.2
αETK	1.17	1.22	1.12	1.06	1.08	1.07	1.05	1.05
Riboflavin (µmol/l)	0.32	0.29	0.33	0.37	0.33	0.31	0.31	0.48
EGR Activity (U/mmol Hb)	79	81	71	86	100	83	93	140
αEGR	1.11	1.19	1.21	1.07	1.01	1.18	1.04	0.95
Pyridoxal phosphate (nmol/l)	16	9	7	7.5	9	17	29	31
EGOT Activity (U/mmol Hb)	70	74	76	61	84	75	83	140
αEGOT	1.79	1.79	1.76	1.76	1.77	1.80	1.85	1.29
Vitamin B_{12} (pmol/l)	264	280	237	294	318	352	365	589
Folacin (S) (nmol/l)	5.3	5.7	5.3	4.8	2.9	5.6	4.6	16.3
Folacin (E) (nmol/l)	392	282	262	318	317	278	367	1054

TABLE 6.3.1. Mean value and standard deviation (SD) of some vitamin status parameters of women from the S-Reference group at week 16 and 34, 6 months pp and in cord blood, according to smoking habits.

Smoking group	Period	I (non-smokers)			II (1-10 sig/day)			III (> 10 sig/day)		
Parameter		$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N
Retinol (µmol/l)	16 w	0.99	0.28	36	1.10	0.44	15	0.99	0.34	18
	34 w	0.74	0.19	37	0.89	0.17	15	0.77	0.33	18
	6mpp	1.25	0.49	29	1.21	0.42	14	1.40	0.55	16
	Cord	0.56	0.18	28	0.46	0.16	14	0.60	0.20	16
25-OH-Vit.D (nmol/l)	16 w	47	18	37	62	27	15	47	19	18
	34 w	59	27	37	46	29	15	46	19	18
	6mpp	62	29	30	69	32	14	56	20	16
	Cord	36	16	30	32	21	13	31	17	17
Riboflavin (µmol/l)	16 w	0.31	0.04	37	0.30	0.03	15	0.30	0.03	18
	34 w	0.31	0.04	36	0.32	0.03	15	0.31	0.04	18
	6mpp	0.32	0.05	31	0.32	0.04	14	0.32	0.03	17
	Cord	0.49	0.08	33	0.42*	0.11	12	0.41*	0.10	17
Pyridoxal phosphate (nmol/l)	16 w	16	5	37	14	6	15	14	5	17
	34 w	8	3	36	7	2	15	8	3	17
	6mpp	22	11	31	31	25	14	20	7	17
	Cord	49	19	33	42	17	12	51	23	18
Folacin (S) (nmol/l)	16 w	6.8	2.6	34	6.4	2.4	15	6.1	2.1	18
	34 w	4.2	1.9	36	3.5	1.9	15	3.2	2.2	17
	6mpp	4.7	2.1	31	4.3	23	14	4.1	3.0	17
	Cord	14.5	7.1	32	15.3	8.5	13	15.5	6.6	15
Vitamin B_{12} (pmol/l)	16 w	400	105	36	392	64	15	373	105	18
	34 w	335	87	37	293	97	15	321	97	18
	6mpp	422	110	28	438	144	13	404	94	15
	Cord	598	154	31	627	207	13	554	147	17
ETK activity (U/mmol Hb)	16 w	9.0	1.6	37	9.8	2.3	15	8.6	1.8	18
	34 w	8.1	2.4	37	7.3	1.2	15	7.8	2.0	18
	6mpp	10.8	2.5	38	11.2	2.2	14	10.5	1.8	17
	Cord	11.3	2.0	30	10.9	2.7	11	10.8	2.0	16
αETK	16 w	1.13	0.09	37	1.17	0.13	15	1.15	0.08	18
	34 w	1.15	0.16	37	1.21	0.12	15	1.17	0.11	18
	6mpp	1.12	0.07	30	1.17	0.08	14	1.12	0.10	17
	Cord	1.06	0.05	30	1.06	0.08	11	1.04	0.10	16

TABLE 6.3.1 continued

Parameter	Smoking group / Period	I (non-smokers) $\bar{x}$	SD	N	II (1-10 sig/day) $\bar{x}$	SD	N	III (> 10 sig/day) $\bar{x}$	SD	N
EGR activity (U/mmol Hb)	16 w	91	20	37	83	14	15	87	22	18
	34 w	85	20	36	82	14	15	75	19	18
	6mpp	102	24	30	99	15	14	96	25	16
	Cord	133	26	30	130	30	11	139	32	16
αEGR	16 w	1.14	0.15	37	1.18	0.17	15	1.18	0.16	18
	34 w	1.15	0.18	36	1.16	0.14	15	1.25	0.28	18
	6mpp	1.13	0.12	30	1.03	0.08	14	1.18	0.19	16
	Cord	0.98	0.16	30	0.97	0.08	11	1.03	0.23	16
EGOT activity (U/mmol Hb)	16 w	66	12	37	63	9	15	59*	8	18
	34 w	68	19	37	51*	10	15	55*	10	18
	6mpp	71	14	30	66	17	14	63*	9	17
	Cord	122	24	30	111	30	11	123	20	16
αEGOT	16 w	1.94	0.15	37	1.99	0.10	15	2.05*	0.15	18
	34 w	1.92	0.26	37	2.18*	0.29	15	2.12*	0.24	18
	6mpp	1.85	0.17	30	1.87	0.24	14	1.90	0.16	17
	Cord	1.39	0.14	30	1.50	0.25	11	1.40	0.14	16

* Significantly different from non-smokers at $p < 0.05$

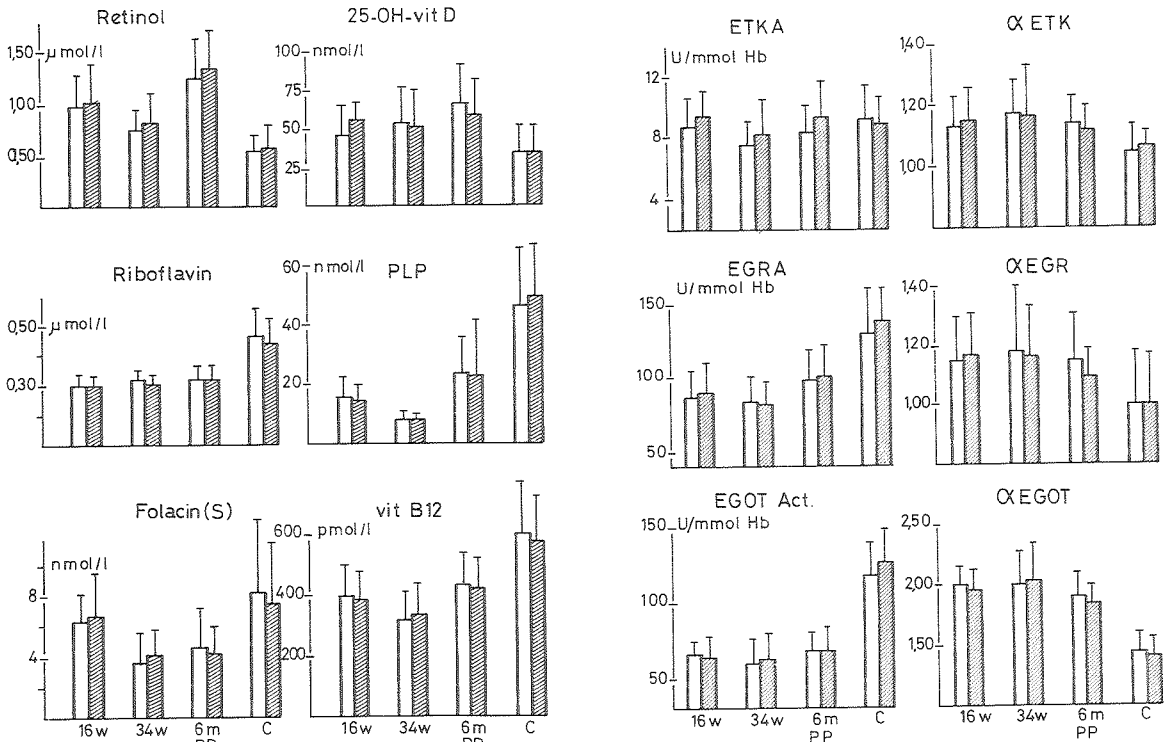


FIGURE 6.4.1 Vitamin status parameters ($\bar{x} \pm SD$) of women from the S-Reference group at 16 and 34 weeks of pregnancy, at 6 months postpartum and in cord blood, according to parity.

□ : parity 0 (n=39)
▨ : parity ⩾ 1 (n=31)

TABLE 6.5.1. Median values of some vitamin status parameters of women from the S-Reference group at different stages of pregnancy, 6 months pp and in cord blood, according to time of sampling.

Parameter	Time	Jan/Febr.		Mar/Apr.		May/June		July/Aug.		Sept/Oct.		Nov/Dec.		All seasons
		P50	(N)	P50	(N)	P50	(N)	P50	(N)	P50	(N)	P50	(N)	P50
Retinol (µmol/l)	16 w	1.10	(12)	0.80	(13)	0.80	(12)	1.10	(11)	1.10	(11)	1.10	(11)	1.10
	34 w	0.90	(11)	0.60	(11)	0.70	(12)	0.80	(14)	0.80	(13)	0.80	(9)	0.84
	Partus	0.40	(13)	0.40	(8)	0.20	(12)	0.40	(14)	0.50	(8)	0.80	(12)	0.45
	6mpp	1.10	(12)	1.70	(9)	0.80	(8)	1.00	(10)	1.00	(9)	1.80	(11)	1.26
	Cord	0.50	(12)	0.50	(8)	0.50	(11)	0.50	(12)	0.50	(7)	0.70	(10)	0.57
25-OHD (nmol/l)	16 w	34	(12)	40	(13)	62	(12)	76	(11)	50	(11)	45	(11)	47
	34 w	29	(11)	30	(11)	63	(11)	67	(14)	65	(13)	46	(9)	51
	Partus	28	(12)	31	(8)	53	(12)	72	(14)	49	(8)	46	(12)	46
	6mpp	50	(13)	51	(9)	53	(10)	86	(10)	65	(9)	55	(11)	56
	Cord	21	(12)	20	(7)	38	(12)	58	(13)	28	(6)	25	(12)	29
Riboflavin (µmol/l)	16 w	0.32	(12)	0.30	(13)	0.30	(12)	0.29	(11)	0.31	(11)	0.29	(10)	0.31
	34 w	0.31	(11)	0.31	(11)	0.33	(11)	0.32	(14)	0.30	(13)	0.30	(8)	0.32
	Partus	0.36	(13)	0.35	(8)	0.35	(12)	0.35	(13)	0.41	(7)	0.33	(11)	0.36
	6mpp	0.33	(13)	0.32	(10)	0.31	(9)	0.31	(10)	0.30	(9)	0.32	(11)	0.32
	Cord	0.45	(12)	0.48	(8)	0.49	(12)	0.47	(13)	0.55	(6)	0.40	(12)	0.47
PLP (nmol/l)	16 w	15	(12)	15	(13)	15	(12)	14	(10)	13	(11)	12	(9)	15
	34 w	7	(11)	6	(11)	9	(12)	7	(13)	9	(12)	7	(9)	8
	Partus	6	(12)	4	(8)	7	(12)	8	(12)	8	(8)	10	(12)	75
	6mpp	18	(13)	26	(10)	24	(9)	27	(10)	17	(9)	18	(11)	19
	Cord	47	(12)	33	(8)	48	(12)	45	(12)	38	(6)	53	(10)	45
Folacin(S) (nmol/l)	16 w	5.5	(12)	7.3	(13)	5.5	(12)	5.4	(11)	6.6	(11)	6.2	(10)	5.9
	34 w	2.9	(11)	4.8	(11)	3.6	(12)	2.0	(14)	3.9	(13)	2.4	(9)	3.4
	Partus	3.2	(12)	3.3	(8)	5.8	(12)	2.6	(13)	3.1	(8)	3.2	(12)	3.5
	6mpp	4.0	(13)	3.7	(7)	3.7	(6)	2.6	(10)	4.0	(9)	4.2	(11)	3.9
	Cord	14.3	(12)	15.0	(7)	20.6	(12)	13.0	(12)	8.9	(6)	13.8	(12)	13.7
Vit.B_{12} (pmol/l)	16 w	362	(12)	408	(12)	335	(12)	418	(11)	426	(11)	385	(11)	397
	34 w	284	(11)	290	(11)	326	(12)	356	(14)	317	(13)	328	(9)	306
	Partus	296	(13)	301	(8)	365	(12)	331	(12)	358	(7)	263	(12)	331
	6mpp	373	(12)	394	(8)	339	(10)	454	(10)	351	(9)	429	(11)	418
	Cord	546	(10)	698	(7)	619	(11)	530	(12)	587	(5)	515	(12)	554
αETK	16 w	1.10	(12)	1.10	(13)	1.11	(12)	1.12	(11)	1.19	(11)	1.17	(11)	1.13
	34 w	1.17	(11)	1.15	(11)	1.07	(12)	1.13	(14)	1.12	(13)	1.28	(9)	1.14
	Partus	1.14	(13)	1.13	(8)	1.11	(12)	1.07	(13)	1.14	(8)	1.19	(12)	1.13
	6mpp	1.12	(12)	1.18	(8)	1.08	(10)	1.11	(10)	1.10	(8)	1.12	(11)	1.13
	Cord	1.05	(10)	1.07	(7)	1.04	(11)	1.06	(12)	0.97	(5)	1.07	(12)	1.06
αEGR	16 w	1.14	(12)	1.01	(13)	1.21	(12)	1.23	(11)	1.25	(11)	1.10	(11)	1.16
	34 w	1.17	(11)	1.14	(11)	1.16	(12)	1.11	(14)	1.15	(13)	1.28	(9)	1.17
	Partus	1.15	(13)	1.05	(8)	1.12	(12)	1.06	(13)	1.02	(8)	1.12	(12)	1.11
	6mpp	1.11	(13)	1.07	(8)	1.15	(10)	1.04	(10)	1.10	(9)	1.12	(11)	1.11
	Cord	0.98	(10)	0.88	(7)	0.98	(10)	0.97	(12)	1.08	(5)	0.97	(12)	0.96
αEGOT	16 w	2.04	(12)	1.96	(13)	1.91	(12)	1.99	(11)	1.96	(10)	2.08	(10)	1.99
	34 w	2.21	(11)	2.00	(11)	1.94	(12)	1.87	(14)	1.99	(12)	2.30	(9)	2.00
	Partus	2.12	(12)	1.85	(7)	2.04	(11)	1.78	(12)	1.85	(6)	2.07	(11)	1.99
	6mpp	1.89	(13)	1.87	(8)	1.84	(9)	1.90	(10)	1.85	(9)	1.90	(11)	1.87
	Cord	1.41	(6)	1.40	(4)	1.37	(5)	1.39	(7)	1.38	(2)	1.45	(7)	1.41

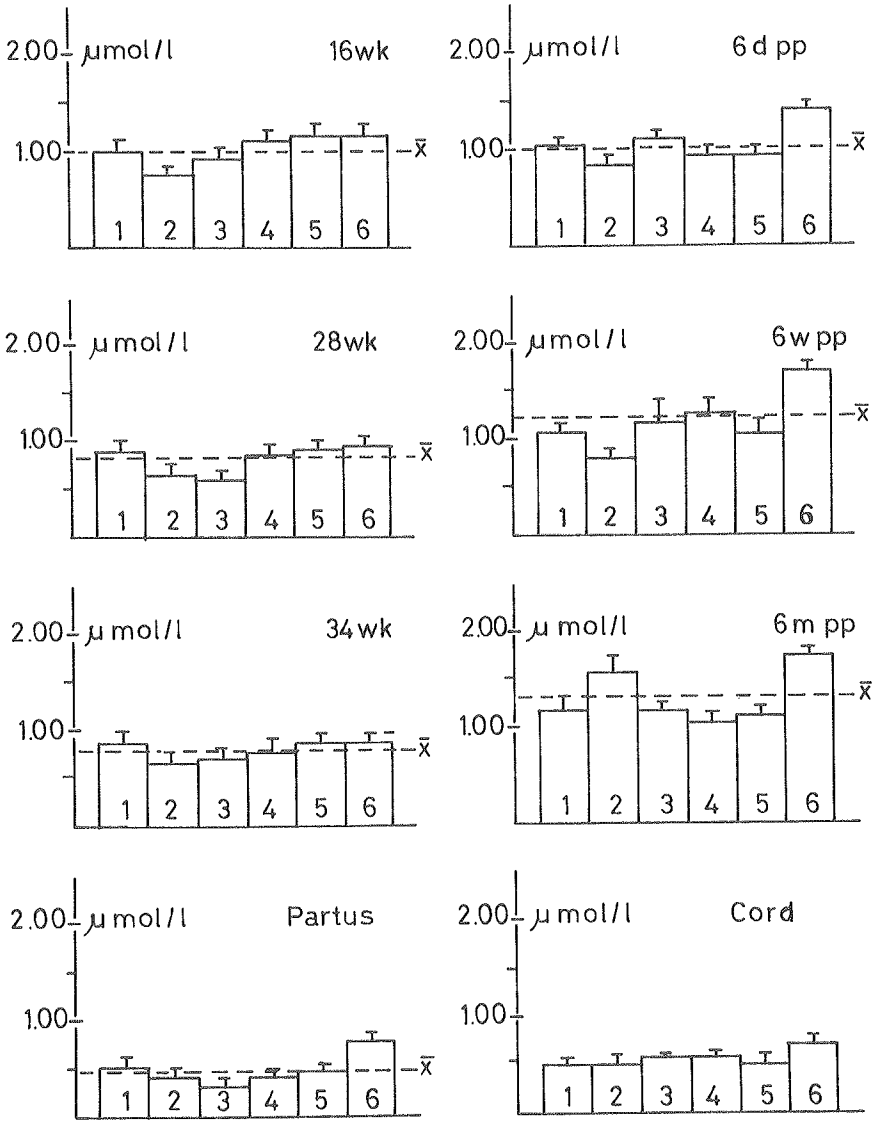


FIGURE 6.5.1 Mean value and standard error of the mean (SEM) of serum retinol content at the various stages of pregnancy and postpartum, according to time of sampling:
1) January/February; 2) March/April; 3) May/June; 4) July/August;
5) September/October; 6) November/December

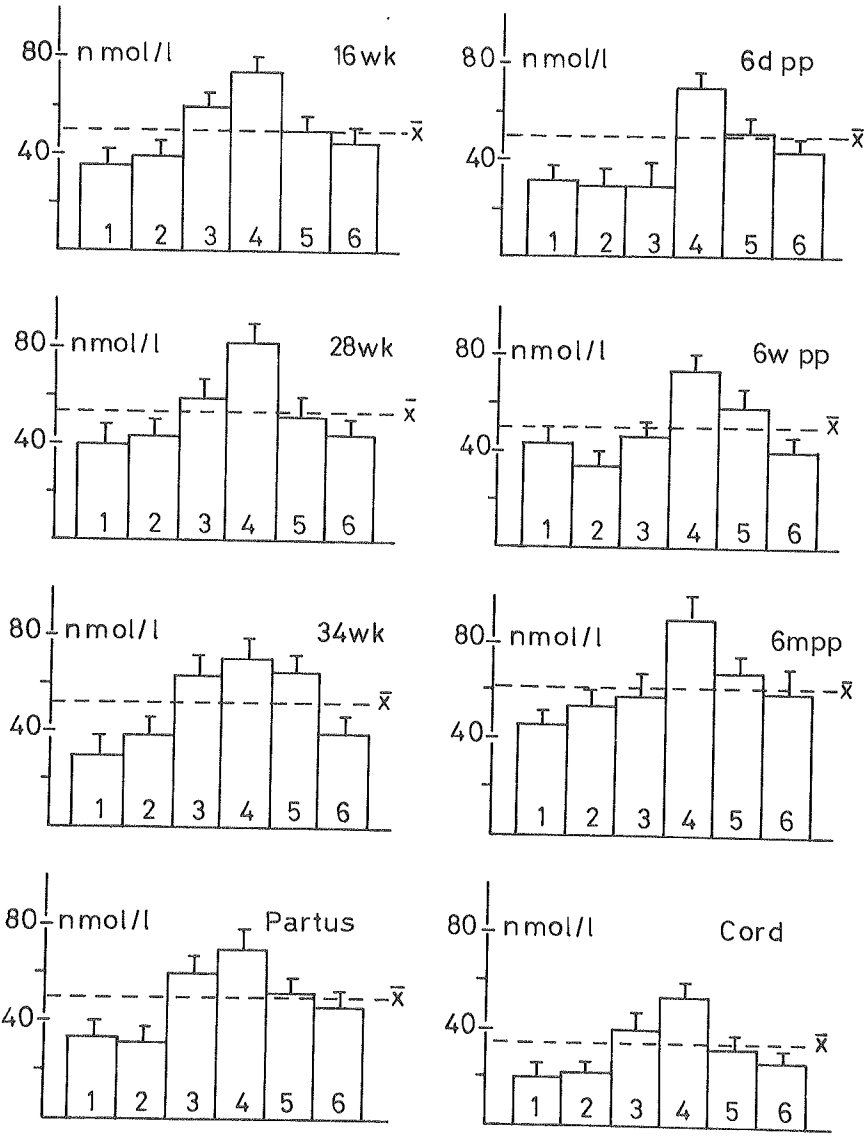
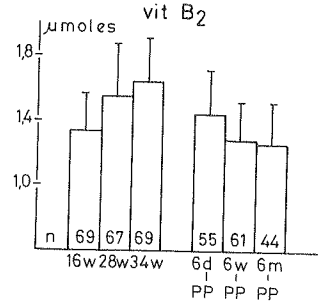


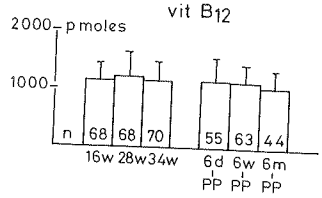
FIGURE 6.5.2 Mean value and standard error of the mean (SEM) of serum 25-OH-vitamin D content at the various stages of pregnancy and postpartum, according to time of sampling:

1) January/February; 2) March/April; 3) May/June; 4) July/August; 5) September/October; 6) November/December

TABLE 6.6.1 Mean, standard deviation and median value of total circulating amount of some vitamins during pregnancy and in the postpartum period (S-Reference group).
S: serum; P: plasma; B: blood.

Parameter		16 w	28 w	34 w	6dpp	6wpp	6mpp
Retinol (S) (µmoles)	$\bar{x}$	0.30	0.28	0.28	0.30	0.31	0.32
	SD	0.12	0.10	0.10	0.11	0.14	0.12
	P50	0.29	0.28	0.27	0.30	0.29	0.12
	N	68	68	70	55	55	43
Riboflavin (B) (µmoles)	$\bar{x}$	1.33	1.55	1.65	1.45	1.29	1.27
	SD	0.24	0.33	0.26	0.26	0.23	0.25
	P50	1.30	1.50	1.62	1.62	1.39	1.25
	N	69	67	69	55	61	44
PLP (P) (µmoles)	$\bar{x}$	0.04	0.03	0.03	0.02	0.05	0.06
	SD	0.02	0.01	0.01	0.01	0.02	0.02
	P50	0.04	0.03	0.03	0.02	0.04	0.05
	N	68	67	68	55	60	44
Folacin (S) (nmoles)	$\bar{x}$	19	16	14	11	11	12
	SD	8	7	7	4	6	6
	P50	17	16	11	11	11	10
	N	66	67	68	53	62	44
Vitamin B_{12} (S) (nmoles)	$\bar{x}$	1.1	1.2	1.2	1.2	1.1	1.0
	SD	0.3	0.4	0.4	0.4	0.3	0.3
	P50	1.1	1.2	1.1	1.1	1.0	1.0
	N	68	68	70	55	63	44





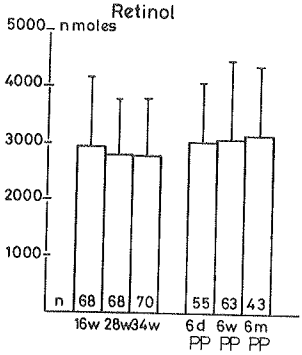
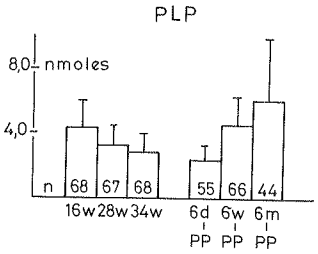


FIGURE 6.6.1 Total circulating amounts of some vitamins during and after pregnancy for women from the S-Reference group (mean value, SD and number are indicated).



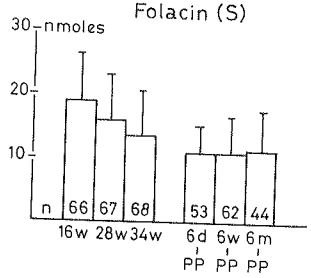


TABLE 6.6.2 Significance of differences between measurements of total circulating amounts for some vitamins during and after pregnancy. +: significant $p < 0.05$; NS: not significant. T_1 : 16 wk; T_2 : 28 wk; T_3 : 34 wk; T_5 : 6dpp; T_6 : 6 wpp; T_7 : 6mpp.

	T_1-T_2	T_1-T_3	T_1-T_5	T_1-T_6	T_1-T_7	T_2-T_3	T_5-T_6	T_5-T_7	T_6-T_7
Retinol	NS	NS	NS	NS	NS	NS	NS	NS	NS
Riboflavin	+	+	+	+	+	+	+	+	NS
PLP	+	+	+	NS	+	+	+	+	+
Folacin	+	+	+	+	+	+	NS	NS	NS
Vitamin B_{12}	+	NS	NS	NS	NS	NS	NS	+	NS

TABLE 6.6.3 Linear correlations between some dietary and biochemical parameters of the vitamin status during pregnancy.

A. Estimated vitamin intake and biochemical values at the 16th week of pregnancy (M_I vs T_1).

		r (N)	Significance
Retinol intake	- serum retinol	0.19 (33)	NS
Thiamin intake	- αETK	-0.40 (29)	<0.05
Riboflavin intake	- αEGR	-0.37 (29)	<0.05
Vitamin B_6 intake	- αEGOT	-0.11 (29)	NS
Vitamin B_6 intake	- plasma PLP	0.33 (33)	<0.05
Energy intake	- serum folacin	0.01 (33)	NS

B. Estimated vitamin intake and biochemical values at the 34th week of pregnancy (M_{II} vs T_3).

		r (N)	Significance
Retinol intake	- serum retinol	0.17 (51)	NS
Thiamin intake	- αETK	-0.28 (29)	NS
Riboflavin intake	- αEGR	-0.22 (29)	NS
Vitamin B_6 intake	- αEGOT	-0.25 (29)	NS
Vitamin B_6 intake	- plasma PLP	0.07 (51)	NS
Energy intake	- serum folacin	0.01 (51)	NS

C. Difference of biochemical measures between the 16th and 34th week value $[\Delta(T_1-T_3)]$ and dietary vitamin intake at the 16th week (M_1) and 34th week (M_{II}) respectively.

		M_1	M_{II}	Significance
Retinol intake	- Δ(serum retinol)	0.19 (33)	0.12 (33)	NS
Vitamin B_6 intake	- Δ(plasma PLP)	0.18 (33)	0.28 (33)	NS
Energy intake	- Δ(serum folacin)	0.05 (33)	0.10 (53)	NS

TABLE 6.6.4 Sensitivity and specificity of dietary history data to identify abnormal values of biochemical indices of the vitamin status.
M_I: dietary intake at wk 16; M_{II}: dietary intake at wk 34;
T_1: value at wk 16; T_3: value at wk 34.

		Sensitivity N/N (%)	Specificity N/N (%)
Retinol intake vs serum retinol level			
Cut-off points	M_I vs T_1 value	1/15 (7)	45/53 (85)
Serum retinol : ≤0.7 µmol/l	M_{II} vs T_3 value	10/21 (48)	25/46 (54)
Retinol intake : 950 µg/day	$M_I + M_{II}$ vs T_3 value	6/10 (60)	27/42 (64)
Thiamin intake vs αETK			
Cut-off points	M_I vs T_1 value	3/21 (14)	43/47 (91)
αETK : >1.25	M_{II} vs T_3 value	8/22 (30)	31/40 (77)
Thiamin intake : 1.1. mg/day	$M_I + M_{II}$ vs T_3 value	3/14 (21)	24/33 (73)
Riboflavin intake vs αEGR			
Cut-off points	M_I vs T_1 value	3/14 (21)	48/54 (89)
αEGR : >1.30	M_{II} vs T_3 value	6/19 (31)	42/48 (88)
Riboflavin intake : 1.6 mg/day	$M_I + M_{II}$ vs T_3 value	3/8 (38)	41/52 (78)
Riboflavin intake vs whole blood riboflavin			
Cut-off points	M_I vs T_1 value	1/14 (7)	52/54 (96)
Whole blood riboflavin : 0.25 µmol/l	M_{II} vs T_3 value	0/19 (0)	47/48 (98)
Riboflavin intake : 1.6 mg/day	$M_I + M_{II}$ vs T_3 value	0/8 (0)	51/52 (99)

TABLE 6.6.4 continued

		Sensitivity N/N (%)	Specificity N/N (%)
Vitamin B6 intake vs αEGOT			
Cut-off points	M_I vs T_1 value	3/19 (16)	47/49 (96)
αEGOT : >2.20	M_{II} vs T_3 value	5/26 (19)	33/41 (80)
Vit. B_6 intake : 1.2 mg/day	$M_I + M_{II}$ vs T_3 value	5/11 (45)	38/46 (83)
Vitamin B6 intake vs plasma PLP			
Cut-off points	M_I vs T_1 value	9/19 (47)	23/49 (47)
Plasma PLP : <15 nmol/l	M_{II} vs T_3 value	25/26 (96)	3/41 (7)
Vit. B_6 intake : 1.2 mg/day	$M_I + M_{II}$ vs T_3 value	11/11 (100)	2/46 (4)

TABLE 6.6.5 Variables available for selection in stepwise multiple regression analysis and sub-set selection analysis to predict the fall in serum(plasma)level between the 16th and 34 th week of pregnancy for retinol, PLP, folacin and vitamin B_{12}.

Independent variables	Multiple Regression Analysis Δ (wk 16-wk 34) (N=34)				Sub-set Analysis Δ (wk 16-wk 34) (N=60)			
	serum retinol	serum PLP	serum folacin	serum vit.B_{12}	serum retinol	plasma PLP	serum folacin	serum vit.B_{12}
Birthweight	x	x	x	x	x	x	x	x
Maternal weight gain Δ (wk 34 - wk 16)	x	x	x	x	x	x	x	x
Prepregnancy weight	x	x	x	x				
Absolute increase in plasmavolume Δ (wk 34 - wk 16)	x	x	x	x	x	x	x	x
Relative increase in plasmavolume Δ (wk 34 - wk 16)/wk 16	x	x	x	x	x	x	x	x
Serum retinol level at wk 16	x				x			
Plasma PLP level at wk 16		x				x		
Serum folacin level at wk 16			x				x	
Serum vitamin B_{12} level at wk 16				x				x
Dietary retinol intake at wk 34	x				x			
Dietary vitamin B_6 intake at wk 34		x				x		
Estimated energy intake at wk 34			x				x	
Free thyroxine index (FTI) at wk 34	x	x	x	x	x	x	x	x
Serum estradiol (E_2) level at wk 34	x	x	x	x	x	x	x	x
Serum estriol (E_3) level at wk 34	x	x	x	x	x	x	x	x
Serum cortisol level at wk 34	x	x	x	x	x	x	x	x
Serum progesterone level at wk 34	x	x	x	x	x	x	x	x
Serum HPL level at wk 34	x	x	x	x	x	x	x	x

TABLE 6.6.5 Continued.

Independent variables	Multiple Regression Analysis Δ (wk 16-wk 34) (N=34)				Sub-set Analysis Δ (wk 16-wk 34) (N=60)			
	serum retinol	serum PLP	serum folacin	serum vit.B_{12}	serum retinol	plasma PLP	serum folacin	serum vit.B_{12}
Serum prolactin level at wk 34	x	x	x	x	x	x	x	x
Serum creatinine level at wk 34	x	x	x	x	x	x	x	x
Change in serum estradiol level between wk 16 and wk 34	x	x	x	x				
Change in serum estriol level between wk 16 and wk 34	x	x	x	x				
Change in serum cortisol level between wk 16 and wk 34	x	x	x	x	x	x	x	x
Change in serum progesterone level between wk 16 and wk 34	x	x	x	x				
Change in serum HPL level between wk 16 and wk 34	x	x	x	x				
Change in serum prolactin level between wk 16 and wk 34	x	x	x	x				
Change in serum albumin level between wk 16 and wk 34	x	x	x	x	x	x	x	x
Change in serum alk. phosphatase activity between wk 16 and wk 34	x	x	x	x	x	x	x	x
Change in serum cholesterol level between wk 16 and wk 34	x							
Change in serum uric acid level between wk 16 and wk 34	x	x	x	x				

TABLE 6.7.1 Mean value and standard deviation of some vitamin status parameters of women from the S-Reference group at 6 weeks and 6 months postpartum according to length of lactational period.

Length of lactation		< 5 days			2-6 weeks			⩾ 6 weeks		
Parameter	time PP	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N
Retinol	6w	1.16	0.47	32	1.18	0.60	16	1.12	0.48	12
(µmol/l)	6m	1.35	0.49	31	1.33	0.44	13	1.03*	0.54	11
25-OH-vitamin D	6w	54	21	32	46	16	16	38*	7	12
(nmol/l)	6m	64	32	33	63	26	14	56	17	10
Riboflavin	6w	0.31	0.04	30	0.31	0.04	16	0.34	0.06	12
(µmol/l)	6m	0.32	0.05	33	0.32	0.03	14	0.32	0.06	11
Pyridoxal phosphate	6w	16	6	31	20	7	15	20	6	11
(nmol/l)	6m	23	18	33	22	8	14	28	15	11
Folacin (S)	6w	4.3	2.3	32	4.4	2.6	15	3.6*	1.2	12
(nmol/l)	6m	4.5	2.1	33	5.2	3.1	14	3.4*	1.6	11
Vitamin B_{12}	6w	414	100	32	545	146	16	436	109	12
(pmol/l)	6m	395	96	32	460	145	13	494*	81	8
ETK activity	6w	9.3	1.8	32	9.0	2.2	16	9.6	2.0	12
(U/mmol Hb)	6m	11.0	2.6	33	10.8	1.9	13	10.8	1.6	11
αETK	6w	1.10	0.07	32	1.07	0.09	16	1.11	0.09	12
	6m	1.14	0.09	32	1.13	0.08	13	1.11	0.09	11
EGR activity	6w	94	20	37	85	26	16	101	19	12
(U/mmol Hb)	6m	100	21	31	97	24	13	99	28	11
αEGR	6w	1.16	0.16	32	1.10	0.17	15	1.03	0.12	12
	6m	1.13	0.17	32	1.08	0.10	12	1.03	0.12	12
EGOT activity	6w	63	17	31	63	14	16	57	11	12
(U/mmol Hb)	6m	70	17	33	67	10	13	63	11	11
αEGOT	6w	1.99	0.23	31	1.95	0.23	16	2.00	0.20	12
	6m	1.85	0.21	33	1.90	0.17	13	1.91	0.15	11

* significantly different from non-lactating (<5 days) group at $p < 0.05$

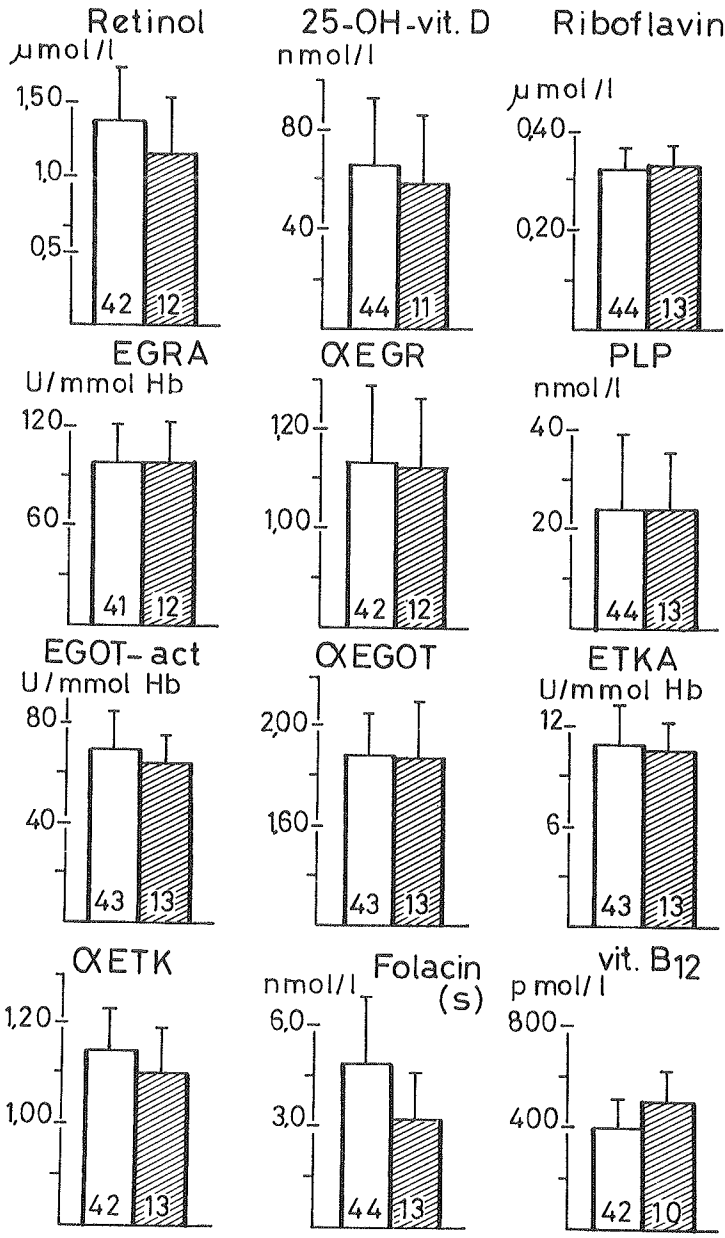


FIGURE 6.7.1 Vitamin status parameters ($\bar{x} \pm SD$) of women from the S-Reference group at 6 months postpartum, according to oral contraceptive (OCA) use.

□ : OCA-users; ▨ : non OCA-users

The number of persons in each group is indicated.

TABLE 6.7.2 The relationship between dietary and biochemical measures of the vitamin status at 6 months postpartum. M_{III}: dietary intake 6 months pp.; T_7: value at 6 months pp.

A Linear correlations between some dietary and biochemical parameters of the vitamin status. (M_{III} vs T_7).

		r (N)	Significance
Retinol intake	- serum retinol	-0.38 (30)	<0.05
Thiamin intake	- αETK	-0.19 (30)	NS
Riboflavin intake	- αEGR	-0.01 (30)	NS
Vitamin B_6	- αEGOT	-0.40 (30)	<0.05
Vitamin B_6	- plasma PLP	0.12 (30)	NS
Energy intake	- serum folacin	-0.15 (30)	NS

B Sensitivity and specificity of dietary history data to identify abnormal values of biochemical indices of the vitamin status.

		Sensitivity N/N (%)	Specificity N/N (%)
Retinol intake vs serum retinol level			
Cut-off points	M_{III} vs T_7 value	1/9 (11)	44/49 (89)
Serum retinol : ⩽ 0.7 µmol/l			
Retinol intake : 850 µg/day			
Thiamin intake vs αETK			
Cut-off points	M_{III} vs T_7 value	1/7 (14)	49/55 (89)
αETK : > 1.25			
Thiamin intake : 0.8 mg/day			
Riboflavin intake vs αEGR			
Cut-off points	M_{III} vs T_7 value	1/14 (7)	45/48 (94)
αEGR : > 1.30			
Riboflavin intake : 1.3 mg/day			

TABLE 6.7.2 continued

		Sensitivity N/N (%)	Specificity N/N (%)
Riboflavin intake vs whole blood riboflavin			
Cut-off points	M_{III} vs T_7 value	0/14 (0)	48/48 (100)
Whole blood riboflavin : < 0.25 µmol/l			
Riboflavin intake : 1.3 mg/day			
Vitamin B6 intake vs αEGOT			
Cut-off points	M_{III} vs T_7 value	1/19 (5)	40/40 (100)
αEGOT : > 2.20			
Vit. B_6 intake : 1.0 mg/day			
Vitamin B6 intake vs plasma PLP			
Cut-off points	M_{III} vs T_7 value	5/19 (26)	33/40 (82)
Plasma PLP : < 15 nmol/l			
Vit. B_6 intake : 1.0 mg/day			

TABLES AND FIGURES

CHAPTER 7

FIGURE 7.1.1 Serum iron levels of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group; NP = non-pregnant reference range.

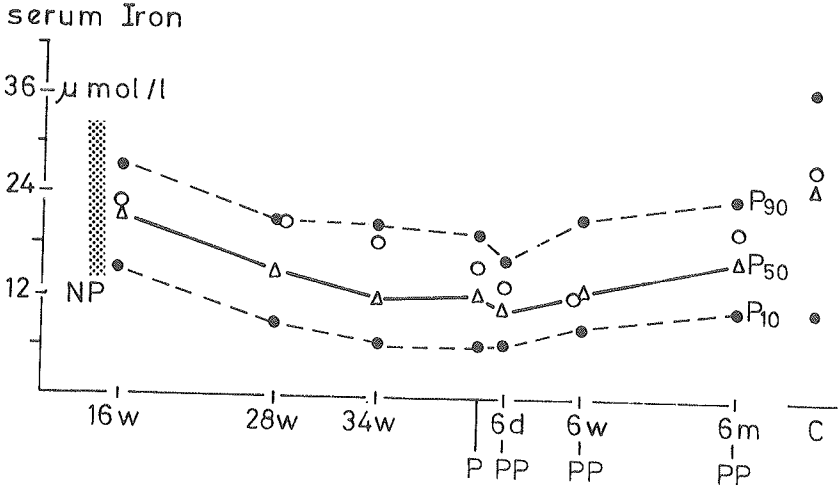


TABLE 7.1.1 Number, mean, S.D. and range of iron values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	68	67	60	64	62	64
$\bar{x}$	21	15	13	13	11	14	17	26
SD	6	5	5.5	5	4	5	6	8
Min.	5	4	5	6	6	2	5	6
Max.	42	25	31	27	27	28	36	45

TABLE 7.1.2 Significance of differences in serum iron measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	+	NS	+	+
6dpp					0	+	+	+
6mpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 7.1.2 Total iron binding capacity (TIBC) of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group; NP = non-pregnant reference range.

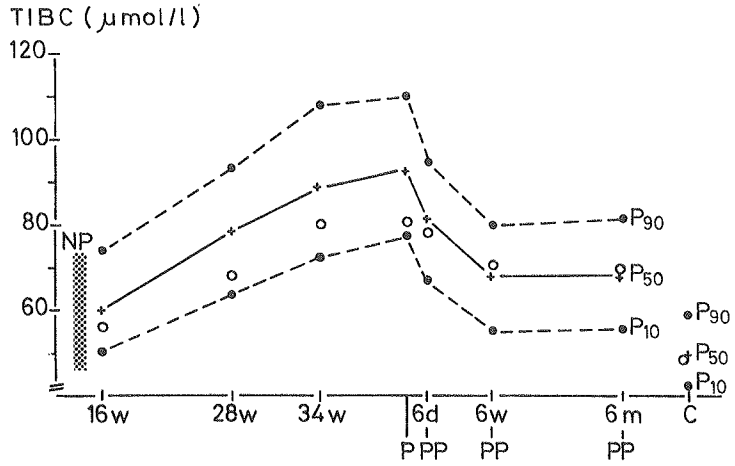


TABLE 7.1.3 Number, mean, SD and range of the TIBC of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	62	64
$\bar{x}$	61	78	89	92	82	67	68	51
SD	10	12	13	12	11	10	10	12
Min.	44	53	68	71	57	51	46	37
Max.	99	109	131	122	112	101	100	117

TABLE 7.1.4 Significance of differences of the TIBC measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6mpp						0	NS	NS

+: significant $p < 0.05$
NS: not significant
ND: no significance determined

FIGURE 7.1.3 Serum transferrin values of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

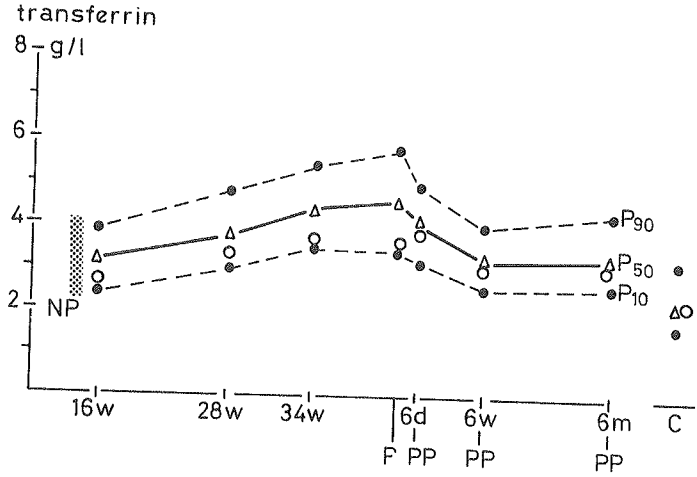


TABLE 7.1.5 Number, mean, SD and range of transferrin values of maternal and cord serum from the S-Reference group group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	70	67	60	64	63	65
$\bar{x}$	3.12	3.84	4.44	4.64	4.06	3.28	3.40	2.41
SD	0.55	0.67	0.68	0.69	0.70	0.55	0.62	0.65
Min.	2.1	2.5	3.0	3.25	2.15	2.25	2.4	1.55
Max.	4.8	5.3	6.4	6.45	5.85	4.95	5.55	5.7

TABLE 7.1.6 Significance of differences in serum transferrin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	NS	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	+	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	+	+
6mpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 7.1.4 Percentage saturation during and after pregnancy in maternal and cord serum from the S-Reference group (10th, 50th and 90th centiles of measurements are shown). Open circles represent the median of the < P10 group NP: non-pregnant reference range.

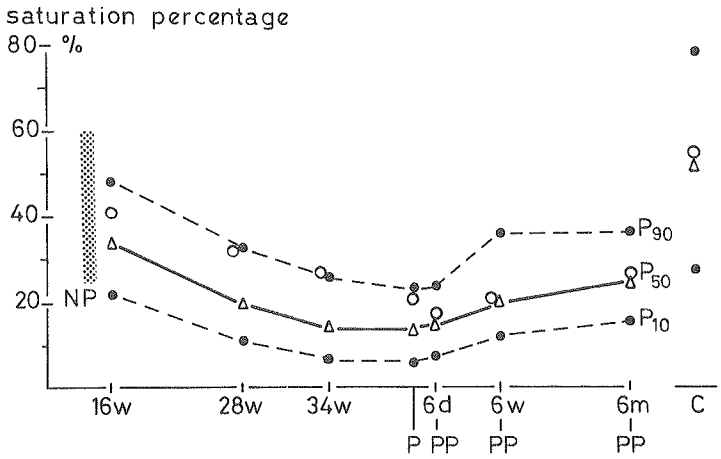


TABLE 7.1.7 Number, mean, SD and range of percentage saturation of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
N	70	70	69	67	60	63	62	64
$\bar{x}$	35	20	15	14	14	21	25	52
SD	11	8	7	6	5	9	9.5	18
Min.	6	4	5	5	7	2	9	8
Max.	68	38	41	32	32	47	59	88

TABLE 7.1.8 Significance of differences in percentage saturation measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	+
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	NS	+	+	+
6dpp					0	+	+	+
6mpp						0	+	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

FIGURE 7.1.5 Serum ferritin levels of women during and after pregnancy and of cord serum from the S-Reference group (10th, 50th and 90th centiles are shown). Open circles represent the median of the < P10 group. NP: non-pregnant reference range.

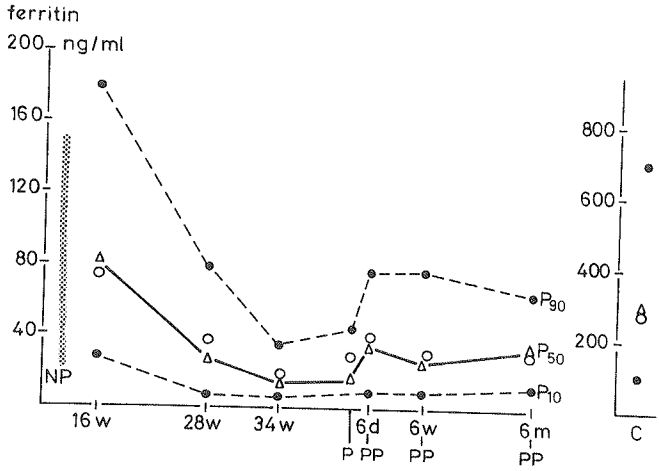


TABLE 7.1.9 Number, mean, SD and range of ferritin values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6 mpp	Cord
N	70	70	70	66	60	64	62	62
$\bar{x}$	91	32	18	21	42	34	38	342
SD	60	32	14	13	23	29	17	210
Min.	11	4	1	6	5	5	12	10
Max.	277	151	65	64	109	153	73	903

TABLE 7.1.10 Significance of differences in serum ferritin measurements from the S-Reference group.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	+	+	+	+	+	+	ND
28 w		0	+	+	ND	ND	ND	ND
34 w			0	NS	ND	ND	ND	+
P				0	+	+	+	+
6dpp					0	+	NS	+
6mpp						0	NS	ND

+: significant $p < 0.05$

NS: not significant

ND: no significance determined

TABLE 7.1.11. Number, mean and SD of serum iron, TIBC, percentage saturation, serum ferritin and serum transferrin from women of the < P10 group and number and median of the same parameters from women of the > P90 group.

			16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Serum iron µmol/l	<P10	N	10	10	9	10	9	10	9	10
		$\bar{x}$	24	20	20	17	13	13	18	23
		SD	7	6	8	7	5	7	8	11
	>P90	N	5	5	5	4	5	5	2	4
		MED	19	15	8	9	7	17	13	27
TIBC µmol/l	<P10	N	10	10	9	10	9	10	9	10
		$\bar{x}$	55	69	78	82	76	67	69	47
		SD	7	10	11	16	13	12	18	10
	>P90	N	5	5	5	4	5	5	2	4
		MED	60	76	92	100	82	66	59	57
% Saturation	<P10	N	10	10	9	10	9	10	9	10
		$\bar{x}$	45	30	27	23	18	20	29	51
		SD	17	12	13	12	6	12	19	24
	>P90	N	5	5	5	4	5	5	2	4
		MED	35	22	9	9	9	23	23	47
Ferritin ng/ml (S)	<P10	N	10	10	9	10	9	10	9	9
		$\bar{x}$	86	47	28	38	49	25	41	284
		SD	58	36	20	31	36	16	30	107
	>P90	N	5	4	5	3	5	5	3	3
		MED	73	16	7	10	26	25	31	206
Transferrin g/l	<P10	N	10	10	9	10	9	10	9	10
		$\bar{x}$	2.79	3.45	3.80	3.91	3.62	3.21	3.43	2.07
		SD	0.25	0.68	0.76	0.89	0.66	0.68	0.78	0.58
	>P90	N	5	5	5	4	5	5	3	4
		MED	3.30	3.60	4.50	5.30	4.45	3.30	3.00	2.63

TABLE 7.1.12. Pearson correlation coefficients between maternal and cord serum iron values from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	0.34***	0.09	0.14	0.29**	0.26*	0.31**	0.06
28 w		0	0.33***	0.41***	0.29**	0.22*	0.17	0.08
34 w			0	0.43***	0.44***	0.16	0.07	0.12
P				0	0.60***	0.26*	0.08	0.07
6dpp					0	0.43***	0.26*	0.29*
6wpp						0	0.17	-0.001
6mpp							0	0.05

TABLE 7.1.13 Pearson correlation coefficients between the TIBC of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	0.68***	0.50***	0.57***	0.53***	0.27*	0.28**	0.23*
28 w		0	0.63***	0.76***	0.64***	0.35***	0.52***	0.13
34 w			0	0.74***	0.60***	0.26*	0.33**	0.29**
P				0	0.71***	0.25*	0.46***	0.29**
6dpp					0	0.47***	0.33**	0.30**
6wpp						0	0.37***	0.05
6mpp							0	0.0001

* P < 0.05

** P < 0.01

*** P < 0.001

TABLE 7.1.14 Pearson correlation coefficient between the percentage saturation of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	0.55***	0.22*	0.41***	0.35**	0.24*	0.38***	0.10
28 w		0	0.38***	0.53***	0.44***	0.29**	0.29**	0.09
34 w			0	0.49***	0.49***	0.16	0.11	0.03
P				0	0.63***	0.28**	0.16	0.13
6dpp					0	0.39***	0.36**	0.23*
6wpp						0	0.38***	0.13
6mpp							0	0.06

TABLE 7.1.15. Pearson correlation coefficients between ferritin values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	0.69***	0.46***	0.25*	0.29**	-0.07	0.44***	0.03
28 w		0	0.68***	0.31**	0.02	-0.14	0.34**	-0.09
34 w			0	0.46***	0.15	0.08	0.18	-0.03
P				0	0.16	0.14	0.17	0.11
6dpp					0	0.18	0.17	-0.007
6wpp						0	0.41***	0.16
6mpp							0	-0.05

TABLE 7.1.16. Pearson correlation coefficient of transferrin values of maternal and cord serum from the S-Reference group during and after pregnancy.

	16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
16 w	0	0.53***	0.44***	0.44***	0.37***	0.24*	0.30**	-0.04
28 w		0	0.53***	0.46***	0.31**	0.14	0.28**	-0.03
34 w			0	0.66***	0.56***	0.17	0.30**	0.25*
P				0	0.71***	0.33**	0.37***	0.31**
6dpp					0	0.37***	0.34**	0.23*
6wpp						0	0.36***	0.03
6mpp							0	-0.05

* P < 0.05

** P < 0.01

*** P < 0.001

TABLE 7.3.1 Red cell volume, hemoglobin and iron status parameters values of women from the S-Reference group divided according to iron medication. Mean and SD are given.
Group 0 (n=50) no iron medication
Group 1 (n= 7) iron medication during pregnancy
Group 2 (n=10) iron medication postpartum

			16 w	28 w	34 w	P	6dpp	6wpp	6mpp	Cord
Red cell volume/l	0	$\bar{x}$	1.544	1.712	1.785	-	1.532	1.572	1.553	-
		SD	0.234	0.234	0.247	-	0.215	0.214	0.218	-
	1	$\bar{x}$	1.479	1.551	1.750	-	1.698	1.484	1.493	-
		SD	0.211	0.196	0.237	-	0.312	0.108	0.257	-
	2	$\bar{x}$	1.469	1.601	1.597	-	1.304	1.396	1.487	-
		SD	0.311	0.230	0.240	-	0.290	0.240	0.380	-
Hb mmol/l	0	$\bar{x}$	7.9	7.6	7.6	8.1	7.7	8.4	8.7	10.1
		SD	0.7	0.6	0.5	0.6	0.6	0.6	0.6	0.9
	1	$\bar{x}$	7.1	6.7	6.9	7.8	8.0	8.4	8.4	9.6
		SD	0.8	0.8	0.5	0.5	0.8	0.6	0.8	1.2
	2	$\bar{x}$	8.0	7.5	7.5	7.9	6.9	8.3	8.9	10.7
		SD	0.6	0.4	0.5	0.3	0.5	0.4	0.4	0.9
Serum iron µmol/l	0	$\bar{x}$	21.7	15.6	22.6	13.1	12.2	13.4	16.7	26.0
		SD	5.9	4.6	4.8	5.0	4.6	5.5	5.6	8.3
	1	$\bar{x}$	14.7	13.4	15.7	12.6	10.6	14.9	14.3	27
		SD	5.6	5.3	4.8	4.0	3.0	4.0	7.2	10.6
	2	$\bar{x}$	22	12.8	7.4	10.5	8.8	15.7	18.0	24.2
		SD	2.7	5.0	1.7	2.9	1.8	5.9	7.5	7.9
Percentage saturation	0	$\bar{x}$	37	21	15	15	15	20	26	52
		SD	11	8	7	7	6	9	10	17
	1	$\bar{x}$	24	17	18	13	14	23	21	56
		SD	11	8	6	4	3	7	11	23
	2	$\bar{x}$	36	16	8	11	11	25	25	51
		SD	6	9	2	4	3	10	9	20
Ferritin ng/ml	0	$\bar{x}$	97	35	18	21	41	27	39	346
		SD	63	34	14	13	23	22	19	207
	1	$\bar{x}$	95	36	24	25	51	61	50	367
		SD	59	36	16	18	29	43	19	267
	2	$\bar{x}$	72	23	16	16	39	43	26	278
		SD	42	22	8	5	27	41	14	136
Transferrin g/l	0	$\bar{x}$	3.1	3.7	4.3	4.6	4.1	3.3	3.4	2.4
		SD	0.6	0.6	0.6	0.7	0.7	0.6	0.6	0.7
	1	$\bar{x}$	3.2	4.3	4.4	4.4	3.5	3.2	3.2	2.3
		SD	0.6	0.4	0.4	0.5	0.9	0.5	0.7	0.3
	2	$\bar{x}$	3.2	4.1	4.8	5.0	4.4	3.1	3.7	2.4
		SD	0.3	0.8	0.9	0.6	0.6	0.5	0.9	0.7

TABLES AND FIGURES

CHAPTER 8

TABLE 8.9.1. The independent variables used in the stepwise forward multiple regression analysis and in the subset selection regression analysis. Birth centile and Ponderal-index are the dependent variables.

Variables	Stepwise forward multiple regression	Subset selection regression analysis
Maternal height	x	x
Maternal age	x	x
Prepregnant weight	x	x
Maximal weight gain	x	x
Weight gain up to 34 weeks	x	
Paternal height	x	
Plasma volume 16 weeks	x	
Plasma volume 34 weeks	x	x
Plasma volume 34 w - 6 w pp	x	
Blood volume 16 weeks	x	
Blood volume 34 weeks	x	
Blood volume 34 w - 6 w pp	x	
% increase plasma volume 34 w - 6 w pp	x	
% increase blood volume 34 w - 6 w pp	x	
Hemoglobin 34 weeks	x	
Serum iron 34 weeks	x	
EGOT-activity 34 weeks	x	
EGOT-stimulation effect 34 weeks	x	
Energy intake per kg at 34 weeks	x	
Fat intake per kg at 34 weeks	x	
HPL level at 34 weeks	x	
Smoking	x	x