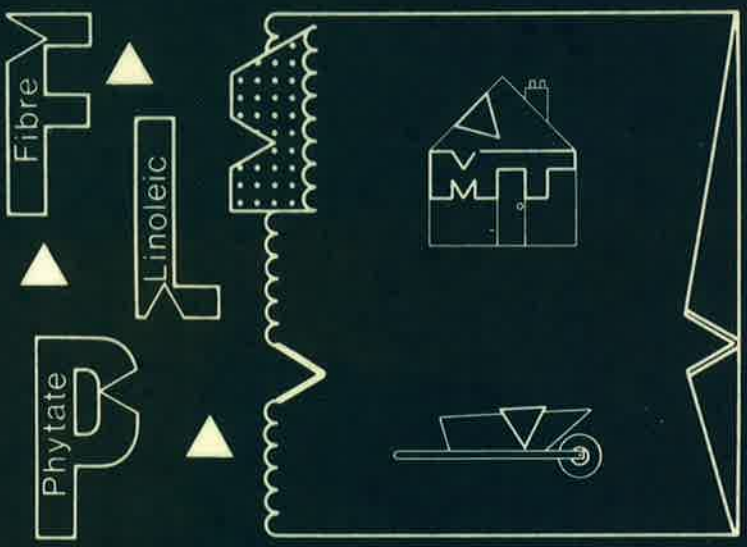
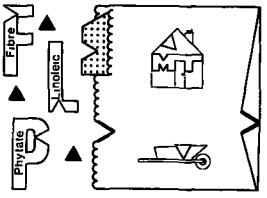
B 5

DIETARY RECOMMENDATIONS AND MINERAL UTILIZATION



W. VAN DOKKUM

DIETARY RECOMMENDATIONS AND MINERAL UTILIZATION



This schematic drawing illustrates the suggested interactions of minerals (▲) with some components of the diet (dietary fibre, phytate and linoleic acid) in the intestinal lumen. All components mentioned may bind with minerals, resulting in complexes which cannot enter the mucosal cell. In addition, if the minerals have actually been taken up by the cell, they can either be transported through the cell by a carrier protein or interact with a mineral binding protein (MT = metallothionein). In the latter case the minerals cannot pass through the cell and will therefore not be absorbed.

Details of the absorption mechanisms are presented in chapter 7 of this thesis.

1. Bij het vaststellen van aanbevolen hoeveelheden van mineralen en spoorelementen is het gewenst te streven naar een gecombineerde aanbeveling, waarbij de onderlinge verhoudingen binnen bepaalde grenzen worden vastgelegd.
   Dit proefschrift.

2. Het is noodzakelijk de aanbevelingen voor mineralen en spoorelementen af te stemmen op een bepaald voedingssysteem in verband met mogelijke verschillen in de benutbaarheid.
   Dit proefschrift.

3. Een aanbevolen voedingspatroon voor de Nederlander is slechts realistisch bij behoud van het typische karakter van de voedingsgewoonten in Nederland.

4. Voorzichtigheid zij geboden met het suppleren van de voeding met mineralen en spoorelementen tijdens zwangerschap, lactatie en gedurende de eerste levensjaren: interacties tussen mineralen kunnen door ongunstige onderlinge verhoudingen leiden tot een onverwachte marginale mineralenstatus.
   L.C. Hurley et al. (1983), Fed. Proc. 42, 1735-1739.

5. Voor het vaststellen van de mineralen- en spoorelementen-status is het gewenst een combinatie van criteria te hanteren. Afgaan op de meting van slechts een parameter is voor het vaststellen van een marginale status onvoldoende.

6. Niet de additieven en contaminanten in onze voeding vormen een bedreiging voor de volksgezondheid, het is meer de onjuiste voedselkeuze die zorgen behoort te baren.

7. De door Linus Pauling gepropageerde opneming van megadoses vitamine C gaat voorbij aan de ongunstige invloed die dit kan hebben op de koperabsorptie en op het ontstaan van nier- en blaasstenen.
   E.B. Finley and F.L. Cerklewski (1983), Am. J. Clin. Nutr. 37, 553-556.
   L. Alhadeff et al. (1984), Nutr. Rev. 42 nr. 2, 33-40.

8. Een door biochemische parameters geïndiceerde marginale vitaminestatus kan leiden tot een vermindering van het lichamelijk functioneren.
   E.J. van der Beek, W. van Dokkum and J. Schrijver (1984)
   Nederl. Milit. Geneesk. T. 37 nr. 2, 47-52

9. Hoewel een laag serumcholesterolgehalte ongetwijfeld bij kan dragen tot een verlaging van het risico voor hart- en vaatziekten is het niet zo, dat een wetenschappelijk (voedings)onderzoek pas compleet is als ook het serumcholesterolgehalte wordt gemeten.

10. Voor het verzamelen van 24-uurs urine is de bottle-neck vaak de bottle-neck.

11. Voor het welslagen van internationale cursussen is aandacht voor de niet-wetenschappelijke aspecten tenminste even belangrijk als de zorg voor een goed cursusprogramma.

12. Uit een oogpunt van voedingsgezondheid zou het aanbeveling verdienen de uitdrukking "een grote hoop is een blijde boodschap" door middel van een verhoogde voedingsvezelconsumptie algemeen eigen te maken.

13. De bekende compositie Dr. Jazz doet vermoeden, dat deze muziekvorm gepromoveerd is tot een algemeen aanvaarde vorm van kunst. Dit vermoeden is helaas onjuist.

14. Het is eenvoudiger te goochelen voor een populatie van hoogleraren dan voor een groep kinderen.

Stellingen behorende bij het proefschrift van W. van Dokkum.

DIETARY RECOMMENDATIONS AND MINERAL UTILIZATION

ACADEMISCH PROEFSCHRIFT

Ter verkrijging van de graad van doctor in de Geneeskunde
aan de Universiteit van Amsterdam, op gezag van de
Rector Magnificus, Dr. D. W. Bresters, hoogleraar in
de Faculteit der Wiskunde en Natuurwetenschappen,
in het openbaar te verdedigen in de aula der Universiteit
(tijdelijk in het Wiskundegebouw, Roetersstraat 15)
op donderdag 27 september 1984 te 16.00 precies.

door

Willem van Dokkum

geboren te Maartensdijk

krips repro meppel

Promotores: Prof. Dr. R. Luyken
Prof. Dr. Ir. R. J. J. Hermus

Financial support by the Netherlands Heart Foundation
for the publication of this thesis is gratefully acknowledged.

Scientae Caput Referentia Est Dei

aan myn ouders

voor Joke,
Margreet,
Femke

Contents

Voorwoord

De in dit proefschrift beschreven experimenten met vrijwilligers konden uiteraard slechts uitgevoerd worden met behulp van velen.
Als eersten wil ik mijn beide promotores Prof.Dr. R. Luyken en Prof.Dr.Ir. R.J.J. Hermus dank zeggen voor de begeleiding gedurende de afgelopen jaren; de bescheiden, maar zeker motiverende wijze waarop Rijk Luyken immer blijk gaf van zijn interesse voor de voedingsproeven zijn erg stimulerend geweest; de waardevolle kritische opmerkingen van Ruud Hermus in vooral de "schrijffase" zijn zeer gewaardeerd. De Directie van het CIVO-TNO complex te Zeist ben ik erkentelijk voor de geboden faciliteiten de onderzoekresultaten in dit proefschrift vast te leggen.
De diëtisten Frances Cloughley, Karin Hulshof en Anneke Wesstra hebben een essentieel aandeel gehad in de vele voorbereidende werkzaamheden ten aanzien van de verzorging van de "intake" van de proefpersonen, waarvoor veel dank is verschuldigd.
De proefpersonen zouden en masse zijn weggelopen wanneer de voeding niet op zo'n uitstekende wijze en in zo'n genietbare vorm zou zijn klaargemaakt door Liesbeth de Groot!
Het analytische deel van het onderzoek is vaak niet de meest in het oog lopende activiteit, overigens geheel ten onrechte. Dank zij de volharding van de analistes en analisten bij de analyses van de zeer vele voedings-, urine- en faecesmonsters zijn de experimenten tot een goed einde gebracht; ik wil hier speciaal noemen Leen van Ginkel, Francien Schippers, Gemma ten Holter, Hillie van Steenbrugge, Lien Wansink en Joke IJsseling.
De noeste ijver waarmede Niek Pikaar zich vervolgens op de getallen stortte werd immer als grote steun ervaren; voor de plezierige samenwerking en de nuttige discussies over vooral de analytische aspecten van de proeven ben ik hem zeer erkentelijk.
Alle proefpersonen werden regelmatig door Henk van de Weerd onder andere in de tang genomen; veel gegevens over anthropometrie en bloeddruk waren het resultaat, door allrounder Henk op uitstekende en wetenschappelijk verantwoorde wijze vergaard; veel dank hiervoor!
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Het moeten honderden pagina's tekst zijn geweest die voor, tijdens en na de proeven door Tineke van der Vaart zijn getypt; Tineke bedankt voor dit onmisbare onderdeel van de experimenten!
De statistische begeleiding was in voortreffelijke handen van Jacques Thissen en Michel Wedel, die de wellicht ondankbare taak hadden de meetgegevens in een dusdanige vorm te gieten dat een eenvoudig auteur de significantie van een en ander kon aangeven.
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Veel medewerkers van andere afdelingen binnen het CIVO-complex hadden een belangrijk aandeel in de analyses van de bloed- en urinemonsters (vooral de afdeling Klinische Biochemie) en de voedingsmonsters (afdeling Algemene Levensmiddelenchemie en afdeling Oliën, Vetten en Margarine). De standaard bal gehakt en andere vleeswaren waren van uitstekende kwaliteit dank zij de zorg van de afdeling Nederlands Centrum voor Vleestechnologie.
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De heer Vermeer heeft als arts van de Bedrijfsgeneeskundige Dienst TNO ertoe bijgedragen dat de proefpersonen uiteindelijk het praedikaat "goedgekeurd" konden verwerven.
De ondersteuning van de service afdelingen Technische Dienst, Bibliotheek en Documentatie, Algemeen Secretariaat, Financiële Administratie en Inkoop en Magazijn tijdens en na de proeven is steeds erg op prijs gesteld!
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Uiteraard was uitvoering van de experimenten onmogelijk geweest zonder de 46 proefpersonen. Hen ben ik erg dankbaar voor de volharding en medewerking die ertoe geleid hebben dat de proeven zonder grote problemen zijn verlopen.
Tenslotte dank ik mijn vrouw Joke voor de stimulans bij het voltooien van dit proefschrift.

1. Introduction

1.1 DIETARY RECOMMENDATIONS

In the last ten to twenty years growing importance has been attached to studying the health aspects of the lifestyle, including nutrition, in industrialized countries. In this connection an increasing recognition of the role of nutrition in the etiology of modern diseases in western society is observed. On the one hand, this is based on the results of various epidemiological investigations suggesting interaction between nutrition and health, on the other hand on the experience gained in a large number of animal and human experimental nutrition studies, supporting the epidemiological findings.

The results of this research work enabled national Nutrition Councils and other official bodies to establish dietary recommendations, which may lead to recommended intakes of nutrients. However, there seems to be confusion about the definition of recommendations.

According to the Dutch Nutrition Council (27) dietary recommendations are to be interpreted as desirable amounts of energy and nutrients for programming the food supply of a population group; recommendations also serve to evaluate the actual consumption figures. Moreover, it seems increasingly important to use recommendations for the nutrient labeling of foods.

The U.S. National Academy of Sciences (Committee on Dietary Allowances of the Food and Nutrition Board of the National Research Council) defines the recommended dietary allowance (RDA) as: the levels of intake of essential nutrients considered, on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons (17).

The U.K. Department of Health and Social Security defines the recommended dietary intake (RDI) for nutrients as the amounts sufficient or more than sufficient for the nutritional needs of practically all healthy persons in a population (29). The U.S. and U.K. definitions are almost identical, except that "intake" refers to what is eaten and "allowance" to what is provided.

It should be emphasized that both RDA and RDI are not identical with requirements, which is a physiological concept: the amount of a nutrient needed to maintain health.

In a discussion on the transition from requirements to recommendations, Waterlow (29) mentions two factors: The individual variability of requirements and the need for recommendations to be practical within a social context.

The coefficient of variation of e.g. the individual protein requirement has been estimated at 15 % (20). An intake equal to the mean + 30 % should cover the needs of 97.5 % of the individuals in a population. At this intake the number of people who remain at risk (2.5 %) is small enough to be acceptable from a public health point of view. This is the justification of the term safe level of intake adopted by FAO/WHO (20): "the amount of protein considered necessary to meet the physiological needs and maintain the health of nearly all persons in a specified group.

The term "safe" has the advantage of introducing the idea of risk and hence of probability; a person who has an intake lower than the safe level is not necessarily deficient, but the lower the intake, the greater the probability to become deficient.

Recommendations should also be practical. They must not be out of line with established dietary patterns and prevailing eating habits. As the actual protein intake in the U.S. is much higher than the safe level of protein intake, a National Academy of Sciences report (17) states that RDA should not be unnecessarily used as justifications for reducing habitual intakes of nutrients.

Although estimates of human requirements may be the same in different countries, it seems reasonable that recommendations should differ from one country to another on account of practical considerations.

Waterlow (29) arrives at the conclusion that both RDA and RDI appears to be unusable for the purpose of prescription, planning and diagnosis. He suggests the term reference amounts of a nutrient, which is the average amount, which, on the basis of current knowledge and experience, should be provided or consumed per person, to meet the needs of a group. If an individual consumes less than the reference amount he would be considered potentially at risk, but there would be no implication that a government recommendation was not being fulfilled. This would only be the case if a group received less than the reference amount.

It is interesting to look at the developments in the dietary recommendations in The Netherlands in the last 30 years. Table 1 summarizes the Dutch recommendations for energy and macronutrients in 1954, 1973 and 1981.

TABLE 1 - DUTCH DIETARY RECOMMENDATIONS FOR ENERGY AND MACRONUTRIENTS IN 1954, 1973 and 1981*
(for male adults of 20-35 year, low activity pattern)

	1954	1973	1981
Energy (kcal)	2800	2600	2600
Protein (g)	75	65	65
Protein (en. %)	11	10	10
Fat (g)	75	85-100	85-115**
Fat (en. %)	24	30- 35	30- 40**
Carbohydrate (g)	445	360-390	360-390
Carbohydrate (en. %)	64	55- 60	55- 60

* source: Dutch food tables for the years indicated
** the desirable fat intake is approximately 33 en. % (= appr. 95 g)

Apart from the definitions of RDA, RDI and safe levels of intake, a recommended pattern of nutrient intake arrives at a more overall concept, aiming at changes of the current dietary pattern towards a diet which is in line with the RDA (RDI). For The Netherlands the concept has changed from the "basic five food groups" approach ("schijf van vijf"), in which it was recommended to consume an adequate amount of each food group daily, to an approach in which it is generally advised to consume more vegetable products and less of animal products ("Maaltijdschijf").
The recommended pattern of nutrient intake is summarized in the so called "dietary goals" in the USA (7,23). In The Netherlands the Nutrition Council has drawn up recommendations for the improvement of our diet with a view to nutritional health (26); in other countries similar nutrition statements have been formulated.

Evaluation of our current diet, which is discussed in detail in 1.2, has led to the following recommendations regarding macronutrients (26):

- Reduction of total fat intake.
- Reduction of saturated fat intake.
- Increase of the amount of dietary linoleic acid (or generally polyunsaturated fat).
- Increase of dietary fibre intake.
- Shift from largely animal protein towards vegetable protein.

In various countries there is a more or less common opinion regarding the advisable direction of the recommended dietary changes, although the recommended dietary nutrient intake differs from country to country, as can be concluded from table 2.

TABLE 2 - RECOMMENDED STANDARDS ON PROTEIN, FAT AND CARBOHYDRATE DENSITY IN THE NATIONAL DIET OF SOME EUROPEAN COUNTRIES (31) AND THE USA (17)

Nutrient	Density (% of total energy)	Country
Protein	10	United Kingdom
	10-12	The Netherlands
	10-15	Sweden
	12	USA
	12-13	W-Germany
Fat	25-35	Sweden
	30	USA
	30-33	W-Germany
	30-35	Norway, The Netherlands
Carbohydrate	50-60	Sweden
	55-60	The Netherlands, USA

The desired ratio between animal and vegetable protein also differs, e.g. 30-33 % of total protein intake as animal protein in Czechoslovakia and 54-70 % in E-Germany. As far as the type of fat is concerned, in The Netherlands the Nutrition Council recommends a ratio between polyunsaturated fatty acids (specifically linoleic acid) and saturated fatty acids (particularly 12-16 carbon atoms) of 1:2 to 1:1 in a diet with 30-35 energy % total fat.

In W-Germany an energy % of saturated dietary fat of less than 15 is recommended (8).
The required dietary concentration of linoleic acid varies between 1 and 6 energy %, however, these figures pertain to physiological requirements (31). In The Netherlands a recommendation of 8-10 energy % of linoleic acid has been formulated (26); US dietary goals recommend a figure of 10 energy % for polyunsaturated fats.
No recommended intake values for dietary fibre have been proposed as yet, partly because of too many uncertainties with respect to the knowledge of the action of different dietary fibre components in the human gut.

1.2 THE DUTCH DIETARY PATTERN

In order to evaluate the significance of dietary recommendations and the impact these recommendations may have on actual dietary practice it is of great importance to investigate the dietary pattern of the population regularly.
The dietary pattern in The Netherlands can be characterized on the basis of the "daily gross intake per capita of energy and some nutrients", derived from data on available food supply published by the Ministry of Agriculture and Fisheries (3). As to the macronutrients and energy, table 3 summarizes the development of the Dutch dietary pattern since 1936.
The proportion of protein in the available energy supply remained constant throughout the years at a level of 11-12 energy %. However, the gradual shift from vegetable protein to animal protein is apparent. The contribution of fat has increased to approximately 40 energy %; the contribution of total carbohydrates decreased to 45 energy % and the alcohol contribution reached a level of almost 5 energy %.
The same development can be seen in e.g. the USA as summarized in table 4.

An essential question is whether the figures of table 3 can be used to describe the Dutch dietary pattern, since they present the gross average of the whole population from 0-80 years and over. In addition, the availability of food does not mean that the actual intake is similar.

TABLE 3 - PERCENTAGE CONTRIBUTION OF THE MACRONUTRIENTS TO THE AVAILABLE ENERGY SUPPLY IN THE NETHERLANDS FROM 1936-1981
(per caput per day)

	1936-1938	1950	1960	1970	1975	1978	1979	1980	1981
Total protein	11.9	11.0	10.9	11.0	11.5	11.5	11.5	11.5	11.6
Animal protein	5.3	5.8	6.5	7.3	8.0	7.8	7.8	7.9	7.9
Vegetable protein	6.6	5.2	4.4	3.7	3.5	3.7	3.7	3.6	3.7
Total fat	33.7	32.6	36.8	38.3	38.0	38.5	38.6	39.1	39.1
Total carbohydrates	54.4	55.2	50.8	47.9	46.0	45.6	45.3	45.1	45.0
Alcohol	?	1.2	1.5	2.8	4.5	4.4	4.6	4.3	4.7

TABLE 4 - SOURCES OF ENERGY IN THE US FOOD SUPPLY (30)
(% of total energy supply)

	1909-1913	1947-1949	1980*
Total protein	12	12	12
Animal protein	6.2	7.7	8.2
Vegetable protein	5.8	4.3	3.8
Total fat	32	39	43
Animal fat	27	29	25
Vegetable fat	5	10	18
Total carbohydrates	56	49	46

*preliminary data

TABLE 5 - INTAKE OF ENERGY (kcal) AND MACRONUTRIENTS (en. %) IN THE NETHERLANDS (1970-1980) FROM FOOD CONSUMPTION STUDIES OF VARIOUS AGE GROUPS (source: Dutch Nutrition Council (26))

Age group	sex	no. of subjects	energy (kcal)	total protein (en. %)	total fat (en. %)	satur. fat (en. %)	PUFA (en. %)	linoleic acid (en. %)	total carboh. (en. %)	mono + disacch. (en. %)	poly sacch. (en. %)	alcohol (en. %)
0-1	m + f	131	780	15	28				58			
4-6	m + f	85	1640	12	37				50			
5	m + f	705	1703	13	40	19	4	4	47	27	20	
6	m + f	378	1700	13	39				48	28	20	
6	m + f	327	1676	13	38				48	28	20	
6-10	m	121	2041	14	38	18	6		47	26	20	
6-10	f	99	1852	13	39	19	6		46	26	20	
6-10	m	105	1891	12	42	18	5		49	28	22	
6-10	f	89	1788	12	41	18	6		48	28	20	
6-10	m	94	2103	14	40	19	6		44	23	21	
6-10	f	68	1848	13	40	19	6		45	25	21	
7	m + f	840	1894	14	37	17	5	4	48	26	22	
8	m	37	2413	12	40			5	47	11	36	
8	f	36	2074	12	40			4	47	13	34	
8-9	m	64	1974	12	36				53			
8-9	m	85	2072	12	38				50			
8-9	f	96	1871	12	39				49			
6-12	m + f	159	2065	12	40	17	6		47			
12-13	m	5	2340	12	40				46			
12-13	m	4	2430	12	39				48			
12-13	f	7	2365	11	41				47			
12-13	f	7	2350	14	44				43			
14	f	30	2790	12	42			5	47	24	23	
15-16	m	6	3170	13	41				45			
15-16	m	6	2935	14	38				47			
15-16	f	6	2770	12	46				41			
15-16	f	5	2370	14	40				46			
15-17	m	79	2916	12	38				49	23	26	
15-17	f	57	2215	13	37				49	24	25	
15-18	m	39	3584	11	33			5	51	22	29	5
16-19	m	15	3560	14	39				42			5
16-19	f	21	2450	13	41				44			2
17	m	49	3342	12	37				47			4
17	m	43	3373	12	42				44			3
17	m	30	3397	12	41			5	44	10	34	
17	m	30	2903	12	42			6	44	10	34	
17	f	20	2383	13	42			5	43	12	31	
17	f	18	2497	12	41			7	46	11	35	
16-64	m	266	2880	14	39				42			
16-64	f	31	2090	14	41				41			

TABLE 5 - INTAKE OF ENERGY (kcal) AND MACRONUTRIENTS (en. %) IN THE NETHERLANDS (1970-1980) FROM FOOD CONSUMPTION STUDIES OF VARIOUS AGE GROUPS, CONTINUED (source: Dutch Nutrition Council (26))

Age group	sex	no. of subjects	energy (kcal)	total protein (en. %)	total fat (en. %)	satur. fat (en. %)	PUFA (en. %)	linoleic acid (en. %)	total carboh. (en. %)	mono + disacch. (en. %)	poly sacch. (en. %)	alcohol (en. %)
19	m	208	4320	11	36			4	48			5
19-31	m	30	2950	11	37	16		5	45	22	23	7
19-31	m	30	2933	12	37	17		5	44	21	23	7
19-31	m	22	2885	13	39	17		5	43	23	20	5
19-31	m	22	2883	13	41	18		6	42	23	19	5
19-31[1]	f	42	1997	14	37	16		5	47	23	24	2
19-31	f	42	1953	14	38	17		5	46	23	23	2
19-31	f	25	1883	16	38	17		4	45	24	21	2
19-31	f	25	1837	15	39	18		4	44	23	21	2
20-40	m	11	2423	13	38			4	45			3
20-40	f	23	1986	14	38			4	44			4
20-59	m	212	3305	12	42	19	7		44	8	36	2
20-59	m	212	2899	13	40	18	7		44	7	37	3
20-59	m	212	3057	13	41	17	8		44	5	39	3
20-59	m	166	2926	13	39	17	7		43	6	37	5
20-59	m	166	2920	13	39	18	6		41	5	36	7
22-50	m	22	2476	14	32		4		48			6
22-50	f	27	2004	15	34		4		46			5
25-55	m	50	2466	12	38	17	5	5	40	19	21	10
25-55	m	50	2635	12	40	18	6	5	39	19	20	10
25-65	m	44	2790	13	38				43			6
25-65	f	56	2040	14	41				41			3
30-35	m	31	2676	12	36	16	7	6	51			
30-35	m	41	2650	12	38	17	7	6	41			10
30-35	f	43	2015	12	36	16	7	6	51			
30-35	f	62	2074	13	37	16	6	5	45			3
50-55	m	42	2278	12	35	15	7	6	52			
50-55	m	28	2494	12	36	16	6	5	46			6
50-55	f	74	1865	13	35	15	7	6	57			
50-55	f	39	2190	13	37	16	6	5	46			4
56-67	m	50	2875	12	42			6	42	11	31	4
64-70	m	72	2300	13	42				41			
64-70	f	70	1790	14	43				41			
70-75	m	33	2345	13	43				42			
70-75	f	54	1755	15	42				42			
>75	m	39	2180	13	43				41			
>75	f	24	1600	13	44				41			
64-82	m	35	2554	13	38	14	6	6	47			2
56-88	f	53	1826	15	38	25	6	6	45			1
>75	m	15	1460	15	35				50			
>75	f	20	1767	16	37				48			
>75	m	18	1811	14	38	19	5	4	44	23	21	4
>75	f	22	1581	14	39	19	5	4	46	26	20	
means				13.0	38.9	17.4	6.0	5.1	45.7	18.5	25.9	4.5
standard deviation				1.1	2.8	1.8	1.0	0.8	3.6	8.3	6.7	2.4

Recently the results of a large number of food consumption surveys carried out in The Netherlands in the period 1970-1980, were compiled by Breedveld (26). In table 5 the intake of energy and macronutrients (as a percentage of total energy intake) is presented for most age categories. It is remarkable that the overall means for the contribution of protein, fat, carbohydrates and alcohol to the energy intake is of the same order as calculated from the data on available food supply. Moreover it is surprising that for most of the age groups studied, the same pattern for the macronutrients is observed.

Based on the additional data regarding type of fat and carbohydrate intake (see table 5), an estimation of the Dutch food pattern can be given.

These data from the period 1970-1980, presented in table 6, are not supposed to be basically different from those expected for 1984.

TABLE 6 - DIETARY PATTERN FOR MACRONUTRIENTS IN THE NETHERLANDS IN THE PERIOD 1970-1980
(source: Dutch Nutrition Council and Ministry of Agriculture and Fisheries)
(all data as percentage of total energy intake)

Total protein	12
Animal protein	8
Vegetable protein	4
Total fat	39
Saturated fat	17
Poly-unsaturated fat	6
Total carbohydrates	45
Mono + disaccharides	18
Polysaccharides	26
Alcohol	4

Values calculated from figures given in the FAO productions yearbook (10), summarized by Wretlind (32) show that in almost all European countries the protein content in the national diet is remarkably constant at 10-12 energy %. In most western European countries the fat content amounts from 38 to 43 energy %.

The dietary pattern as presented in table 6 can be evaluated in the following way:

A. The energy contribution by fat is considered too high; the high fat content of the diet is regarded a risk factor in the development of cardiovascular diseases (CVD) (5, 26) and possibly cancer (2, 9, 11).

B. The energy percentage of saturated fat is considered too high, which is evaluated as a risk factor for CVD as well, largely through the influence on the level of serumcholesterol (5, 26). On the other hand, the amount of linoleic acid, relative to the amount of saturated fat, is too low; increased levels of linoleic acid in the diet may lower serum cholesterol, although decrease in saturated fat intake is twice as effective in lowering serum cholesterol (13, 14).

C. The dietary fibre intake - not included in the tables as such - or generally the amount of complex carbohydrates in the diet, is considered to be too low. Although conclusive evidence is lacking, dietary fibre may protect against various diseases of the colon (4, 12); also a relation of a low fibre intake and diabetes as well as CVD is suggested (25).

D. The total protein content of our diet is at least sufficient; FAO/WHO arrive at figures of a "safe and adequate intake" of approximately 0.6 g protein per kg body weight per day, which is less than 10 % of total energy intake. The actual ratio animal: vegetable protein of 2:1 is considered as less desirable, partly because of a high (saturated) fat intake which very often accompanies a high "western style" protein intake (27).

Dietary changes are, however, of a complex nature: reduction of fat intake may lead to an increase in protein intake, as is observed in vegetarians (21).

If the figures of the current dietary pattern are compared with those of the recommended diet it can be generally concluded that an adjustment of the actual intake to the recommended intake seems apparent for particularly fat and carbohydrate.

1.3 ADDITIONAL ASPECTS OF THE RECOMMENDED DIET
(aim of our studies)

The overall concept of dietary goals or recommended dietary patterns is generally accepted, the primary aim being to reduce the incidence of cardio-vascular diseases. The possible relation between dietary fat and several types of cancer as well as the role dietary fibre might play in protection against colorectal cancer, are still partly speculative and at most promising; conclusive evidence cannot yet be supplied.
However, it is necessary to consider these aspects when discussing the recommended dietary pattern.
In addition, this discussion is mainly limited to macronutrients; although RDA's have been formulated for micronutrients as well, vitamins and minerals/trace elements seem to be of minor importance in the concept of dietary goals.
For some minerals and trace elements RDA's have been established, merely presenting the amount to be ingested. In table 7 the RDA's for a few minerals (for male adults) in various countries are presented. From this table especially the differences in the RDA for calcium are apparent. The relatively low values in the United Kingdom, Finland and Italy (500 mg Ca/d) are of the same level as those in many tropical countries. It has been suggested that a high dietary fat intake could inhibit calcium absorption through the formation of unsoluble calcium soaps in the intestinal tract (18, 24, 28); a high protein intake might also have a negative influence on calcium utilization caused by an increased urinary calcium excretion (1, 6, 13, 15, 16). This might be a justification for the relatively high calcium recommendation in The Netherlands and other industrialized countries, compared with tropical countries, where the fat and the protein intakes are usually lower.
FAO/WHO arrive at a "practical allowance" of 400-500 mg Ca per day for adults because there appears to be no evidence of calcium deficiencies in countries in which calcium intakes are of this order (17). It is recognized that persons consuming less than the customary US intake of protein and phosphorus remain in calcium balance with intakes considerably below the recommended allowance (17); adaptation to lower calcium intake eventually occurs (31).

TABLE 7 - RECOMMENDED DIETARY ALLOWANCES FOR Ca, Fe, Mg AND Zn FOR MALE ADULTS*

"Country"	Year	Ca	Fe	Mg	Zn
FAO/WHO	1974	400-500	5-9	-	-
Australia	1970	400-800	10	-	12-16
United Kingdom	1979	500	10	-	-
Finland	1980	500	5	-	-
Italy	1978	500	10	350	15
Spain	1980	600	10	350	15
Scandinavia	1980	600	10	-	-
East Germany	1980	600	10	250	12
Canada	1975	800	10	300	10
West Germany	1975	800	12	260	-
France	1981	800	10	350	-
USA	1980	800	10	350	15
The Netherlands	1978**	800	10	-	-

* source: Recommended Dietary Intakes around the world.
A report by Committee 1/5 of the International Union of Nutritional Sciences (1982). Nutr. Abstr. Rev. 53 (1983) no. 11

** The RDA's for 1984 are similar

-= RDA not established

The recommendations for iron are based on a suggested absorption of 10 % from a mixed diet (17). Modern knowledge of the factors that might either inhibit or enhance iron absorption would make it possible to estimate the absorption from any meal more precisely. It has been indicated that an increased dietary fibre intake, which can be brought about by increasing the amount of vegetable products (one of the dietary recommendations), may inhibit the absorption of divalent minerals (19, 22). This possible interference of mineral absorption or mineralavailability by dietary fibre has led to general concern regarding the recommendations to increase fibre in the diet.

The results of the various studies on the influence of dietary factors on the utilization of minerals and trace elements are, however, not always identical, nor do they lead to similar conclusions. Moreover, specifically the influence of macronutrients on the utilization of minerals and trace elements under "normal dietary practices" (a habitual "normal" food intake) has hardly been the basis for the various experiments carried out. It is therefore essential not only to include micronutrients as such in the discussion of a recommended dietary pattern, but also to consider the possible influence of the suggested dietary changes on the utilization of minerals and trace elements.

The aspects outlined above form the basis of the various studies described in this thesis.

In order to study any unexpected or undesirable consequences of the recommended dietary pattern for nutritional health, a series of experiments with human subjects was carried out. The aim was the following:

To evaluate the effects of the recommended dietary changes on mineral utilization and bowel function in the perspective of the desirable effects, e.g. on blood lipids, more precisely: to study the effects of amount and type of respectively dietary fat, fibre and protein on mineral balance and bowel function (fibre and protein only); the recommended dietary pattern will be compared with present dietary practices.

Since we intended to apply the results directly in human nutrition, we undertook experiments with human subjects; although the importance of animal studies is certainly recognized, a final appraisal of the role of nutrition on human health can only be made through studies with humans.

In this thesis the results are compiled of four studies performed with healthy male volunteers under strictly controlled metabolic ward conditions.

Chapter 2 presents the results of two studies in which the consequences of lowering dietary fat intake on the retention of calcium, magnesium and iron are described; in the same chapter the influence of increasing the linoleic acid intake from 4 to 16 energy % on the retention of these minerals is discussed.

Chapter 3 deals with the effect of an increased intake of dietary fibre through bread on mineral retention.

In chapter 4 the emphasis is laid on the effect of dietary fibre from bread on colonic function. In this chapter the factors that might contribute to the waterholding capacity of the colon are also discussed; in addition, the digestion of dietary fibre by the intestinal microflora is dealt with.
In chapter 5 the results are presented of an experiment in which the influence of the amount and kind of protein in the diet on mineral utilization and colonic function was studied.
Chapter 6 comprises a description and an evaluation of the methods applied in our human nutrition studies; to this effect some results are also presented of a study with four healthy male volunteers of the intra-individual variability of some (biochemical) parameters on a constant and adequate diet.
Finally in chapter 7 the data from the four studies are summarized and discussed in view of the dietary goals or recommended dietary pattern.

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2. Effect of Variations in Fat and Linoleic Acid Intake on the Calcium, Magnesium and Iron Balance of Young Men*

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Key Words. Fat · Linoleic acid · Mineral balance · Calcium · Magnesium · Iron

Abstract. In one study a group of 10 young adult male volunteers were given two experimental diets, differing in fat content. In a second study another group of 12 such volunteers received two experimental diets differing in linoleic acid content. The retention of calcium, magnesium and iron was measured during the dietary periods, each lasting 1 month. Decreasing the fat intake from 42 to 22 energy % did not result in statistically significant changes of the mineral balance. An increase in linoleic acid intake from 4 to 16 energy % (at a constant level of fat intake of 42 energy %) caused a decrease in the iron balance from 3.3 to 2.3 mg/day ($p < 0.01$), while the calcium and magnesium retention did not change significantly. During the high linoleic acid dietary period haemoglobin levels decreased from 9.6 to 9.1 mmol/l and packed cell volume from 0.48 to 0.46 l/l ($p < 0.01$). This effect of linoleic acid on iron utilization needs further investigation.

Introduction

The influence of dietary fat on mineral utilization, i.e., more specifically availability and absorption, seems to be rather complex and only partially understood [1, 2]. Because of the high fat intake in the affluent western world, the question is relevant whether such a high fat consumption might inhibit mineral absorption, possibly by formation of insoluble soaps or complexes with fatty acids or partially digested fats.

It is generally recommended by most nutrition experts to reduce total fat intake, particularly saturated fat, whereas increase of the consumption of poly-unsaturated fatty acids is often advised in order to reduce the risk of coronary heart diseases [3, 4]. The influence of this dietary change on mineral absorption is largely unknown.

Various human nutrition studies have been published on the interrelationship of dietary fat and calcium absorption. Besides the results being often conflicting, the diets fed were far from being composed of regular mixed foods [5–11]. The influence of amount and kind of dietary fat on the absorption of minerals other than calcium in man consuming common food items has hardly been studied.

Because knowledge of the various interactions of dietary factors will contribute to the

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formation of recommended dietary allowances and dietary guidelines, two nutrition experiments were carried out with young male volunteers in order to investigate the effect of decreasing the amount of total fat intake as well as of increasing the amount of linoleic acid intake on the retention of calcium, magnesium and iron.

Materials and Methods

Experimental Plan and Subjects

Two studies were performed: In study A, special attention was given to the effect of the quantity of dietary fat on mineral balance and in study B to the effect of the quantity of dietary linoleic acid on mineral retention. The experimental design is presented in table I. Each study was carried out with 4 volunteers, but replicated two times (with other subjects), resulting in three experiments with 4 volunteers each. In the first study (A) the results of 2 volunteers had to be omitted for various reasons not related to the experimental design.

In studies A and B, 10 and 12 healthy male volunteers participated, respectively (mean age 23 ± 2 years, weight 67 ± 6 kg and height 183 ± 7 cm). They gave informed consent and were housed in the institute's controlled metabolic ward, but they continued their normal daily routines. They passed a clinical examination and a nutritional evaluation; the routine blood parameters were all within normal ranges. This research was approved from an ethical standpoint by a working group responsible for human nutrition studies.

Diets

The composition of the experimental diets is given in table II. Table III shows the fatty acid composition in study B. In both studies the subjects received their habitual diet during the general adaptation period preceding the actual experiments (table I). In all the experimental periods a constant basal diet was served for breakfast, lunch and the cooked meal (see table IV).

Consumption of tea, coffee, sugar, alcoholic and non-alcoholic beverages was permitted, though restricted and carefully controlled throughout the study.

Table I. Experimental design

Days n	Study A (10 subjects)	Study B (12 subjects)
8	general adaptation	general adaptation
8	adaptation period high-fat diet	adaptation period low-linoleic acid diet
20	experimental period high-fat diet	experimental period low-linoleic acid diet
8	adaptation period low-fat diet	adaptation period high-linoleic acid diet
20	experimental period low-fat diet	experimental period high-linoleic acid diet

The diets were adapted to individual energy needs based upon a dietary history and maintenance of a constant body weight was aimed at. The foods were prepared in the institute's dietary kitchen according to standard procedures; the food items were packed in individual portions and deep-frozen when necessary. Only demineralized drinking water was allowed. A special low-calcium tooth paste was provided. All meals were served at the institute. In study A, the main difference between the two diets was the quantity of fat (and consequently the carbohydrate content at a constant protein intake) which was effected for example by replacing part of the margarine by sugar, jelly and soft drinks. The linoleic acid content as a percentage of total dietary fat remained constant at the 18% level; the contribution of the other fatty acids to total fat was kept constant as well. In study B, the total amount of dietary fat was constant at a level of 42 energy %. The only difference between the low-linoleic and the high-linoleic acid periods was the fatty acid composition of the diets (see table III), which was effected by switching for example the type of margarine served.

In both studies dietary fibre intake was constant. All the meals were composed according to habitual Dutch food patterns.

As calcium absorption may be influenced by the vitamin D status, the subjects were not permitted to excessively expose themselves to sunlight, moreover a constant 'normal' pattern of daily activities (including outdoors) was aimed at throughout the study.

Table II. Diet characteristics (means per day)

	Study A (n = 10)		Study B (n = 12)	
	high-fat diet	low-fat diet	low-linoleic acid diet	high-linoleic acid diet
Energy intake, MJ[1]	14.0	13.4	12.9	12.8
As percentage of energy: fat	42	22	41	42
protein	11	11	10	11
total carbohydrates	46	65	46	45
linoleic acid	7	4	4	16
Linoleic acid, percentage of total fat	18	18	10	38

[1] Energy intake was adjusted in order to maintain a constant body weight. Alcohol contributed 1–3% to the total energy intake.

Sample Collection

During the last 20 days of each dietary period 24-hour urine samples were collected in polyethylene bottles with HCl as preservative. Stools were collected in 3-litre plastic buckets, one for every 4 days, and stored at 4 °C. 4-day composites of urine and stools were mixed for analysis. Carmine and polyethylene glycol were used as faecal markers [12, 13].

Blood was withdrawn from the antecubital vein before breakfast at the beginning and end of each experimental period. In each dietary period individual duplicate samples of the daily diet were homogenized. The various servings were prepared, weighed and combined in the same way as the food for the volunteers.

Analytical Procedures

Urine. From the 4-day composites, samples were taken for calcium, magnesium and iron analysis by means of atomic absorption spectrophotometry (AAS) (Perkin-Elmer 303).

Faeces. The stools from each 4-day period were weighed and homogenized, using a modified method of *Massion and McNeely* [14]. AAS was applied for determination of calcium, magnesium and iron. Polyethylene glycol was determined according to *Malawer and Powell* [15].

Diets. Aliquots of the homogenized duplicate diets were saved for the analyses of calcium, magnesium and iron (all by AAS), protein, calculated from the Kjeldahl nitrogen determination, using the automated Kjellfoss [16], fat, according to the Weibull-Stoldt method by extraction of the sample with light petroleum (b.p. 60–80 °C) [17], total (available) carbohydrates [18] and fatty acid composition by gas-liquid chromatography of the fatty acids methyl ester [19].

Table III. Fatty acid composition of the diet in study B (% of total dietary fat)

Fatty acid	Low-linoleic acid diet	High-linoleic acid diet
Capric C10:0	1	1
Lauric C12:0	9	3
Myristic C14:0	7	4
Palmitic C16:0	25	17
Stearic C18:0	10	10
Oleic C18:1	30	23
Linoleic C18:2	10	38
Linolenic C18:3	0.4	0.5
Other fatty acids	< 1 each	< 1 each

Mineral balance figures were calculated for each subject individually from the means of 2 duplicate diets and 5 4-day faecal and urinary excretion values (the last 20 days of each 28-day dietary period). From these data the means of 10 (study A) and 12 (study B) were calculated.

Blood. The venous blood samples were analysed for calcium [20], haemoglobin (cyanmethaemoglobin method), packed cell volume (micro method), iron [21], cholesterol (method of *Huang* et al. [22]) and total lipids [23].

Body Composition. Estimates of percent total body fat (table V) were obtained by densitometric [24] and skinfold thickness measurements at four sites (biceps, triceps, subscapula and suprailiaca) [25].

Statistical Analyses. All available data were analysed statistically by analysis of variance, using a t test for paired observations [26].

Results

The balance data for calcium, magnesium and iron are presented in table VI. At an almost constant dietary calcium level, faecal calcium did not change significantly during the low-fat period (study A) but decreased significantly ($p < 0.01$) during the period when the high-linoleic acid diet was consumed (study B). Urinary calcium was constant in each study separately. The calcium balance differences observed in both study A and study B were not significant ($p > 0.05$). When expressed as a percentage of the intake, the calcium retention appears to be relatively low, but normal (6–14%). 65–77% of the calcium intake is lost in the faeces.

For magnesium all differences found were small and not significant at the 5% level. The magnesium intake appeared to be

Table IV. Composition of the diets given

Food item	Study A		Study B	
	high-fat diet	low-fat diet	low-linoleic acid diet	high-linoleic acid diet
Orange juice, g	250	250	250	250
White bread, g	200	200	200	200
Brown bread, g	80	80	80	80
Cheese, g	45	45	45	45
Jelly, g	40	52	40	40
Honey cake, g	25	25	25	25
Rye bread, g	35	35	35	35
Cream, g	60	–	60	60
Margarine, g[1]	90	30	70	70
Smoked beef, g	15	30	15	15
Corned beef, g	20	–	20	20
Ground beef, g	75	75	75	–
Ground pork, g	–	–	–	75
String beans, g	125	125	125	125
Apple sauce, g	100	100	100	100
Potatoes, g	250	250	250	250
Custard, g	100	100	125	125
Cookies, g	35	35	35	35
Coffee (instant), g	8	8	8	8
Tea (instant), g	1.2	1.2	1.2	1.2
Sugar or equivalent, g[2]	75	162	45	45

[1] Fatty acid composition of the margarine was different in both diets of study B and identical in study A.
[2] Soft drinks, alcoholic beverages, extra amount of jelly or sweets.

higher in the first study as compared to the second study; this is reflected in faecal magnesium and, to a lesser extent, in urinary magnesium, which is fairly constant. The magnesium balance was almost constant during both study A and study B. In both studies magnesium retention as percentage of intake is low, but normal (1–4%); 53–59% of dietary magnesium is excreted in the faeces while, as compared to calcium, a much higher proportion is excreted in the urine.

In study A, no significant difference in both iron balance and faecal iron excretion could be observed between the high-fat diet and the low-fat diet. In the second study, however, iron balance decreased significantly ($p < 0.01$) during the high-linoleic acid period. Iron retention was calculated to be 19–26% of the iron intake; urinary iron was low and constant in both studies.

Tables VII and VIII show the results of some chemical measurements in blood serum in both studies. In study A, all parameters appeared to be constant, i.e., no significant changes were detectable. In the second study statistically significant lower levels ($p < 0.01$) of haemoglobin, packed cell volume, serum cholesterol and serum total lipids were observed during the high-linoleic acid period as compared to the low-linoleic acid period. The percentage of body fat of the volunteers, as assessed by densitometry and skinfold thickness measurements, increased significantly ($p < 0.01$) during the low-fat diet in study A, whereas the total body weight remained constant. The values obtained in study B, during which the total fat intake remained constant throughout the experiment, did not indicate significant changes in body composition (see table V).

Table V. Body weight and fat mass of the volunteers in study A (n = 10) and study B (n = 12), means ± SD

	Initial value	After 28 days high-fat diet	After 28 days low-fat diet
Study A			
Body weight, kg	66.1 ± 4.8	66.3 ± 4.2	66.9 ± 4.5
% body fat (densitometry)	8.5 ± 2.3	7.8 ± 2.6	9.7 ± 2.6*
Σ 4 skinfold thicknesses, mm[1]	26.0 ± 4.2	26.2 ± 5.6	29.4 ± 6.8*
	Initial value	After 28 days low-linoleic acid diet	After 28 days high-linoleic acid diet
Study B			
Body weight, kg	68.9 ± 8.2	68.6 ± 8.0	69.0 ± 7.6
% body fat (densitometry)	10.5 ± 5.4	10.2 ± 5.9	11.1 ± 6.6
Σ 4 skinfold thicknesses, mm[1]	34.2 ± 14.0	30.5 ± 9.0	33.1 ± 11.2

* Statistically significant difference ($p < 0.01$) from the high-fat period. In study B none of the differences was statistically significant ($p > 0.05$).

[1] Skinfolds: biceps, triceps, subscapula and crista iliaca.

Table VI. Intake, excretion and balance of calcium, magnesium and iron during the last 20 days of each dietary period (mean/day ± SD)

	Study A (n = 10)		Study B (n = 12)	
	high-fat diet	low-fat diet	low-linoleic acid diet	high-linoleic acid diet
Dietary calcium, mg	832±63	844±90	848±32	829±19
Urinary calcium, mg	142±40	138±38	176±70	172±72
Faecal calcium, mg	622±44	652±72	585±79	539±79*
Calcium balance, mg	68±40	54±35	87±73	118±77
Dietary magnesium, mg	389±34	367±25	312±24	319±26
Urinary magnesium, mg	156±19	148±20	136±23	140±17
Faecal magnesium, mg	217±31	216±30	166±19	172±22
Magnesium balance, mg	16±23	3±15	10±15	7±13
Dietary iron, mg	14.4±1.2	14.8±1.7	12.9±1.2	12.4±1.3
Urinary iron, mg	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01
Faecal iron, mg	11.2±0.4	11.6±0.8	9.4±0.5	9.9±0.7
Iron balance, mg	3.0±1.4	3.0±1.4	3.3±1.3	2.3±1.0*

* Significantly different ($p < 0.01$) from the low-linoleic acid diet.

Table VII. Serum biochemical data, study A (n = 10), means ± SD

	Initial value	After 28 days high-fat diet	After 28 days low-fat diet
Haemoglobin, mmol/l	9.7±0.6	9.7±0.9	9.8±0.8
Packed cell volume, %	47±3	48±3	47±2
Serum iron, mmol/l	19±5	22±5	22±6
Serum calcium, mmol/l	2.50±0.10	2.50±0.05	2.50±0.08
Serum cholesterol, mmol/l	5.1±0.5	5.3±0.5	5.3±0.2
Serum total lipids, g/l	7.0±0.6	7.2±0.7	7.2±0.6

None of the differences was statistically significant ($p > 0.05$).

Discussion

A possible effect of dietary fat on mineral absorption may depend on the quantity of dietary fat, the chain length of the (dietary) fatty acids and/or the degree of saturation of the fatty acids. In study A, the fatty acid composition of dietary fat was constant throughout the experiment.

The relatively high standard deviation of the mineral intakes (table VI) are mainly due to the fact that each study consisted of three experiments: although the diets were basically the same, the composition of several

Table VIII. Serum biochemical data, study B (n = 12), means ± SD

	Initial value	After 28 days low-linoleic acid diet	After 28 days high-linoleic acid diet
Haemoglobin, mmol/l	9.4 ± 0.8	9.6 ± 0.8	9.1 ± 0.7*
Packed cell volume, %	47 ± 2	48 ± 3	46 ± 3*
Serum iron, mmol/l	19 ± 6	20 ± 4	21 ± 4
Serum calcium, mmol/l	2.50 ± 0.12	2.50 ± 0.10	2.50 ± 0.12
Serum cholesterol, mmol/l	5.6 ± 1.0	6.0 ± 1.3	5.2 ± 0.9*
Serum total lipids, g/l	7.7 ± 1.1	7.7 ± 1.4	7.2 ± 1.4*

* Significantly different ($p < 0.01$) from the low-linoleic acid period.

food items was slightly different in each experiment. The overall results are not influenced as each subject served as his own control. The differences in the mean intakes between the independent studies A and B can be explained in the same way.

The calcium balance data suggest that decreasing the quantity of dietary fat does not seem to influence calcium absorption. Since a low-fat diet corresponds to a high-carbohydrate diet and vice versa, the question is relevant whether carbohydrates might have an effect on calcium utilization. Only lactose is known to improve calcium absorption [27]. In our study the lactose intake was low and constant.

Although in the second study the calcium balance difference did not reach statistical significance, the slightly higher balance value during the high-linoleic acid period as compared to the low-linoleic acid diet confirms the results of previous findings [11]. Any influence of type and quantity of dietary fat on serum calcium levels could not be detected.

Both studies lead to the conclusion that decreasing the amount of dietary fat and increasing the amount of linoleic acid does not seem to influence magnesium retention.

As far as iron is concerned, decreasing the quantity of dietary fat did not show any effect on iron utilization. Although enhanced absorption of non-haeme iron by a high carbohydrate diet has been reported [28–30], such an effect was not observed during the low-fat high-carbohydrate period in study A.

The results of study B, however, indicate an inhibitory effect of linoleic acid on iron absorption. Because the basal diet was constant in both periods, the ratio haeme iron:non-haeme iron did not change either. Dietary factors do not seem to have much influence on haeme iron absorption [31, 32]. The lowered iron retention during the high-linoleic acid period is therefore even more significant when expressed as a percentage of non-haeme iron intake. At a constant level of total dietary fat, a shift in the amount of linoleic acid necessarily means a different fatty acid composition during both dietary periods in study B. However, changes in fatty acids other than linoleic acid, as a percentage of total fat, were small (see table III). It seems likely therefore that the results can be mainly interpreted as having been derived from the difference in the linoleic acid content in both diets.

Other dietary factors that might either impair or improve (non-haeme) iron absorption remained constant during the study.

The significant decrease of the haemoglobin levels and the packed cell volumes observed during the high-linoleic acid period cannot easily be explained, but this observation seems to be in line with the decreased iron balance. It should, however, be noted that serum iron concentrations remained constant. Because it does not seem likely that a fall in haemoglobin levels will occur during a period of 28 days in persons having a normal iron status, this observation needs further investigation.

The mineral balance data are not to be interpreted as absolute values, because miscellaneous losses (skin, hair, etc.) that were not measured might result in lower retention figures. Because of the constant activity pattern of the volunteers during the experiments, these losses may be considered constant as well. The conclusions concerning the effect of dietary fat on mineral balance will therefore not be considerably influenced.

Dermal magnesium losses are reported to be less important as compared to calcium [33], thus the magnesium retention figures are closer to reality than those of calcium. Since no negative magnesium balance was induced by the consumption of 300–400 mg Mg/day, this dietary intake seems to be adequate for maintaining the Mg balance; e.g., it compares very well to the suggested RDA values of 350 mg Mg/day in for example the USA [34].

The large amount of linoleic acid in the 2nd month of study B appeared to be effective in decreasing both serum cholesterol concentrations and serum total lipid levels; halving of fat intake with identical fatty acid proportions induced identical blood lipid levels, although increased levels of total lipids could possibly be expected on an increased carbohydrate (sugar) intake.

Body composition was assessed by two different methods. The changes in body composition in study A seem interesting as the main difference in the two diets is the ratio dietary fat/dietary sugar; the conclusion that dietary sugar is more 'fattening' than dietary fat may be too speculative.

The results of this study do not support the view that a high fat content might impair calcium absorption and should consequently lead to higher calcium recommendations. The unexpected findings of a decreased iron balance, decreased haemoglobin levels and decreased packed cell volume during the high-linoleic acid period require further study.

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3. Physiological effects of fibre-rich types of bread

1. The effect of dietary fibre from bread on the mineral balance of young men

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1. Twelve young adult male volunteers were given a low-fibre white bread diet (9 g neutral-detergent fibre (NDF)/d) and a medium-fibre coarse-bran bread diet (22 g NDF/d), each lasting 20 d. In a third period of 20 d the volunteers were subdivided in groups of four, consuming a high-fibre coarse-bran bread diet (35 g NDF/d), a medium-fibre fine-bran bread diet (22 g NDF/d, bran particle size < 0·35 mm) or a wholemeal bread diet (22 g NDF/d). Retention of calcium, magnesium, iron, zinc and copper were determined during each 20 d period.

2. An increase of the amount of dietary fibre (through bran in bread) from 9 g to 22 g NDF/d resulted in a significantly increased mineral intake, but also faecal excretion increased significantly; mineral retention remained almost constant.

3. Both intake and faecal excretion of all minerals studied, except faecal Ca, increased further ($P < 0·05$) on the diet providing 35 g NDF/d; only Fe balance decreased significantly. No significant differences with respect to intake, excretion (except urinary Ca) and balance of the minerals could be detected between the coarse-bran bread and fine-bran bread diets providing 22 g NDF/d. Faecal Fe, Cu balance and Mg balance increased significantly during the wholemeal bread period compared to the coarse-bran bread diet providing 22 g NDF.

4. Serum cholesterol increased significantly, i.e. by 0·3 mmol/l, during the coarse-bran bread diet providing 22 g NDF, compared to the white-bread diet.

5. It is concluded that increasing the amount of bran in bread does not appear to affect mineral balance considerably but there seems to be an influence on mineral availability. The increased intake was accompanied by increased faecal excretion.

There is an increasing interest in the interaction of dietary fibre and minerals in the gastrointestinal tract. Some authors have already reported that dietary fibre might lower the availability of minerals which may lead to decreased absorption (Reinhold *et al.* 1976; Ismail-Beigi *et al.* 1977; Cummings, 1978; Sandstead *et al.* 1978; Drews *et al.* 1979; Kelsay *et al.* 1979). Dietary fibre is suggested to be effective in preventing the incidence of several diseases (Burkitt *et al.* 1974; Trowell, 1976); specifically the role of dietary fibre from cereal sources on colonic function seems to be of importance (Kelsay, 1978; Spiller *et al.* 1978). Since bread is one of the staple foods in many industrialized countries, the generally recommended increase of dietary fibre consumption might well be reached through fibre-rich bread. However, an over-estimation of the value of dietary fibre could lead to an excessive intake, which may have a negative influence on the mineral status owing to the possibly inhibitory effect of dietary fibre on mineral absorption.

As part of a larger project concerning the significance of bread in human nutrition, we have studied the consequences for human physiology of an increased intake of fibre from bread. The criteria studied included the colonic function, the digestibility of dietary fibre (components) by the intestinal microflora and the balance of calcium, magnesium, iron, zinc and copper. The results of the mineral balances are reported in this paper.

METHODS

The experimental design is shown in Table 1. Twelve male volunteers (mean age 23 ± 2 years, weight 68 ± 6 kg, height $1·82 \pm 0·06$ m and $14 \pm 3\%$ body fat) were given two experimental

Table 1. *Experimental design**

	General adaptation	First experimental period	Second experimental period	Third experimental period	
Period...	A	B	C	D	
Duration (d)...	8	20	20	20	
Type of bread	White bread (entirely white flour)	White bread (entirely white flour)	Bread made from 850 g white flour and 150 g coarse bran/kg	1 Bread made from 680 g white flour and 320 g coarse bran/kg (*n* 4) 2 Bread made from 850 g white flour and 150 g fine bran/kg (*n* 4) 3 Wholemeal bread (*n* 4)	
Approximate total daily fibre intake (g NDF)	9	9	22	1 2 3	35 22 22

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).

* The study was carried out with four volunteers at a time and replicated twice (with other subjects), resulting in three experiments with four volunteers each. Only the last periods of 20 d were different.

diets with different amounts and types of dietary fibre for 20 d each. Results were compared with those on a low-fibre (white bread) diet with 9 g dietary fibre/d.

Experimental procedure

All volunteers consumed the white-bread diet (periods A and B) and the 150 g coarse-bran/kg bread diet (period C). The amount of dietary fibre in the latter type of bread was the same as that in conventional wholemeal bread; the organoleptic properties were different however. In the third experimental period (period D), three other types of bread were consumed, each type by four of the twelve volunteers. The 150 g fine-bran/kg bread was included to study the effect of the bran particle size on e.g. mineral balance. The 320 g coarse-bran/kg bread contained twice the amount of dietary fibre as compared to conventional wholemeal bread, which was included as well. The particle size of the coarse and fine bran were $> 0{\cdot}35$ mm and $< 0{\cdot}35$ mm respectively.

The volunteers gave informed written consent according to the Institute's procedures and were housed in the Institute's controlled metabolic ward, but they continued their normal daily routines. They passed beforehand a clinical examination and a nutritional evaluation. The routine haematological values were all within normal ranges. The basal diet, which was constant throughout the study, consisted of conventional low-fibre foods and provided 7 g NDF/d (see Table 2). The diet characteristics, based on analyses of individual duplicate daily samples, are given in Table 3.

In practice an increased bran consumption automatically results in a higher mineral intake. The objective being to study the mineral availability under these conditions, no supplementation of minerals (e.g. in the white-bread period) was applied.

The energy intake was adjusted to the individual energy requirements based on a constant body-weight during the study and a dietary history before the experiment. Reduction of body-weight by more than 2% was corrected for by sugar, soft drinks and other carbohydrate equivalents. This procedure was necessary for four volunteers.

The food was prepared in the diet kitchen according to standard procedures, weighed to the nearest g, packed in individual portions and deep-frozen when necessary.

Only demineralized water was allowed in restricted amounts (maximum approximately 200 ml daily, apart from water for coffee and tea). All types of bread were prepared from

Table 2. *Composition (g) of the basal diet*

(Mean daily values)

Bread*	240	Ground beef	100
Cheese	60	Ice cream	50
Smoked beef	15	Whipped cream	25
Ham	15	Vegetables†	
Orange juice	250	Instant tea	0·9
Vegetable margarine	30	Instant coffee	3
Custard (low-fat)	150	Sugar	20
Jelly	30	Soft drinks	400
Potatoes	200	Whisky	35

* Various types; for details, see Table 1.

† Each 4 d the following rotating order: day 1: 75 g string beans, 5 g margarine, 100 g apple sauce; day 2: 30 g lettuce, 40 g carrot salad, 40 g celeriac salad; day 3: 75 g sliced beans, 5 g margarine, 100 g apple sauce; day 4: 200 g tomatoes, 5 g margarine, 5 g rusk.

one batch of wheat flour. All meals were served at the Institute. The basal diet and the breads were analysed separately.

Urine samples (24 h) were collected in polyethylene bottles with hydrochloric acid as preservative. Stools were collected in 3 l plastic buckets, one for every 4 d, stored at 4°. Composites (4 d) of urine and stools were made for analysis and stored at −20°.

Blood was withdrawn from the antecubital vein before breakfast at the beginning and at the end of each period.

Analytical procedures

The analytical procedures included mineral determinations by means of atomic absorption spectrophotometry (Perkin-Elmer 303). As wheat bran dietary fibre is low in water soluble components and in addition the basal diet (without bread) was low in dietary fibre, neutral-detergent fibre analyses were carried out according to the Van Soest method (Van Soest & Wine, 1967), as an approximation for dietary fibre, applying predigestion with pancreatin to remove residual starch (Terry & Outen, 1973). Blood haemoglobin levels were determined with the cyanmethaemoglobin method; for packed cell volume the micromethod was used; erythrocyte count by a Coulter counter; serum cholesterol was determined by means of the Huang method (Huang *et al.* 1961); serum triglycerides, enzymically, with the Eggstein (1968) method. For serum Mg, Zn and Cu, atomic absorption spectrophotometry was applied, serum Fe determination was carried out according to the Führ (1965) method and serum Ca with a Ca titrator (Raman & Chang, 1974).

Mineral balance was calculated from 20 d dietary intake and urinary+faecal excretion in each experimental period (subdivided in five 4 d periods). For statistical evaluation the results of each dietary treatment were compared with the preceding treatment by means of analysis of variance (Snedecor & Cochran, 1967), using Student's *t* test for paired observations. The means for period D (four subjects) were compared with those of the corresponding subjects in period C.

This research was approved from an ethical standpoint by a working group responsible for human nutrition studies.

RESULTS

The mineral balance data are presented in Tables 4–7.

An increased cereal-fibre intake (through bran in bread) resulted in statistically significantly ($P < 0·01$) increased intake of all minerals studied, as shown by the values of the 150 g coarse-bran/kg bread period (period C) and those of the white-bread period (period B).

Table 3. *Diet characteristics of the various experimental periods**

(Mean daily values with values for the contribution of bread (%) to the total daily intake in parentheses)

Experimental period...		B White bread (*n* 12)	C 150 g coarse-bran/kg bread (*n* 12)	D_1 320 g coarse-bran/kg bread (*n* 4)	D_2 150 g fine-bran/kg bread (*n* 4)	D_3 Wholemeal bread (*n* 4)
Energy	(MJ)	11·1 (26)	11·1 (24)	11·5 (21)	9·9 (27)	11·5 (24)
Total fat	(energy %)	36 (8)	36 (8)	41 (8)	35 (10)	36 (9)
Total available carbohydrate	(energy %)	49 (40)	48 (37)	42 (33)	49 (39)	50 (33)
Total protein	(energy %)	12 (29)	12 (30)	12 (33)	13 (30)	12 (31)
Linoleic acid	(energy %)	5·5 (5)	5·5 (5)	5·5 (5)	5·5 (5)	5·5 (5)
Alcohol	(energy %)	3 (0)	4 (0)	5 (0)	3 (0)	2 (0)
NDF	(g)	8·7 (25)	21·2 (69)	34·9 (83)	22·8 (65)	22·0 (71)
Calcium	(mg)	956 (4)	1003 (6)	1087 (12)	966 (6)	1022 (8)
Magnesium	(mg)	213 (23)	373 (55)	537 (68)	384 (56)	397 (59)
Iron	(mg)	8·3 (26)	12·2 (51)	12·2 (77)	12·8 (52)	15·0 (55)
Zinc	(mg)	9·0 (14)	11·3 (31)	13·5 (43)	11·6 (32)	12·7 (38)
Copper	(mg)	1·24 (30)	1·48 (44)	1·95 (49)	1·24 (52)	1·76 (49)

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).

* For details, see Table 1.

Table 4. *Intake, excretion and balance of calcium, magnesium, iron, zinc and copper (mg/d) of human subjects on a white-bread diet and a 150 g coarse-bran/kg bread diet during 20 d*

(Mean values and standard deviations)

Experimental period...	B (*n* 12)		C (*n* 12)	
Type of bread...	White bread		150 g coarse-bran/kg bread	
NDF intake (g/d)...	9		22	
	Mean	SD	Mean	SD
Ca				
Intake	956	40	1003	90*
Urinary	225	79	209	90
Faecal	717	99	836	99*
Balance	14	118	−42	109
Mg				
Intake	213	13	373	15*
Urinary	116	8	136	14*
Faecal	105	16	240	24*
Balance	−8	15	−3	22
Fe				
Intake	8·3	0·6	12·2	0·5*
Urinary	0·1	0·05	0·1	0·02
Faecal	7·4	1·1	11·4	0·9*
Balance	0·8	1·2	0·7	0·7
Zn				
Intake	9·0	0·2	11·3	0·2*
Urinary	0·7	0·3	0·6	0·3
Faecal	8·9	1·5	11·1	0·9*
Balance	−0·6	1·7	−0·4	1·0
Cu				
Intake	1·24	0·23	1·48	0·19*
Urinary	0·05	0·01	0·05	0·01
Faecal	0·97	0·16	1·18	0·11*
Balance	0·22	0·29	0·25	0·24

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period B: * $P < 0·01$.

This change in the type of bread consumed also resulted in significantly ($P < 0·01$) increased faecal mineral excretion; urinary excretion of Ca, Fe, Zn and Cu remained constant ($P > 0·05$), whereas urinary Mg excretion increased significantly ($P < 0·01$) during the 150 g coarse-bran/kg bread period; no significant differences in any of the mineral balance values could be detected however.

During the 320 g coarse-bran/kg bread period (period D_1, see Table 5) all mineral intakes were higher when compared with the 150 g coarse-bran/kg bread period; except for Ca, faecal mineral excretion increased significantly ($P < 0·05$) as well, whereas urinary mineral output did not change significantly. As to the mineral balance values, only Fe balance decreased significantly ($P < 0·01$) during period D_1. However, except for Cu, all mineral balance values were negative in the 320 g coarse-bran/kg bread period.

Apart from a significant decrease ($P < 0·01$) in urinary Ca in period D_2 (150 g fine-bran/kg bread) as compared with period C (150 g coarse-bran/kg bread), intakes of all minerals as well as excretion in urine and faeces were not changed significantly (see Table 6). Although all mineral balance values seem to have improved on the fine-bran bread diet, none of the differences compared with the 150 g coarse-bran/kg bread diet was statistically significant.

Table 5. *Intake, excretion and balance of calcium, magnesium, iron, zinc and copper (mg/d) of four human subjects on a 150 g coarse-bran/kg bread diet and a 320 g coarse-bran/kg bread diet during 20 d*

(Mean values and standard deviations)

Experimental period...	C (*n* 4)		D_1 (*n* 4)	
Type of bread...	150 g coarse-bran/kg bread		320 g coarse-bran/kg bread	
NDF intake (g/d)...	22		35	
	Mean	SD	Mean	SD
Ca				
Intake	1019	30	1087	30**
Urinary	224	53	211	43
Faecal	816	155	906	98
Balance	−21	123	−30	42
Mg				
Intake	377	8	537	8**
Urinary	128	12	130	9
Faecal	234	12	410	22**
Balance	15	16	−3	14
Fe				
Intake	11·8	0·6	12·2	0·6**
Urinary	0·1	0·02	0·1	0·03
Faecal	10·5	0·6	15·0	0·8**
Balance	1·2	0·9	−2·9	0·4**
Zn				
Intake	11·2	0·2	13·5	0·2**
Urinary	0·5	0·2	0·5	0·2
Faecal	10·8	1·3	13·6	0·9*
Balance	−0·1	1·6	−0·6	0·8
Cu				
Intake	1·64	0·06	1·95	0·06**
Urinary	0·05	0·01	0·05	0·01
Faecal	1·11	0·11	1·41	0·10*
Balance	0·48	0·17	0·49	0·12

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period C: * $P < 0·05$, ** $P < 0·01$.

During the wholemeal-bread period (D_3) all mineral intakes, except the Ca intake, were significantly higher than those during the 150 g coarse-bran/kg bread period (see Table 7); a significant increase in faecal Fe excretion ($P < 0·01$) could be detected whereas faecal excretions of the other minerals and all urinary mineral excretions did not differ significantly from the 150 g coarse-bran/kg period. The balance values of Ca, Zn and Fe were also similar in both periods; Cu balance and Mg balance however, increased significantly ($P < 0·05$) during the wholemeal-bread period.

Table 8 shows the results in blood serum obtained at the beginning and at the end of the 20 d experimental periods B and C.

Small, but significant increases ($P < 0·05$) in haemoglobin levels, packed cell volume and the erythrocyte count were observed during the white-bread period. The mineral concentrations in blood serum appeared to be constant in most instances; though small differences were found, none reached statistical significance.

A significant increase of serum cholesterol ($P < 0·05$) during the 150 g coarse-bran/kg

Table 6. *Intake, excretion and balance of calcium, magnesium, iron, zinc and copper (mg/d) of four human subjects on a 150 g coarse†-bran/kg bread diet and a 150 g fine†-bran/kg bread diet during 20 d*

(Mean values and standard deviations)

Experimental period...	C (*n* 4)		D_2 (*n* 4)	
Type of bread...	150 g coarse-bran/kg bread		150 g fine-bran/kg bread	
NDF intake (g/d)...	22		22	
	Mean	SD	Mean	SD
Ca				
Intake	964	25	966	25
Urinary	130	30	160	22*
Faecal	841	40	758	91
Balance	−7	57	48	94
Mg				
Intake	374	15	384	15
Urinary	130	13	134	10
Faecal	247	29	240	37
Balance	−3	16	10	22
Fe				
Intake	12·4	0·3	12·8	0·3
Urinary	0·1	0·02	0·1	0·02
Faecal	12·1	0·2	11·4	0·8
Balance	0·2	0·3	1·3	0·8
Zn				
Intake	11·4	0·2	11·6	0·2
Urinary	0·6	0·2	0·6	0·1
Faecal	11·5	0·2	10·8	0·8
Balance	−0·7	0·3	0·2	1·0
Cu				
Intake	1·24	0·04	1·24	0·04
Urinary	0·05	0·01	0·05	0·01
Faecal	1·21	0·05	1·11	0·11
Balance	−0·02	0·04	0·08	0·07

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period C: * $P < 0·01$.
† Coarse-bran, particle size > 0·35 mm, fine-bran, particle size < 0·35 mm.

bread period (compared with the white-bread period) was observed. During the periods D none of the measured parameters in blood were statistically different from the corresponding periods C (results not presented).

DISCUSSION

McCance & Widdowson (1942) suggested a possible relation between the type of bread consumed and mineral retention or absorption. The availability for absorption of minerals from wholemeal bread was considered to be less than that from white bread. The first explanation of an impaired mineral retention following the consumption of the darker types of bread seemed to be the presence of phytates which might make divalent electrolytes unavailable for absorption by the formation of insoluble complexes. Reinhold *et al.* (1976) and Ismail-Beigi *et al.* (1977) came to the conclusion that particularly dietary fibre from

Table 7. *Intake, excretion and balance of calcium, magnesium, iron, zinc and copper (mg/d) of four human subjects on a 150 g coarse-bran/kg bread diet and a wholemeal bread diet during 20 d*

(Mean values and standard deviations)

Experimental period...	C (*n* 4)		D_3 (*n* 4)	
Type of bread...	150 g coarse-bran/kg bread		wholemeal bread	
NDF intake (g/d)...	22		22	
	Mean	SD	Mean	SD
Ca				
Intake	1033	170	1022	114
Urinary	273	110	203	40
Faecal	850	97	829	91
Balance	−90	141	−10	64
Mg				
Intake	369	21	397	23*
Urinary	151	3	145	16
Faecal	238	30	240	32
Balance	−20	19	12	12*
Fe				
Intake	12·6	0·2	15·0	0·9**
Urinary	0·1	0·02	0·1	0·02
Faecal	11·6	0·9	12·7	0·9**
Balance	0·9	0·7	2·2	0·2
Zn				
Intake	11·2	0·2	12·7	0·4**
Urinary	0·8	0·3	0·7	0·3
Faecal	10·9	0·8	11·4	0·4
Balance	−0·5	0·9	0·6	0·3
Cu				
Intake	1·54	0·12	1·76	0·12*
Urinary	0·05	0·01	0·05	0·01
Faecal	1·21	0·14	1·29	0·12
Balance	0·28	0·18	0·42	0·16*

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period C: * $P < 0·05$, ** $P < 0·01$.

bread could have an inhibitory effect on mineral absorption, possibly by mechanisms adsorbing the minerals to fibre. The presence of the enzyme phytase (*EC* 3.1.3.8) during the baking process of bread and in the intestinal tract of man might however increase the availability of the minerals by splitting the phytate–mineral complexes. A decreased mineral balance as a result of a more Western type of diet was also reported by Sandstead *et al.* (1978). Our results did not indicate an apparent influence on the mineral balance when the dietary fibre content was increased from 9 g NDF/d (white-bread diet) to 22 g NDF/d (bread with 150 g coarse-bran). The increased amount of minerals, which accompany the increased amount of bran (and thus of dietary fibre) did not appear to be available for absorption since faecal excretions of the minerals studied also increased. The significantly decreased Fe balance during period D_1 with 35 g NDF/d, together with the negative balance values for Ca, Mg, Fe and Zn as well as the significant further increase of faecal mineral excretions (except for Ca), not only shows the influene of dietary fibre on mineral absorption, but also indicates that by increasing the quantity of bran in bread, the mineral utilization becomes less favourable.

Table 8. *Blood constituents values and serum biochemical criteria of human subjects on a white-bread diet and a 150 g coarse-bran/kg bread diet during 20 d*

(Mean values and standard deviations)

Experimental period†...		A (*n* 12) White bread 9		B (*n* 12) White bread 9		C (*n* 12) 150 g coarse-bran/kg bread 22	
		Mean	SD	Mean	SD	Mean	SD
Haemoglobin	(mmol/l)	9·3	0·4	9·6	0·4*	9·8	0·5
Packed cell volume		0·45	0·02	0·46	0·02*	0·47	0·02
Erythrocyte count	(10^{12}/l)	4·6	0·3	4·8	0·3*	4·7	0·4
Serum Iron	(μmol/l)	21	6	17	4	18	6
Calcium	(mmol/l)	2·40	0·10	2·45	0·07	2·45	0·09
Magnesium	(mmol/l)	0·95	0·14	0·91	0·12	0·85	0·14
Zinc	(μmol/l)	16·8	4·6	15·3	6·1	18·4	3·1
Copper	(μmol/l)	17·3	6·3	17·3	4·7	15·7	4·7
Total cholesterol	(mmol/l)	4·6	1·0	4·5	0·6	4·8	0·8**
Triglycerides	(mmol/l)	1·33	0·53	1·30	0·54	1·27	0·44

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
† Blood was withdrawn at the end of each period indicated; for details, see Table 1.
Mean values significantly different from those for period A: *$P < 0·05$.
Mean value significantly different from that for period B: **$P < 0·05$.

No apparent influence of the bran particle size on mineral retention could be demonstrated, although the increased balance values during the fine-bran bread period indicate some effect; however, the inter-individual variations for the balance values were such that the mean differences were not statistically significant. During the wholemeal-bread period the dietary fibre intake was the same as during the 150 g coarse-bran/kg bread period. The significant increase of the Cu and Mg balances could possibly be explained by the higher intakes during the wholemeal-bread period as excretion of both minerals remained almost constant.

For an explanation of the effects observed, we have to distinguish between: (1) the availability of the minerals for absorption and (2) the actual absorption step. The mineral balance (retention) is dependent on both availability and absorption.

Dietary fibre does not only seem to reduce the availability of minerals but we might also assume that the absorptive capacity of the intestinal wall is limited: excessive amounts cannot be absorbed. In other words, the intestinal wall only absorbs such amounts of minerals to maintain homoeostasis, which seems to be indicated by a rather constant urinary output. Finally, one should not underestimate the interactions of the minerals themselves when accounting for the findings observed, both during the digestion step (competition as to the availability) and during the actual absorption step (competition as to the transport mechanisms through the intestinal wall) (Davies, 1974).

Whether the suggested decreased mineral availability (whatever the cause may be) is a real problem in the Western type of diet is not easy to indicate. It is not impossible that in the long-term a physiological adaptation of the body to the increased consumption of dietary fibre (from bread) will occur, until, in a new equilibrium the absorption of the minerals will meet the requirements (Anderson *et al.* 1980). Besides, the risk of an impaired mineral absorption seems to be higher in countries where phytate-rich bread is consumed and where the daily diet is less varied as compared to the possibilities in the Western World. The negative balance for Ca, Mg, Fe and Zn in period D_1 in which the amount of bran consumed is high, however, is an indication that even on a Western type of diet a too high

wheat-fibre intake bears the risk of insufficient mineral absorption due to decreased availibility. No effects of alterations in the wheat-fibre intake on the mineral concentrations in blood serum of our subjects were observed, although it is questionable whether any changes are to be expected within 20 d.

Increased serum cholesterol levels on a high wheat-fibre intake (through bran in bread) were also reported by other authors (Jenkins *et al.* 1975; Kay & Truswell, 1977; Stasse-Wolthuis *et al.* 1980). Some investigators did not find elevated levels (Heaton & Pomare, 1974; Dixon, 1978; Van Berge-Henegouwen *et al.* 1979). However, one may wonder whether the increase of serum cholesterol will be of any significance in a mixed diet with fruit and vegetables (in our study only low-fibre vegetables and no fresh fruit were consumed) since dietary fibre from fruit and vegetables (pectins) have been reported to decrease serum cholesterol levels (Grande *et al.* 1974; Stasse-Wolthuis *et al.* 1980).

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4. Physiological effects of fibre-rich types of bread

2. Dietary fibre from bread: digestibility by the intestinal microflora and water-holding capacity in the colon of human subjects*

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1. Twelve young adult male volunteers were given a low-fibre white bread diet (9 g neutral-detergent fibre (NDF)/d) and a medium-fibre coarse-bran bread diet (22 g NDF/d), each lasting 20 d. In a third period of 20 d the volunteers were subdivided in groups of four, consuming a high-fibre coarse-bran bread diet (35 g NDF/d), a medium-fibre fine-bran diet (22 g NDF/d, bran particle size > 0·35 mm) or a wholemeal bread diet (22 g NDF/d). Digestion of dietary fibre and its components hemicellulose, cellulose and lignin were determined as well as colonic function.

2. An increase of the amount of dietary fibre (through bran in bread) from 9 to 22 g NDF/d resulted in the following significant changes ($P < 0·01$): increase in faecal wet weight of 63 g/d, decrease in the percentage of faecal dry weight from 27 to 24, increase in defaecation frequency of 0·2 stools/d and reduction of the intestinal transit time of 36 h.

3. Further significant changes with regard to all factors mentioned were observed during the high-fibre diet. Faecal wet weight was significantly ($P < 0·05$) lower with the fine-bran bread diet than with the coarse-bran bread on a similar fibre intake of 22 g NDF/d. Results obtained in the wholemeal-bread period did not show significant differences compared with those from the coarse-bran bread period of 22 g NDF/d.

4. Mean digestibilities for the fibre from bread were: for NDF 0·34, for hemicellulose 0·46, for cellulose 0·20 and for lignin 0·04.

5. The results obtained suggest that the theory of sponge activity of the fibre matrix structure is the predominant factor accounting for the water binding capacity of fibre in the colon.

Many papers have been published on the significance of dietary fibre in human nutrition. It is generally recommended that in the western world dietary fibre intake should be increased, because several diseases are believed to be associated with the consumption of refined carbohydrate-rich foods (Burkitt *et al.* 1972; Burkitt & Trowell 1975; Trowell 1976). However, conclusive evidence of the relative importance of fibre in our diet in the aetiology of diseases, such as appendicitis, cancer of the colon and diabetes, is still lacking.

Because of the beneficial influence dietary fibre, particularly from cereal sources, seems to have on colonic function (Cummings *et al.* 1976*a*; Mitchell & Eastwood 1976), an increased cereal fibre intake is desirable and this can best be effected by means of bread.

In various review articles (Kelsay 1978; Spiller *et al.* 1978) the following effects of increasing wheat-fibre intake are mentioned: increase in stool volume, shorter intestinal transit time, increase in stool frequency and decrease in absorptions of energy, fat, nitrogen and minerals. The influence of wheat fibre on serum lipids is not clear; both increased and decreased levels have been reported as a result of increased wheat-fibre intake and the effects observed on bile acid excretion are also rather contradictory. Although the definition of dietary fibre states that digestion does not take place in the (human) gastrointestinal tract by alimentary enzymes, it does not imply that dietary fibre or its components (cellulose, hemicellulose, lignin and pectin, the latter not present in wheat fibre) or both are not digested at all. Several of the previously mentioned physiological effects are brought about by the

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action of the bacterial flora of the colon, resulting in partial digestion of dietary fibre components. However, there is little information available on the extent of digestion of the various dietary fibre components (Holloway *et al.* 1978; Heller *et al.* 1980).

In order to determine some of the physiological effects of wheat fibre, as well as the digestibility of dietary fibre components by the bacterial flora of the colon, we carried out a study with subjects who were given three experimental diets, each lasting 20 d, differing in amount and particle size of bran incorporated in the bread. The mineral-balance results and the effects on some serum biochemical indices have been reported previously (Van Dokkum *et al.* 1982). In the present paper the effects are reported of wheat fibre on stool weight, stool frequency, intestinal transit time, bile acid excretion, excretion of volatile fatty acids (VFA), excretion of faecal N and phosphorus and the apparent digestibility by the intestinal flora of hemicellulose, cellulose, lignin and of the total amount of dietary fibre.

METHODS

The experimental design is shown in Table 1. Twelve male volunteers (means and standard deviations: age 23 (2)years, weight 68 (6)kg, height 1·82 (0·06)m, body fat 14 (3)%) were given two experimental diets with different amounts and types of dietary fibre for 20 d each. Results were compared with those on a low-fibre (white bread) diet with 9 g dietary fibre/d.

The volunteers gave informed written consent according to the Institute's procedures and were housed in the Institute's controlled metabolic ward, but they continued their normal daily routines. They passed beforehand a clinical examination and a nutritional evaluation. The routine haematological values were all within normal ranges.

Experimental procedure

The basal diet (the total daily diet without bread), which was constant for each subject throughout the study, consisted of conventional low-fibre foods and provided 7 g neutral-detergent fibre (NDF)/d (see Table 2).

In addition to the basal diet, all volunteers consumed bread, i.e. white bread in the adaptation period A and in the experimental period B and 150 g coarse-bran/kg bread in period C. The amount of dietary fibre in the latter type of bread was the same as that in conventional wholemeal bread; the organoleptic properties were different however. In the third experimental period (period D), three other types of bread were consumed, each type by four of the twelve volunteers. The 150 g fine-bran/kg bread was included to study the effect of the bran particle size on, for example, colonic function. The 320 g coarse-bran/kg bread contained twice the amount of dietary fibre as compared with conventional wholemeal bread, which was also included. The particle size of the coarse and fine brans were $> 0{\cdot}35$ mm and $< 0{\cdot}35$ mm respectively.

The diet characteristics (Table 3) are based on analyses of individual, duplicate, daily samples; the basal diet and the breads were analysed separately.

The energy intake was adjusted to the individual energy requirements derived from a dietary history before the experiment. A constant body-weight during the study was aimed at; reduction of body-weight of more than 2% was corrected by the addition of sugar, soft drinks and other carbohydrate equivalents. This procedure was necessary for four volunteers.

The food was prepared in the diet kitchen according to standard procedures, weighed to the nearest g, packed in individual portions and deep-frozen, when necessary.

Only demineralized water was allowed in restricted amounts (maximum approximately 200 ml daily, apart from water for coffee and tea). All types of bread were prepared from one batch of wheat flour. All meals were served at the Institute.

Table 1. *Experimental design**

Period ...	General adaptation A	First experimental period B	Second experimental period C	Third experimental period D
Duration (d)	8	20	20	20
Type of bread	White bread (entirely white flour)	White bread (entirely white flour)	Bread made from 850 g white flour and 150 g coarse bran/kg	(1) Bread made from 680 g white flour and 320 g coarse bran/kg (*n* 4) (2) Bread made from 850 g white flour and 150 g fine bran/kg (*n* 4) (3) Wholemeal bread (*n* 4)
Approximate total daily fibre intake (g NDF)	9	9	22	(1) 35 (2) 22 (3) 22

NDF, neutral-detergent fibre (Van Soest & Wine, 1967).

* The study was carried out with four volunteers at a time and replicated twice (with other subjects), resulting in three experiments with four volunteers each.

Table 2. *Composition (g) of the basal* diet*

(Mean daily values)

Cheese	60
Smoked beef	15
Ham	15
Orange juice	250
Vegetable margarine	30
Custard (low-fat)	150
Jelly	30
Potatoes	200
Ground beef	100
Ice cream	50
Whipped cream	25
Vegetables†	
Instant tea	0·9
Instant coffee	3
Sugar	20
Soft drinks	400
Whisky	35

* In addition to the basal diet, 240 g bread/d of various types were consumed; for details, see Table 1.

† Rotating order for every 4 d: day 1: 75 g string beans, 5 g margarine, 100 g apple sauce; day 2: 30 g lettuce, 40 g carrot salad, 40 g celeriac salad; day 3: 75 g sliced beans, 5 g margarine, 100 g apple sauce; day 4: 200 g tomatoes, 5 g margarine, 5 g rusk.

Stools were collected in 3 l plastic buckets, one for every 4 d, stored at 4°. Composites (4 d) of stools were made for analysis and stored at −20°.

Analytical procedures

As wheat bran dietary fibre is low in water-soluble components and the basal diet (without bread) was low in dietary fibre, analyses were carried out according to the method of Van Soest & Wine (1967) as an approximation for dietary fibre, applying predigestion with pancreatin to remove residual starch (Terry & Outen, 1973). The method of Van Soest & Wine (1967) was also used for the analysis of acid-detergent fibre (ADF), cellulose and lignin. The hemicellulose content was calculated as the difference between NDF and ADF. Total available carbohydrates were determined, using pancreatic amylase to transform starch into soluble carbohydrates and subsequent hydrolysis of the carbohydrates with hydrochloric acid to glucose; glucose and other monomeric sugars were analysed using the Luff–Schoorl reagent (van de Kamer, 1941). Fat was analysed according to the Weibull-Stoldt method by extraction of the sample with light petroleum (b.p. 60–80°) (Schormüller, 1969). Protein was calculated from the Kjeldahl N determination, using the automated KjellFoss (Noel, 1976) and applying the factor 6·25. Phosphorus was analysed gravimetrically as ammonium molybdophosphate (Schormüller, 1967).

In all stool composites the moisture content was analysed by drying on sand. Stool frequency was computed from information obtained from the diaries of the volunteers; intestinal transit time was measured using radio-opaque pellets (Hinton *et al.* 1969) towards the end of each experimental period and calculated as 80% recovery. In the last two 4 d composites of the stools in each period of 20 d, total bile acid excretion was measured enzymically with 3-α-hydroxy-steroid-dehydrogenase (*EC* 1.1.1.50) in a faecal extract which was prepared as follows. The faeces were boiled with methanol, the extracted matter was saponified by boiling with 1 M-KOH in ethylene glycol; the free bile acids were extracted

Table 3. *Diet characteristics of the various experimental periods**

(Mean daily values, with values for the contribution of bread (%) to the total daily intake in parentheses)

Experimental period ...	B	C	D_1	D_2	D_3
Type of bread ...	White bread (*n* 12)	150 g coarse-bran/kg bread (*n* 12)	320 g coarse-bran/kg bread (*n* 4)	150 g fine-bran/kg bread (*n* 4)	Wholemeal bread (*n* 4)
Energy (MJ)	11·1 (26)	11·1 (24)	11·5 (21)	9·9 (27)	11·5 (24)
Total fat (energy %)	36 (8)	36 (8)	41 (8)	35 (10)	36 (9)
Total available carbohydrates (energy %)	49 (40)	48 (37)	42 (33)	49 (39)	50 (33)
Total protein (energy %)	12 (29)	12 (30)	12 (33)	13 (30)	12 (31)
Phosphorus (mg)	1163 (17)	1530 (36)	1905 (48)	1867 (31)	1622 (40)
NDF (g)	8·7 (25)	21·2 (69)	34·9 (83)	22·8 (65)	22·0 (71)
NDF (g/4·2 MJ)	3·3 (25)	8·0 (69)	12·8 (83)	9·6 (65)	8·0 (71)
ADF (g)	5·8 (21)	9·6 (52)	13·5 (69)	10·2 (53)	10·7 (52)
Cellulose (g)	4·2 (19)	6·9 (51)	10·2 (67)	6·8 (48)	7·1 (51)
Hemicellulose (g)	2·9 (31)	11·6 (83)	21·4 (92)	12·6 (75)	11·3 (90)
Lignin (g)	1·6 (25)	2·7 (56)	3·3 (78)	3·4 (62)	3·6 (53)

NDF, neutral-detergent fibre (Van Soest & Wine, 1967); ADF, acid-detergent fibre (Van Soest & Wine, 1967).

* For details, see Table 1.

Table 4. *Defaecation pattern of twelve human subjects consuming a white bread diet and a 150 g coarse-bran/kg bread diet for a period of 20 d*

(Mean values and standard error of differences)

Experimental period ...	B	C	
Type of bread ...	White bread	150 g coarse-bran/kg bread	
NDF intake (g/d) ...	9	22	
	Mean	Mean	SED
Faecal wet wt (g/d)	77	140**	5
Faecal dry wt (%)	27	24**	0·8
Defaecation frequency (no. of stools/d)	0·9	1·1**	0·1
Intestinal transit time (h)	88	52**	8
Faecal VFA (mmol/g wet faeces)	0·10	0·11	0·01
Faecal VFA (mmol/d)	8·3	15·2**	1·5
Faecal bile acids:			
(μmol/g wet faeces)	12·6	6·2**	0·8
(μmol/d)	936	806	108
Faecal N (g/d)	1·11	1·58**	0·12
Faecal P (mg/d)	323	590**	36

VFA, volatile fatty acids.
NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period B: $^{**}P < 0{\cdot}01$.

with ethyl ether, purified by partitioning between petroleum ether and aqueous methanol (H_2O–CH_3OH, 30:70 v/v) and dissolved in methanol.

Faecal P was determined gravimetrically as ammonium molybdophosphate (Schormüller, 1967), faecal N was analysed (Noel, 1976) and faecal VFA (C_1–C_6) were determined by gas–liquid chromatography (van de Kamer *et al.* 1955); the modified method of Van Soest & Wine (1967) was applied to determine the amount of residual dietary fibre components in the stools.

The apparent digestibilities of dietary fibre and its components were estimated by regression analysis, using the values for intake and faecal excretion (see pp. 69–71).

For statistical evaluation the results, with respect to the defaecation pattern of each dietary treatment, were compared with those of the preceding treatment by means of analysis of variance (Snedecor & Cochran, 1967) using Student's *t*-test for paired observations. Each subject thus served as his own control. The means for period D (four subjects) were compared with those of the corresponding subjects in period C.

This research was approved from an ethical standpoint by a working group responsible for human nutrition studies.

RESULTS

The defaecation pattern is indicated by results presented in Tables 4–7. Substituting 150 g coarse-bran/kg bread (period C) for white bread (period B) resulted in the following significant ($P < 0{\cdot}01$) changes: an increase in faecal wet weight of 63 g/d, a decrease in the percentage of faecal dry weight of 3, an increase in defaecation frequency from 0·9 to 1·1 stools/d, a decrease in the intestinal transit time of 36 h, an increase in faecal VFA from 8·3 to 15·2 mmol/d and increases in faecal N (by 0·47 g/d) and faecal P (by 0·27 g/d) (see Table 4).

During the 320 g coarse-bran/kg bread period (period D_1, see Table 5) a further significant ($P < 0{\cdot}01$) increase of faecal wet weight was observed, the defaecation frequency

Table 5. *Defaecation pattern of four human subjects consuming a 150 g coarse-bran/kg bread diet and a 320 g coarse-bran/kg bread diet for periods of 20 d*

(Mean values and standard error of differences)

Experimental period...	C	D_1	
Type of bread...	150 g coarse-bran/kg bread	320 g coarse-bran/kg bread	
NDF intake (g/d)...	22	35	
	Mean	Mean	SED
Faecal wet wt (g/d)	137	202**	8
Faecal dry wt (%)	25	23	1
Defaecation frequency (no. of stools/d)	1·1	1·4**	0·03
Intestinal transit time (h)	77	45*	11
Faecal VFA (mmol/g wet faeces)	0·08	0·09	0·01
Faecal VFA (mmol/d)	10·0	16·9	2·7
Faecal bile acids:			
μmol/g wet faeces	7·3	4·6**	0·5
μmol/d	812	888	63
Faecal N (g/d)	1·30	1·84	0·19
Faecal P (mg/d)	524	891*	79

VFA, volatile fatty acids.
NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean values significantly different from those for period C: $*P < 0·05$, $**P < 0·01$.

Table 6. *Defaecation pattern of four human subjects consuming a 150 g coarse†-bran/kg bread diet and a 150 g fine†-bran/kg bread diet for periods of 20 d*

(Mean values and standard error of differences)

Experimental period...	C	D_2	
Type of bread...	150 g coarse-bran/kg bread	150 g fine-bran/kg bread	
NDF intake (g/d)...	22	22	
	Mean	Mean	SED
Faecal wet wt (g/d)	126	102*	8
Faecal dry wt (%)	26	29	1
Defaecation frequency (no. of stools/d)	1·2	1·1	0·05
Intestinal transit time (h)	44	62	15
Faecal VFA (mmol/g wet faeces)	0·10	0·09	0·01
Faecal VFA (mmol/d)	12·8	10·9	2·2
Faecal bile acids:			
μmol/g wet faeces	6·4	6·7	0·6
μmol/d	818	788	121
Faecal N (g/d)	1·67	1·63	0·16
Faecal P (mg/d)	592	621	17

VFA, volatile fatty acids.
NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
Mean value significantly different from that for period C: $*P < 0·05$.
† Coarse-bran, particle size > 0·35 mm, fine-bran, particle size < 0·35 mm.

Table 7. *Defaecation pattern of four human subjects consuming a 150 g coarse-bran/kg bread diet and a wholemeal bread diet for periods of 20 d*

(Mean values and standard error of differences)

Experimental period...	C	D_3	
Type of bread...	150 g coarse-bran/kg bread	Wholemeal bread	
NDF intake (g/d)...	22	22	
	Mean	Mean	SED
Faecal wet wt (g/d)	158	143	11
Faecal dry wt (%)	22	24	1
Defaecation frequency (no. of stools/d)	1·1	1·2	0·1
Intestinal transit time (h)	35	45	10
Faecal VFA (mmol/g wet faeces)	0·14	0·14	0·01
Faecal VFA (mmol/d)	22·8	18·8	3·0
Faecal bile acids:			
μmol/g wet faeces	4·8	4·8	0·3
μmol/d	790	645	60
Faecal N (g/d)	1·82	1·48	0·12
Faecal P (mg/d)	653	549	15

VFA, volatile fatty acids.
NDF, neutral-detergent fibre (Van Soest & Wine, 1967).
None of the differences is statistically significant.

increased to 1·4 ($P < 0·01$) and the intestinal transit time was significantly ($P < 0·05$) shorter than during the 150 g coarse-bran/kg bread period. The further increase of faecal VFA and faecal N on the 320 g coarse-bran/kg bread diet was not statistically significant while faecal P increased significantly ($P < 0·05$), which reflects the increased P intake.

A marked influence of the particle size of the bran on the water-holding capacity was observed. The amount of faecal wet weight was significantly ($P < 0·05$) lower on the fine-bran bread (period D_2) than on the coarse-bran bread (period C, see Table 6); the increase of the percentage of faecal dry weight and the intestinal transit time, as well as the decrease of the excretion of faecal VFA on the fine-bran bread diet, were not statistically significant.

No significant differences of the various indices were detectable between the wholemeal bread period (period D_3) and the 150 g coarse-bran/kg bread period (period C, see Table 7), both providing a similar dietary fibre intake of 22 g NDF/d.

Throughout the study no significant changes in the daily excretion of faecal bile acids could be demonstrated; the concentration of total bile acids in the faeces decreased significantly ($P < 0·01$) on an increasing dietary fibre intake (Tables 4 and 5).

Although the amount of faecal VFA increased on the higher bran intake, the concentration in the faeces and the VFA pattern (of which 70% was acetic acid) remained constant.

The levels of intake and faecal excretion of dietary fibre and its components for each volunteer are shown in Figs. 1–4.

The total intake of NDF in the white bread period (period B) was 9 g. Of the total NDF intake 75% originated from the basal diet (see Table 3). In eight subjects NDF was almost completely digested, as shown by the low faecal excretions. For the lower digestibility of NDF in four subjects, cellulose and lignin seem to be responsible; hemicellulose, however, was almost completely degraded in all subjects. When 150 g coarse-bran/kg bread (period C) was substituted for white bread (period B), the NDF intake increased by 13 g (through bran in bread). It appears that in eleven subjects this increase in NDF intake resulted in approximately equal increments in faecal excretion of NDF, independent of a low

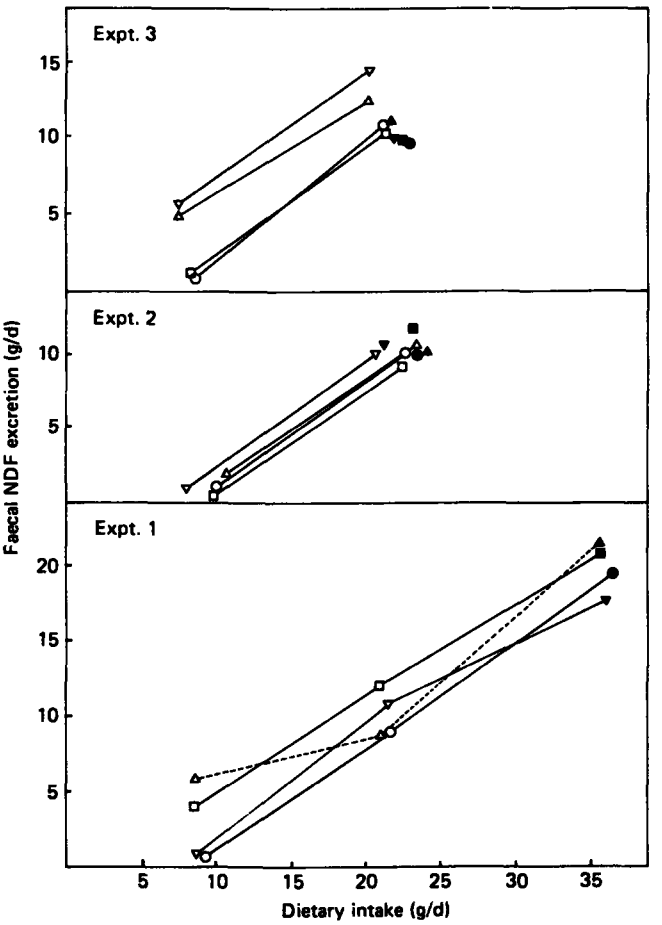

Fig. 1. Dietary intake and faecal excretion of neutral-detergent fibre (NDF). Each symbol indicates one subject. Open symbols on the left side of the graphs relate to the white-bread period (n 12) and those on the right side to the 150 g coarse-bran/kg bread period (n 12). Closed symbols refer in Expt 1 to the 320 g coarse-bran/kg bread period (n 4), in Expt 2 to the 150 g fine-bran/kg bread period (n 4) and in Expt 3 to the wholemeal-bread period (n 4).

or high faecal excretion in period B; this seems to be applicable also for hemicellulose, cellulose and lignin. For one subject (Δ in Expt 1) the results were contradictory, suggesting an analytical error.

The increase in fibre intake in period D_1 (320 g coarse-bran/kg bread diet) resulted in almost similar increments in faecal fibre excretions (four subjects) when compared with those after the substitution of 150 g coarse-bran/kg bread for white bread (Figs. 1–4).

When 150 g fine-bran/kg bread (period D_2) was substituted for 150 g coarse-bran/kg bread, the total NDF intake remained unchanged (22 g NDF/d). The faecal excretion of the fibre components was also similar for the four subjects.

When wholemeal bread was substituted for 150 g coarse-bran/kg bread the faecal excretion pattern of NDF and the components was not essentially altered, although small differences were observed in the digestion of all fractions of dietary fibre.

Faecal excretion of fibre components with the faeces can be expressed as a fraction of the intake. These coefficients of indigestibility were estimated by regression analysis. For

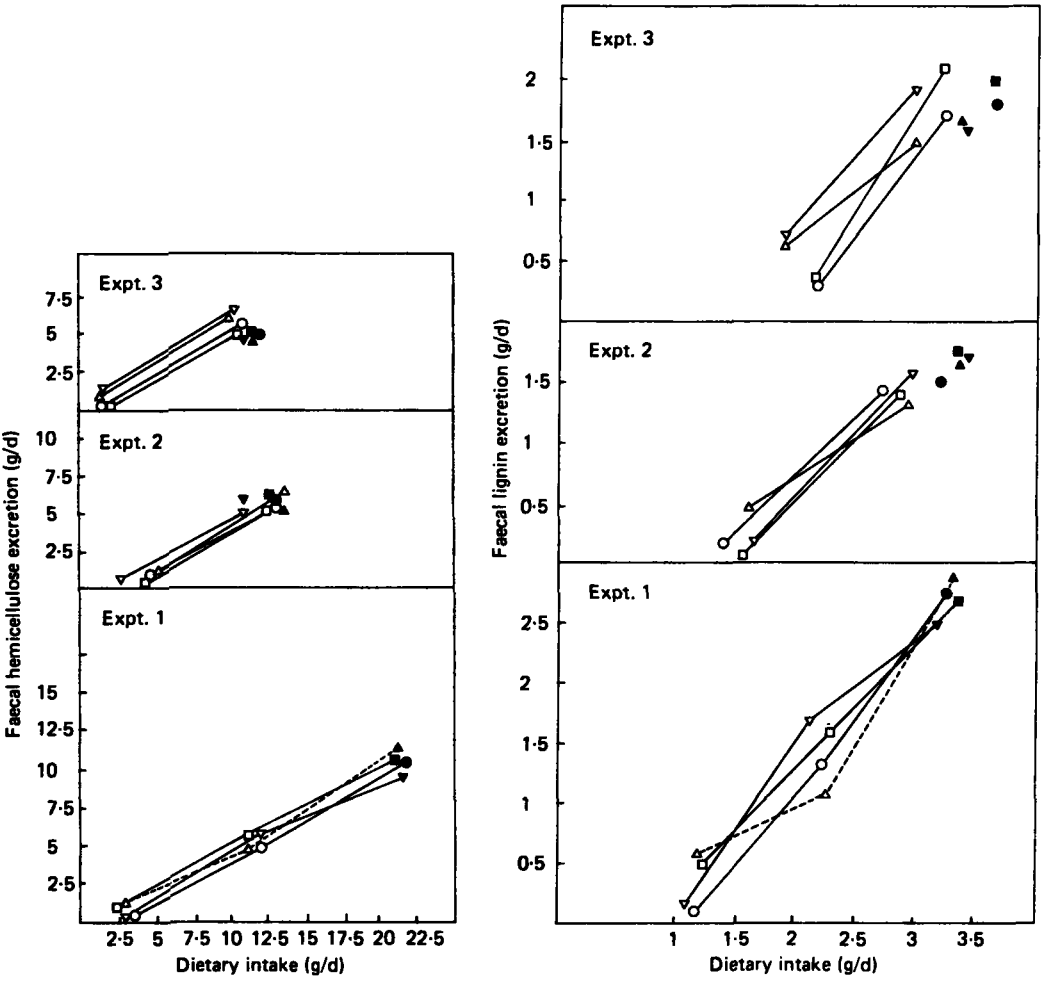

Fig. 2. Dietary intake and faecal excretion of hemicellulose. For details, see legend to Fig. 1.

Fig. 3. Dietary intake and faecal excretion of lignin. For details, see legend to Fig. 1.

every subject in each period the following data were available:

Amount in the basal diet	(x_0)
Amount in the bread	(x_1)
Amount excreted with the faeces	(y)

The excretion can be described by the regression equation:

$$y_i = \beta_0 x_{0_i} + \beta_1 x_{1_i} + e_i$$

where $i = 1, \ldots, 36$, β_1 is the coefficient of indigestibility of fibre from the bread and β_0 is the coefficient of indigestibility of fibre from the basal diet. In this simplified model, β_1 is assumed to be the same for all types of bread and β_0 and β_1 are assumed to be subject-independent.

Estimates of β_0 and β_1 and their confidence intervals for NDF, hemicellulose, lignin and cellulose are given in Table 8. These values were based on the analysis of the regression

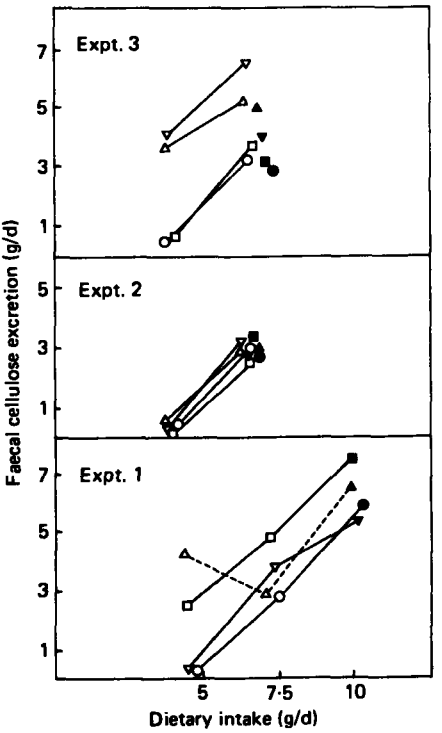

Fig. 4. Dietary intake and faecal excretion of cellulose. For details, see legend to Fig. 1.

Table 8. *Coefficients of indigestibility and digestibility of dietary fibre and the components hemicellulose, lignin and cellulose from the basal diet and from bread*

	NDF		Hemicellulose		Lignin		Cellulose	
		95% confidence intervals		95% confidence intervals		95% confidence intervals		95% confidence intervals
β_0	0·09	(0·02, 0·37)	0·06	(0·01, 0·33)	0·0	(0·0, 0·0)	0·23	(0·08, 0·53)
β_1	0·66	(0·59, 0·73)	0·54	(0·51, 0·57)	0·96	(0·83, 0·99)	0·80	(0·50, 0·94)
$1-\beta_1$	0·34		0·46		0·04		0·20	
RMS	3·1		0·33		0·081		1·6	
PVA	91		97		87		57	

β_0 = coefficient of indigestibility of fibre from the basal diet.
β_1 = coefficient of indigestibility of fibre from the bread.
$1-\beta_1$ = coefficient of digestibility of fibre from the bread.
RMS = Residual mean squares.
PVA = Percentage variance accounted for.

equation in the form:

$$y_i = \frac{1}{1+\exp(-\gamma_0)} \cdot x_{0_i} + \frac{1}{1+\exp(-\gamma_1)} \cdot x_{1_i} + e_i$$

where $\gamma_j = \log(\beta_j/1-\beta_j)$ and $j = 0, 1$. This approach was chosen because β_0 and β_1 must lie between 0 and 1. Different coefficients of indigestibility for the different types of bread did not result in a better fit for all four types of fibre. The relatively large 95% confidence

intervals for β_0 suggest a large variability in the excretion of fibre originating from the basal diet between subjects. Applying a variability between subjects in the regression analysis yields individual β_0's and actually results in a better description of the excretion for NDF ($P < 0.05$) and cellulose ($P < 0.01$), but not for hemicellulose and lignin. Similarly, assuming a variability between subjects for β_1 does not result in a better fit for all four types of fibre. In addition, in Table 8 the coefficients of digestion for the different types of bread are presented, calculated as the complement of the coefficients of indigestibility.

DISCUSSION

The changes in colonic function following the ingestion of wheat fibre observed in the present study are not in all respects in agreement with the results of other investigators. The observed significant increase in the percentage of faecal water after adding fibre to the diet was also reported by Thomas & Elchazly (1976), whereas other investigators found no significant change (Cummings *et al.* 1976*b*; Floch & Fuchs, 1978). Fantus *et al.* (1941) were unable to find any influence of particle size of the bran on faecal weight, while we observed a smaller faecal weight with the ground bran. In more recent studies, Heller *et al.* (1980) and Smith *et al.* (1981) also found a more significant effect of coarse-bran than of fine-bran on colonic function. The longer intestinal transit time associated with a smaller bran particle size was also observed by Kirwan *et al.* (1974). An increasing wheat fibre intake does not always seem to result in a shorter intestinal transit time; other factors, both physical and psychological ones, are also likely to influence the transit time (Cummings *et al.* 1976*b*; Tucker *et al.* 1981). The influence of dietary fibre on defaecation frequency is also contradictory; the significant increase with an increased wheat fibre intake in our experiment was similar to that reported by Kay & Truswell (1977), while others did not find significant changes (Payler *et al.* 1975; Wymann *et al.* 1976; Heller *et al.* 1980).

The results obtained with the ground bran with respect to colonic function may give some indications as to the mechanism by which dietary fibre from bread effects increase in stool weight. An increased stool weight depends on the production of VFA in the colon (Williams & Olmsted, 1936) as a result of bacterial digestion of cellulose and hemicellulose, suggesting an osmotic action of the VFA (Bustos Fernandez *et al.* 1971; Forsyth *et al.* 1978). The constant concentration of VFA in wet faeces observed by us and also reported by others (Bustos Fernandez *et al.* 1971; Fordtran, 1971; Cummings *et al.* 1976*a*; Forsyth *et al.* 1978), supports the hypothesis of equilibrium concentration. VFA are, however, reported to be absorbed in the human colon (Dawson *et al.* 1964; McNeil *et al.* 1978). Van Soest & Robertson (1977) estimated that bran consumption leads to the absorption of 65% of the VFA produced by the bacterial flora. Applying the same method of calculation, we found 77% VFA absorbed by the colon. Consequently, the VFA in the colon can only partly account for the observed increase in water binding on an increased wheat fibre intake.

Another suggested reason for the increase in water binding is that undigested dietary fibre promotes gel-formation in the colon. The hemicellulose fraction appears to be particularly important in this respect (Jelaca & Hlynka, 1971; Eastwood, 1974). When we compare the 150 g coarse-bran/kg bread with the 150 g fine-bran/kg bread, the hemicellulose intake and the faecal output appear to be the same; the colonic function was, however, different.

The increased stool weight associated with an increased wheat fibre intake could also be accounted for by the increased bacterial mass (as a result of fibre fermentation) which may hold water as well (Stephen & Cummings, 1979). Since a similar degradation of dietary fibre by the bacterial flora was observed in the coarse-bran period and in the fine-bran bread period, the difference in faecal wet weight is not likely to be caused by a difference in bacterial mass, since the faecal N output did not change either.

Water binding is finally ascribed to the capillary structure and sorptive properties of

dietary fibre. Eastwood & Kay (1979) concluded that the matrix of the fibre particles is responsible for sponge action, which is partly destroyed when the particle size is small. This fits well with our findings.

From the present study it can be concluded that both the VFA hypothesis and the non-digested hemicellulose theory (gel formation) may be important factors in accounting for faecal bulk, but that the matrix structure of dietary fibre seems to be the most important determinant of the observed increase in stool weight. The other indices of colonic function (transit time and defaecation frequency) are probably a result of water binding.

Although the concentration of total bile acids in the faeces decreased with an increasing amount of bran in bread, the total daily excretion of bile acids did not change significantly, which reflects the dilution of the bile acids in the increased faecal bulk. Some authors indicate an increase in faecal bile acid excretion in man, others found decreased values following wheat bran feeding (Kay, 1982).

By means of regression analysis an estimate can be made of the apparent digestibility of fibre in the basal diet and in bread separately. The digestibility of the fibre components in the basal diet appears to differ from that in bread; moreover, the digestibility of NDF and cellulose in the basal diet is variable in different subjects. Differences in the degradability of fibre fractions from different sources have been observed by other investigators. Van Soest & Robertson (1977) suggested that the cell wall of vegetables is more fermentable than bran. Holloway *et al.* (1978) observed a bacterial degradation of 78% for cellulose and 96% for hemicellulose on a normal, low-fibre diet. Heller *et al.* (1980) reported a digestibility of 0·35 for NDF and of 0·50 for hemicellulose on a coarse-bran diet; their findings for cellulose (digestibility 0·06) and lignin (a strongly negative digestion) differ from our observations.

Inter-individual variations have been reported as well, e.g. by Dintzis *et al.* (1979). Furthermore, it is remarkable that the degradation of fibre from bread appears to be the same in (almost) all subjects, independent of the amount of wheat fibre in the diet and also independent of the intestinal transit time, the latter being shortest in the period in which 320 g coarse bran/kg bread was consumed. This suggests a maximal digestion of wheat fibre under the various circumstances, possibly because of the typical physical structure of wheat fibre which does not enable bacteria to ferment the fibre component further than to the maximal value observed by us. In experiments with rats (Sinkeldam, unpublished results) similar values for the digestibility of dietary fibre from wheat were found using the same types of bread as in our human experiments. In addition, it is surprising that the digestibility is the same for all types of bread tested in the present study, viz. white bread with practically no visible fibre, wholemeal bread and bread prepared from coarse and fine brans. In particular, a more extensive degradation might have been expected for fine bran, because of the larger surface area of the particles.

Summarizing, we observed a nearly complete degradation in the gut of the small amount of fibre in our basal diet in most of the subjects, whereas in all subjects approximately two-thirds of the fibre from bread were recovered from the faeces. The sorptive properties of fibre in the large intestine seem to be mainly responsible for the bulking effect, which is considered to be beneficial to the colon function (Kay, 1982). Consequently, the importance of including an ample amount of bread, preferably high in coarse fibre, in the diet should be emphasized.

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5. The effect of quantity and kind of dietary protein on mineral balance, bowel function and blood pressure of young men

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1. Twelve young adult male volunteers were given three mixed diets: a low protein (LP) diet (9 energy % total protein, 67 % animal protein), a high-animal protein (HA) diet (16 energy % protein, 67 % of animal origin) and a high-vegetable protein (HV) diet (16 energy % protein, 67 % of vegetable origin), each diet period lasting 20 d. Retention of calcium, magnesium, iron, zinc and copper as well as blood pressure and various bowel function parameters were investigated during each dietary period.
2. An increased urinary Ca-excretion and a lower percentage of urinary Ca-reabsorption ($P < 0.001$) on the HA diet compared with the LP diet was found, suggesting a lower Ca-utilization when the amount of dietary protein is raised. No change was observed when the HV diet was substituted for the HA diet, which indicates that there is no effect of the type of protein on Ca-utilization.
3. Retention of Ca, Mg, Zn and Fe did not change significantly when protein intake increased (LP to HP diet) nor when the type of dietary protein was changed (HA to HV diet). However, the increased faecal excretions indicate an influence on mineral availability.
4. Substituting the HV diet for the HA diet did not result in significant changes in serumcholesterol values; systolic blood pressure, however, increased significantly ($P < 0.05$).
5. Substituting the HV diet for the HA diet resulted in significant increases in faecal wet weight (of 15 g/d), defaecation frequency (of 0.1 stools/d), faecal volatile fatty acids (of 2.6 mmol/d) and a decrease in faecal bile acids (of 129 µmol/d). It is, however concluded

that a high protein diet, rather than the quantity or the kind of the protein as such affects bowel function.

It has been postulated that incidence of osteoporosis might be influenced by enhanced urinary calcium-excretion and a negative Ca-balance as a result of increased protein intake (Hegsted et al., 1981; Linkswiler et al., 1981; Wachman and Bernstein, 1968). Animal studies suggest that increasing animal protein intake may cause a rise in blood pressure (Engen and Swenson, 1969; Handler and Bernheim, 1950). Not very many data are available on the influence of type and quantity of protein on mineral balance other than Ca. On assessing recommended dietary allowances (RDA) for various minerals it is of importance to look into dietary factors that might affect mineral retention or availability.

Epidemiological studies suggest that a high-animal protein diet might influence bowel function and faecal composition with a possible relationship to the incidence of cancer of the colon (Armstrong and Doll, 1975; Drasar and Irving, 1973; Hill et al., 1971). In previous papers we reported the influence of increasing the amount of dietary fibre from bread on mineral balance and bowel function (Van Dokkum et al., 1982; Van Dokkum et al., 1983). In the present paper special emphasis is put on the effect on various parameters of mineral utilization and bowel function as well as on blood pressure when: a. the quantity of dietary protein is increased at a constant ratio animal:vegetable protein of 2:1 and b. this ratio changes to 1:2 at a constant amount of total protein, as it is generally recommended to increase the amount of vegetable protein products in the diet.

METHODS

The experimental design is shown in table 1. Twelve male volunteers (mean age 23 $\pm$ 2 years, weight 72 $\pm$ 7 kg, height 1.82 $\pm$ 0.08 m and 15 $\pm$ 2 % body fat) were given three experimental diets with different amounts and types of dietary protein for 20 d each. As we were primarily interested in the dietary changes indicated in the introduction (and not so much vice versa) the order of consumption of the diets was the same for all volunteers.

A general 8 d adaptation, preceding the experimental periods was introduced in order to assess the energy requirements of the volunteers. The balance study was carried out in 3 periods of 20 d each. During each period

Table 1. Experimental design*

Experimental period	low protein diet LP	high animal protein diet HA	high-vegetable protein diet HV
duration (d)	20	20	20
protein intake (% of total energy)	9	16	16
ratio animal : vegetable protein	2:1	2:1	1:2
blood sampling	xx	xx	xx
blood pressure	twice a week	twice a week	twice a week
urine and faecal collections	20 d.	20 d.	20 d.

* The study was carried out with four volunteers at a time and replicated twice (with other subjects), resulting in three experiments with four volunteers each. Only pooled data are given of all 12 volunteers

On two days at the end of each period

the mineral balance was determined by analyzing individual duplicate daily dietary samples (for each subject twice per 20 d) and for each subject 20 d faecal and urinary mineral excretion (subdivided in 5 4 d periods of sample collection). The subjects gave informed written consent according to the Institute's procedures and were housed in the Institute's controlled metabolic ward, but they continued their normal daily routines.
They passed beforehand a clinical examination and a nutritional evaluation. The routine haematological values were all within normal ranges.

Experimental procedure

During each 20 d period the diet was constant and similar for all volunteers with minor differences between subjects regarding sugar, soft drinks and jelly. These products were also supplied when reduction in body weight of more than 2 % compared with the weight in the general adaptation period was observed. The composition of the three experimental diets is given in table 2, the diet characteristics, based on analysis of individual duplicate daily samples, in table 3.
For the LP diet a special low-protein bread was prepared; a higher protein intake in the HA period was realized by increasing the amount of meat and meat products. In the last period (HV) bread was prepared with 15 % wheat gluten and 20 % soy-protein isolate (both on dry weight basis), in the hamburger 17 % soy-protein concentrate ("textured procon") was incorporated. In the HA and HV periods the diets were adapted in order to reach the ratios of animal protein:vegetable protein of 2:1 and 1:2 respectively. The dietary changes with respect to quantity and kind of protein, as indicated in practice automatically result in a different mineral intake. The objective being to study the mineral balance under these conditions, no suppletion of minerals was applied to achieve similar levels of mineral intake in the 3 diets. The food was prepared in the diet kitchen, according to standard procedures, weighed to the nearest g, packed in individual portions and deep-frozen, if necessary.
Only demineralized water was allowed, e.g. for coffee and tea. All meals were served at the Institute. Urine samples (24 h) were collected in polyethylene bottles with hydrochloric acid as preservative. Stools were collected in 3 l plastic buckets, one for every 4 d, stored at 4 °.

Table 2. Composition (g) of the experimental diets
(mean daily values)

Experimental period	low protein diet	high-animal protein diet	high-vegetable protein diet
Breakfast			
2 rolls	2 x 40	2 x 40	2 x 40
Cheese	30	-	30
Pork roast	-	30	-
Peanutbutter	-	14	14
Orange juice	125	150	150
Lunch			
3 rolls	3 x 40	3 x 40	3 x 40
Cheese	30	30	30
Ham (raw)	15	-	15
Ham (smoked)	-	20	-
Chicken meat	-	25	-
Jelly	30	-	14
Orange juice	125	-	150
Custard (low fat)	150	150	150
Curd shake	-	145	-
Dinner			
Potatoes	200	200	200
Vegetables*			
Hamburger	100	150	165
Margarine	15	15	15
Icecream	50	-	-
Curd and custard	-	185	-
Canned fruit	-	-	100
1 roll	40	40	40

Table 2. Composition (g) of the experimental diets, continued (mean daily values)

Experimental period	low protein diet	high-animal protein diet	high-vegetable protein diet
During the day			
Margarine	35	30	30
Whisky	70	70	70
Coffee (instant)	3	3	3
Tea (instant)	0.9	0.9	0.9
Sugar	25	25	25
Whipped cream	25	-	25
Rye bread	33	-	-
Jelly	14	-	-
Fibre biscuits	-	15	-
Cake	-	-	25
Raisins	-	-	35
Soft drinks	2 x 200	2 x 200	2 x 200
Canned fruit	-	100	-

* Rotating order for every 4 d; day 1: 100 g string beans, 7 g margarine, 100 g apple sauce; day 2: 30 g lettuce, 45 g carrot salad, 45 g celeriac salad, 20 g dressing; day 3: 100 g sliced beans, 7 g margarine, 100 g apple sauce; day 4: 100 g carrots, 7 g margarine, 100 g apple sauce.

hamburger: LP and HA diet: 30 % beef, 30 % pork; 20 % fat (beef), 10 % eggprotein, 10 % water. HV diet 40 % beef, 17 % soy-concentrate, 13 % lard, 30 % water.

Table 3. Diet characteristics of the various experimental diets (mean daily values)

Experimental period		low protein diet	high-animal protein diet	high-vegetable protein diet
Energy	(MJ)	12	12	13
Total protein	(energy %)	9	16	16
Total fat	(energy %)	36	35	35
Total available carbohydrates	(energy %)	52	46	45
Linoleic acid	(energy %)	9	8	8
Saturated fat	(energy %)	15	13	14
Alcohol	(energy %)	3	3	3
Dietary fibre	(g NDF)	15	15	21
Cysteine + methionine	(g)	1.9	4.0	3.7
Sodium	(g)	3.7	3.7	4.3
Phosphorus	(mg)	1158	1688	1668
% of total protein:				
Meat protein		29	36	15
Milk protein		38	33	17
Vegetable protein		33	31	68

The stools from each 4 d period were weighed and carefully homogenized in each bucket, securing that no faecal loss or mineral contamination took place. Composites (4 d) of urine and stools were made for analysis and stored at -20 °. Blood was withdrawn from the antecubital vein before breakfast on two consecutive days at the end of each period. Blood pressure was assessed twice a week directly after breakfast with a London School of Hygiene sphygmomanometer (Rose et al., 1964), using a cuff of 34 x 12.5 cm. Blood pressure was measured at the right upperarm of the subject in sitting position. Each blood pressure observation consisted of two measurements within a few minutes time; when the differences of the

duplicate values of systolic pressure or diastolic pressure were more than 10 and 8 mm respectively, the measurements were repeated. Body composition was assessed at the beginning and at the end of each dietary period and included height, weight and skinfold thickness measurements (Weiner and Lourie, 1969). The percentage of body fat was calculated from the sum of 4 skinfold thicknesses (Durnin and Womersley, 1974).

Analytical procedures

Urine. From the 4 d composites, samples were taken for Ca, Mg, Fe, Zn, Na and Cu analysis by means of atomic absorption spectrophotometry (AAS) (Perkin-Elmer 303). For 8 subjects pH of the (non-acidified) urine was determined. From the creatinine clearance data the percentage of Ca-reabsorption was calculated (Licata et al., 1979).

Faeces. From the 4 d composites, samples were taken for mineral determinations by AAS and moisture determinations by drying on sand. In the last two 4 d composites of each dietary period volatile fatty acids (VFA) were determined by gas-liquid chromatography (Van de Kamer et al., 1955) and total bile acids by an enzymatic method (Van Dokkum et al., 1983). Stool frequency was computed from information obtained from the diaries of the volunteers.

Diets. Aliquots of the homogenized (individual) duplicate diets were saved for analysis of the minerals (by AAS), protein using an automated KjellFoss (Noel, 1976), fat (Schormüller, 1969), total available carbohydrates (Van de Kamer, 1941), fatty acid composition by GLC (IUPAC, 1979), dietary fibre (Van Soest and Wine, 1969) and amino-acid composition with an automated amino-acid analyzer; cysteine and methionine were pretreated with performic acid (Moore, 1963).

For mineral analysis faecal and dietary samples were carefully dried, ashed at 55 ° for 2 h and another 1 h at 550 ° after addition of 1 ml hydrochloric acid (5N) in order to prevent splashing. The ash was dissolved in HCl for the final analysis of the minerals by AAS. This procedure was carried out in duplicate for each sample; for both faecal and dietary samples the coefficient of variation of the duplicates ranged from 2-3 %.

Blood. The venous blood or serum samples were analysed for haemoglobin (cyanmethaemoglobin method), packed cell volume (micro method), erythrocyte count by a Coulter counter, calcium (Raman and Chang, 1974), phos-

phorus (Zilversmit and Davis, 1950), total cholesterol (Huang et al., 1961), triglycerides (Eggstein, 1968), total protein (Am. Ass. Clin. Chem., 1953), albumin (Doumas et al., 1971), urea (Fawcett and Scott, 1960), ferritin (Miles et al., 1974), magnesium, copper and zinc (by AAS). Mineral balance was calculated from 20 d dietary intake and urinary + faecal excretion in each experimental period (subdivided in five 4 d periods).

For statistical evaluation the results of each dietary treatment were compared with the preceding treatment by means of analysis of variance (Snedecor and Cochran, 1967), using student's t-test for paired observation. Thus, subjects acted as their own control.

A working group responsible for human nutrition studies performed at the Institute approved of this research being carried out.

RESULTS

The defaecation pattern is indicated by the results presented in figure 1. Increasing the amount of protein (substituting the HA diet for the LP diet) only resulted in a significant ($P < 0.002$) increase in faecal wet weight of 17 g/d; the other differences observed were statistically not significant. Substituting the HV diet for the HA diet resulted, however, in the following significant changes: an increase in faecal wet weight of 15 g/d ($P < 0.01$), an increase in defaecation frequency from 0.9 to 1.0 stools/d ($P < 0.05$), an increase in faecal VFA of 2.6 mmol/d ($P < 0.05$) and a decrease in faecal bile acids of 1.3 µmol/g wet faeces ($P < 0.001$) and of 129 µmol/d ($P < 0.05$). The VFA composition was similar in all experimental periods; 66 % of the VFA is acetic acid, 14 % propionic acid and 11 % butyric acid.

Increasing the amount of protein in the diet (LP to HA period) resulted in a significant decrease in fractional renal tubular reabsorption of Ca as calculated from the glomerular filtration rate (measured as endogenous creatinine clearance) (table 4). This decrease in Ca reabsorption was accompanied by a significant increase in urinary Ca excretion. pH of urine decreased significantly; the excretion of Na remained constant when substituting the HA diet for the LP diet. On the HV diet the percentage of Ca reabsorption was equal to that on the HA diet, although the quantity of Ca reabsorbed was significantly lower on the HV diet. Urinary pH increased significantly on the HV diet and reached the level of the LP diet.

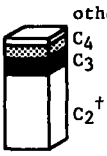
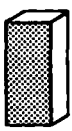
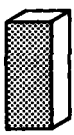
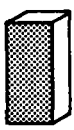
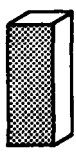
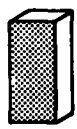
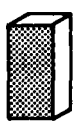
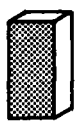
Table 4. Urine parameters of 12 human subjects on the low protein diet (LP),the high-animal protein diet (HA) and the high-vegetable protein diet (HV)
(mean values of the last 4 d in each period of 20 d and standard deviations)

Experimental period		LP		HA		HV	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
Glomerular filtration rate	(ml/min)	116	7	122	9*	112	6***
Ca filtered	(mg/min)	5.50	0.31	5.83	0.38*	5.38*	0.23**
Ca excreted	(mg/min)	0.12	0.04	0.17	0.05****	0.15	0.04
Ca reabsorbed	(mg/min)	5.38	0.32	5.66	0.36*	5.23	0.24****
% Ca reabsorption		97.7	0.8	97.1	0.8****	97.2	0.8
pH urine		6.30	0.14	5.87	0.10****	6.38	0.19****
Na excreted	(g/24 h)	3.43	0.27	3.47	0.28	4.36	0.95**

Mean values significantly different from the preceding period:
* $P < 0.05$, ** $P < 0.005$, *** $P < 0.002$, **** $P < 0.001$

The increased amount of Na excreted in the HV diet ($P < 0.005$) as compared with the HA diet reflects the higher Na intake from the HV diet.
Figure 2 shows the mineral balance data. Increasing the amount of dietary protein (LP to HA diet) resulted in significant increases of urinary Ca and Mg ($P < 0.01$) and in faecal Ca, Mg, Fe and Zn. The balance of the four minerals however did not change significantly. Cu balance increased significantly on the HA diet compared with the LP diet. Substituting the HV diet for the HA diet resulted in significant increases in urinary Mg and faecal Mg, Fe and Cu; also this dietary change did not influence the mineral balance significantly, only Cu balance was higher on the HV diet ($P < 0.001$). The increased dietary intake of the minerals seems to be partly responsible for the higher excretion figures observed. Urinary excretion of Fe, Zn and Cu was low and constant throughout the study.

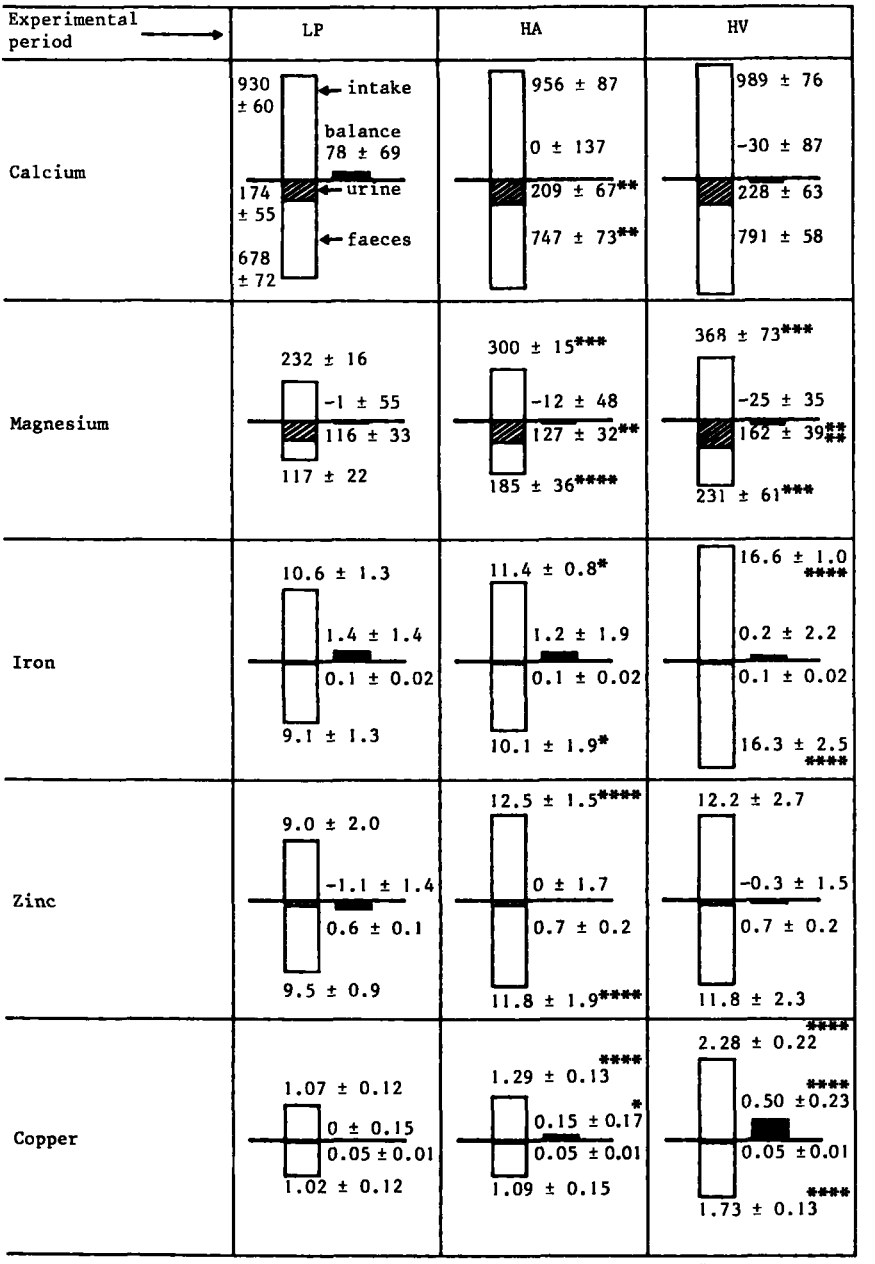
FIGURE 1 - FAECAL PARAMETERS OF 12 HUMAN SUBJECTS ON THE LOW PROTEIN (LP) DIET, THE HIGH-ANIMAL PROTEIN (HA) DIET AND THE HIGH-VEGETABLE PROTEIN (HV) DIET (MEAN VALUES AND STANDARD DEVIATIONS)

Experimental period →	LP	HA	HV
Faecal wet weight (g/d)	104 ± 25	121 ± 25***	136 ± 28**
% of faecal dry weight	 22 % ± 2	 22 % ± 3	 22 % ± 2
Defaecation frequency (no. of stools/d)	0.86 ± 0.32	0.88 ± 0.27	1.00 ± 0.37*
(mmol/d total VFA)	11 ± 5	13 ± 4	15 ± 5*
Volatile fatty acids (% composition)			
Volatile fatty acids (mmol/kg wet faeces)	102 ± 29 	105 ± 24 	111 ± 24 
Bile acids (mmol/d)	601 ± 110 	710 ± 140 	582 ± 123* 
Bile acids (mmol/kg wet faeces)	5.7 ± 1.5 	5.8 ± 1.4 	4.5 ± 1.4**** 

mean values significantly different from the preceding period: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$, **** $P < 0.001$

† C_2 = acetic acid, C_3 = propionic acid, C_4 = butyric acid, other = sum of other VFA

FIGURE 2 - INTAKE, EXCRETION AND BALANCE OF CALCIUM, MAGNESIUM, IRON, ZINC AND COPPER (mg/d) OF 12 HUMAN SUBJECTS ON A LOW PROTEIN (LP) DIET, A HIGH-ANIMAL PROTEIN (HA) DIET AND A HIGH-VEGETABLE PROTEIN (HV) DIET DURING 20 d (MEAN VALUES AND STANDARD DEVIATIONS)



mean values significantly different from the preceding period: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$, **** $P < 0.001$

Table 5. Blood constituents values and serumbiochemical criteria of 12 human subjects at the end of the general adaptation period (A) and on a low protein (LP) diet, a high-animal protein (HA) diet and a high-vegetable protein (HV) diet (mean values and standard deviations)

Experimental period		A (8 d)		LP (20 d)		HA (20 d)		HV (20 d)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Haemoglobin	(mmol/l)	9.1	0.5	9.3	0.5	9.2	0.4	9.3	0.5
Packed cell volume		0.45	0.02	0.45	0.02	0.44	0.02	0.42	0.03
Erythrocyte count	(10^{12}/l)	4.8	0.3	4.8	0.4	4.8	0.3	4.9	0.3
Serum calcium	(mmol/l)	2.4	0.1	2.4	0.1	2.4	0.1	2.4	0.1
magnesium	(mmol/l)	0.82	0.06	0.81	0.06	0.82	0.04	0.82	0.05
zinc	(µmol/l)	16.9	4.0	16.5	3.4	17.3	3.5	16.9	3.8
copper	(µmol/l)	14.3	2.4	14.4	3.3	13.8	2.0	13.4	5.1
phosphorus	(mmol/l)	1.2	0.2	1.2	0.1	1.3	0.2	1.3	0.1
Ferritin	(µg/l)	172	112	163	117	158	114	135	104
Total protein	(g/l)	68	3	68	4	68	3	70	4*
Albumin	(g/l)	46	2	46	2	46	2	47	2
Urea	(mmol/l)	5.1	1.2	4.8	0.8	6.3	1.0***	6.6	1.2
Total cholesterol	(mmol/l)	4.2	0.7	3.7	0.5**	3.6	0.4	3.7	0.4
Triglycerides	(mmol/l)	1.18	0.40	1.12	0.51	0.90	0.23*	0.92	0.28

Mean values significantly different from the preceding period:

* $P < 0.05$, ** $P < 0.002$, *** $P < 0.001$

Blood was withdrawn at the end of each period indicated

In table 5 the results in blood (serum) obtained at the end of each experimental period are presented. Almost all parameters were constant in all periods; significant changes were observed only in a few cases. No significant decrease in serum cholesterol was seen when the HV diet was substituted for the HA diet. Although various significant changes of the mineral balance data were observed, serum mineral concentrations were not affected. The increase in serum protein concentration in the HV period was small, but significant. The increase in total protein intake (LP to HA diet) resulted in a significant increase in serum urea values; serum triglycerides concentrations decreased significantly during the HA period. As to the blood pressure of the subjects, only a slight, but significant increase in systolic blood pressure on the HV diet as compared with the HA diet was measured (see table 6).

Table 6. Systolic and diastolic blood pressure of 12 human subjects on the three experimental diets (mean values and standard deviations)

Experimental period	LP		HA		HV	
	Mean	SD	Mean	SD	Mean	SD
Systolic pressure (mm Hg)	113	5	114	5	117	7*
Diastolic pressure (mm Hg)	59	8	57	8	59	5

Mean value significantly different from HA period: * $P < 0.05$

DISCUSSION

The increase in the amount of dry weight of the diet (555 g, 600 g and 630 g respectively in the LP, HA and HV diet) might partly explain the increase in faecal wet weight. The higher amount of dietary fibre in the HV period seems to be responsible for the increase in faecal wet weight as well. The VFA is considered as a factor accounting for the waterbinding capacity in the colon (Forsyth et al., 1978; Hellendoorn, 1978); the increase in VFA observed in our study corresponds to this assumption.

Morris et al. (1977) and Saunders and Betschart (1980) mentioned the significance of protein as a component of dietary fibre; they particularly consider partially digested protein of vegetable origin as a factor in fibre physiology.

The percentage composition of the VFA is in good agreement with that found in a previous study (Van Dokkum et al., 1983). The effect of protein on bile acid excretion has hardly been studied. Cummings et al. (1979) found a non-significant increase of 11 % replacing a low-animal protein diet (63 g/d) by a high-animal protein diet (136 g/d); a significant increase in bile acid excretion of 100 % resulted from an increase in dietary fibre intake from 22 to 53 g/d. In our study the increase in dietary fibre intake from 15 to 21 g/d did not result in an increase in bile acid excretion. As other macronutrients than dietary protein, which are involved in bile acid metabolism, are almost constant in the HA and HV periods, the observed decrease in bile acid excretion in the high-vegetable protein period could partly be attributed to the type of protein in the diet. A low concentration of bile acids in the faeces, together with a low excretion per 24 h may contribute to the prevention of colo-rectal cancer (Zaridze, 1981).

A higher sulphur and phosphorus intake in the HA period, resulting in a higher anion-concentration in the urine may be partly responsible for the significant decrease in urinary pH in the HA period compared with the LP period. As the S and P intake in the HA and HV periods do not differ much, the higher pH in the HV period can be accounted for by a higher cation concentration, of which the increased Na- and Mg-excretion in urine is an indication. The increased urinary Ca-excretion and the decreased percentage of Ca-reabsorption on the HA diet compared with the LP diet can be considered as less favourable and confirms the results of other studies (Hegsted and Linkswiler, 1981; Zemel et al., 1981; Allen et al., 1979). The higher anion concentrations resulting from the increased S and P intake may explain these effects (Schuette et al., 1980; Zemel et al., 1981). Although the retention of Ca, Mg, Fe and Zn did not change significantly during the study, the amount of mineral faecal excretion, as percentage of mineral intake reached a maximum in the high-vegetable protein period; the apparent absorption seems therefore to be less than in the other periods.

Haeme-iron absorption is known to be better than non-haeme-iron absorption (Hallberg, 1981); moreover haeme-iron facilitates the absorption of non-haeme-iron (Finch, 1977; Hallberg, 1981). The diets with a relatively high ratio animal protein:vegetable protein therefore seem to be more favourable with respect to iron absorption than a high-vegetable protein diet. For Zn, the retention is negative in 10 of the subjects on the low protein diet, possibly because of a marginal Zn intake. The US recommended dietary allowance for Zn is 15 mg/d for male adults; in the Netherlands no officially adopted allowance for Zn exists.
Concerning the interpretation of the mineral balance data the following remarks can be made:

- The fact that the balance of Ca, Mg, Fe and Zn did not change significantly suggests that apparently the diet does not influence mineral retention.
- The increased faecal excretion of the minerals on a higher mineral intake suggests a decreased availability. Interactions of the minerals with other food components (fibre, protein, other minerals) might play an important role.
- Although the mineral intake in our study generally is to be considered as adequate (except for Zn in the LP period), the observed decrease in mineral availability, when substituting a high-vegetable protein diet for a high-animal protein diet, seems to be significant for persons with a marginal or low mineral status.
- The suggested decreased mineral availability on a higher mineral intake is in agreement with previous findings in which dietary fibre was the variable component (Van Dokkum et al., 1982).

The significant decrease in serumcholesterol during the first period of 20 d can be accounted for by a higher linoleic acid intake and a lower intake of saturated fat compared with the habitual dietary pattern of the volunteers. Moreover it might be of interest to speculate about the possibility of a more moderate and regular life-style and probably less stress during the study compared with their habitual way of life, causing a decrease in serumcholesterol. The small differences in linoleic acid intake and saturated fat intake between the LP and HA diets compensate each other and will result in a constant serumcholesterol value. The same can be said when comparing the HA and HV periods; this means that the in-

creased dietary fibre intake in the HV diet did not result in a lower serumcholesterol level.

Soy protein being part of the protein in the HV diet did not result in decreased serumcholesterol levels either, which is in agreement with recent findings in healthy male volunteers (Van Raay et al., 1982). The decreased serumtriglycerides concentrations during the HA period are probably the result of a lower carbohydrate intake. The significant increase in serum-urea values in the HA period compared with the LP period supports the positive correlation with the amount of dietary protein intake (Eggum, 1970; Taylor et al., 1974). It is remarkable that the mean systolic blood pressure in the high-vegetable protein period was higher than in the high-animal protein period. Our blood pressure measurements took place under constant circumstances; nevertheless blood pressure may be influenced by various factors. The lowering effect on the bloodpressure of a vegetarian diet, as observed in several studies (Sack et al., 1974; Rouse et al., 1983), is often attributed to the increased consumption of vegetable protein, dietary fibre and polyunsaturated fat and to a decreased consumption of saturated fat (Wright et al., 1979; Sacks et al., 1981). It is suggested that dietary sodium does not account for the observed differences in blood pressure between vegetarians and non-vegetarians (Armstrong et al., 1979). In our experiment, the total fat intake as well as the intake of saturated and polyunsaturated fat was almost equal in the HA and HV diets. However, the amount of dietary fibre, vegetable protein and also the sodium content of the HV diet were higher than in the HA diet. In a recent study (Brussaard et al., 1981) dietary fibre, protein and fat had no demonstrable effect on blood pressure in young normotensive persons. It seems therefore likely that the difference in sodium intake (HA 9,5 g NaCl/d, HV 11.1 g NaCl/d) is responsible for the difference in mean systolic blood pressure we observed. Birkenhäger and De Leeuw (1982) suggested that the mean bloodpressure will decrease 1 mm Hg on a decrease of 1 g NaCl intake.

In our study a high-animal protein diet was actually not found to cause increase in blood pressure.

Conclusions

From the results of this study we have drawn the following conclusions. The observed changes in urinary calcium excretion and pH of the urine provide an indication of a high-animal protein diet probably being a risk

factor for developing of osteoporosis.
We did not find a basis for the suggestion that a high (animal) protein diet causes a rise in blood pressure. The observed decrease in apparent absorption of Ca, Mg, Fe and Zn, indicating a decreased availability of the minerals, is of significance in the discussion of the recommended dietary allowances. A diet with a high amount of vegetable protein does not seem to induce a lower serumcholesterol value than a similar diet with a high amount of animal protein.
Considering a possible relation between consumption of a protein-rich diet and the incidence of colo-rectal cancer (through changes in bowel function and faecal composition), more emphasis has to be put on the effects of a high-protein diet (with dietary fibre, phytate etc.) than on the protein as such.

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6. Metabolic balance studies at the CIVO-Institutes TNO, Zeist

6.1 INTRODUCTION

In the previous chapters various results have been described of human nutrition studies performed at the TNO Institute at Zeist. In interpreting these results it is at least necessary to take into account the methods, techniques and analytical procedures used. The external conditions under which experiments with humans have to be performed are often the basis of the scientific design. In this connection, the maximum capacity of metabolic rooms, the available personnel and last but not least the financial aspects have to be considered.
When visiting several centres in the USA where human nutrition studies are being carried out on a regular scale, large differences in set up, execution, analytical procedures etc. became evident. This is particularly a disadvantage since it would be useful to be able to compare the results of the expensive metabolic balance studies and to exchange experiences. In this way a certain standardization of methods could be effected which would certainly lead to increased significance of the conclusions.

In this chapter the methods and techniques which were applied in our experiments are discussed in detail. In addition, some results are presented of a study carried out with 4 male subjects on a constant and adequate diet for 60 days, to evaluate the intra-individual variability of some biochemical parameters in blood and urine.

6.2 DESIGN OF THE STUDIES

In the years that the experiments described in this thesis were performed, we had a maximum capacity for live-ins of four. From the literature and the discussions in the USA it became apparent that for metabolic balance studies a number of 10-12 subjects is at least necessary to arrive at conclusions based on adequate statistics. For this purpose we had to repeat each experiment two times under circumstances which were as ident-

ical as possible. This (practical) approach has the disadvantage that the nutrient intake in the 3 experiments with 4 subjects cannot be identical, because the foodstuffs are purchased separately for each experiment; seasonal variations have to be reckoned with as well.
The set up of our studies has been adapted to the general aim of the programme outlined in chapter 1: to look into the consequences of changing the present diet in the direction of the recommended dietary pattern and not so much vice versa. This means that we programmed the same order of diets for all subjects in each study (consisting of 3 experiments). Each subject served as his own control.

6.3 SUBJECTS, SELECTION PROCEDURE, CONDUCT AND MOTIVATION

For all experiments described, healthy male subjects were recruited from the student population of Utrecht University. The selection procedure comprised the following steps:

1. General information (by telephone) given to candidates who answered an advertisement in the University journal.
2. Detailed information, a dietary history and a routine blood-haematological test at the Institute.
   In almost all cases this stage appeared to be decisive in the selection procedure. In view of group composition sociopsychological aspects were also taken into account.
3. A final clinical examination of candidates who passed stage 2 of the selection procedure.

The following criteria were applied:

- Male Caucasian subjects, age 20-30 years.
- Routine haematological values within the reference range.
- Habitual energy intake between 2500 and 3000 kcal (10-12 MJ).
- No drug users, no or moderate smoking habits.
- No or moderate consumption of alcoholic beverages.
- Moderate daily activity pattern.
- Normal bowel habits.

These criteria were applied in order to obtain for each study a group that is as homogeneous as possible and consists of 12 subjects. Before participation the subjects acquainted themselves with the various rules and regulations, partly of a general character (living in a metabolic ward), partly specifically for the study concerned. All subjects gave written informed consent prior to the beginning of the experiments, after having received an explanation of the purpose of the study and the procedures to be followed.

Daily contacts of the "metabolic ward team" with the subjects served to prevent undesirable changes in the procedures and to maintain motivation. During none of the studies a single case of drop-out occurred; all subjects cooperated well in all stages of the experiments. The subjects were enabled as much as possible to continue the normal daily routine of their (university) study programme.

In table 1 the general characteristics of all 46 subjects are summarized.

TABLE 1 - GENERAL CHARACTERISTICS OF THE SUBJECTS OF ALL STUDIES DESCRIBED (means ± standard deviation). N = 46, unless otherwise specified

Age (years)	22.9 ± 2.5
Height (cm)	182.4 ± 6.9
Weight (at the start of the exp.) (kg)	68.8 ± 6.9
Weight (at the end of the exp.) (kg)	68.1 ± 6.4
% Body fat (at the start)	13.7 ± 3.5
% Body fat (at the end)	13.2 ± 2.8
Haemoglobin (at the start) (mmol/l)	9.5 ± 0.6
Packed cell volume (at the start) (%)	46.5 ± 2.3
Red blood cell count (at the start) ($N \times 10^{12}/l$)	5.0 ± 0.3
Serum cholesterol (at the start) (mmol/l)	5.0 ± 1.0
Serum triglycerides (at the start) (mmol/l)	1.26 ± 0.66 (N = 24)

In table 1 the differences in body weight and percentage of body fat between the beginning and the end of the experiments are not statistically significant.

The conclusion can be drawn that our subjects formed a rather homogeneous group with respect to age, body composition and some serum/blood parameters. The 46 subjects represented in total 15 different (university) study disciplines.

6.4 EXPERIMENTAL DESIGN OF A STUDY TO EVALUATE THE INTRA-INDIVIDUAL VARIABILITY OF SOME BIOCHEMICAL PARAMETERS IN BLOOD AND URINE OF HUMAN SUBJECTS

In order to gain a clear insight into the several aspects of our experiments (diet, urine and faeces, blood, anthropometry), it appeared to be suitable to make use of the results of a study - not previously described - with four male subjects on a constant diet for 60 days. The aim of that study was to evaluate the intra-individual variability of some biochemical parameters in blood and urine. Moreover, the validity of the diet sampling procedure in terms of variability was assessed and the variability of various anthropometric data as measured by one observer was taken into account.

In this sub-chapter the design and procedures of the study are described. The results are presented in the following sub-chapters as part of the discussion of the respective methods and techniques.
The study design is presented in table 2.

TABLE 2 - INTRA-INDIVIDUAL VARIABILITY OF SOME BIOCHEMICAL PARAMETERS IN BLOOD AND URINE
STUDY DESIGN

- Duration of the study: 60 days on a constant diet
- Subjects: 4, healthy male, age 21-25 years, weight 60-69 kg, height 170-182 cm, % body fat 13-15.
- Blood sampling: 1. twice a week (a total of 20 samples)
  2. on day 47: every two hours from 08.00-22.00.
- Urine collections: 24 h samples over 60 days.
- Diet sampling: for each subject 6 homogenized duplicate diets.
- Anthropometric measurements: 7 times over 60 days.

The selection of the subjects took place as described in 6.3. The diet was constant for the entire period of the study (60 days), consisted of normal foodstuffs and was adequate with respect to all macro- and micronutrients. The composition of the daily diet is presented in table 4. The energy and nutrient composition of this diet was calculated using food tables;

results are presented in table 3 for subject no. 4 and compared with the means of the analysed values for the same subject.

TABLE 3 - CALCULATED VALUES OF ENERGY AND NUTRIENTS IN THE DIET OF SUBJECT 4 DURING THE VARIABILITY STUDY, COMPARED WITH THE MEANS OF THE ANALYSED VALUES (daily intake)
(see also table 5)

		Calculated	Analysed
(Metabolisable) energy	(MJ)	11.4	12.1
Protein, % of total energy		12.7	13.4
Fat, % of total energy		39.6	37.2
Available carbohydrates, % of total energy		47.8	49.3
Calcium	(mg)	984	1002
Iron	(mg)	11.4	16.1
Sodium	(g)	3.23	4.01
Vitamin B_1	(mg)	1.95	1.62
Vitamin B_2	(mg)	1.79	1.62
Vitamin B_6	(mg)	1.28	0.93

From this table it appears that using food tables the vitamin intake was overestimated and (particularly) the iron intake lower than analysed.

The various samples of diet, blood and urine were analysed as described in previous chapters. Sample collection also took place as outlined before and is discussed in the following sub-chapters.

6.5 FOOD PURCHASES, PREPARATIONS, SUPPLY OF THE DAILY DIETS AND ANALYSES

For all our studies the food products were as much as possible purchased in bulk for the entire experiment, to ensure that all individual food items came from one batch. This means that the nutrient intake of the basal diet was constant in each experiment. The complete diet was provided by the Institute; minor individual preferences with respect to coffee, tea, sugar, soft drinks and alcohol were made possible beforehand,

TABLE 4 - COMPOSITION OF THE DIET DURING THE VARIABILITY STUDY

Breakfast	2 rolls (brown) of	40	g
	cheese	15	g
	corned beef	30	g
	orange juice	150	g
Lunch	3 rolls (brown) of	40	g
	cheese	15	g
	ham	30	g
	jelly	30	g
	orange juice	150	g
	custard (low fat)	150	g
Cooked meal	potatoes	200	g
	vegetables	*	
	ground beef/pork	150	g
	ice cream	50	g
	1 roll (brown) of	40	g
During the day	dietary margarine	30	g
	tea (instant)	0.9	g
	coffee (instant)	3	g
	sugar	32	g
	whisky	35	g
	whipped cream	25	g
	cake	25	g
	canned fruits	100	g
	chocolate drink	200	g
	soft drink	200	g

*rotating order for every 4 days: day 1 100 g string beans and 100 g apple sauce
day 2 100 g pease
day 3 200 g cabbage and 125 g apple sauce
day 4 115 g asparagus and 100 g apple sauce

TABLE 5 - COMPOSITION OF THE DAILY DIET AS CONSUMED BY 4 SUBJECTS DURING A 60-DAY EXPERIMENT ON A CONSTANT DIET
(Means, standard deviations and coefficients of variation (C.V., %) calculated from the analyses of 6 homogenized duplicate diets, per subject)

		subject 1			subject 2			subject 3			subject 4		
	unit	means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.
Weight dupl. diet	g	2113	14.0	0.7	2115	9.0	0.4	2080	5.0	0.2	2012	5.0	0.2
Total avail. carboh.	g	381	14.3	3.8	382	9.4	2.5	382	16.9	4.4	356	11.1	3.1
Fat	g	117	4.0	3.4	117	3.4	2.9	124	3.0	2.5	119	3.6	3.0
Protein	g	94	0.8	0.9	92	1.6	1.7	91	1.0	1.1	97	2.6	2.7
Total energy	MJ	12.96	0.37	2.8	12.96	0.21	1.6	12.93	0.23	1.8	12.11	0.30	2.5
Total avail. carboh.	en. %	49.3	0.6	1.2	49.5	0.7	1.4	49.6	1.4	2.8	49.3	0.7	1.4
Fat	en. %	34.0	0.6	1.8	34.1	0.7	2.0	36.2	1.1	3.0	37.2	0.5	1.3
Protein	en. %	12.2	0.4	3.3	12.0	0.3	2.5	11.9	0.2	1.7	13.4	0.4	3.0
Alcohol	en. %	4.4	0.1	2.3	4.4	0.1	2.3	2.2	0.1	4.5	-	-	-
Calcium	mg	1044	26	2.5	1012	25	2.5	1015	22	2.2	1002	28	2.8
Magnesium	mg	377	8	2.1	385	5	1.3	394	2	0.5	389	7	1.8
Iron	mg	16.9	0.6	3.6	16.7	0.6	3.6	16.5	0.5	3.0	16.1	0.9	5.6
Zinc	mg	11.2	0.2	1.8	11.2	0.3	2.7	10.7	0.1	0.9	10.9	0.3	2.8
Copper	mg	2.05	0.07	3.4	2.08	0.28	13.5	2.07	0.19	9.2	1.66	0.19	11.4
NDF*	g	19.5	1.1	5.8	18.0	1.5	8.6	19.8	1.1	5.7	19.5	2.0	10.5
Sodium	g	3.89	0.20	5.1	3.74	0.17	4.5	3.83	0.20	5.2	4.01	0.10	2.5
Vitamin B_1	mg	1.51	0.07	4.6	1.50	0.11	7.3	1.55	0.04	2.6	1.62	0.07	4.3
Vitamin B_2	mg	1.71	0.06	3.5	1.62	0.04	2.5	1.76	0.08	4.6	1.62	0.08	4.9
Vitamin B_6	mg	0.95	0.08	8.4	1.18	0.17	14.4	1.29	0.10	7.8	0.93	0.06	6.5

*NDF = Neutral Detergent Fibre (12)

but remained constant throughout the study. Only in case of a change in body weight of more than 2 % of the initial weight, the diet was corrected, as described in the previous chapters. Foodstuffs requiring preparation were prepared according to standard procedures and stored at -20 °C if necessary. The foods were weighed to the nearest gram for each subject and provided in individual packages. All meals had to be consumed in the dining room of the Institute (7 days per week); coffee, tea and other beverages, included in the diet, could be consumed elsewhere. In order to control the mineral intake as accurately as possible, a low-Ca toothpaste was provided. Apart from the daily diet, an ample amount of distilled water was allowed.

As one of the disadvantages of balance studies, overestimation of the food intake is sometimes mentioned (6). In reality less than 100 % of the daily diet is consumed, as there are always small visible or invisible returns, e.g. in the various packages after final heating and emptying, or on the dishes after the meals. Therefore, the real food intake was determined according to the following procedure: For each subject, individual daily duplicate samples were prepared, heated and made ready for consumption in the same way as was done by the subjects; these duplicate diets were homogenized and stored at -20 °C until required for analysis. This procedure was followed at least twice for each experimental diet. All individual homogenized samples were analysed separately. For the calculation of the (mineral) balance, the individual intake figures were used.

The results of the variability study (see 6.4) with respect to the food intake give an indication of the validity of the procedure applied. Table 5 summarizes the results of the analyses of 6 individual duplicate diets (for each of the 4 subjects), prepared and homogenized as described above, on 6 different days at constant intervals throughout the experiment.

In a limited number of samples (3 duplicate diets for subject 1 and 3) the fatty acids were analysed as well. The amounts of dietary linoleic acid as percentage of total energy intake were 6.9, 6.9 and 7.1 for subject 1 and 7.4, 7.8 and 8.0 for subject 3.

As shown in table 5, the coefficient of variation (C.V.) for most analyses proved to be quite acceptable; for the relatively high C.V. observed for the vitamins in a few cases, storing/thawing conditions might be responsible. The general conclusion can be drwan that for almost all nutrients, which were taken into account in the various studies described in this thesis (macronutrients and minerals), the method applied for the determi-

nation of the food intake is a reliable one as regards variability. Since the duplicate diet is composed in such a way that it is close to the real food intake by the subjects, the nutrient intake as calculated from the duplicate diet analyses will be close to reality too.

6.6 URINE AND FAECES, COLLECTION, HOMOGENIZATION, SAMPLING AND ANALYSES

6.6.1 Urine

In all studies urine of 24-hr was collected in polyethylene 2 l bottles, preserved with 20 ml 5N HCl. Small bottles (500 ml) were provided to be used outside the Institute. After homogenizing the (invidual) 24-hr urines, the volume was measured and samples were taken for creatinine analyses (1). The urine collected during 4 days was combined and mixed, samples were taken and stored at -20 °C until required for analysis.
In various studies the creatinine excretion in urine is used as a control for completeness of collection; there is extensive literature on this subject but the views on the correctness of this procedure are contradictory (8).
In the variability study we measured creatinine and sodium in all 60 24-hr urine samples. The results are presented in table 6.

TABLE 6 - CREATININE AND SODIUM IN 24-HR URINE SAMPLES OF 4 SUBJECTS DURING A 60-DAY STUDY ON A CONSTANT DIET
(Means and coefficient of variation (C.V., %) of 60 samples per subject)

Subject no.	1		2		3		4	
	means	C.V.	means	C.V.	means	C.V.	means	C.V.
Creatinine (mmol/24 hr)	14.7	5	15.0	8	16.7	6	16.5	5
Sodium (mmol/24 hr)	168	15	144	15	170	18	177	15
Sodium (mmol/mmol creatinine)	11	14	10	13	10	17	11	14

The coefficients of variation for creatinine can be considered as acceptable, although it is questionable whether small losses of urine can be detected even under this low C.V. circumstances. The sodium intake was constant throughout the 60-day study (use of "table salt" was not allowed); however the variations in sodium excretion in urine were considerable.

No improvement was observed when the sodium excretion was expressed per mmol creatinine. Although a constant activity pattern of the subjects during the variability study was aimed at, the high C.V. of Na-excretion can probably be explained by a high variability of dermal losses of Na (sweat). In any case, the results indicate that the use of 24-hr urinary Na-excretions for estimating Na-intake is imprecise.

The creatinine excretion figures of the studies described in chapters 2-5 are summarized in table 7.

TABLE 7 - CREATININE EXCRETION IN URINE OF 46 SUBJECTS TAKING PART IN THE STUDIES DESCRIBED IN CHAPTERS 2,3,4 AND 5
(mean, standard deviation, range and coefficient of variation)

Mean excretion (mmol/24 hr)	15.13
Standard deviation (mmol/24 hr)	1.32
Range (mmol/24 hr)	11.68-17.70
Mean coefficient of variation (%)	14.4
Range of the coefficient of variation (%)	5-27

From these results - particularly the relatively high mean coefficient of variation - it is concluded that the creatinine excretion in urine can not be used as control for completeness of urine collections of our subjects. The use of creatinine values for correcting mineral excretion is questionable for this same reason. Hartley et al. (5) likewise found hardly any effect of the creatinine correction on the coefficient of variation of Na, Mg, Cu and Zn excretion in a study with 11 subjects on a constant diet for more than 2 weeks.

6.6.2 Faeces

All faecal collections took place directly in 3 l plastic buckets, which were stored in a refrigerator. Every subject used one bucket for 4 days. The collection started one day after the beginning of a dietary period and ended one day after the last day of the experimental dietary period. Each 4 days composite was homogenized in an all-plastic environment in order to exclude mineral contamination. From each 4 days homogenate samples were taken for analysis and stored at -20 °C.

In metabolic balance studies various markers are often used to separate faecal periods. Especially for short balance periods (e.g. 5 days) this seems the only way to estimate faecal mineral excretions since day to day variations in faecal output may be high. This is not so much caused by variation in mineral absorption but reflects irregularities in the bowel movements (8).

Still the application of faecal markers can even be criticized. Lutwak and Burton (10) came to the conclusion that markers do not facilitate accurate separations. However, with an increasing length of a balance period, the mean balance could come close to the true balance, since errors in the demarcation of faeces cancel out (7). A mineral balance study, therefore, cannot be evaluated if the balance period (dietary period) is too short. Furthermore, the method of faecal marking presupposes a complete mixing of the minerals and the (inert) indicator, which is difficult to prove (7,11). In the studies described in chapter 2 we have used three methods to calculate the faecal excretion figures: a) by the "one day after" method, as given above; b) by corrections applying polyethyleneglycol (PEG) as a continuously administered marker and c) by calculating the mean faecal output per 4 days from the overall 20 day stool production (per subject) and multiplying this value by the mineral concentrations in the stools, as analysed in each 4-day period. In table 8 the mean balance for calcium, magnesium and iron is given, calculated by means of these 3 methods for monitoring faecal mineral excretions.

As shown in this table the mean values and the standard deviations appear to be of the same magnitude for each mineral; no statistical difference between the 3 methods could be demonstrated. The significant difference in Fe-balance between the low-linoleic acid period and the high-linoleic acid period was found with all 3 methods for calculating faecal mineral output.

TABLE 8 - MEAN BALANCE OF Ca, Mg AND Fe OF 4 DIETARY PERIODS, EACH LASTING 20 DAYS, APPLYING 3 METHODS FOR CALCULATING FAECAL MINERAL OUTPUT (from the studies described in chapter 2).
(mean ± standard deviation in mg/d, N = 10 or 12 subjects)

	high-fat period	low-fat period	low-linoleic acid period	high-linoleic acid period
Ca-balance				
"one day after" method	68 ± 40	54 ± 35	87 ± 73	118 ± 77
PEG corrected faeces	82 ± 46	58 ± 73	88 ± 70	131 ± 70
mean faecal output method	62 ± 41	48 ± 41	81 ± 79	109 ± 74
Mg-balance				
"one day after" method	16 ± 23	3 ± 15	10 ± 15	7 ± 13
PEG corrected faeces	21 ± 23	4 ± 26	10 ± 16	11 ± 11
mean faecal output method	15 ± 24	1 ± 18	9 ± 16	4 ± 13
Fe-balance				
"one day after" method	3.0 ± 1.4	3.0 ± 1.4	3.3 ± 1.3	2.3 ± 1.0*
PEG corrected faeces	3.3 ± 1.6	3.1 ± 1.6	3.3 ± 1.1	2.5 ± 0.9*
mean faecal output method	3.0 ± 1.4	2.9 ± 1.4	3.2 ± 1.1	2.1 ± 1.0*

* significantly different from the low-linoleic acid period: $P < 0.01$

+ PEG = Polyethyleneglycol

From these results and the above considerations on the use of faecal markers together with an extra analytical error possibly involved in using a marker, we concluded that for our balance studies with a sufficient number of days for each dietary period (20 days), the "one day after" method was the method of choice for estimating faecal mineral excretions. Finally, it can be remarked that faecal loss on sanitary paper (not collected) may amount to e.g. 10 mg calcium per day (7), illustrating the assumption that faecal excretions will usually be underestimated (6).

6.7 CALCULATION OF THE MINERAL BALANCE; MISCELLANEOUS LOSSES

The mineral balance data were collected in the following way:

a. For each subject a mean intake figure was calculated from the individual duplicate diet analyses for each experimental period. From these data a mean for 10 or 12 subjects was calculated.
b. A mean urinary and faecal excretion figure was obtained in the same way i.e. the subject mean was calculated from the excretion figures of the five 4 days periods in each experimental period.
c. The balance of the minerals (= apparent absorption) was obtained by applying the formula:

   mineral balance = mean dietary intake - (mean urinary excretion + mean faecal excretion)

The term "apparent absorption" is applicable, because by this technique the faecal mineral excretions cannot be differentiated in non-absorbed minerals of exogenous origin and lost minerals from endogenous origin.
Miscellaneous losses (skin, hair, etc.) are not taken into account on a routine basis either.
In a few cases an estimate was made of skin calcium losses by measuring the concentration of calcium in sweat applying the pilocarpine iontophoresis test (3). The measurements were performed in the study described in chapter 2 for 11 subjects. The results are presented in table 9.

TABLE 9 - DERMAL CALCIUM LOSSES. CONCENTRATION OF CALCIUM IN SWEAT OF 11 SUBJECTS OF THE STUDY DESCRIBED IN CHAPTER 2; DAILY CALCIUM LOSS CALCULATED ON THE ASSUMPTION OF 500 ml SWEAT PRODUCED. (means, standard deviations and range)

	mean	s.d.	range
Ca-concentration in sweat (mg/100 ml)	4.1	4.2	0.4-14.2
Ca-loss on the assumption of 500 ml sweat produced/d (mg)	20.6	20.8	2-72

These results show that the daily sweat calcium loss can be considerable and that there is a high inter-individual variability. Values reported in literature included mean losses of 0.7 mg Ca, 0.1 mg Mg, 0.1 mg Fe and 0.1 mg Zn per day for preadolescent boys (4). Jacob et al. (9) estimated mean daily whole body surface losses in male adults for zinc, copper and iron of 0.5 mg, 0.3 mg and 0.3 mg respectively. This indicates that dermal losses are not to be neglected. However the procedure for measuring these losses in metabolic (mineral) balance studies will generally be too elaborate to be applied on a routine basis. Moreover, the subjects in our studies were instructed to aim at a constant activity pattern in order to achieve as much as possible a constant magnitude of dermal (sweat) loss. In addition, each subject served as his own control.
Another factor which should be taken into account is the small loss of minerals when blood samples are taken; for calcium this means an extra loss of approximately 2 mg during each venapunction of 20 ml blood (concentration of 2.5 mmol Ca/l). As blood is sampled only one or two times in each dietary period of 20 days, the influence of the loss of calcium through blood can be neglected.

6.8 BLOOD

In all studies blood was withdrawn from the antecubital vein, after an overnight fast, at the end of each experimental period.
The validity of a single blood sample for each subject was evaluated in the protein study (described in chapter 5). In this study blood was withdrawn on two consecutive days. For a number of parameters the coef-

ficients of variation of these "duplicate" samples, for each experiment with 4 subjects separately, are presented in table 10. The coefficient of variation was calculated according to the formulas:

$$S^2 = \frac{\sum_{i=1}^{n} (x_{1i} - x_{2i})^2}{2n} \quad \text{and Coeff. of var.} = \frac{S}{\text{mean value}};$$

x_1 and x_2 represent the duplicate values. The coefficients of variation can be considered as quite acceptable for almost all parameters listed. This conclusion could be confirmed in more recent studies executed in our Institute.

The variability within subjects was further evaluated in the study with 4 subjects on a constant diet for 60 days (see 6.4). During these 60 days, 20 blood samples were taken on each Monday and Thursday. Moreover blood was sampled on day 47 every 2 hours between 08.00 and 22.00 (8 blood samples per subject) in order to evaluate the variability during the day.

TABLE 10 - COEFFICIENT OF VARIATION (%) OF DUPLICATE BLOOD SAMPLES FOR SOME PARAMETERS IN 3 EXPERIMENTS WITH 4 SUBJECTS IN THE PROTEIN STUDY (chapter 5)

	protein 1, April-June (n = 15 duplicates)	protein 2, April-June (n = 20)	protein 3, October-December (n = 20)
Heamoglobin	1.6	2.2	2.9
Packed cell volume	1.9	2.5	3.7
Red blood cells	4.1	3.8	2.8
White blood cells	13.2	7.4	11.3
Calcium	1.7	0.7	1.8
Phosphorus	6.8	6.1	4.4
Total protein	2.5	0.8	3.0
Albumin	2.6	1.6	3.8
Serum cholesterol	3.1	2.4	4.1

TABLE 11 - VARIABILITY OF HAEMOGLOBIN, PACKED CELL VOLUME AND RED BLOOD CELL COUNT WITHIN SUBJECTS, IN A STUDY WITH 4 SUBJECTS ON A CONSTANT DIET FOR 60 DAYS
(means, standard deviations and coefficients of variation (%))
A = 20 samples in 60 days, B = 8 samples on one day (day 47)

		subject 1			subject 2			subject 3			subject 4		
		means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.
Haemoglobin (mmol/l)	A	9.74	0.21	2.2	8.88	0.40	4.5	9.99	0.27	2.7	9.86	0.42	4.3
	B	9.82	0.15	1.5	9.01	0.22	2.4	10.16	0.15	1.5	9.92	0.24	2.4
Packed cell volume (%)	A	46.7	0.8	1.7	42.2	1.3	3.0	47.0	1.1	2.3	45.8	1.3	2.9
	B	46.4	0.8	1.7	41.4	1.0	2.5	46.4	0.9	2.0	44.8	0.8	1.7
Red blood cell count	A	4.8	0.24	5.0	4.2	0.26	6.2	5.1	0.27	5.3	5.1	0.36	7.1
($n \times 10^{12}/l$)	B	4.9	0.14	2.9	4.2	0.13	3.1	5.2	0.12	2.3	5.2	0.14	2.7

FIGURE 1

HDL AND TOTAL CHOLESTEROL IN SERUM
OF THE FOUR SUBJECTS IN THE VARIABILITY STUDY

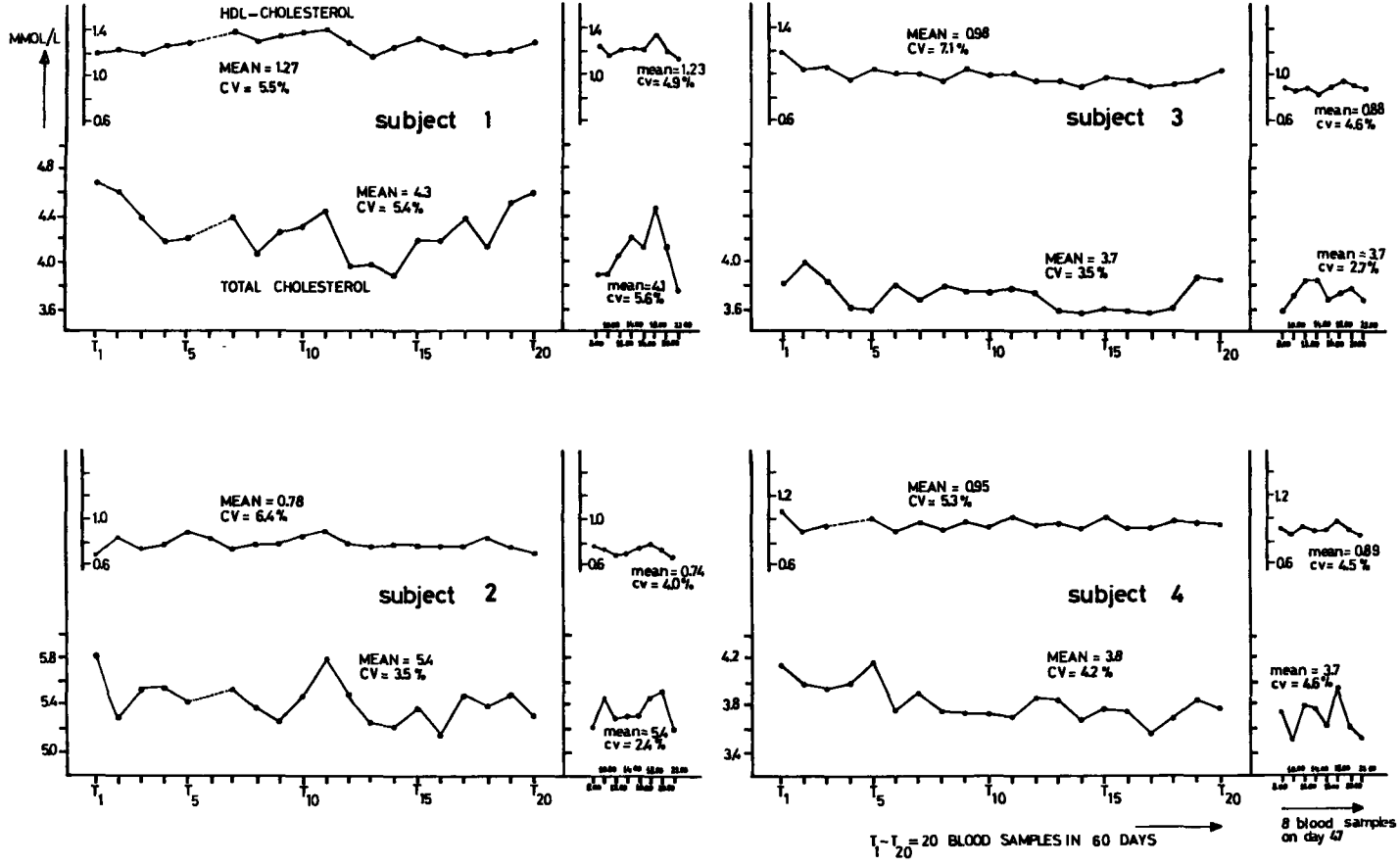

Table 11 shows the results of some haematological analyses, while figure 1 presents the findings with respect to serum HDL-cholesterol and total cholesterol.
The haematological parameters were analysed within 3 hours after the blood had been taken; the other samples were stored at -20 °C (as serum) and analysed in one batch after the study.
For all 4 subjects the haematological values were all within normal ranges; the intra-individual variability is low in most cases, only for the red cell count a few times higher values of the coefficient of variation were observed, although still at an acceptable level.
The variability over one day also appears to be low for the haematological parameters analysed. From figure 1 it can be concluded that also the coefficients of variation for both total- and HDL-cholesterol are acceptable; as regards these parameters it is interesting to observe two peaks on the day on which 8 samples were taken at 2-hour intervals. These peaks do not appear simultaneously for all 4 subjects; there is a tendency of minimum values early in the morning and later in the evening and for all subjects a maximum value for total-cholesterol between 18.00 and 20.00 h. At 18.00 h a maximum value for HDL-cholesterol was observed for all subjects. This illustrates the necessity of standardizing the time of the day for blood sampling (e.g. in epidemiological studies).
The general conclusion can be drawn that the design for blood sampling and analyses applied in our experiments leads to an adequate interpretation of the results, in accordance with the aim of our studies.

6.9 ANTHROPOMETRIC MEASUREMENTS

During all our studies various anthropometric data were collected according to the methods and techniques described in previous chapters.
In order to evaluate these and other anthropometric measurements in terms of within-subject variability, as measured by one observer, we included them in our variability study.
In the course of 60 days on a constant diet each subject was measured 7 times under identical circumstances with respect to time of day and methodology.

TABLE 12 - ANTHROPOMETRIC DATA OF 4 SUBJECTS DURING THE VARIABILITY STUDY; 7 MEASUREMENTS IN 60 DAYS ON A CONSTANT DIET

(means, standard deviations and coefficients of variation (%))

	subject 1			subject 2			subject 3			subject 4		
	means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.	means	s.d.	C.V.
Height (cm)	181.5	0.34	0.2	170.0	0.39	0.2	181.05	0.44	0.2	175.4	0.26	0.1
Wrist width (cm)	10.9	0.10	0.9	11.0	0.11	1.0	11.1	0.38	3.4	12.1	0.18	1.5
Knee width (cm)	18.6	0.09	0.5	17.9	0.08	0.4	19.0	0.39	2.1	20.0	0.10	0.5
Shoulder width (cm)	36.6	2.14	5.8	36.1	0.19	0.5	39.2	1.38	3.5	40.0	0.45	1.1
Chest width (cm)	26.4	0.35	1.3	28.4	0.19	0.7	26.9	0.35	1.3	29.0	0.41	1.4
Pelvic width (cm)	26.2	0.39	1.5	24.1	0.19	0.8	24.9	0.27	1.1	28.4	0.48	1.7
Arm circumference (cm)	20.9	0.56	2.7	22.4	0.46	2.1	22.0	0.14	0.6	23.3	0.19	0.8
Chest circumference (cm)	81.7	0.46	0.6	85.2	0.68	0.8	83.8	0.91	1.1	86.4	0.95	1.1
Abdomen circumference (cm)	66.2	1.14	1.7	69.4	1.33	1.9	67.9	0.63	0.9	71.2	3.28	4.6
Pelvic circumference (cm)	86.8	0.84	1.0	82.6	0.81	1.0	86.6	0.69	0.8	91.8	0.50	0.5
Thigh circumference (cm)	44.4	1.40	3.2	48.6	1.10	2.3	47.1	1.29	2.7	49.5	0.74	1.5
Calf circumference (cm)	31.8	1.18	3.7	35.3	0.08	0.2	34.6	0.48	1.4	35.2	0.15	0.4
Skinfold biceps (mm)	3.7	0.24	6.5	4.0	0.30	7.5	3.5	0.19	5.4	4.3	0.17	4.0
Skinfold triceps (mm)	7.3	0.50	6.8	6.0	0.46	7.7	6.2	0.58	9.4	7.5	0.42	5.6
Skinfold subscapular (mm)	8.9	0.38	4.3	8.9	0.48	5.4	8.9	0.27	3.0	9.0	0.31	3.4
Skinfold suprailiac (mm)	13.1	1.30	9.9	16.4	0.63	3.8	13.6	1.78	13.1	9.9	0.58	5.9
Skinfold subcostalis (mm)	7.7	1.21	15.7	6.3	0.40	6.3	6.7	0.28	4.2	6.0	0.13	2.2
Skinfold parsumbilicus (mm)	7.4	0.66	8.9	5.9	0.70	11.9	5.2	0.42	8.1	6.1	1.10	18.0
Weight (kg)	60.1	1.01	1.7	60.9	0.39	0.6	64.5	0.87	1.3	68.6	0.66	0.9
Σ 4 skinfolds* (mm)	32.7	1.91	5.8	35.4	0.99	2.8	32.1	2.09	6.5	30.7	0.99	3.2
% body fat (Durnin) (%)	13.9	0.75	5.4	14.9	0.41	2.8	13.7	0.73	5.3	13.3	0.40	3.0
Skeleton weight (kg)	11.6	0.17	1.5	10.6	0.11	1.0	11.9	0.28	2.4	12.6	0.15	1.2
Lean body mass (kg)	52.2	0.73	1.4	51.8	0.21	0.4	56.1	1.02	1.8	59.5	0.51	0.9
LBM - skeleton weight (kg)	40.6	0.78	1.9	41.3	0.29	0.7	44.2	1.02	2.3	46.9	0.50	1.1
Fat mass (Σ4 skinfolds) (kg)	8.4	0.56	6.7	9.1	0.29	3.2	8.8	0.57	6.5	9.1	0.31	3.4

* biceps, triceps, subscapular and suprailiac

The various measurements were executed according to methods published by Weiner and Lourie (13) with minor modifications (2). The measurements took place as far as applicable at the left side of the body. All skinfold thicknesses were measured in duplicate; if the duplicate values differed more than 10 %, a second series of two measurements was carried out. Table 12 presents the results obtained.

The coefficient of variation for the various circumferences and diameters (widths) are low, with only one or two exceptions. The intra-individual variabilities of the skinfold thicknesses are generally much higher, which shows that these thicknesses are actually difficult to measure. On the other hand, small changes in body composition during the 60-day study will partly be responsible for the high coefficients of variation.

In the various studies described in this thesis (chapters 2-5) the variability of the skinfold thicknesses was (much) less, possibly because of the periods on a constant diet being shorter, in most cases 20 days.

It is remarkable that the coefficient of variation of the sum of the 4 skinfold thicknesses (biceps, triceps, subscapular and suprailiac) is not higher than the C.V. of the separate skinfold thicknesses. Probably the errors involved in measuring skinfold thicknesses are random and counterbalance one another when the sum is calculated.

6.10 STATISTICAL ANALYSES OF THE DATA

In all our studies, the statistical analysis was performed in the same way, in that the effect of each dietary treatment was compared with the preceding one and/or that in the following period.

Analysis of variance was used to detect statistically significant differences between periods. The level of significance was calculated applying a t-test for paired observations.

Each subject served as his own control.

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7. General discussion

7.1 INTRODUCTION

As outlined in chapter 1, one of the aims of our studies was to evaluate the effect of diet composition on the utilization of minerals and trace-elements.

For a better understanding of the various processes that take place after an element has been ingested, some definitions are presented with respect to mineral utilization.

Discussed are in addition:

- In brief, with zinc as example, the various steps during which interactions of macro-nutrients with micro-nutrients might occur.
- In the light of these interactions, the results of the studies described in this thesis, as well as some recent findings of other investigators, including results of animal experiments carried out in our Institute.
- The results obtained in our studies from the point of view of the recommended dietary pattern.

7.2 TERMINOLOGY

Mineral utilization comprises all processes taking place between ingestion and excretion of a mineral. The following subdivision of the utilization process is suggested:

1. Chemical and/or physical availability: the proportion of an ingested mineral that is available for absorption. During the digestion process various intraluminal interactions occur, such as adsorption of minerals to e.g. macro-nutrients, binding of minerals to other compounds (of a chemical or complex character), inclusions, reductions and oxidations. The net result of all these interactions is conclusive as to a mineral being available for the actual absorption process.
2. Mineral uptake into the mucosal cell (= adsorption).
3. Transport of the mineral across the cell.
4. Transfer of the mineral into the circulation.

Absorption is actually a combination of these last 3 steps. When step 4 does not take place the mineral will return into the intestinal lumen by sequestration or desquamation.

5. Transport of the mineral to its site of action.
6. Conversion into the physiologically (or toxicologically) active species.
7. Metabolic action.
8. Excretion/secretion (urine, faeces, gastrointestinal juices and cells, skin, blood, hair, nails etc.); reabsorption from the gastrointestinal tract or renal tubular reabsorption causes the mineral to re-enter the circulation.

At all levels interactions might lead to a decrease or an increase in mineral utilization (antagonism or synergism).
The term bioavailability seems to be applicable to steps 1-7. The balance or retention is the net difference between intake and excretion of e.g. a mineral.

7.3 ABSORPTION MECHANISMS AND INTERACTIONS

In this subchapter the general concept of the interactions involved in the various steps of pre-absorption and absorption of zinc is put forward.
The overall concept presented hereafter will be applicable to other minerals than zinc as well (25, 49) although there may be detailed differences in interactions.

7.3.1 Intraluminal interactions

Intraluminal interactions (48, 49) can be ascribed to the various reactions during the chemical/physical availability stage. Zinc and other minerals can compete for a compound that acts as a binding-ligand favouring the zinc absorption. An excess of the competing mineral would displace zinc from the ligand and reduce its uptake. On the other hand, another mineral may compete with zinc for a complexing substance which forms an insoluble complex; examples are phytate and dietary fibre.
In the following descending order Zn, Cu, Ca en Fe form less stable com-

plexes with phytate (6); Zn also forms complexes with cellulose (2), hemicellulose (2, 16) and lignin (30, 43), in this case more avidly than e.g. Cu (43).

Apart from this competition for common binding ligands or complexing agents, zinc and other minerals might interact directly, forming a complex with one another or together with a third moiety that alone would complex neither. Two minerals might also interact in oxidation-reduction reactions, in which the preferred oxidation state for absorption of one or both minerals is altered. The stability of the Zn^{2+} state is such that it acts neither as an oxidizing nor as a reducing agent in the intestinal tract. In this respect it is of interest that ascorbic acid is presumed to reduce Fe^{3+} to Fe^{2+} or to maintain Fe^{2+} in its reduced state which is more easily available for absorption. On the other hand ascorbic acid can reduce Cu^{2+} to Cu^{+}, the less readily available form of copper (19, 31). Hill and Matrone (25) put forward the hypothesis that elements with similar physical and chemical properties will affect each other antagonistically from a biological point of view. On the basis of similar electron orbital configurations they were able to predict in which cases mineral interactions might take place. The hypothesis was confirmed in many animal and human experiments. This theory of mineral interactions is not only applicable to the intraluminal process, but also to the actual absorption steps. The necessity for similar physical and chemical properties regarding mineral interactions is illustrated for iron and zinc: interactions between these minerals have been demonstrated when both are in the ionic form (48, 49), but haem-iron was shown not to reduce zinc availability (50).

7.3.2 Mucosal interactions

Mucosal interactions or interactions during the mucosal uptake can also take place (49): Zinc might compete with another mineral for a common receptor mechanism, involved in the uptake of both minerals across the mucosal membrane. As a consequence high concentrations of the competitor might reduce the transfer of zinc from the intestinal lumen into the cell. As an alternative in this competition for a common receptor site, also externalized carrier-protein competition with minerals might be involved in the translocation of minerals across the membrane into the cell.

7.3.3 Intracellular interactions

Intracellular interactions or interactions during the transport of the minerals across the mucosal cell have been described as well (12, 48, 49). Zinc and other minerals might compete within the cell for a common carrier mechanism, which is essential in transporting zinc toward the serosal surface of the mucosal cell (14, 25, 29).

Apart from this competition for a common internal carrier-protein also common intracellular binding-proteins are involved.

It has been suggested (11, 12) that intestinal metallothionein (MT), a sulphur-rich protein, containing approximately 30 % cysteine, acts as a trap, capturing the zinc that enters the cell from the lumen (or from the bloodstream) and holding it until the zinc re-enters the faecal stream after the normal exfoliation of intestinal mucosa (43). Since MT binds other minerals as well, e.g. Cu (36), mineral interactions within the cell might influence the actual absorption (32). The situation is even more complex as zinc and copper may induce the synthesis of MT (3, 12, 41); copper, however, binds more strongly to this protein than zinc (49).

High intakes of zinc relative to copper may cause anaemia which can be accounted for by (14): a) the induction of MT by zinc within the mucosal cell, b) the binding of copper by MT or displacement of zinc by copper in the Zn-MT complex, c) reduced Cu-absorption resulting in a decrease of ceruloplasmin in blood, d) as ceruloplasmin is involved in the oxidation of Fe^{2+} to Fe^{3+}, the formation of haemoglobin may be reduced, which might finally cause anaemia. From the results of various human studies on the effect of zinc on copper absorption (4, 22, 53), it is suggested that Zn:Cu ratios greater than 10:1 might affect Cu status (49).

It has been hypothesized that a metabolic imbalance of zinc and copper might also be a major factor in the etiology of ischemic heart disease: either a relative or absolute deficiency of copper, characterized by a high ratio of zinc to copper, may result in hypercholesterolemia and increased mortality (34).

Ascorbic acid might promote hypercholesterolemia because of the inhibitory effect on copper availability (33).

7.3.4 Serosal interactions

Serosal interactions or interactions during the transfer of the mineral into the circulation might be applicable (49).
Apart from competition for a common serosal receptor, competition for transport by a circulating carrier-protein might play a role in the final exit of a mineral from the mucosal cell (42). Both transferrin (18) and albumin (45, 46) might be involved, but the presence of excess minerals that bind to the same site on the portal transport protein as zinc, would reduce the passage of zinc into the circulation (49). It is suggested that the zinc-iron interaction can be partly explained on account of the latter competition mechanism (49).

7.4 GENERAL DISCUSSION OF THE STUDIES CARRIED OUT

7.4.1 Fat and linoleic acid intake and mineral balance (chapter 2)

It is assumed that a possible interaction of dietary fat and dietary linoleic acid with minerals may be of significance during the step which has been defined as chemical/physical availability. As no detectable change in Ca-, Mg- and Fe-balance was observed when dietary fat decreased from 42 energy % to 22 energy %, it seems likely that any formation of insoluble complexes of fat or fatty acids with the minerals studied is similar at both levels of fat intake. The ratio of the three minerals in the diet being almost constant, the actual absorption will probably not be influenced by mineral-mineral interaction; on the basis of electron configuration it cannot be expected that these interactions will occur between Fe and Mg or Ca. Animal experiments carried out in our Institute (Luyken, et al., unpublished results) did not indicate an influence of the amount of dietary fat on calcium utilization as well: when rats were fed diets containing 2 % and 27 % of fat respectively, at a constant calcium intake, the ash content of the bones and serum alkaline phosphatase activity were almost similar in both groups. When the amount of calcium was increased from 0.06 to 0.6 % in both the 2 % and the 27 % fat diet, it appeared that the influence of dietary calcium was much more pronounced than the influence of fat: bone ash content was approximately

40 % higher and serum alkaline phosphatase activity approximately 50 % lower on the high calcium diet compared with the low calcium diet.
The decrease in iron balance on an increased linoleic acid intake suggests that iron might interact with linoleic acid, forming less available complexes than with Ca or Mg (no significant change of the Ca and Mg balance). Since also in this study the ratio of the minerals was almost constant, the mineral-mineral interactions are not likely to explain the results obtained.
It has been indicated that the fall in haemoglobin levels during the high-linoleic acid period needs further investigation.
In two animal experiments performed in our Institute the same influence of (mainly) linoleic acid on haemoglobin levels was found: in one experiment with male albino rats haemoglobin was significantly lower on a soybean oil diet (50 % linoleic acid) compared with a hydrogenated soybean oil diet (5 % linoleic acid). No significant difference of liver-iron content was observed. In a second experiment these findings were confirmed with normal as well as with anaemic rats; moreover, in this case liver-iron content was lower on the non-hydrogenated soybean oil diet (13, 54). Although no clear explanation can be given for the effects observed, the actual findings seem to be relevant in a discussion of the recommended increase of linoleic acid intake. Although polyunsaturated fatty acids might be useful for reduction of plasma lipids, there is a growing consensus that large quantities of these fats should be avoided (56). Possible long-term effects ascribed to large amounts of polyunsaturated fats in the diet include increased risk for gallstones (23, 51) and for carcinogenenis (5).

7.4.2 Dietary fibre intake and mineral balance (chapter 3)

As in almost all dietary alterations the retention and the urinary excretion of the minerals studied were constant on an increased dietary fibre intake through bread, the influence of fibre on mineral utilization seems to be apparent during the chemical availability step and/or the actual absorption. During the chemical availability stage dietary fibre (from bread) may adversely affect mineral absorption by (31): a) diluting the mineral concentration in the intestinal chyme, b) decreasing the time for absorption by decreasing faecal transit time, c) trapping minerals within particles containing fibre and d) providing surfaces for mineral

adsorption and thus hindering absorption through the intestine.
The fact that the ratio of the intake figures of the minerals studied did not change significantly on an increased dietary fibre intake, does not suggest that mineral-mineral interactions will be different during the actual absorption step. However, it is possible that competition as to the formation of unavailable complexes of minerals with dietary fibre (components) will result in other ratios before absorption takes place. In that case, mucosal and intracellular interactions could be different after the consumption of different amounts of dietary fibre. Nevertheless, it is questionable whether the suggested changes in mineral ratios are thus that the mineral absorption is considerably influenced.
Therefore, we arrive at the conclusion that the effects observed in our dietary fibre study are mainly due to the various intraluminal interactions, in which dietary fibre seems to play an essential role.
It should be noted that the influence of phytate is apparent only when raw bran is consumed. In bread the action of phytase from yeast will have considerably reduced the mineral-binding capacity of the phytate during fermentation. In addition, it has been demonstrated that phytate in foods is completely or partially hydrolyzed in baking, soaking, cooking or heat processing (39, 52).
Recent studies on the effects of bread on mineral balance confirm our findings (1).

7.4.3 Dietary fibre and colonic function (chapter 4)

As far as wheat fibre is concerned we have demonstrated the marked influence on colonic function when bran as a source of dietary fibre is incorporated in bread. Our conclusion that coarse bran is to be preferred to ground bran was confirmed by recent studies by Wrick et al. (57), who found that grinding of bran significantly reduced faecal output bacause of reduced faecal water. Not only particle size of bran is of importance to colonic function, but also individual dietary fibre components have specific physiological effects. Hillman et al. (28) did not find statistically significant changes in faecal wet weight and intestinal transit time when diets were supplemented with pectin or lignin; cellulose, on the other hand decreased transit time and increased stool

weight. Thus the biological significance of any fibre containing food will depend on the specific fibre composition.
As to the mechanism which is held responsible for the waterholding capacity of dietary fibre in the colon, the results of Fleming and Rodriquez (21) (cf our study) did not support the VFA theory either: their data were not in line with the suggestion that faecal water is partially regulated by the concentration of VFA in the colonic lumen.
Eastwood et al. (17) support our hypothesis of the predominant influence of the fibre-matrix structure accounting for the waterbinding capacity of fibre in the colon. Fleming et al. (20) suggest that fibre components that are only marginally fermented cause large faecal outputs and frequent defaecations.

Although it is generally recommended to increase dietary fibre intake, the possible relationship between dietary fibre ingestion and e.g. colorectal cancer is complex. Our findings that increasing the amount of bran in bread results in decreased concentrations of faecal bile acids could be an indication of a positive effect: a high bile acid concentration in faeces has been associated with a high incidence of colorectal cancer (15, 26, 27, 40).

7.4.4 Dietary protein versus mineral balance and colonic function (chapter 5)

In the study in which we varied the amount and the origin of dietary protein no significant changes of the mineral balance could be detected. However, we concluded from the increased faecal excretion that the (chemical) availability of the minerals, as defined in 7.2, was influenced by the dietary changes.
It has been suggested that the small increase in dietary fibre intake in the high-vegetable protein period of 6 g NDF, compared with the high-animal protein period, could be partly responsible for the increased faecal mineral excretions. Moreover, phytate from the soy-protein concentrate might also be responsible for binding minerals.
Changes in the (weight) ratios of the mineral intake figures might also contribute to the effects observed; however most differences in these ratios in the three diets are probably too small to account for the

changes in faecal mineral excretions: the Fe:Zn ratio ranges from 1.4:1 to 0.9:1, the Zn:Ca ratio from 5.4:1 to 9.7:1 and the Ca:Mg ratio from 2.7:1 to 4:1. From the possible mineral-mineral interactions only the Ca:Zn ratio might be of significance: in the low-protein diet this ratio was 103:1, whereas in the other diets it amounted to 80:1. This might partly explain the negative zinc balance during the low-protein diet period, although the zinc intake as such may also be considered as marginal and may thus contribute to the negative zinc balance observed. Snedeker et al. (47), however, did not find an effect of dietary calcium on the utilization of zinc, although the Ca-Zn antagonism has been demonstrated in animals (24). The positive influence of haem-iron on iron absorption (8) is illustrated by the lower faecal iron excretions and thus higher apparent absorption in the low-protein and the high-animal protein periods (both 67 % animal protein), when expressed as percentage of iron intake, compared with the high-vegetable protein period. In this respect it is of interest to indicate that one gram of meat is reported to be roughly equivalent to 1 mg ascorbic acid in enhancing iron absorption (9). Colin et al. (7) did not find any effect of dietary protein on zinc and copper retention either; in their study faecal zinc also paralleled zinc intake. Dietary zinc did not affect copper excretion or retention when the Zn:Cu ratio varied from approximately 5:1 to 10:1.

As to the possible effect of dietary fibre and phytate on mineral utilization, Shah (44) came to the conclusion that no appreciable adverse effects can be expected when the protein content of a mixed diet is high. Cossack and Prasad (10) concluded from their studies on 5 men that 15 mg of dietary zinc may not be sufficient to meet the daily zinc requirement (for adults) if soyprotein is the major source of protein, because of the effect of phytate on zinc bioavailability. Also other investigators have expressed concern about a possible unfavourable effect of the consumption of foods based on soy, on zinc bioavailability (35, 38). Solomons (48) states that it is safe to conclude that both phytate and dietary fibre (present in leguminous and wholegrain foodstuffs) contribute to the reduction of Zn bioavailability in man. The situation is even more complex as the (molar) ratio Ca:Mg:phytate seems to play a predominant role in zinc bioavailability (37, 48, 55).

As to the influence of diet on colonic function, the changes observed when the high-vegetable protein diet is substituted for the high-animal protein diet, lead to the conclusion that a high-vegetable protein diet (not the vegetable protein as such) is to be preferred to a high-animal protein diet regarding risk factors for the incidence of colorectal cancer.

7.5 THE RELATIVE IMPORTANCE OF OUR RESULTS FOR EVALUATING THE RECOMMENDED DIETARY PATTERN

From the results of the studies described in chapter 2-5 the following overall conclusions are drawn:

- The (recommended) reduction of total fat intake does not seem to influence Ca, Mg and Fe utilization.
- The (recommended) increase of dietary linoleic acid seems to have an adverse effect on Fe utilization, not on Ca or Mg utilization.
- The (recommended) increase of dietary fibre intake, through bran incorporated in bread, is considered beneficial to colonic function, but seems to be unfavourable for mineral utilization.
- The (recommended) shift from an animal protein diet to a diet with more vegetable protein leads to an improved bowel function, although mineral availability may be lower.

The question is relevant whether the effects observed are of significance when considering the recommended dietary pattern.
In the first instance, it has to be stated that the recommendations as outlined in chapter 1 can be justified on the basis of the present knowledge regarding the relation nutrition-health; the results of our study of dietary fibre intake and colonic function (chapter 4) support this hypothesis.
As to the suggested decrease in mineral availability when the present diet is shifted in the direction of the recommended dietary pattern, it is not impossible that in the long run an adaptation of human physiology takes place leading to a new homeostasis with mineral balance in equilibrium (31, 32, 48); although the periods on a constant diet in our studies were relatively short (20-30 days), the changes in mineral balance observed in most cases being not statistically significant is an indication of this theory.

However, assessment of the nutritional status of population groups on a regular basis is necessary to ascertain whether the suggested decrease in mineral availability is of significance in the long run. Solomons recently stated that impaired zinc nutritional state in vegetarians is rarely detected despite the large content of zinc inhibitors in plant-based diets; therefore a true intestinal adaptation to the quantity and quality of dietary zinc ingested may be operative (48).
Moreover, it seems possible to counterbalance the negative influence of some dietary factors on mineral availability, taking the enhancing dietary factors into account (e.g. vitamin C for iron).
It is, however, important to mention that much research has been carried out on individual dietary factors which influence mineral availability and absorption. Prediction of combined effects, e.g. the relative significance of competitive inhibitors versus binding substances, is of more realistic importance for human mineral nutrition. Thus, caution is urged considering levels of either trace element supplements (especially during pregnancy, lactation or early childhood (29)), because of various mineral interactions, or known absorption promoting agents (cf. the effects of ascorbic acid on Fe and Cu absorption, see 7.3.1).

Finally we arrive at the following statements:

1. There seems to be no compelling reason to criticize the overall concept of the recommended dietary pattern for The Netherlands.
2. Some concern regarding mineral utilization when the present diet will be shifted into the direction of the recommended dietary pattern seems justified.
3. More research is needed to understand and comprehend the effects of the simultaneous interactions of dietary factors that inhibit or enhance mineral availability and/or mineral absorption. Moreover, study of long term effects and adaptation to dietary alterations is highly recommended.

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Summary

In this thesis results are described of 4 experiments with healthy male volunteers, carried out in the metabolic ward of the Institute CIVO-Toxicology and Nutrition TNO at Zeist.

The objective of the studies was to evaluate the effects on mineral utilization when the present Dutch diet would be shifted towards the recommended dietary pattern.

Chapter 1 deals with various aspects of the dietary recommendations of macronutrients; in addition, the development of the average intake of macronutrients in The Netherlands is described. It is remarkable that regarding macronutrients data from the Ministry of Agriculture and Fisheries (derived from data on available food supply) and those collected by the Nutrition Council (derived from a great number of food consumption studies, carried out in age categories of 0-80 years) are not substantially different. Moreover this pattern is almost similar in all age categories. The primary aim in the concept of recommended dietary patterns is to reduce the incidence of cardiovascular diseases and the influence of macronutrients on the risk factors involved; in this chapter it is emphasized that other aspects, particularly mineral utilization, should be considered as well when discussing a recommended dietary pattern.

In Chapter 2 results are presented of a study with 10 subjects who in two consecutive periods were given a diet of 42 energy % of fat, followed by a diet of 22 energy % of fat at a constant fatty acid composition; each dietary period lasted 28 days. This decrease in the amount of dietary fat did not cause significant changes of the Ca- , Mg- and Fe-balance.

In the second experiment - again two 28-day periods - with 12 subjects the amount of linoleic acid in the diet was increased from 4 energy % to 16 energy %, at a constant total fat intake of 40 energy %.

The Ca- and Mg-balance did not change significantly. The Fe-balance showed a slight, significant decrease when the amount of dietary linoleic acid increased; moreover a significant decrease in haemoglobin was observed, which was confirmed twice in animal experiments carried out at the Institute. One of the conclusions drawn from these results is that the amount of fat and the fatty acid composition (in the diet) has no influence on Ca-absorption. As a consequence the relatively high recommended Ca-intake (800 mg/d for adults in The Netherlands) seems not

to be justified on account of an assumed influence of dietary fat on Ca-absorption. The effects of a high-linoleic acid diet on iron utilization require further study.

In Chapter 3 emphasis is laid on the effect on mineral balance of an (recommended) increased dietary fibre intake. Twelve subjects were given different diets in 20-day dietary periods; in addition to a constant basal diet, bread with different amounts of dietary fibre was consumed. In most cases the Ca- , Mg- , Fe- , Zn- en Cu-balance did not change significantly on an increased amount of dietary fibre. The increased dietary fibre intake, however, did result in an increased mineral intake, causing increased faecal mineral excretion. It was concluded that increasing dietary fibre in our diet does not affect mineral retention, whereas the availability of the minerals - defined as the stage before absorption - seems to be impaired.

In Chapter 4 the influence of increasing dietary fibre in the diet (through bran in bread) on colonic function is described. Data were obtained in the same study as mentioned in chapter 3 (i.e. with the same 12 subjects). The observed increase in faecal wet weight, the increased defaecation frequency and the shorter intestinal transit time as a result of an increased dietary fibre intake is generally considered as beneficial to nutritional health. Coarse bran appeared to have a slightly more favourable effect on colonic function than the fine bran (both incorporated in bread). From calculations of the digestibility of dietary fibre (components) by the intestinal flora, it is concluded that the theory of sponge activity of the fibre matrix structure is predominant in accounting for the water binding capacity of fibre in the colon.

In Chapter 5 results are presented of a study with 12 subjects who were given diets differing in amount of dietary protein and in the origin of the protein. The mineral balance did not change significantly, neither when a high-animal protein diet (16 energy % total protein, two thirds of animal origin) was substituted for a low-protein diet (9 energy % total protein, two thirds of animal origin), nor when a high-vegetable protein diet (16 energy % total protein, two thirds of vegetable origin) was substituted for the high-animal protein diet. From the increased faecal mineral excretion, a decreased mineral availability is concluded however, when the 16 energy % high-vegetable protein diet is substituted for the 16 energy % high-animal protein diet. This dietary change did not result in a lower serumcholesterol concentration; what we did observe was a

small significant increase in systolic blood pressure. It seems that the increased blood pressure can be accounted for by different sodium intakes. From the changes in colonic function parameters in this study it is concluded that a high protein diet (with phytate and dietary fibre) rather than the quantity of the kind of protein as such affects bowel function.

In Chapter 6 the methods and techniques are discussed which were applied in the metabolic balance studies at the Institute. In addition and in support of the various data, some results are presented of a study carried out to evaluate the biological variability of a number of biochemical parameters in blood and urine of four subjects who were given a constant diet for 60 days.

In Chapter 7 various aspects of mineral utilization are discussed. The emphasis is laid on interactions of minerals with macronutrients and on mineral-mineral interactions. The results of the various studies are discussed in the light of this review; more recent data from literature than those presented in chapters 2-5, are taken into account as well. Finally the results and conclusions are discussed in view of the recommended dietary pattern as outlined in chapter 1.

It is generally concluded that there seems to be no compelling reason to criticize the overall concept of the recommended dietary pattern for The Netherlands, but some concern regarding mineral utilization in case of shifting the present diet into the direction of the recommended dietary pattern seems justified. Emphasis on the study of the simultaneous interactions of dietary factors inhibiting or enhancing mineral availability and/or mineral absorption is highly recommended, as well as the study of long term effects and adaptations to dietary alterations.

Samenvatting

In dit proefschrift worden de resultaten beschreven van vier experimenten die in de metabole unit van het Instituut CIVO-Toxicologie en Voeding TNO met gezonde mannelijke proefpersonen werden uitgevoerd. Doelstelling was na te gaan welke veranderingen in de benutting van mineralen kunnen optreden wanneer het huidige Nederlandse voedingspatroon zich zodanig wijzigt dat het meer overeenkomst vertoont met hetgeen voor een prudente voeding wordt aanbevolen.

In hoofdstuk 1 wordt ingegaan op enkele aspecten van de voor macronutrienten aanbevolen hoeveelheden; tevens wordt de gemiddelde macronutrientenopname in Nederland globaal geschetst. Het is opmerkelijk dat gegevens van het Ministerie van Landbouw en Visserij - bruto verbruikscijfers - en van de Voedingsraad - verzameld uit een groot aantal voedselconsumptie-onderzoeken bij diverse leeftijdsgroepen van 0-80 jaar - goed overeenstemmen. Bovendien blijkt het patroon ten aanzien van de macronutrienten vrijwel onafhankelijk van leeftijd te zijn.

Daar bij het formuleren van een aanbevolen voeding vooral wordt gelet op de risicofactoren voor hart- en vaatziekten en de invloed van de macronutrienten hierop, wordt in dit hoofdstuk aangegeven dat ook aan andere factoren dan de primair beoogde aandacht dient te worden besteed; de mineralenbenutting staat in dit opzicht centraal.

In hoofdstuk 2 worden resultaten van een onderzoek bij 10 proefpersonen beschreven, die gedurende twee maal 28 dagen een voeding kregen aangeboden met 42 energie % vet respectievelijk 22 energie % vet, bij een constante vetzuursamenstelling. Er bleek bij deze verlaging van de hoeveelheid vet in de voeding geen significante verandering van de Ca-, Mg- en Fe-balans op te treden. In een tweede experiment van eveneens twee maal 28 dagen werd bij 12 proefpersonen de hoeveelheid linolzuur in de voeding verhoogd van 4 energie % tot 16 energie %, bij een constante hoeveelheid totaal vet (van 40 energie %). De Ca- en Mg-balans veranderde bij deze overgang niet significant. De Fe-balans vertoonde echter een kleine doch significante daling bij verhoging van de hoeveelheid linolzuur in de voeding; tevens werd een significante daling van het haemoglobinegehalte geconstateerd, hetgeen tot tweemaal toe door dierexperimenteel onderzoek (CIVO-TNO) werd bevestigd. Uit de in dit hoofdstuk gerapporteerde resultaten werd onder meer geconcludeerd dat de hoeveelheid vet en de samen-

stelling van het vet in de voeding geen invloed heeft op de Ca-absorptie. De relatief hoge Ca-norm in Nederland (800 mg/d voor volwassenen) lijkt derhalve niet gerechtvaardigd op grond van een vermeende invloed van voedingsvet op de Ca-absorptie. De geconstateerde effecten van een linolzuurrijke voeding ten aanzien van de ijzerbenutting dienen tenminste nader onderzocht te worden.

In hoofdstuk 3 valt de nadruk op de invloed van een (gewenste) verhoging van de hoeveelheid voedingsvezel in onze voeding op de mineralenbalans. 12 proefpersonen kregen hiertoe gedurende steeds 20 dagen, naast een constante basisvoeding, wisselende hoeveelheden voedingsvezel (via zemelen in brood) aangeboden. De balans van Ca, Mg, Fe, Zn en Cu bleek bij toenemende hoeveelheden voedingsvezel in de meeste gevallen niet significant te veranderen. Wel werd bij de verhoogde consumptie van voedingsvezel een grotere hoeveelheid van de meeste mineralen opgenomen, hetgeen echter resulteerde in een verhoogde uitscheiding met de faeces. Hieruit werd de conclusie getrokken dat een verhoogde hoeveelheid voedingsvezel geen invloed heeft op de retentie van de mineralen, maar dat de "availability" (gedefinieerd als de stap voor absorptie) wel (negatief) wordt beinvloed.

De gevolgen van de verhoging van de hoeveelheid voedingsvezel (via zemelen in brood) voor de darmfunctie worden in hoofdstuk 4 beschreven. Metingen werden uitgevoerd bij dezelfde 12 proefpersonen als in de in hoofdstuk 3 besproken studie. De geconstateerde verhoging van de hoeveelheid natte faeces, de hogere defaecatiefrequentie en de kortere darmpassagetijd bij toenemende hoeveelheid voedingsvezel wordt algemeen als gunstig voor de voedingsgezondheid beschouwd. Grove zemelen in brood bleken hierbij iets beter voor de darmfunctie dan gemalen zemelen. Uit berekeningen van de vertering van voedingsvezel (componenten) door de darmflora van het colon en de verandering van de darmfunctieparameters werd geconcludeerd dat de waterbinding van onverteerde voedingsvezel (componenten) het best verklaard kan worden uit sponswerking ten gevolge van de matrixstructuur van voedingsvezel.

Hoofdstuk 5 geeft resultaten van een studie bij 12 proefpersonen die voedingen kregen verstrekt die verschilden in hoeveelheid totaal eiwit en soor eiwit. De mineralenbalans veranderde ook in deze studie niet significant, enerzijds bij een overgang van 9 energie % eiwit (waarvan 2/3 dierlijk) naar 16 energie % eiwit (eveneens 2/3 dierlijk eiwit), anderzijds bij een overgang van de laatstgenoemde naar een voeding met 16 energie % eiwit, maar nu 2/3 deel plantaardig eiwit. Uit de verhoogde

uitscheiding van de mineralen met de faeces werd evenwel een verminderde "availability" van de mineralen afgeleid indien van de 16 energie % voornamelijk dierlijk-eiwit-voeding wordt overgegaan op de 16 energie % voornamelijk plantaardig-eiwit-voeding; deze overgang resulteerde niet in een verlaging van het serumcholesterolgehalte, wel werd een lichte verhoging van de systolische bloeddruk waargenomen, die voor een belangrijk deel kan worden verklaard uit het verschil in Na-opneming met de voeding. De veranderingen van de darmfunctieparameters bij beide overgangen kunnen voornamelijk worden toegeschreven aan stoffen, zoals voedingsvezel en fytaat, die een hoogplantaardige eiwitvoeding "begeleiden", dus in veel mindere mate aan het type eiwit in de voeding als zodanig.

In hoofdstuk 6 worden de methoden en technieken besproken die bij de balansstudies binnen de CIVO Instituten TNO werden toegepast. Ter ondersteuning van deze gegevens worden tevens enkele resultaten gepresenteerd van een onderzoek waarin de biologische variabiliteit van enkele biochemische parameters in bloed en urine werd nagegaan bij 4 proefpersonen die gedurende 60 dagen een constante voeding kregen aangeboden.

In hoofdstuk 7 wordt ingegaan op de diverse aspecten van mineralenbenutting, waarbij interacties met macronutrienten en vooral met andere mineralen aan de orde komen. In het licht hiervan worden alle experimentele resultaten behandeld, ondersteund door recentere literatuurgegevens dan in de reeds verschenen publikaties (hoofdstukken 2 tot en met 5) zijn verwerkt. Tenslotte wordt een en ander bezien tegen de achtergrond van de in hoofdstuk 1 besproken aanbevolen hoeveelheden c.q. het aanbevolen voedingspatroon.

Als algemene conclusie wordt gesteld dat er geen reden is de uitgangspunten van de aanbevolen voeding te kritiseren, maar dat enige zorg voor een juiste mineralenvoorziening op zijn plaats is bij verschuiving van het huidige voedingspatroon in de richting van het gewenste patroon. Aandacht voor onderzoek naar gecombineerde effecten van alle factoren die de mineralenabsorptie negatief of positief beïnvloeden wordt sterk bepleit, evenals onderzoek naar de lange termijn effecten en adaptaties aan voedingsinterventies.

Curriculum vitae

Wim van Dokkum werd op 9 maart 1939 te Maartensdijk geboren.
Na het behalen van het diploma HBS-B aan het Christelijk Lyceum Populierstraat in 's-Gravenhage en het vervullen van zijn militaire dienstplicht werd in 1960 begonnen met de studie Scheikunde aan de Rijks Universiteit in Leiden. In april 1967 werd het doctoraal diploma behaald (hoofdvak organische chemie, bijvak levensmiddelenchemie) en trad hij dezelfde maand in dienst van het Centraal Instituut voor Voedingsonderzoek TNO te Zeist, aanvankelijk op de afdeling Oliën, Vetten en Margarine.
Na vervolgens twee jaar te zijn belast met de opleiding van chemici uit Saudi Arabië (onder meer voedingsmiddelenanalyse ten behoeve van een tweetal Keuringsdiensten van Waren), werd hij door TNO gedurende vier jaar (1970 tot en met 1973) beschikbaar gesteld als cursusleider van de "International Course in Food Science and Nutrition" van de NUFFIC (Netherlands Universities Foundation for International Cooperation).
Van 1973 tot op heden is hij werkzaam op de afdeling Voeding van het TNO-complex in Zeist, waar hij als hoofd van de "metabole unit" belast is met de uitvoering van voedingsproeven bij de mens.
In zijn vrije tijd is de auteur een enthousiast amateur goochelaar en als drummer actief in het dixieland orkest Dixie Daddies.