Smoking and health from adolescence into adulthood

Claire M Bernaards

Results from the Amsterdam Growth and Health Longitudinal Study

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The study presented in this thesis was performed at the Institute for Research in Extramural Medicine (EMGO Institute) of the VU University Medical Center, the Netherlands. The EMGO Institute participates in the Netherlands School of Primary Care Research (CaRe), which was acknowledged in 1995 by the Royal Netherlands Academy of Arts and Sciences (KNAW).

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De Boelelaan 1105

door

Claire Monique Bernaards

geboren te 's-Gravenhage

promotoren:	prof.dr. H.C.G. Kemper
	prof.dr. W. van Mechelen
copromotoren:	dr. J.W.R. Twisk
	dr. J. Snel

Contents

Het schrijven van een proefschrift is als het maken van een trektocht door IJsland.
Zodra het einde in zicht is, ben je er nog lang niet.

Chapter 1

General introduction

The history of tobacco

Tobacco was first smoked in Central America and it is believed that at least 2500 years ago the Mayans already inhaled the smoke of burning tobacco leaves. At first, tobacco was used in religious ceremonies and for healing purposes. Later on, tobacco started to be used for pleasure.[1] The Spaniards and Portuguese brought tobacco to Europe after Columbus first came into contact with it in 1492. In Europe tobacco was claimed to have a medicinal value. The tobacco plant was named after Jean Nicot in 1585 ('Nicotiana'), after he had introduced it to the royal court of Paris.

Originally, tobacco was chewed, smoked in pipes[2] or taken nasally as a powder,[1] but gradually cigars and cigarettes became popular. After the cigarette-making machine had been introduced, cigarettes became incredibly popular. They were no longer exclusively smoked by the upper class, but became affordable for the working class as well.[3] In Western Countries, the use of cigarettes rose dramatically during the First World War, since cigarettes were largely distributed among soldiers. Consequently, a large number of men were addicted to cigarettes after the war. For women, on the other hand, it remained socially unacceptable to smoke until the 1920s, when a significant number of women started smoking in the USA and Britain.[1] Women started smoking at different times in different countries. The increase in tobacco smoking among women first started in the Northern European Countries, whereas women from the Southern European countries only took up smoking in the 1980s.[4] Nowadays, 1.1 billion people (one in three adults) smoke worldwide, of whom approximately 80% live in low- and middle-income countries. In the rich countries, the affluent people are giving up smoking, whilst poorer people are continuing. Globally, the prevalence of smoking is four times higher in men than in women, but this is mainly due to the striking gender differences in low and middle-income countries.[5]

Smoking in the Netherlands

In the Netherlands, the smoking prevalence among men aged 15 years and older decreased considerably from 90% in 1958 to 33% in 2001. Most of this decrease occurred before the 1980s and since then, the prevalence of smoking has been relatively stable.[6,7] In women aged 15 years and older, the prevalence of smoking was 29% in 1958. It showed a peak at the end of the 1960s when it rose to 42% in 1967 and 1970, and became relatively stable between 1980 and 2001. In 2001, the prevalence of smoking among females aged 15 years and older, was 27%. In 1999, the number of deaths from smoking in the Netherlands was estimated at 14379 males and 8101 females.[6]

Research on smoking and health

At the beginning of the twentieth century, tobacco smoking was no longer believed to be beneficial and in several countries, anti-tobacco movements tried to discourage smoking. The anti-tobacco movements, however, had no sound medical evidence of the harmful effects of smoking. The available evidence came from: 1. Clinical observations on patients, 2. Ecological observations of national trends, 3. Comparative studies of the smoking habits of patients with different diseases or no disease at all, and 4. Laboratory experiments.[1]

The first Scientific Institute for the Research into the Hazards of Tobacco was founded on 5 April 1941 at the Friedrich-Schiller University in Jena (Germany). The institute was (financially) supported by Adolf Hitler who believed tobacco to be one of the most dangerous poisons that threatened mankind.[8] The pathologist Professor Eberhard Schairer performed animal experiments with the support of the Tobacco Institute and also conducted an epidemiological study on smoking in relationship to lung cancer together with Erich Schöniger. Despite the fact that the work of Schairer and Schöniger marks an important phase in the development of knowledge on the harmful effects of tobacco, as Richard Doll wrote in 2001, 'it failed the recognition that it deserves'.[9] In 1950, the authors of the first two large case-control studies on smoking and lung cancer[10,11] did not refer to the work of Schairer and Schöniger, because they simply did not know of its existence.[9] Despite strong evidence from these two cross-sectional studies and three other ones published in 1950,[12-14] the relationship between smoking and lung cancer was not accepted until the first cohort studies were published in 1954 and 1956. These cohort studies did not only show a dose-response relationship between the amount of tobacco smoked and lung cancer, but also between the amount of tobacco smoked and other causes of death, such as coronary thrombosis and chronic bronchitis.[15,16] Since then, many other cohort studies have shown the association between smoking, morbidity and mortality.[17-21] Furthermore, the International Agency for Research on Cancer Research has identified 55 (out of 2000) chemical compounds in tobacco leaf that show evidence of carcinogenity.[5]

Smoking cessation

Since the prevalence of smoking decreased substantially in the United Kingdom between 1950 and 1990, Peto *et al.*[22] were able to investigate the effects of prolonged smoking cessation. They found that smoking cessation at the age of 30, coincides with a 90% reduction in the risk of lung cancer attributable to tobacco and that even people who stop smoking at the age of 50 or 60 avoid most of their subsequent risk. Similar results were found in the British Doctors' study, in which British doctors were followed-up for 40 years between 1951 and 1991.[23] This study showed that those

who had stopped smoking before the age of 35, had similar life expectancies than those who had never smoked. Furthermore, for those who had stopped later, the survival was intermediate between that of non-smokers and continuing smokers.

Low yield cigarettes

After the dose-response relationship between smoking and lung cancer became more and more evident, the tobacco industry started to change the makeup of commercial cigarettes in order to reduce the exposure to toxins and carcinogens.[2] Commercial cigarettes with filter tips, lower tar yields and lower nicotine yields were introduced. Unfortunately, the introduction of these 'low-yield' cigarettes did not have the beneficial effect to the extent that was expected. An explanation for this finding is that smokers of low and medium yield cigarettes tend to compensate for the reduced nicotine intake by increasing the frequency of puffs, the puff volume and by a deeper inhalation of cigarette smoke into the lungs.[24] Although several early studies found a reduction in the risk of lung carcinoma in subjects who switched from non-filter cigarettes to filter cigarettes,[25,26] more recent studies have shown that this only counts for squamous cell carcinoma and not for adenocarcinoma of the lung.[24,27]

Adolescence and young adulthood

Since 1950, many smoking related causes of death have been identified.[1] Most of the information on smoking related causes of death comes from studies in middle-aged smokers or older, with a relatively long smoking history and a high number of pack-years.[19,20,23] Relatively few studies have investigated the association between smoking and health parameters in adolescents and young adults,[28-30] who usually smoke fewer cigarettes per day and have a lower number of pack-years. Furthermore, little is known on the development of the association between smoking and health parameters between adolescence and adulthood.

Amsterdam Growth and Health Longitudinal Study (AGAHLS)

The AGAHLS is an ongoing cohort study that started in 1977 with about 600 pupils from the first and second grade of two secondary schools in Amsterdam and Purmerend, The Netherlands.[31,32] The pupils from the school in Amsterdam (i.e. the "longitudinal group") were measured annually between 1977 and 1980 at a mean age of 13, 14, 15 and 16 years respectively. The pupils from the school in Purmerend were used as controls and were measured only once during this four-year period. Every year, about a quarter of the control group was measured. In adulthood, the "longitudinal group" was measured at the age of 21 (1985), 27 (1991), 29 (1993), 32/33 (1996/1997) and 36 (2000), whereas the control group was measured at the age of 32/33 (1996/1997) and 36 (2000) only. Within the AGAHLS, lifestyle parameters

(i.e. smoking, alcohol consumption, physical activity pattern and dietary habits), psychological parameters and many biological (health) parameters have been measured longitudinally.

Smoking

During the first four years of the AGAHLS, subjects were asked in a confidential interview about the number of cigarettes and the number of packages of own-rolled tobacco smoked per week. In later years, subjects filled out a questionnaire about their current use of cigarettes, the number of packages of own-rolled tobacco, the number of cigars/cigarillos and the number of packages of pipe tobacco. Tobacco smoking was expressed in total gram of tobacco per week (1 cigarette = 1 gram, 1 package of own-rolled tobacco = 40 gram, 1 cigar/cigarillo = 3 gram, 1 package of pipe tobacco = 50 gram). Subjects were counted as smokers if they reported to smoke minimally 7 gram of tobacco per week (i.e. 1 cigarette a day on average). Each year the questionnaire contained questions about current smoking as well as smoking in the recent past and quitting efforts. At the ninth and latest measurement of the AGAHLS in the years 2000, the questions about smoking in the past and quitting attempts were more extensive in order to calculate pack-years retrospectively.[33] Furthermore, a relatively simple biochemical dipstick method (NicCheck 1®) to assess nicotine intake in the previous 20 hours was added to the measurements.

Prevalence of smoking

Figure 1 presents the prevalence of smoking among the participants of the AGAHLS between the age of 13 and 36 years. Only 1.0% of the 13-year-old male participants and 0.9% of the 13-year-old female participants of the AGAHLS met the definition for "smoker" in 1977. In 1978, when the participants were 14-years old on average, smoking prevalence was 5.4% in boys and 2.9% in girls. In 1984, Zwart *et al.*[34] reported a prevalence of daily smoking in the Netherlands of 6 and 7% among 12- to 13-year-olds boys and girls respectively, and of 14 to 19% among 14- to 15-year-old boys and girls. Nowadays, the prevalence of smoking seems to be much higher among 13- and 14-year-olds. The Dutch Organisation for Tobacco Control reported a smoking prevalence of 20% among 13- and 14-year-old boys and girls in 2001.[6] However, the Dutch Organisation for Tobacco Control used "smoking at least one cigarette in the previous month" as the definition for "smoker", whereas we used "smoking at least 7 gram of tobacco per week (i.e. one cigarette per day on average)". The definition for "smoker" used in this thesis may not be the best definition to be used in adolescents, since adolescents are believed to go through several stages before they become addicted to smoking and start smoking on a daily basis.[35] Therefore, we used a different definition for "smoker" in *Chapter* 4, which deals with

the development of smoking behaviour during adolescence. With this different definition for "smoker", smoking prevalence rose to 2.6% and 2.8% in 13-year-old boys and girls respectively, and to 10.2% and 11.6% in 14-year-old boys and girls.

Although the prevalence of smoking in the AGAHLS rose rapidly during adolescence in both boys and girls, a considerable higher number of girls than boys reported smoking at the age of 16. Female participants reached their maximum smoking prevalence at the age of 21 (i.e. 34.9%) and males at the age of 27 (i.e. 34.5%). From that age onwards, smoking prevalence dropped to 20.5% in 36-year-old males and 17.9% in 36-year-old females.

Tobacco consumption

Figure 2 and 3 show the development of tobacco consumption in gram per week (median and interquartile range) between calendar age 13 and 36 in male (figure 2) and female smokers (figure 3) within the AGAHLS. The development of tobacco consumption in smokers shows a similar pattern as the development of smoking prevalence. The median tobacco consumption was low during adolescence, but rose until it reached its maximum at calendar age 21 in females and calendar age 27 in males. After these ages, tobacco consumption became relatively stable.

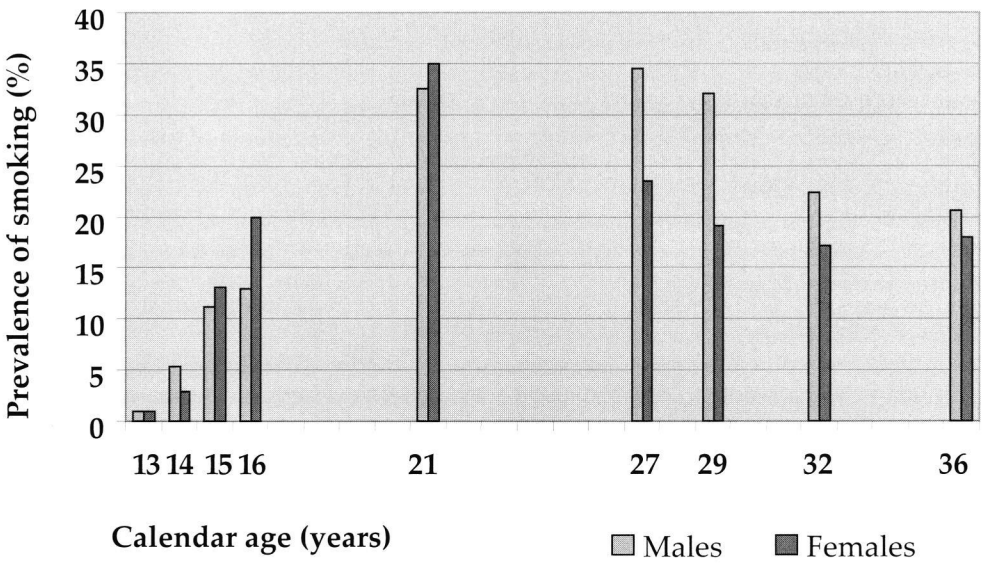

Figure 1. Prevalence of smoking between the age of 13 and 36 years in male and female participants of the Amsterdam Growth and Health Longitudinal Study. Participants were considered smokers when they reported to smoke at least 7 gram of tobacco per week (i.e. one cigarette per day on average).

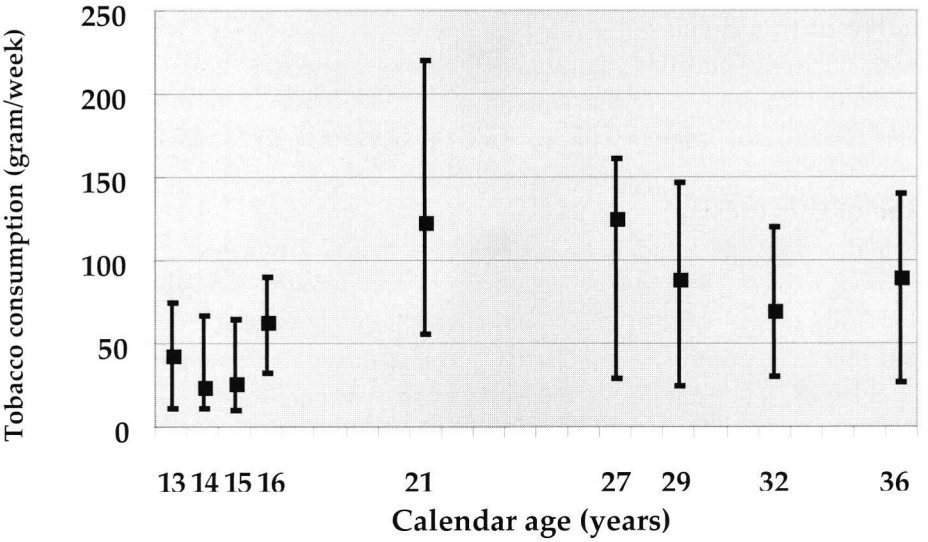

Figure 2. Median tobacco consumption (and interquartile range) between the age of 13 and 36 years in male smoking participants of the Amsterdam Growth and Health Longitudinal Study.

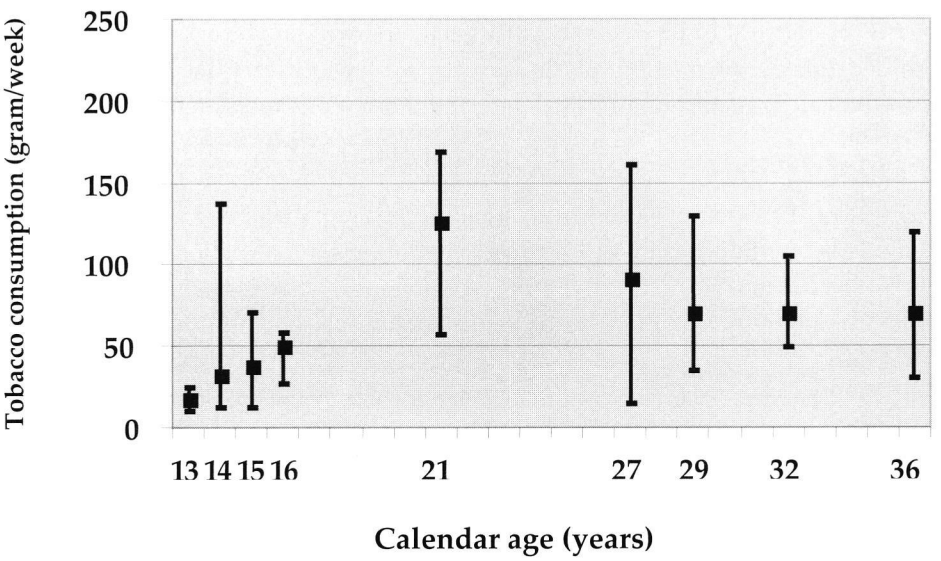

Figure 3. Median tobacco consumption (and interquartile range) between the age of 13 and 36 years in female smoking participants of the Amsterdam Growth and Health Longitudinal Study.

Objective of this thesis

The main objective of this thesis was to investigate the cross-sectional and longitudinal association between smoking and health parameters in young and relatively healthy males and females between the age of 13 and 36 years.

Outline of this thesis

Chapter 2 describes the comparison between two methods to assess nicotine intake: self-reported tobacco consumption *versus* a colorimetric dipstick method (NicCheck 1®). This study was performed to gain more insight into the association between self-reported tobacco consumption and actual nicotine intake. Although several studies have compared self-report with biochemical methods to assess nicotine intake, NicCheck 1® is a promising instrument to be used in large epidemiological studies because it is quick and relative inexpensive. Furthermore, instead of measuring cotinine (the primary metabolite of nicotine) only, NicCheck 1® reacts with nicotine and all its metabolites in urine, which makes it less dependent on inter-individual differences in nicotine metabolism.

Chapter 3 describes a study that aims to investigate the (relative) validity of retrospectively calculated pack-years. By calculating pack-years retrospectively (py-retro), researchers are forced to rely on the memory of their subjects who might have poor recall capabilities or the tendency to underrate tobacco consumption.[36] In *Chapter* 3, py-retro is compared with prospectively calculated pack-years (py-pro). Py-pro depends on the memory of the subjects to a lesser extent than py-retro, since it is based on all available longitudinal data on tobacco consumption between the age of 13 and 36.

Chapter 4 describes the development of smoking behaviour in boys and girls during adolescence in relationship to four estimates of biological maturation. In addition, it describes the extent to which timing of biological maturation (early or late maturation) can predict smoking status in late adolescence and adulthood.

Chapter 5 describes the longitudinal relationship between tobacco smoking, cardiovascular fitness and heart rate response to exercise. Moreover, it shows to what extent these relationships become stronger over time (i.e. between the age of 13 and 36).

Chapter 6 presents the results of a study on changes in tobacco consumption in relationship to changes in several biological risk factors for cardiovascular disease during a four-to-six year follow-up period.

Prior studies on changes in tobacco consumption have mainly been restricted to smoking cessation, and/or have used relatively short follow-up periods (i.e. 4-17 weeks) under controlled experimental conditions, sometimes with use of nicotine replacement therapy.

Chapter 7 shows the results of a study on smoking and bone strength parameters in 36-year-old males and females, measured with quantitative ultrasound (QUS) and dual-energy X-ray absorptiometry (DXA).

Finally, in *Chapter 8*, the results of all studies presented in this thesis are discussed.

Chapters 2 to 7 were originally written as separate articles. Consequently, the content of these chapters show some overlap, especially with regard to the methods section.

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Chapter 2

A comparison between self-report and a dipstick method (NicCheck 1®) to assess nicotine intake

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Submitted

Abstract

Objectives. The purpose of this study was to investigate the agreement between self-reported tobacco consumption and NicCheck 1® (Dynagen Inc. Cambridge, MA, USA) regarding smoking status and nicotine intake. NicCheck 1® is a dipstick that changes colour in the presence of urinary nicotine metabolites.

Methods and procedures. Smoking was assessed by self-report and NicCheck 1® in 169 males and 191 females (mean age 36.0 SD 0.7).

Results. Self-report and NicCheck 1® agreed highly on smoking status, especially in moderate to heavy smokers. With regard to nicotine intake, there was a large overlap in self-reported tobacco consumption between NicCheck 1® levels, despite a relatively high correlation coefficient between self-report and NicCheck 1® in smokers (i.e. 0.74). No effect-modification by gender or BMI was found. When both methods were validated against two blood lipid parameters, self-report seemed to do equally well as NicCheck 1®, in assessing nicotine intake.

Conclusions. This study provides no evidence that self-report does worse than NicCheck 1® in assessing tobacco consumption.

Introduction

The sensitivity and specificity of self-reported smoking status are generally high,[1,3] but contradictory results have been published on the validity of self-reported tobacco consumption as an indicator of actual nicotine intake. Studies that correlated self-reported number of cigarettes with cotinine levels have reported large variations in correlation coefficients.[4-7] The utility of cotinine as an indicator of nicotine intake, however, is limited due to inter-individual differences in the fractional conversion of nicotine to cotinine (55-92%) and the rate of cotinine elimination.[8] As a consequence, several methods have been developed to assess nicotine and all its metabolites.[9-12]

Methods to assess nicotine metabolites, however, are generally either costly or time consuming. NicCheck 1® (Dynagen Inc. Cambridge, MA, USA), however, is a quick and inexpensive dipstick method to measure nicotine intake in the previous 20 hours. It is based on the colorimetric method described by Peach *et al.*[9] Because of its relatively low costs, NicCheck 1® seems to be a promising instrument for the assessment of nicotine intake in the previous 20 hours in large epidemiological studies. Leischow *et al.*[13] found NicCheck 1® to be sensitive to differences in nicotine intake but also reported low correlation coefficients between self-reported number of cigarettes and NicCheck 1® (i.e. 0.29 and 0.23). In the present study, we used an improved version of NicCheck 1® and included non-smokers and light smokers besides moderate to heavy smokers in contrast to Leischow *et al.*[13] This is important because many biochemical methods lack sensitivity at low levels of tobacco consumption.[2] Furthermore, we studied the role of possible effect-modifiers in the association between self-reported tobacco consumption and NicCheck 1®. Gender, for instance, might affect the relationship between self-reported tobacco consumption and NicCheck 1® by differences in nicotine intake per cigarette between men and women, e.g. due to differences in puffing behaviour.[14] Body mass index (BMI), on the other hand, might do so by providing a larger distribution volume.[5]

The aim of our study was to investigate the agreement between self-report and NicCheck 1® with regard to smoking status and nicotine intake. Since NicCheck 1® has been developed as a simple method to discriminate between non-smokers, light smokers and moderate to heavy smokers, we also studied the agreement between self-report and NicCheck 1® after both estimates had been transformed into categorical variables. Finally, we tried to validate both estimates of nicotine intake by associating them with blood lipid parameters that are known to be associated with smoking.

Methods

Study population

The study population consisted of 179 male and 200 female participants of the Amsterdam Growth and Health Longitudinal Study (AGAHLS).[15,16] The AGAHLS is an ongoing study in which smoking behaviour has been assessed repeatedly over a period of 23 years with the help of a smoking questionnaire. At the latest measurement, when the participants were on average 36.0 (SD 0.7) years old, smoking behaviour was additionally assessed with NicCheck 1®. Subjects were excluded from the study if they reported to use alternative nicotine delivery systems. Mainly all subjects were Caucasians (96.8%). The AGAHLS was approved by the Medical Ethical Committee of the VU University Medical Center and subjects provided written informed consent.

Smoking interview

Participants arrived at our laboratory between 8.00 and 9.00 in the morning and were directly interviewed about their tobacco consumption in the previous 48 hours. Participants were considered as self-reported smokers when they reported to have smoked minimally one gram of tobacco (i.e. one cigarette) in the previous 24 hours. Furthermore, participants were asked about the use of alternative nicotine delivery systems (i.e. nicotine chewing gum, nicotine skin patches) and the time spent in a smoky room in the previous 24 hours. In a written questionnaire, participants were asked about their regular smoking behaviour and whether or not they were living together with a smoker.

Urine collection

Urine was collected directly after the smoking interview. All urine samples were collected within a small range of time (08:15 to 09:15 in the morning) and stored in the refrigerator before analysis or frozen at -20 to -40 °C when the samples could not be analysed on the measurement day. All samples were analysed at least within two months after collection.

Possible effect-modifiers

Gender and body mass index (BMI) were investigated for effect-modification in the relationship between self-reported tobacco consumption and NicCheck 1®. BMI was calculated by dividing body weight by body height raised to the square. Body weight was measured with a spring balance (Van Vucht, Amsterdam, The Netherlands).

Test procedure of NicCheck 1®

The urine samples were brought to room temperature and mixed thoroughly.

Of each urine sample, 0.75mL was transferred to a 13 mm × 100 mm test tube after which the test strip was placed directly from the canister into the test tube with a clean pair of tweezers. Each NicCheck 1® test strip is composed of 7.5 cm × 0.5 absorbent paper with chloramine-T, potassium thiocyanate, citrate buffer and diethylthiobarbituric acid spotted along the length of the strip at definite intervals. When urine diffuses up the test strip, the potassium thiocyanate mixes with chloramines-T on the strip, after which the releasing cyanogen chloride reacts with the pyridine ring of nicotine and its metabolites in urine. Diethylthio-barbituric acid reacts with the resulting glutaconaldehyde to produce a pale pink to dark pink colour along the length of the test strip. The darkness of the colour is dependent on the level of urinary nicotine metabolites. After 15 minutes, the colour on the test strip was compared with a colour chart provided by the manufacturer. Each colour on the test strip corresponds to one of the NicCheck 1® levels on the colour chart (ranging from 0 to 14). Absence of colour corresponds to NicCheck 1® level 0 (i.e. non-smoker), whereas dark pink corresponds to NicCheck 1® level 14 (i.e. very heavy smoker). All tests were performed 'blind' (i.e. without reference to self-reported tobacco consumption) by the same person. A disadvantage of NicCheck 1® is that it does not provide quantitative data regarding concentrations of urinary nicotine metabolites. Similar concentrations of cotinine and nicotine, for instance, may not give rise to the exact similar pink colour. This might be due to differences in the availability of the pyridine ring, the other bonds by which it is attached to the rest of the molecule and differences in optimal conditions for the reaction between metabolites.

Serum cholesterol levels

Since cigarette smoking is associated with "atherogenic" lipoprotein cholesterol profiles,[17-20] we used high-density lipoprotein cholesterol (HDL-C) and the ratio between total serum cholesterol and HDL-C (TC/HDL-C) as means to validate self-report and NicCheck 1®. We expected better estimates of nicotine intake to show stronger dose-response relationships with HDL-C and TC/HDL-C. Blood serum was obtained from a sample of approximately 10-ml of venous blood, taken from the vena antecubitis in the non-fasting state. Both blood sampling and serum preparations were done between 08:00 and 12:00 in the morning. TC and HDL-C were measured in serum with standard methods. External quality control took place with target samples from a World Health Organisation reference laboratory (Lipid Standardization Laboratory, Atlanta, Georgia).

Statistical analyses

Agreement between self-report and NicCheck 1®. The agreement between self-report and NicCheck 1® was estimated with tobacco smoking as a dichotomous variable (i.e. smoking status), a categorical variable (i.e. smoking groups) and a continuous variable (i.e. nicotine intake). The agreement on smoking status was estimated by calculating positive and negative predictive value of self-report, and by calculating (relative) sensitivity and specificity of self-report, with NicCheck 1® as the reference. The agreement on smoking groups was studied with Cohen's Kappa. Categorisation based on NicCheck 1® resulted in the following smoking groups: non-smokers (NicCheck 1® =0), light smokers (NicCheck 1® < 3), and moderate to heavy smokers (NicCheck 1® ≥ 3). Categorisation based on self-report resulted in the following smoking groups: non-smokers (< 1 gram of tobacco), light smokers (≤ 10 gram of tobacco) and moderate to heavy smokers (> 10 gram of tobacco). Categorisation into the light and moderate to heavy smoking groups was based on the median values of self-report (i.e. 10 g of tobacco) and NicCheck 1® (i.e. level 3). To indicate the agreement on nicotine intake between self-report and NicCheck 1®, we correlated self-reported tobacco consumption with NicCheck 1® level in smokers, by using Spearman's correlation coefficient. In this analysis, both self-reported tobacco consumption and NicCheck 1® level were treated as continuous variables. Self-reported non-smokers were excluded to prevent tied observations (i.e. self-report=0 and NicCheck 1®=0).

Effect-modification. To investigate possible effect-modification by gender and BMI, we studied the interaction between self-reported tobacco consumption on the one hand, and gender and BMI on the other hand, using multiple linear regression analysis with NicCheck 1® level as the outcome variable. Gender and BMI were considered effect-modifiers if the interactions reached p-values below 0.10.

Association with blood lipid parameters. One-way analysis of variance (ANOVA) was used to analyse the relationship between categorised NicCheck 1® and categorised self-reported tobacco consumption on the one hand, and TC and TC/HDL-C on the other hand.

Results

From the initial 379 participants, 360 participants (169 males, 191 females) were included in the study. Two females were excluded from the study for using alternative nicotine delivery systems. The others were excluded for not being able to collect urine directly after the smoking interview. Based on the smoking interview, 23.7% of the males (N=40) and 18.3% (N=35) of the females were classified as

smokers. Table 1 presents the characteristics of the self-reported smokers and self-reported non-smokers. Cigarettes were smoked by most of the self-reported smokers, own-rolled tobacco by a considerable lower number of smokers, cigars and cigarillos by two smokers and pipe tobacco by none of the smokers. Ten participants reported smoking more than one tobacco product.

Table 1. Characteristics of self-reported smokers and self-reported non-smokers

	Self-reported smokers (N=75)	Self-reported non-smokers (N=285)
Gender (% male)	53.3	45.3
Tobacco smoked in previous 24 hours (gram)[1]	12.1 (8.9)	-
Type of tobacco (% Yes)		
Cigarettes	71.6	-
Own-rolled tobacco	35.1	-
Cigars/Cigarillos	2.7	-
Pipe	0.0	-
Body mass index[1], males	25.0 (3.0)	24.7 (2.6)
Body mass index[1], females	23.7 (4.0)	23.4 (3.2)

[1]expressed as mean & (standard deviation)

Agreement between self-report and NicCheck 1®: smoking status

Table 2 presents the number of participants categorised as smokers based on NicCheck 1® and self-report. When smoking status based on NicCheck 1® was used as the reference, positive predictive value of self-report was 88% (66 out of 75) and negative predictive value 98% (279 out of 285). Sensitivity of self-report was 0.92 (66 out of 72) and specificity 0.97 (279 out of 288). Nine participants reported smoking despite a negative score (level=0) on NicCheck 1®. Seven of these participants reported having smoked one cigarette in the previous 24 hours, whereas two participants reported having smoked two and seven cigarettes. Six participants reported no smoking in the previous 24 hours, but did score positive on NicCheck 1® (i.e. level 1 or 2). None of these participants reported smoking in the previous 48 hours, two reported living together with a smoker and one reported to have been in a smoky room in the previous 24 hours. One 'false negative' came from a dark urine sample and another 'false negatives' showed a purple colour between the arrows at the top of the test strip.

Table 2. Number of participants categorised as smokers based on NicCheck 1® and self-report

		NicCheck 1®		
		+	-	Total
Self-report	+	66	9	75
	-	6	279	285
Total		72	288	360

+ = smoker, - = non-smoker

Agreement between self-report and NicCheck 1®: smoking groups

Table 3 presents the categorisation of subjects into three smoking groups based on self-report (horizontal axis) and NicCheck 1® (vertical axis). The methods agreed in 326 out of 360 cases (90.6%). The agreement was highest regarding the category of non-smokers. Cohen's Kappa was 0.73.

Table 3. Categorisation of subjects into three smoking groups based on NicCheck 1® (level) and self-reported tobacco consumption (gram)

	Self-reported tobacco consumption			
	0	≤10	>10	Total
NicCheck 1®				
0	279	9	0	288
<3	6	23	14	43
≥3	0	5	24	29
Total	285	37	38	360

Cohen's Kappa = 0.73

Agreement between self-report and NicCheck 1® among smokers: nicotine intake

Spearman's correlation coefficients between self-reported tobacco consumption and NicCheck 1® level was 0.74 (N=75). Figure 1 shows a scatter plot in which self-reported tobacco consumption is plotted against NicCheck 1® level. NicCheck 1® levels in this scatter plot ranged from 0 to 10, since levels higher than 9 were not observed. This figure clearly shows that NicCheck 1® rises linearly with increasing self-reported tobacco consumption, but also that there is a large overlap in self-reported tobacco consumption between NicCheck 1® levels.

Effect modification

We found no effect modification by gender and BMI in the relationship between self-reported tobacco consumption and NicCheck 1® level.

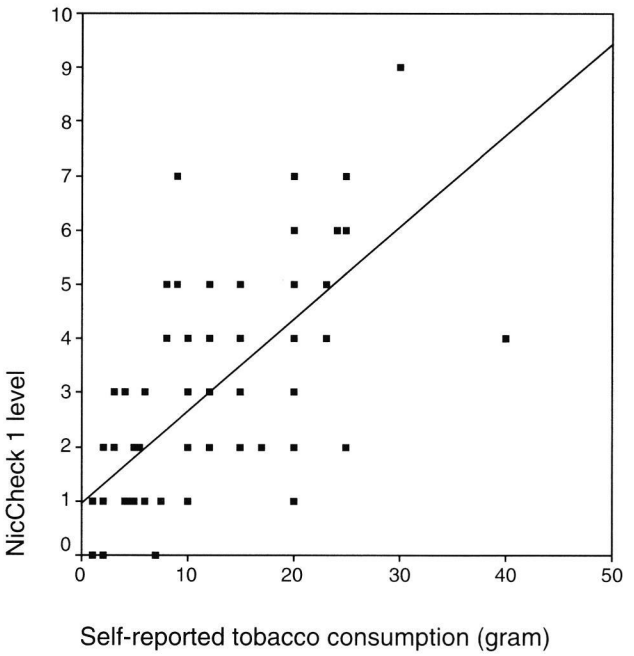

Figure 1. Self-reported tobacco consumption in the previous 24 hours plotted against NicCheck 1® level without stratification for gender. Each point may represent more than one participant. Number of smokers in each NicCheck 1® level: 0 (N=9), 1 (N=13), 2 (N=14), 3 (N=10), 4 (N=9), 5 (N=10), 6 (N=4), 7 (N=5), 8 (N=0), 9 (N=1), 10 (N=0). The equation of the line: y (NicCheck 1® level) = 0.94 + 0.17 * self-reported tobacco consumption.

Association with blood lipid parameters

Table 4 shows mean values and standard deviations of HDL-C and TC/HDL-C in three smoking groups based either on self-report or on NicCheck 1®. In males, we found a dose-response relationship between smoking groups based on NicCheck 1® and TC/HDL-C. However, when smoking groups were categorised according to self-report, we found dose response relationships with both TC/HDL-C and HDL-C. In females, no dose-response relationships were found between smoking groups and blood lipids. In both males and females, differences in blood lipid parameters between smoking groups were non-significant.

Table 4. Comparison between three smoking groups (based either on NicCheck 1® or on self-report), in mean levels of high density lipoprotein cholesterol (HDL-C) and the ratio between total serum cholesterol and HDL-C (TC/HDL-C) (and standard deviation)

	Groups based on NicCheck 1® level				Groups based on self-report			
	0	< 3	≥ 3	p-value*	0	≤ 10	> 10	p-value*
Males	N=133	N=17	N=19		N=129	N=22	N=18	
HDL-C	1.23 (0.29)	1.13 (0.23)	1.14 (0.35)	0.21	1.23 (0.28)	1.19 (0.25)	1.08 (0.35)	0.11
TC/HDL-C	4.40 (1.36)	4.78 (1.34)	4.92 (1.27)	0.19	4.39 (1.35)	4.61 (1.43)	5.14 (1.20)	0.08
Females	N=155	N=16	N=20		N=156	N=15	N=20	
HDL-C	1.59 (0.34)	1.46 (0.35)	1.58 (0.44)	0.41	1.60 (0.35)	1.46 (0.47)	1.53 (0.31)	0.30
TC/HDL-C	3.17 (0.83)	3.51 (1.31)	3.30 (1.05)	0.32	3.16 (0.84)	3.67 (1.31)	3.29 (1.00)	0.11

* p-value based on one-way ANOVA

Discussion

Our results indicate a high agreement between self-reported tobacco consumption and NicCheck 1® with regard to smoking status, but are contradictive with regard to nicotine intake. Although we found a relatively high correlation coefficient between self-report and NicCheck 1® in smokers, we found a large overlap in self-reported tobacco consumption between NicCheck 1® levels. When two blood lipid parameters (known to be associated with smoking) were used to validate self-report and NicCheck 1®, self-report seemed to do equally well as NicCheck 1®, in assessing nicotine intake.

Sensitivity and specificity of self-reported smoking status

We found a relatively high sensitivity (i.e. 0.92) and specificity (i.e. 0.97) of self-reported smoking status. A meta-analysis on the results of 26 studies reported sensitivities between 6 and 100 and specificities between 33 and 100.[1] All of these 26 studies, however, used cotinine, thiocyanate, or carbon monoxide as a reference instead of NicCheck 1®. The relatively high sensitivity and specificity of self-report in our study might be due to the fact that NicCheck 1® provides a more complete picture of nicotine intake than cotinine, thiocyanate or carbon monoxide do. Peach *et al.*[9] compared self-report with urinary nicotine metabolites by using two colorimetric methods with diethylthiobarbituric acid (DETB) to detect nicotine metabolites in urine. Although they reported no false negatives, their results should be interpreted with caution since only regular smokers participated in their study. In our study, discrepancies between self-reported smoking status and smoking status based on NicCheck 1® were mainly found in light tobacco smokers, suggesting that NicCheck 1® is less sensitive at low levels of tobacco consumption similar as other biochemical methods.[2]

Correlation between self-reported tobacco consumption and NicCheck 1®

Spearman's correlation coefficient (i.e. 0.74) between self-reported tobacco consumption and NicCheck 1® in the present study was higher than in the former study on NicCheck 1®.[13] This difference might be the result of the improved version of NicCheck 1® that was used in our study, or due to the lower number of NicCheck 1® levels (i.e. three and five) that was used by Leischow *et al.*.[13] Studies that compared self-reported tobacco consumption with cotinine levels (in different body fluids) reported correlation coefficients between 0.15[5] and 0.70.[6] Our relatively high correlation coefficient might be the result of assessing nicotine and all its metabolites instead of cotinine only. Our results are in agreement with Peach *et al.*[9] who presented a high correlation coefficient (i.e. 0.85) between self-report and urinary

nicotine metabolites as well as a large overlap in urinary nicotine metabolites between groups of self-reported tobacco consumption.

Self-report versus NicCheck 1®

It is interesting to know whether or not NicCheck 1® is better than self-reported tobacco consumption in predicting the quantity of nicotine intake. Unfortunately, a gold standard for measuring nicotine intake is absent. Pérez-Stable *et al.*[6] investigated the validity of different predictors of tobacco intake by correlating these predictors with factors that are known to be associated with smoking, such as blood pressure and serum cholesterol levels. Although this not the ideal way of studying the validity of cotinine, it gives at least some indication. We found no indication that self-report does worse than NicCheck 1® in assessing tobacco consumption, in contrast to Pérez-Stable *et al.*[6] who consistently showed that serum cotinine correlated higher with biochemical and physical assessments than self-report. The absence of any dose-response relationship between smoking groups (based either on self-report or on NicCheck 1®) and blood lipid parameters in females, may suggest that neither self-report nor NicCheck 1® are good estimates of nicotine intake, although it may also suggest that blood lipid parameters cannot be used to validate these measures in females.

Study limitations

The first limitation of our study concerns the external validity of our results. It is well known that the validity of self-report is lower in certain populations such as students[1] or pregnant women.[21] As a consequence, our results cannot automatically be translated to populations other than the AGAHLS population. A second limitation is that urine samples were collected in the morning despite the knowledge that blood nicotine and plasma cotinine concentrations are lower in the morning than at other moments of the day.[11,22,23] This might have increased the discrepancy between self-report and NicCheck 1®. However, the advantage of collecting urine samples at the laboratory is that the time of collection differed only slightly between participants. A third limitation of our study is that urine dilution might have affected the concentration of urinary nicotine metabolites. Therefore, it would have been better if we had measured creatinine concentration to correct for urine dilution.[9,24] Unfortunately, this was not possible in the present study.

Conclusions

Self-report and NicCheck 1® seem to agree highly on smoking status as well as on nicotine intake. Nevertheless, with regard to smoking status, we found higher discrepancies between self-report and NicCheck 1® in light tobacco smokers than in

moderate to heavy tobacco smokers. With regard to nicotine intake, there was a large overlap in self-reported tobacco consumption between NicCheck 1® levels. Finally, our study provides no evidence that self-report does worse than NicCheck 1® in assessing tobacco consumption.

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Chapter 3

Is calculating pack-years retrospectively a valid method to estimate lifetime tobacco smoking?

A comparison between prospectively calculated pack-years and retrospectively calculated pack-years

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Abstract

Objectives. To investigate the relative validity of retrospectively calculated pack-years (py-retro) by comparing py-retro with prospectively calculated pack-years (py-pro).

Methods and procedures. 23-year ongoing cohort study (1977-2000). **Participants.** 154 males and females, 13 years old in 1977 and 36 years old in 2000. **Setting.** Amsterdam, The Netherlands. To calculate py-pro, current smoking and quitting efforts were investigated 9 times in a period of 23 years with the help of an interview or a questionnaire. At the age of 36, subjects filled out a comprehensive questionnaire about their smoking history, to calculate py-retro. Individual differences between py-pro and py-retro were calculated. In addition, Cohen's kappa was calculated after categorising py-pro and py-retro into three groups.

Results. 1) Py-retro does not under or overestimate lifetime tobacco smoking. 2) The relative validity of py-retro was moderate due to large individual differences between py-pro and py-retro. 3) The individual differences between py-pro and py-retro became larger, the higher the number of pack-years. 4) Mean difference (and 95% limits of agreement) between py-pro and py-retro was -0.039 [-5.23, 5.32] when average pack-years was < 5.2 and -1.17 [-10.00, 14.65] when pack-years ≥ 5.2. 5) Cohen's kappa between categorised py-pro and py-retro was 0.79.

Conclusions. Future researchers in the field of smoking should be aware of the moderate relative validity of py-retro. Categorising py-retro into smoking groups, results in a misclassification error that is smaller than the quantitative error in continuous py-retro, but goes together with a loss of information.

Introduction

In epidemiology, one is often interested in lifetime tobacco smoking in order to study the cumulative risk of smoking on a certain kind of chronic disease. For this purpose, it is of great importance to estimate lifetime tobacco smoking as precisely as possible. A commonly used method to estimate lifetime tobacco smoking is by calculating the number of pack-years. Pack-years are calculated by multiplying smoking duration with daily tobacco consumption (e.g. the number of cigarettes per day) and are associated with cardiovascular disease,[1-3] death from various forms of cancer and all-causes mortality.[4] Although daily tobacco consumption is also associated with chronic disease,[4,5] it is assumed to predict mainly short term and reversible adverse effects of smoking, since it does not account for changes in smoking behaviour. Pack-years, on the other hand, take ones complete smoking history into account.

Since the early seventies, the popularity of pack-years has grown enormously, mostly because pack-years are relatively easy to assess. When Pubmed is used as a search engine, 16 papers are found in which pack-years have been used to estimate lifetime tobacco smoking between 1975 and 1980. Between 1995 and 2000, this number has increased to 385 publications.

Although pack-years are relatively easy to access, little is known about their validity. Pack-years are mostly calculated from data gathered retrospectively with the help of questionnaires or interviews in which questions are asked about current tobacco smoking as well as tobacco smoking in the past. However, when calculating pack-years retrospectively (py-retro), researchers are forced to rely on the memory of their subjects. It is assumed that pack-years only have an orientation value because of poor recall capabilities of smokers and the tendency to underrate tobacco consumption.[6] The problem of poor recall capabilities can be coped with by studying smoking behaviour prospectively instead of retrospectively. Unfortunately, studying smoking behaviour prospectively is very time consuming and expensive. During adolescence, smoking behaviour should be investigated minimally once a year, since during this period of life, recall for information beyond one year is inconsistent.[7]

In the Amsterdam Growth and Health Longitudinal Study (AGAHLS) smoking behaviour was assessed nine times between calendar age 13 and 36 in a cohort of males and females. During adolescence, smoking behaviour was assessed annually. Every measurement period, information about current smoking as well as information about smoking in the recent past was assessed.[8,9] At the age of 36, subjects filled out a comprehensive questionnaire about their complete smoking history to calculate py-retro. As a consequence, we could calculate pack-years retrospectively as well as prospectively (py-pro) at age 36. By comparing py-retro with py-pro, we could investigate the *relative* validity of py-retro. Furthermore, we investigated the effect of frequent quitting and gender on the difference between py-

pro and py-retro because changes in smoking behaviour as well as gender have shown to affect the accuracy of retrospective information on tobacco smoking.[10]

The main purpose of the present study is to investigate the relative validity of py-retro, as an estimate of lifetime tobacco smoking, by calculating the agreement between py-retro and py-pro at age 36.

Methods

Subjects

The Amsterdam Growth and Health Longitudinal Study (AGAHLS) started in 1977 with the main objective to study the growth and health development of teenagers (Kemper 1985). Boys and girls from the first and second class of a secondary school in Amsterdam participated in this study (n=347). They were measured in 1977, 1978, 1979 and 1980 at the age of 13.0 (SD 0.6), 14.0 (SD 0.7), 15.0 (SD 0.6) and 16.0 (SD 0.6) respectively. The measurements were carried on in 1985 (age 21.1 SD 0.8), 1991 (age 27.1 SD 0.8), 1993 (age 29.2 SD 0.8), 1996 (age 32.4 SD 0.9) and 2000 (age 36.0 SD 0.8).

Inclusion criteria

Subjects were included in the analyses when:

1. they were measured in 2000 (i.e. at age 36),
2. they did not miss more than one measurement during adolescence (1977-1980) and,
3. they did not miss more than one measurement in adulthood (1985-2000).

Of the 346 subjects, 67 males and 87 females (n=154) met all the inclusion criteria.

Smoking

During the first four years of the AGAHLS, subjects were asked in a confidential interview about the number of cigarettes and the number of packages of own-rolled tobacco smoked per week. In later years, subjects filled out a questionnaire about their current use of cigarettes, the number of packages of own-rolled tobacco, the number of cigars/cigarillos and the number of packages of pipe tobacco. Tobacco smoking was expressed in total gram of tobacco per week (1 cigarette = 1 gram, 1 package of own-rolled tobacco = 40 gram, 1 cigar/cigarillo = 3 gram, 1 package of pipe tobacco = 50 gram). Subjects were counted as smokers if they reported to smoke minimally 7 gram of tobacco per week (i.e. 1 cigarette a day). Each year the questionnaire contained questions about current smoking as well as smoking in the recent past and quitting efforts. In 2000, the questions about smoking in the past and quitting attempts were more extensive in order to calculate pack-years retrospectively (see Appendix 1).

Pack-years

In pack-years, smoking duration and tobacco exposure are combined. One pack-year is defined as one packet, or 20 g tobacco, smoked each day over a course of one year.[6] As one cigarette is assumed to contain 1 gram of tobacco,[6] one pack year can also be defined as having smoked 7300 gram of tobacco (365 days * 20 cigarettes each day). From our data, pack years were calculated prospectively as well as retrospectively as follows:

1) *Prospective*

When py-pro were calculated, all prospective data were used. Total gram of tobacco smoked per week, reported at each measurement, was assumed not to change until the following measurement (last value *carries forward*). Corrections for this assumption were made when subjects reported a quitting period at the first following measurement.

Example 1:

A female reported to smoke 30 cigarettes a day in 1991. In 1993 she reported to smoke only 10 cigarettes a day. In addition, she also reported to have quit smoking for four months in the last two years. The calculated number of pack-years between 1991 and 1993 is:

30/20 * (2 years minus 4 months) =

1.5 * $1^{2}/_{3}$ = *2.5 pack-years*

When subjects *started* smoking between two measurement periods, total gram of tobacco smoked per week reported at the last of the two measurements is *"carried backwards"*. This happened frequently between calendar age 16 and calendar age 21.

Example 2:

A 21-year-old male reported to smoke 5 cigarettes a day but he also reported not to smoke at age 16. If our male subject started smoking at age 18, the calculated number of pack-years between age 16 and 21 is:

5/20 * 3 years (21-18) = *0.75 pack-years*

In some cases, we used retrospective information about smoking in the recent past to calculate py-pro. This happened when a subject reported not to smoke at two consecutive measurements but did report a smoking period between these two measurements.

Example 3:

A 27-year-old male reported to have started smoking 20 cigarettes a day from the age of 24, and to have quitted smoking at the age of 26. He reported not to smoke

at the age of 21 and 27, when the actual measurements took place. We calculated py-pro as follows:
20/20 * 2 years (26-24) = *2 pack-years*.

2) *Retrospective*
When py-retro were calculated, only data gathered in the year 2000 were used (appendix 1). These data include current tobacco smoking as well as smoking in the past and quitting efforts.

Example 1 (ex-smoker in 2000)
A 36-year-old male reported not to smoke in 2000 but he reported to have smoked 10 cigarettes a day during 14 years. In addition, he reported to have smoked 5 cigarettes a day before that time. He started smoking at the age of 13 and quitted at the age of 32. Between age 13 and 32, he quitted six times with the quitting periods being 3.5 months on average.
Total smoking time (without quitting time): 19 years
Quitting time: 6 * 3.5/12 = 1.75 years
10/20 * (14 - (14/19 * 1.75)) = 6.36 pack-years
5/20 * (5 - (5/19 * 1.75)) = 1.13 pack-years
Py-retro = 7.49 *pack-years*

Example 2 (smoker in 2000)
A 36-year-old female reported that she has smoked 40 cigarettes a day for the last 15 years. Before that time, she smoked 20 cigarettes a day. She started smoking at age 17 and has never quit smoking.
Total smoking period = 19 years
40/20 * 15 = 30 pack-years
20/20 * 4 = 4 pack-years
Py-retro is = *34 pack-years*

Effect of frequent quitting and gender
To study the effect of frequent quitting, the mean difference between py-pro and py-retro was compared between subjects who quitted less than five times and subjects who quitted five times or more. To study the effect of gender, the mean difference between py-pro and py-retro was compared between men and women.

Statistical Analyses
Py-retro was plotted against py-pro to visualise the association between both estimates of pack-years. In addition, Spearman's rank correlation coefficient was

calculated. The relative validity of py-retro was investigated by calculating the agreement between py-pro and py-retro. Two methods were used to calculate agreement.

1) The mean difference was calculated between py-pro and py-retro with its 95% limits of agreement for all subjects who met the inclusion criteria and did not score zero on both py-pro and py-retro.[11] The 95% limits of agreement were calculated by using the 2.5% and 97.5% percentile. The subjects who scored zero on both py-pro and py-retro were left out of the analysis because they will inevitably show a perfect agreement between both methods. To visualise the individual differences between py-pro and py-retro, they were plotted against the average of py-pro and py-retro.
2) After categorising py-pro and py-retro into three smoking groups, Cohen's kappa was calculated. Cohen's kappa measures the agreement between two categorical assessments and corrects for the agreement that would have occurred anyhow by chance only.[12] The subjects who had never smoked tobacco (pack-years =0) were categorised into group 1,whereas subjects with pack-years > 0 were split into group 2 and 3, based on the median number of py-pro and py-retro. Subjects with pack-years below the median were categorised in group 2, and subjects with pack-years equal to, or higher than the median, were categorised in group 3. As the median number of py-pro and py-retro was 5.7, the groups were as follows:
   Group 1: pack-years = 0,
   Group 2: 0 < pack-years < 5.7,
   Group 3: pack-years ≥ 5.7.

Independent t-tests were used to study differences between frequent quitters and non-frequent quitters and between men and women.

Results

Of the 154 subjects who met the inclusion criteria, 11 missed one measurement during adolescence and 21 missed one measurement during adulthood. Py-pro and py-retro were both zero in 77 of the 154 subjects (34 men, 43 women). Table 1 presents the mean and standard deviation of py-pro and py-retro at the age of 36 in 1) the 154 subjects who met the inclusion criteria and 2) the 77 subjects who did *not* score zero on both py-pro and py-retro.

Table 1. Mean and standard deviation of prospectively calculated pack-years (py-pro) and retrospectively calculated pack-years (py-retro) in 1) subjects who met the inclusion criteria (n=154) and 2) subjects who met the inclusion criteria and did not score zero on py-pro and py-retro (n=77).

	N = 154		N = 77	
	Mean	SD	Mean	SD
Py-pro	3.26	5.62	6.53	6.48
Py-retro	3.56	6.11	7.12	7.03

Association between the prospective and retrospective method

In Figure 1, py-retro is plotted against py-pro at the age of 36. Spearman's rank correlation coefficient is 0.83 when the subjects who scored zero on both py-pro and py-retro were left out of the analysis.

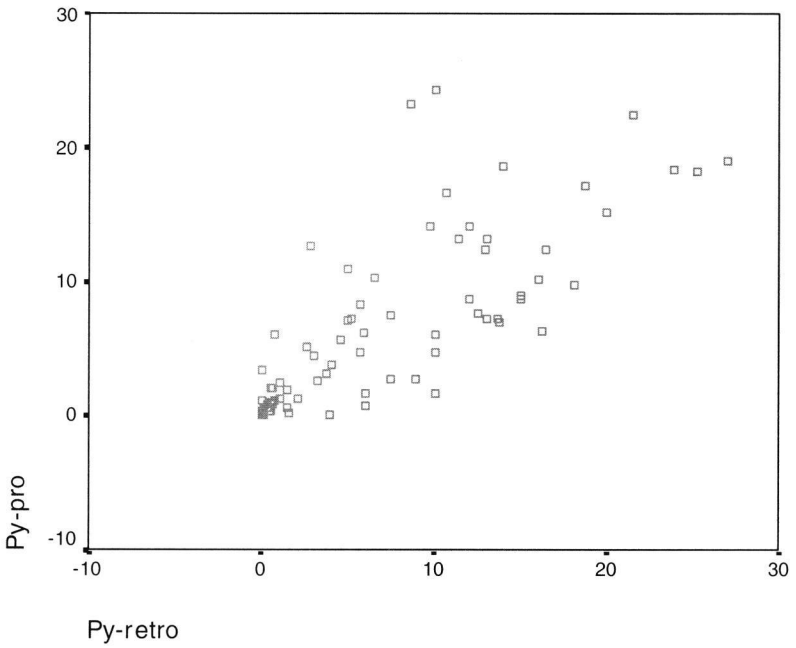

Figure 1. Prospectively calculated pack-years (py-pro) plotted against retrospectively calculated pack-years (py-retro).

Agreement between the prospective and retrospective method

Before calculating agreement between py-pro and py-retro, the individual differences between py-pro and py-retro were plotted against the average of py-pro and py-retro according to Bland & Altman.[11] The difference between py-pro and py-retro is dependent on the average of py-pro and py-retro. The higher the average of py-pro and py-retro, the larger the difference between py-pro and py-retro (Figure 2).

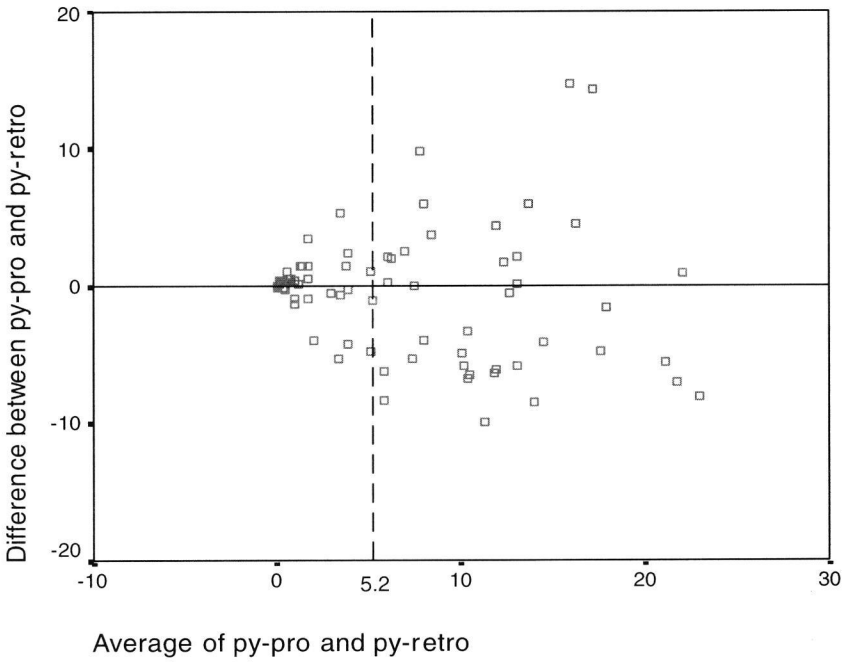

Figure 2. Individual differences between prospectively calculated pack-years (py-pro) and retrospectively calculated pack-years (py-retro), plotted against the average of py-pro and py-retro. The dotted line indicates the median of the average scores of py-pro and py-retro (= 5.2).

To make the difference between py-pro and py-retro less dependent on the average of py-pro and py-retro, the agreement between py-pro and py-retro and its 95% limits of agreement was calculated separately for subjects below and above the median of the average scores of py-pro and py-retro, like in Stensland-Bugge *et al.*[13] The 2.5th and 97th percentile were used to calculate the 95% limits of agreement because the data were not normally distributed, even after splitting the average of py-pro and py-retro into two groups. When subjects who scored zero on both py-pro

and py-retro were excluded from the analysis, the median of the average scores of py-pro and py-retro was 5.2 pack-years. The average differences between py-pro and py-retro (and 95% limits of agreement) in the below median smokers was -0.039 [-5.23, 5.32] and -1.17 [-10.00, 14.65] in the above median smokers. Although py-pro and py-retro agree well on average, individual differences between py-pro and py-retro are high, especially in subjects whose lifetime tobacco exposure is 5.2 pack-years or higher. Finally, py-retro is not systematically lower or higher than py-pro. Instead, py-pro and py-retro are both equally distributed around difference = 0 (Figure 2).

Table 2 presents the distribution of subjects into three smoking groups based on the median number of py-pro and py-retro. The agreement between py-pro and py-retro is the highest subjects who had never smoked tobacco. Only six subjects were misclassified by py-retro. They were categorised into group 2 (i.e. 0< pack-years< 5.2) by py-pro instead of in group 1 (i.e. pack-years=0) by py-retro. Although agreement between py-pro and py-retro, when divided into three groups, is not perfect, Cohen's kappa was 0.79. This indicates a good agreement between the two methods to calculate pack-years.[12]

Table 2. Distribution of subjects into category 1 (pack-years = 0), category 2 (0 < pack-years < 5.7) and category 3 (pack-years ≥ 5.7) according to prospectively calculated pack-years (py-pro) and retrospectively calculated pack-years (py-retro).

		Py-retro			
		1	2	3	Total
Py-pro	1	77	2	0	79
	2	6	26	7	39
	3	0	5	31	36
Total		83	33	38	154

Effect of quitting and gender

Nine subjects from the AGAHLS who met the inclusion criteria, were classified as frequent quitters, since they quitted smoking five times or more between the moment that they started smoking regularly and the age of 36. The agreement between py-pro and py-retro was slightly lower in frequent quitters than in non-frequent quitters but not significantly. Mean difference between py-pro and py-retro (and 95% limits of agreement) was -2.40 [-8.32, 5.32] in frequent quitters and -0.36 [-8.86, 14.39] in non-frequent quitters (n=68). No difference between men and women was found in the agreement between py-pro and py-retro.

Discussion

The aim of the present study was to investigate the relative validity of py-retro, as an estimate of lifetime tobacco smoking, by calculating the agreement between py-pro and py-retro at the age of 36. Furthermore, the effect of frequent quitting and gender was investigated on the agreement between py-pro and py-retro.

The relative validity of py-retro was found to be moderate in comparison to py-pro. Although py-pro and py-retro agree well on average, the individual differences between py-pro and py-retro are large. Py-retro is not systematically lower or higher than py-pro, which suggests that py-retro does not over- or underestimate lifetime tobacco smoking. In frequent quitters py-retro tended to be slightly higher than py-pro, but not significantly. Gender did not affect mean difference between py-pro and py-retro. The misclassification error in categorised py-retro was smaller than the quantitative error in continuous py-retro.

To our knowledge, this is the first study in which prospectively calculated pack-years were compared with retrospectively calculated pack-years. Nevertheless, a few studies investigated the accuracy of retrospective information on cigarette smoking from one point of time by comparing the retrospective information with the original information.[10,14,15] Accuracy was found to be dependent on smoking status at the follow-up measurement. Subjects who had increased cigarette consumption since the first measurement, were inclined to overestimate previous tobacco consumption whereas subjects who had decreased cigarette consumption tended to underestimate previous tobacco consumption.[10,14] In the study of Lee *et al.*,[15] on the other hand, cigarette consumption of eleven years ago was overestimated despite the fact that cigarette consumption had decreased between the baseline measurement and the follow-up measurement. In the AGAHLS, we did not study the effect of increasing or decreasing tobacco smoking on the difference between py-pro and py-retro. Since we measured our subjects nine times over a period of 23 years, subjects could increase smoking in one period and decrease it in another period. Instead, we investigated the effect of frequent quitting on the agreement between py-pro and py-retro and found frequent quitters (i.e. ≥ five attempts) to slightly overestimate py-retro.

In accordance with the study of Krall *et al.*,[14] gender did not influence the agreement between py-pro and py-retro. Persson and Norell,[10] on the other hand, found that men who increased their cigarette use in a six-year period, were more inclined to overestimate their previous cigarette use than women did. However, when interpreting our results, one should be aware of the low number of subjects included in the analyses, especially in the stratified analyses.

A shortcoming in the studies of Krall *et al.*[14], Persson & Norell[10] and Lee *et al.*[15] is that they asked their subjects to recall smoking habits from only one point of time.

To study the health effects of smoking, epidemiologists are primarily interested in *lifetime* tobacco smoking instead of tobacco smoking at one point of time.

The agreement between prospectively calculated pack-years and retrospectively calculated pack-years was found to be dependent on the number of pack-years. The higher the number of pack-years, the lower the agreement between py-pro and py-retro. This finding can be explained in two ways: Firstly, the smoking history to recall is probably large in case the number of high pack-years is high, which increases the risk of making mistakes in the process of recalling. Secondly, because of the large smoking history in subjects with a high number of pack-years, a mistake in tobacco exposure will always be multiplied by a large smoking duration (Pack-years = smoking duration × tobacco exposure). For instance, when a subject reports to have smoked 10 cigarettes per day instead of 12 cigarettes per day, the mistake of 2 cigarettes a day will be multiplied by a large number of smoking years. When the number of pack-years is low, on the contrary, a mistake in 2 cigarettes a day will only be multiplied by a few smoking years.

The individual differences between prospectively calculated pack-years and retrospectively calculated pack-years are high, especially in subjects with a lifetime exposure to tobacco smoking of 5.2 pack-years and higher. The first explanation for the moderate agreement between py-pro and py-retro is the fact that subjects who scored zero on both py-pro and py-retro were excluded from analyses. By excluding these subjects, agreement between the two methods to calculate pack-years appear lower than they really are because one excludes a group with a perfect agreement. When the subjects who scored zero on both py-pro and py-retro were included in the analyses, the mean difference (and 95% limits of agreement) in subjects with a lifetime exposure to tobacco smoking below the median became -0.013 [-4.33, 2.50] instead of -0.039 [-5.23, 5.32]. As pack-years are often categorised into groups (e.g. no exposure, low exposure and high exposure), we also calculated Cohen's kappa after categorisation of py-pro and py-retro. The largest advantage of this method is the possibility to include subjects who scored zero on both py-pro and py-retro because 'pack-years = 0' was one of the categories. As expected, the agreement between categorised py-pro and categorised py-retro was fairly good (Cohen's kappa = 0.79). Categorised py-retro can therefore be considered as a valid estimate of lifetime tobacco smoking. However, although the misclassification error in categorised pack-years is smaller than the quantitative error in continuous pack-years, turning a continuous variable into a categorised variable coincides with a considerable loss of information. As a consequence, continuous pack-years might still be more useful than categorised pack-years. The second explanation for the moderate agreement between py-pro and py-retro is the prospective way of calculating pack-years. Although measuring pack-years prospectively is the most accurate way of estimating

lifetime tobacco smoking, it is not the gold standard. Smoking behaviour can change dramatically in short periods of time, which makes it is difficult to measure prospectively, especially during adolescence.[7] Although tobacco smoking was studied every year between calendar age 13 and 16,[8,16] the measurements in the AGAHLS were performed less frequently after the age of 16. This could have reduced the accuracy of py-pro, especially between calendar age 16 and 21, when the prevalence of smoking rose from 21% to 34%. To cope with the problem of less frequent measurements during adulthood, the AGAHLS included questions about quitting attempts in the recent past. Questions about quitting attempts in the recent past reintroduce the problem of recall bias due to the nature of retrospective measurements. Nevertheless, the recall periods in the prospective method to calculate pack-years cover a much smaller period of time than the recall period in the retrospective method to calculate pack-years, which can cover ones complete smoking history of maximally 23 years. Therefore, we assumed py-pro to be a fairly good approximation of the true lifetime tobacco smoking.

A possible limitation of our study is the fact that all our subjects were recruited from the same school. Furthermore, the question rises whether or not the validity of py-retro is dependent on the compliance of subjects. We only included compliant subjects into our analyses, which is a small percentage of the total number of subjects. However, we needed to do so because many observations are needed to calculate py-pro.

Future researchers in the field of smoking should be aware of the moderate relative validity of py-retro. A possible solution is to categorise pack-years into smoking groups. Categorising py-retro into smoking groups, results in a misclassification error that is smaller than the quantitative error in continuous py-retro, but goes together with a loss of information.

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Appendix 1

Questionnaire 2000

Cigarettes

1. Do you smoke cigarettes? *If yes*
2. How many cigarettes do you smoke each day?
3. During how many years have you smoked this number of cigarettes each day?
4. Before that time, I did not smoke [ ]
   I smoked.....cigarettes a day

5. Do you smoke own-rolled cigarettes? *If yes*
6. How many packages of tobacco (40 grams) do you use each week?
7. During how many years have you used this number of packages each week?
8. Before that time, I did not smoke [ ]
   I smoked.....packages of own-rolled tobacco a week

9. Do you smoke cigars or cigarillos? *If yes*
10. How many cigars/cigarillos do you smoke each week?
11. During how many years have you smoked this number of cigars/cigarillos each week?
12. Before that time, I did not smoke [ ]
    I smoked .....cigars/cigarillos a week

Pipe tobacco

13. Do you smoke pipe tobacco? *If yes*
14. How many packages of pipe tobacco (50 grams) do you use each week?
15. During how many years have you smoked this number of packages each week?
16. Before that time, I did not smoke [ ]
    I smoked .....packages of pipe tobacco a week

17. How old were you when you first started smoking?.....
18. Have you ever tried to quit smoking from the moment you started to smoke regularly? Yes [ ] No [ ]

If yes,

18. How many times did you quit smoking? ..... times
19. How long did these attempt last on average? .....

20. How old were you when you tried to quit smoking for the first time?..... years
21. How old were you when you tried to quit smoking for the last time?.....years

Non-smokers

1. Have you ever smoked? Yes [ ]
No [ ]

2. How many cigarettes did you smoke each day on average? .....
How many years did you smoke this number of cigarettes?.....years

3. How many packages of own-rolled tobacco did you smoke each week on average?
.....packages
How many years did you smoke this number of packages?.....years
4. How many cigars/cigarillos did you smoke each week on average?.....
How many years did you smoke this number of cigars/cigarillos on average?
.....years.
5. How many packages of pipe tobacco did you smoke each week on average?
..... packages.
How many years did you smoke this number of packages?.....years

Before that time, I did not smoke [ ]
I smoked.....cigarettes a day
.....packages of own-rolled tobacco each week
.....cigars/cigarillos each week
.....packages of pipe tobacco each week

6. How old were you when you first started smoking ..... years

7. How old were you when you quit smoking for the last time?
.....years
8. How many times did you quit before?.....
9. How long did these attempts last on average?.....

Chapter 4

Smoking behaviour and biological maturation in males and females; a twenty year longitudinal study

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Abstract

Objectives. 1. Describe the longitudinal smoking behaviour of boys and girls during adolescence in relation to calendar age, skeletal age, years from peak height velocity (PHV) and years from menarche (in girls). 2 & 3. Investigate timing of biological maturation (early or late maturation) in relation to smoking behaviour in adolescence and in adulthood (i.e. calendar age 32/33). We hypothesised skeletal age, years from PHV and years from menarche to be better predictors of smoking than calendar age.
Methods and procedures. This study is part of the Amsterdam Growth and Health Longitudinal Study (AGAHLS) that was started in 1977 with 619 pupils from two secondary schools (mean age 13.0 SD 0.6). Smoking behaviour was assessed four times between 1977 and 1980 and once in 1996/1997. Calendar age and skeletal age were measured annually whereas height and menarche were measured every four months. Maturation rate (skeletal age *minus* calendar age), age at PHV and age at menarche were used to estimate timing of biological maturation. Generalized Estimating Equation (GEE) analysis was used to study maturation rate in relation to smoking during adolescence whereas logistic regression analyses were used to study mean maturation rate, years from PHV and years from menarche in relation to smoking in adulthood.
Results and conclusions. Skeletal age, years from PHV and years from menarche are no better predictors of smoking during adolescence than calendar age. The prevalence of smoking rises gradually with the increase in all four estimates of biological maturation. Timing of biological maturation was positively related to smoking but only at calendar age 13 (O.R.=3.34, 95% C.I. 1.58-7.07). None of the three measures to estimate timing of biological maturation was significantly related to smoking status in adulthood.

Introduction

Smoking is a lifestyle factor that is strongly associated with certain forms of cancer [1-4] and cardiovascular disease. [3,5,6] Yet, the prevalence of smoking in the Netherlands is 34% and has not declined in the past ten years.[7] It is the adolescent period in which children are extremely inclined to start smoking. A rapid increase in the prevalence of smoking during adolescence was found by Wilson *et al.*,[8] Chen and Kandel,[9] Jarallah *et al.*,[10] and Lloyd *et al.*[11] Stressful events such as disagreements with parents, study pressure, concern about their future, mixing with members of the opposite sex might contribute to this rapid increase.[12]

Most research on smoking has focused on calendar age and grade in school as predictors of smoking onset.[13] However, due to differences in the rate of pubertal progression during adolescence, children of the same calendar age can differ in biological maturation.[14] Early puberty was found to be associated with risk taking behaviour in girls,[15] more substance use in girls (i.e. cigarettes, alcohol, marijuana),[16] and with earlier onset of smoking in both boys[17] and girls.[8,15,17] Calendar age is considered to be only a rough estimate of biological maturation whereas skeletal age, years from peak height velocity (PHV) and years from menarche are considered to be more precise estimates of biological maturation. As a consequence, we expect skeletal age, years from PHV and years from menarche to be better predictors of smoking during adolescence than calendar age.

Although timing of biological maturation (early or late maturation) has been found to be related to the initiation of smoking behaviour in early adolescence,[15,16] little is known about the relation between timing of biological maturation and smoking behaviour in late adolescence and in adulthood. In the Amsterdam Growth and Health Longitudinal Study (AGAHLS), smoking behaviour was studied longitudinally between calendar age 13 and 32/33. As a consequence, we have the unique possibility to study timing of biological maturation in relation to smoking in both early and late adolescence and also to predict whether or not timing of biological maturation could predict smoking status (smoking or non-smoking) in adulthood (i.e. at calendar age 32/33).

Most studies on the relation between smoking and biological maturation have concentrated on either boys or girls.[8,15,18] In the present study, data of boys and girls are available separately. This makes it possible to study the role of gender in the relation between biological maturation and smoking.
The aim of the present study is to investigate:

1. The longitudinal development of smoking behaviour in relation to the longitudinal development of calendar age, skeletal age, years from PHV and years from menarche and to study whether or not skeletal age, years from PHV

and years from menarche are better predictors of smoking during adolescence than calendar age;
2. Timing of biological maturation in relation to smoking behaviour during adolescence; and
3. Timing of biological maturation in relation to smoking status in adulthood.

Methods

Subjects

Data of the present study come from the AGAHLS. The AGAHLS was started in 1977 to describe the course of physical and mental development of teenagers.[14] Boys and girls from the first and second class (calendar age 13 to 15) of two different secondary schools in Amsterdam and its suburbs participated in this study (n=619). Longitudinal measurements on biological maturation and smoking behaviour were performed in four consecutive years between 1977 and 1980. However, in subjects from suburban Amsterdam smoking behaviour was not assessed every year. Each year, smoking behaviour was measured in merely a quarter of these subjects, resulting in one smoking measurement per subject between 1977 and 1980. Smoking behaviour of subjects from Amsterdam, on the contrary, was assessed four times between 1977 and 1980 (figure 1). Nevertheless, in both groups height and menarche were measured every four months. In 1996/1997, when the subjects were 32/33 years old, smoking behaviour was assessed again in 429 of the subjects.

Socio-economic background

In the first year of the study the parents of the pupils filled out a questionnaire concerning their level of profession, their level of education and their family income. No difference in non-response rate was found between the two schools. We compared the results from the AGAHLS with the results from a representative sample of the Dutch population. The level of occupation, education and income was higher in Amsterdam and its suburbs than the average for Dutch families in general.[14]

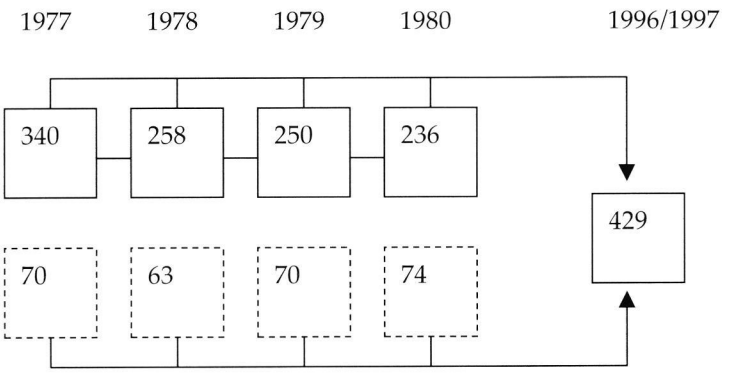

Figure 1. The number of subjects participating in the Amsterdam Growth and Health Longitudinal Study in 1977, 1978, 1979, 1980 and 1996/1997. The upper four boxes (solid lines) present the subjects from Amsterdam, who were measured every year between 1977 and 1980. Note that also in this group some subjects missed one or more measurements. The lower four boxes (dashed lines) present the subjects from suburban Amsterdam, who were measured in only one of the four years.

Smoking

Between January and June of each measurement year, the children were asked in a confidential interview whether or not they smoked cigarettes. At calendar age 32/33 the subjects filled out a questionnaire about their smoking behaviour. Subjects were counted as smokers if they smoked minimally one cigarette a week.

Estimates of biological maturation

1. Calendar age

Calendar age is defined as the age since birth and is considered to be a rough estimate of biological maturation. As our subjects were not selected on the basis of calendar age but on the basis of grade at school, they differed in calendar age between 12 and 15 at the onset of the AGAHLS (1977) and between 15 and 18 at the end of the adolescence period (1980). Calendar age was calculated in same month (September) as the month in which the skeletal age measurements were performed.

2. Skeletal age

Between 1977 and 1980, skeletal age was measured annually from X-ray photographs of the left hand, according to the Tanner-Whitehouse II method,[19] in both boys and girls. Ratings of 20 bones of hand and wrist were assigned by comparing the

ossification stage of each bone with plates, diagrams and descriptions of the bone in question. All X-ray photographs were rated by the same examiner, who had been trained at the Institute of Child Health, University of London, to use the Tanner-Whitehouse II method. When full maturity was reached (skeletal age 16 in girls and skeletal age 18 in boys) skeletal age measurements were discontinued. Note that discontinuing skeletal age measurements did not lead to missing values. Instead, skeletal age was known to be 16 years in girls and 18 years in boys. As a result, a girl could have a skeletal age of 14 in the first year of measurement, and a skeletal age of 16 in the second, third and fourth year of measurement. In the same way a boy could have a skeletal age of 18 for more than one year.

3. Years from peak height velocity

Years from peak height velocity (PHV) was also used as an indicator of biological maturation. To calculate years from PHV, age at PHV was estimated by measuring height every four months between 1977 and 1980. A second degree moving polynomial function analysis was used to determine growth velocity and to establish PHV.[20] A criterion of 5.5 cm per year was used to indicate PHV in boys. In girls a criterion of 3.5 cm per year was used. When this criterion was not met, it was assumed that PHV had been reached before or after the measurement period of four years. It was not possible to estimate PHV in these cases. Even when the criterion had been met, it was not always possible to estimate PHV with certainty (e.g. when the first growth velocity measurement was part of a downward trend or when the last growth velocity measurement was part of an upward trend). Whereas PHV had been estimated with certainty in 155 boys, PHV had only been estimated with certainty in 94 girls. Only the PHV measurements that could be estimated with certainty were included in the analyses.

4. Years from menarche

In order to calculate years from menarche, age at menarche was obtained prospectively. Every four months between 1977 and 1980, the girls were asked if they had experienced menarche in the last four months. In this way age at menarche was elicited rather accurately. If menarche had already occurred before the first measurement, the recall method was used, in which the girls had to recall the date of menarche as exactly as possible.

Timing of biological maturation

Timing of biological maturation was used to study the effect of early or late maturation on smoking behaviour.

Smoking during adolescence

To study timing of biological maturation in relation to smoking during adolescence we used 'maturation rate' as an estimate of biological maturation. Maturation rate was calculated each measurement year between 1977 and 1980 by subtracting calendar age from skeletal age (skeletal age *minus* calendar age). Early maturers had positive scores on maturation rate, whereas late maturers had negative scores on maturation rate.

Smoking status in adulthood

To study timing of biological maturation in relation to smoking status in adulthood, three different estimates of biological maturation were used:

1. Age at menarche
2. Age at PHV
3. Mean maturation rate calculated over the adolescent period by the following formula:

$$\left[\sum_{t=1}^{N} (\text{skeletal age} - \text{calendar age})\right]/n$$

n = number of measurements, t = timepoints

Mean maturation rate was based on between one and four maturation rate measurements.

Statistical analyses

Smoking in relation to four different indicators of biological maturation

Smoking behaviour was studied in relation to 1) calendar age, 2) skeletal age, 3) years from PHV and 4) years from menarche (only in girls). To analyse the relation between the longitudinal development of smoking behaviour and the longitudinal development of biological maturation, Generalized Estimating Equation (GEE) analysis with a logistic link was performed. This was done for all four indicators of biological maturation and for boys and girls separately. With GEE analysis the relation between two longitudinally measured variables can be studied using all longitudinal data simultaneously and correcting for differences in time interval between the measurement periods and also for within person correlations caused by the repeated measurements on each subject.[21] In addition, Pearson correlation coefficients were calculated between a) calendar age and skeletal age and b) age at PHV and age at menarche.

Timing of biological maturation in relation to smoking during adolescence

The relation between maturation rate and smoking behaviour during adolescence was estimated with GEE regression analysis with a logistic link. As maturation rate is not a constant variable like age at menarche and age at PHV, data on maturation rate and smoking from 1977, 1978, 1979 and 1980 were all analysed simultaneously to investigate the longitudinal development of maturation rate in relation to longitudinal smoking behaviour between calendar age 13 and 16. To study the relation between maturation rate and smoking at separate calendar ages (13, 14, 15, and 16) logistic regression analyses between maturation rate and smoking were performed in which only data from specific ages were included.

Timing of biological maturation in relation to smoking status in adulthood

Logistic regression analyses were performed to study mean maturation during adolescence, age at PHV and age at menarche in relation to smoking status at calendar age 32/33. Data of men and women were analysed separately.

Missing data

Not all subjects were measured during all five measurement periods (1977, 1978, 1979, 1980 and 1996/1997). The main reasons for missing data were:

1) Living in suburban Amsterdam;
2) Reaching full maturity before 1980; and
3) Changing schools.

Dropout effect

It was impossible to study the difference in smoking prevalence between dropouts and non-dropouts because most subjects dropped out in the first year of the AGAHLS when only a few subjects had started smoking. Therefore, we defined dropouts in a different way. Subjects were called dropouts when:

1) They lived in Amsterdam and missed two or more measurements between 1977 and 1996;
2) They lived in suburban Amsterdam and missed one measurement between 1977 and 1996/1997.

Odds ratios (95% C.I.) were calculated to investigate the difference in chance of being a smoker between the dropouts and the non-dropouts at all five measurement periods. Logistic regression analyses were performed to study the relation between smoking and dropping out of the study.

Results

Dropout effect

The prevalence of smoking was identical among the dropouts and the non-dropouts in 1977, 1979, 1980 and 1996/1997. Yet, in 1978 the prevalence of smoking was 22.5% among the dropouts and 8.2% among the non-dropouts resulting in an O.R of 3.3 [1.4 – 7.7] (table 1).

Table 1. Prevalence of smoking among the dropouts and the non-dropouts at all years of measurement. Odds ratios (O.R.) and 95% confidence intervals (95% C.I.) present the chance to be a smoker among the dropouts in comparison to the chance to be a smoker among the non-dropouts.

Year of measure-ment	Non-dropouts			Dropouts			O.R.	95% C.I.
	n total	n smokers	Prevalence of smoking (%)	n total	n smokers	Prevalence of smoking (%)		
1977	294	7	2.4	116	4	3.4	1.5	0.4 - 5.1
1978	281	23	8.2	40	9	22.5	3.3	1.4 - 7.7
1979	298	50	16.8	22	4	18.2	1.1	0.4 - 3.4
1980	277	58	20.9	33	7	21.2	1.0	0.4 - 2.5
1996/1997	363	76	20.9	64	16	25.0	1.3	0.7 - 2.3

Smoking in relation to four different indicators of biological maturation

Boys

The prevalence of smoking in boys increased gradually from 4.6% at calendar age 13 to 22.5% at calendar age 17 (figure 2A). Skeletal age (figure 2B) and calendar age (figure 2A) were related rather similarly to the prevalence of smoking. Nevertheless, a decrease in the prevalence of smoking was found at skeletal age 18 whereas no decrease in the prevalence of smoking was found in the relation between calendar age and smoking.

Figure 2C illustrates the relation between smoking behaviour and years from PHV. Mean calendar age at PHV was 14.2 SD 0.7. Before reaching PHV, none of the boys smoked. The prevalence of smoking was 4.9% in the year PHV was reached and levelled off to 16.5% two years after PHV and to 17.2% three years after PHV.

Girls

The prevalence of smoking rises gradually with increasing calendar age (figure 3A) as well as with increasing skeletal age (figure 3B). Most striking is the rapid increase in prevalence of smoking between calendar age 15 and 16 (from 14.1% to 29.5%) in contrast to the modest increase in prevalence of smoking between skeletal age 15 and 16 (18.8% to 23.4%). In addition, the prevalence of smoking at calendar age 17 is much higher than the prevalence of smoking at the end of the other maturational scales. The relation between smoking and years from PHV (figure 3C) is largely identical to the relation between smoking and years from menarche (figure 3D)

Gender

Between calendar age 12 and calendar age 15, boys and girls showed a similar increase in prevalence of smoking. Nevertheless, after calendar age 15 the prevalence of smoking rose sharply in girls (figure 3A) whereas it kept increasing gradually in boys (figure 2A). Three years after PHV the prevalence of smoking levelled off in boys (figure 2C). This levelling off was not found in girls; neither three years after PHV (figure 3C), nor three years after menarche (figure 3D).

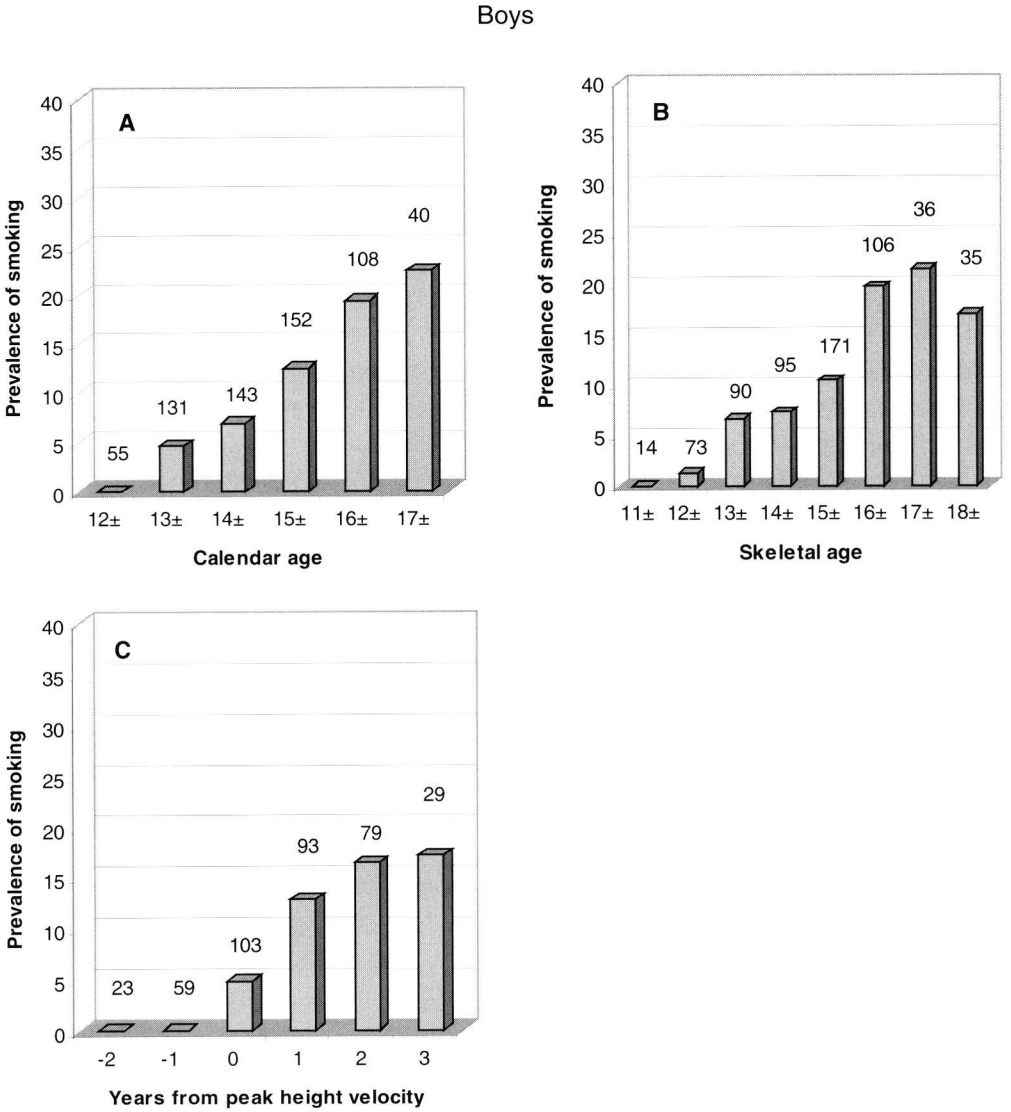

Figure 2. Prevalence of smoking in relation to calendar age (A), skeletal age (B) and years from peak height velocity (C) in boys. The results at calendar age 18 are not presented in figure A because only five boys were measured at this age. The number of participating subjects is presented above the bars.
± indicates a range from –0.5 to + 0.5

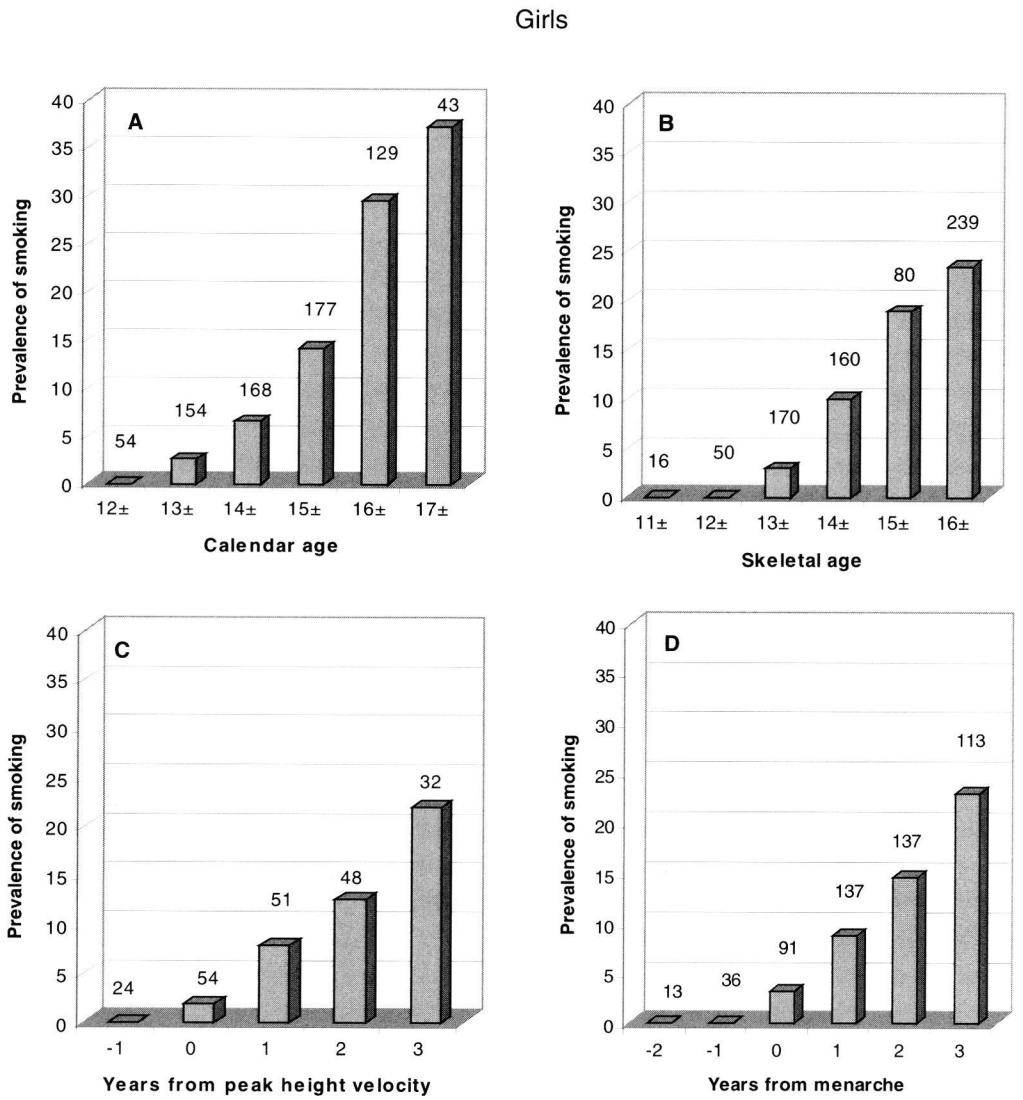

Figure 3. Prevalence of smoking in relation to calendar age (A), skeletal age (B) and years from peak height velocity (C) and years from menarche (D) in girls. The results at calendar age 18 are not presented because only two girls were measured at this age. The number of participating subjects is presented above the bars.
± indicates a range from –0.5 to + 0.5

Timing of biological maturation in relation to smoking during adolescence

When data on maturation rate and smoking from 1977, 1978, 1979 and 1980 were analysed together, no significant association was found between maturation rate and smoking (O.R. 1.0, 95% C.I. 0.8-1.3). Yet, a significant interaction between time and maturation rate was found (p=0.035), indicating that the relation between maturation rate and smoking is time dependent. Logistic regression analyses at separate calendar ages, showed a significant positive association between maturation rate and smoking, but only at calendar age 13 (O.R. 3.34, 95% C.I. 1.58-7.07). Of the 10 smokers between calendar age 12.5 and 13.5, nine were normal to early maturers whereas only one was a late maturer (figure 4). In non-smokers, on the other hand, the distribution of early and late maturers was equal at all calendar ages (figure 5).

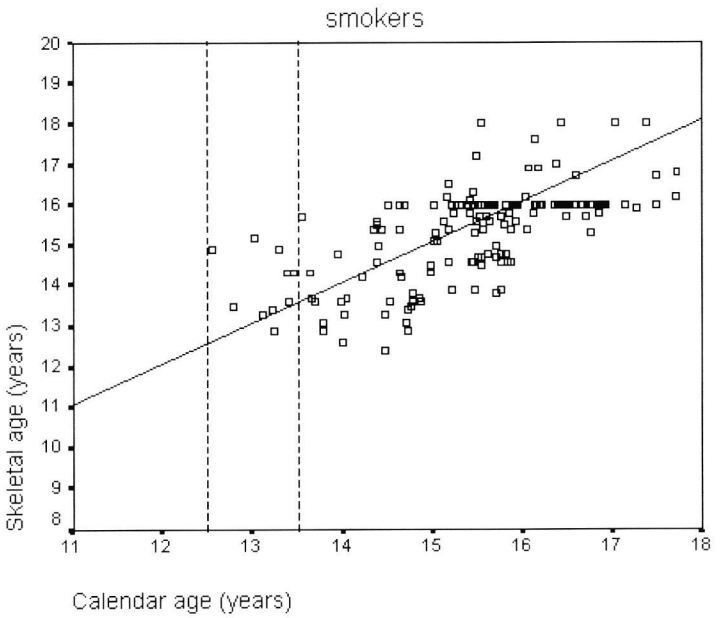

Figure 4. Scatterplot between skeletal age and calendar age in smokers. At the solid line, calendar age equals skeletal age. Points above the solid line represent early maturers and points beneath the solid line represent late maturers. The points between the vertical dashed lines represent the 10 smokers between calendar age 12.5 and 13.5 of whom nine are early maturers and only one is a late maturer.

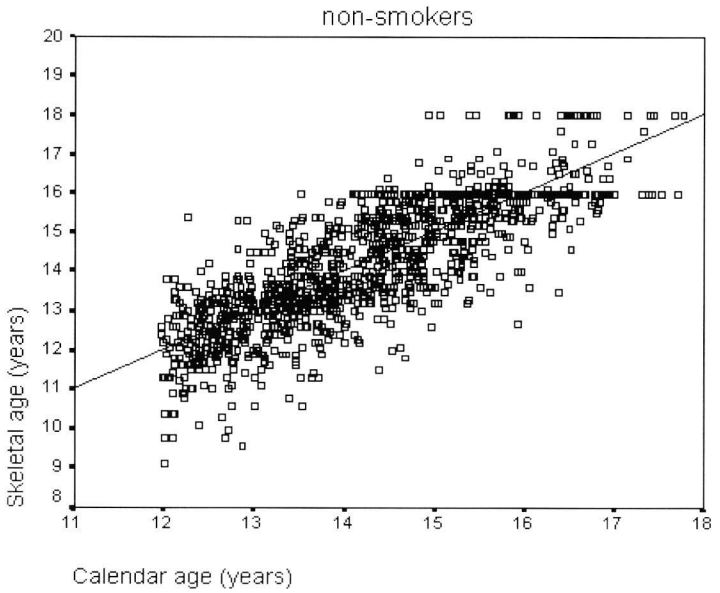

Figure 5. Scatterplot between skeletal age and calendar age in non-smokers. At the solid line, calendar age equals skeletal age. Points above the solid line represent early maturers and points beneath the solid line represent late maturers. The points between the vertical dashed lines represent the non-smokers between calendar age 12.5 and 13.5.

Timing of biological maturation in relation to smoking in adulthood

None of the three measures to estimate timing of biological maturation was significantly related to smoking status in adulthood (table 3). Although not significant, odds ratios in the relation between age at PHV and smoking status at calendar age 32/33 indicate that the higher age at PHV, the higher the chance to be a smoker at calendar age 32/33.

Table 3. Odds ratios (O.R.) and 95% confidence intervals (C.I.) from logistic regression analyses between smoking behaviour in adulthood and 1) mean maturation rate, 2) age at PHV and 3) age at menarche.

	Men			Women		
	O.R.	C.I.	n	O.R.	C.I.	n
Mean maturation rate	0.9	0.6 - 1.2	198	0.9	0.6 - 1.3	231
Age at PHV	1.4	0.8 - 2.7	103	2.4	0.8 - 7.4	74
Age at menarche	-	-		1.0	0.7 - 1.4	183

Discussion

In the present study the development of calendar age, skeletal age, years from PHV and years from menarche was studied in relation to the development of smoking behaviour. In addition, we studied whether or not skeletal age, years from PHV and years from menarche are better predictors op smoking during adolescence than calendar age. Finally, timing of biological maturation was studied in relation to smoking during adolescence and smoking status in adulthood.

The development of biological maturation in relation to the development of smoking behaviour

In both boys and girls, the prevalence of smoking was found to increase gradually with increasing calendar age. This is in accordance with several cross-sectional studies that investigated smoking behaviour in different age groups,[10,22,23] but also with some longitudinal studies in which smoking behaviour was measured repeatedly in the same cohort.[8,24] In contrast to our expectations, calendar age was similarly related to smoking during adolescence than skeletal age, years from PHV and age at menarche. The only difference was the higher prevalence of smoking in girls at calendar age 17 compared to the prevalence of smoking in girls at the end of the other maturational scales. This can be explained by the fact that some biological mature subjects had a relatively low calendar age, which reduced their chance to smoke. The large similarity between calendar age and skeletal age in relation to smoking can be explained by the high correlation between calendar age and skeletal age.

Timing of biological maturation in relation to smoking during adolescence

In several studies an earlier onset of smoking has been found in early maturing children.[8,15,17] This can be explained by a difference in environment between the early and late maturers. An early maturing girl will probably be perceived as an older girl than her peers. As a result she will have older friends and come into contact with substance use at an earlier age than the other girls of her age.[15] In the present study the effect of early maturation on smoking behaviour was only found at calendar age 13. An early maturing child at calendar age 13 had a higher chance to be a smoker at calendar age 13 than an average and late maturing child. In most studies, biological maturation is studied in relation to the onset of smoking, usually taking place in young adolescence (i.e. calendar age 12/13). As a consequence, little is known about biological maturation in relation to smoking behaviour in late adolescence (i.e. calendar ages 15, 16 and 17). Nevertheless, Byckling and Tauri[22] studied sexual maturation and smoking behaviour in 15-year-old boys and girls. The percentage of daily smokers was identical in the early and late maturing girls.

In early maturing boys, the percentage of daily smokers was slightly higher in comparison to the late maturing boys (14% vs. 7%). Harrell *et al.*[17] studied smoking behaviour in relation to pubertal stage by measuring both variables five times between calendar age 9 and 14. A positive relation between levels of pubertal progression and smoking behaviour was found in all five measurement periods. The results of Harrell *et al.*[17] at calendar age 13 are in agreement with our results but differ at calendar age 14. This difference might be explained by the fact that the 14-year-olds in the present study were actually somewhat older. Calendar ages were calculated in September, whereas smoking behaviour was measured three to nine months later. Another explanation might be that the subjects in the study of Harrell *et al.*[17] rated themselves on growth spurt, pubic hear etc., whereas in the present study maturation rate was calculated by subtracting calendar age from skeletal age at all measurement periods.

The present study suggests that maturation rate is only related to smoking behaviour in early adolescence, when children start smoking their first cigarette (around calendar age 13). This is in accordance with the study of Wilson *et al.*[8] where early mature girls started smoking at mean calendar age 12.8 years and late mature girls at mean calendar age 13.4.

Timing of biological maturation in relation to smoking in adulthood

None of the three measures to estimate timing of biological maturation could predict smoking status at calendar age 32/33. Nevertheless, a trend indicated that the higher age at PHV, the higher the chance to smoke at calendar age 32/33. This is in contradiction with the results at calendar age 13, where the early maturers had a higher chance to smoke instead of the late maturers. However, the 13-year-old smokers did not remain smokers in our study. Ten 13-year-olds reported smoking in our study and only seven of these subjects were remeasured at calendar age 32/33. Of these seven subjects only two were still smoking at calendar age 32/33. It seems as if maturation rate can predict smoking at calendar age 13 but whether or not timing of biological maturation can predict smoking status in adulthood is still unclear.

Study limitations

While performing this study we had to deal with the following difficulties:

1. There is a time-lag between the four estimates of biological maturation and smoking behaviour. Smoking behaviour was measured between January and June, whereas skeletal age and calendar age were estimated in September. When PHV or menarche occurred at calendar age 13.6 it was coupled with smoking behaviour at the nearest calendar age (in this case calendar age 14). The lag between the smoking measurements and the biological maturation measurements will have some influence

on the detectability of small differences between the four estimates of biological maturation and smoking behaviour. In the same way rounding up or down calendar ages and skeletal ages in the bar graphs will influence detectability of small differences.

2. Skeletal age could stay unchanged between two years because 1) girls reached full maturity at skeletal age 16 and boys at skeletal age 18 and 2) skeletal ages were rounded up or down to whole numbers. When a skeletal age of a subject remained the same over two or three measurement periods, his or her smoking behaviour was taken into account more than once at that particular skeletal age. This was important for those subjects that did not change in skeletal age but did change in smoking status. The disadvantage is that the results are not independent. The girls that were measured more than once at skeletal age 16 were the relatively early maturers. The early maturers were slightly more likely to smoke at skeletal age 16 than the late maturers, which could have led to a slight overestimation of the number of smokers at skeletal age 16.

3. In figure 3B the number of girls measured at skeletal age 15 was 80, whereas it was 160 at skeletal age 14 and 239 at skeletal age 16. This can be explained by the fact that the mean increase in skeletal age in the year after skeletal age 14 was 1.54 SD 0.55. As a consequence, a substantial number of girls went from skeletal age 14 directly to skeletal age 16.

4. The socio-economic background of our subjects was higher than the average for Dutch families in general. At the same time the prevalence of smoking was lower than in the general Dutch population at every calendar age. Although the high socio-economic background can be a reason for the relatively low prevalence of smoking in our subjects there is no reason to assume that relation between biological maturation and smoking is different in subjects with a high socio-economic back-ground.

5. A substantial number of subjects were excluded from the analysis between years from PHV and smoking because their age at PHV could not be estimated with certainty. Most of these subjects were early maturers who reached PHV before the AGAHLS started. However, whether or not this had any influence on our results cannot be examined because no smoking data is available from the moment these bjects reached PHV.

To conclude, skeletal age, years from PHV and years from menarche are no better predictors of smoking during adolescence than calendar age. The prevalence of smoking rises gradually with the increase in all four estimates of biological maturation. In accordance with other studies[8,15,17] early biological maturation increased the chance to smoke at calendar age 13 but it did not increase the chance to remain a smoker into late adolescence and adulthood (calendar age 32/33). On the

contrary, there was a non-significant trend indicating that late maturation, measured by age at PHV, increases the chance to smoke at calendar age 32/33.

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Chapter 5

A longitudinal study on smoking in relationship to fitness and heart rate response

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Abstract

Objectives. Smoking has been shown to be associated with impaired cardiovascular fitness and reduced heart rate response to exercise. It is not known whether these associations are present in adolescence and young adults and whether they change over time.

Methods. Maximal oxygen uptake ($VO2_{max}$), maximum treadmill slope ($Slope_{max}$), resting heart rate (HR_{rest}), heart rate at sub maximal exercise (HR_{submax}), heart rate reserve (HRR) and maximum heart rate (HR_{max}), were measured one to nine times between age 13 and 36 in 298 male and 334 female participants of the Amsterdam Growth and Health Longitudinal Study (AGAHLS). Generalized Estimating Equation (GEE) analyses were used to study the longitudinal relationship between smoking and cardiovascular fitness and heart rate response to exercise, whereas linear regression analyses were used to study the reversibility of smoking effects at age 36.

Results. Moderate to heavy smoking (≥ 10 g of tobacco/day) was longitudinally and negatively related to $VO2_{max}$, $Slope_{max}$, HR_{submax} and HR_{max}. With increasing age, the negative relationship between smoking and $VO2_{max}$, $Slope_{max}$ and HR_{max} became stronger in males. Cross-sectional analyses suggested that the adverse effects of smoking were reversible in 36-year-old males.

Conclusions. Cardiovascular fitness and heart rate response to exercise are already reduced in young healthy smokers. In men, the adverse effects of smoking become stronger with increasing age but appear to be reversible at age 36.

Introduction

Tobacco smoking has been associated with low cardiovascular fitness[1-3] and an impaired heart rate response to exercise (i.e. chronotropic incompetence),[4-6] which are both important predictors of all-cause mortality.[7-9]

The first shortcoming in almost all previous studies on smoking in relationship to cardiovascular fitness and heart rate response to exercise is that they have been conducted in studies with a cross-sectional design. With a cross-sectional design, however, nothing can be said about possible changes over time. It seems logical to assume that the adverse effects of smoking become stronger with increasing age, as it probably takes some time before detrimental effects of smoking on the cardiovascular system become measurable. The second shortcoming in previous studies on smoking and cardiovascular fitness and heart rate response to exercise is that only very few studies have included women[6,10] or subjects younger than 18 years.[11,12] From a public health perspective it is interesting to know to what extent young healthy smokers already show a reduced cardiovascular fitness and an impaired heart rate response to exercise. From this same perspective it is also interesting to know to what extent the effects of smoking are reversible.

In the Amsterdam Growth And Health Longitudinal Study (AGAHLS), smoking behaviour, cardiovascular fitness and heart rate response to exercise were measured one to nine times between age 13 and 36, in a cohort of 298 men and 331 women. This gave us the opportunity to study smoking in relation to cardiovascular fitness and heart rate response to exercise longitudinally. The reversibility of smoking was investigated by studying smoking status (i.e. never smoker, short-term ex-smoker, long-term ex-smoker and current smoker) in relation to cardiovascular fitness and heart rate response to exercise.

Methods

Subjects and design

All subjects were participants of the Amsterdam Growth And Health Longitudinal Study (AGAHLS) that started in 1977 with 298 boys and 334 girls from the first and second grade of two secondary schools in Amsterdam and Purmerend, The Netherlands.[13] Measurements were performed at calendar ages 13, 14, 15, 16, 22, 27, 29, 32 and 36. Although the number of measurements on each subject varied between one and nine measurements, all pupils were taken into account in the longitudinal analyses. Dropout analyses were performed to investigate whether or not tobacco consumption, fitness parameters, lifestyle parameters and body composition parameters at all measurements predicted dropout at the next measurement. No dropout effects were found between age 13 and 16.[14] From the age of 27 onwards, single dropout effects were found with HR_{rest}, body weight, $Slope_{max}$ and tobacco

consumption. These dropout effects were not consistent over time, were not found in both sexes and could be due to multiple testing. The AGAHLS was approved by the Medical Ethical Committee of the VU University Medical Center. Written informed consent was provided by the parents when the subjects were 13 to 16 years of age. At the ages 21 to 36, subjects provided written consent themselves. Subjects who reported to use β-blockers (n=5) were excluded from the analyses in that particular year of measurement.

Smoking

At each measurement year, subjects filled out a questionnaire about their use of cigarettes, own-rolled tobacco, cigars/cigarillos and pipe tobacco. From this data, tobacco smoking was expressed in total gram of tobacco smoked per week (1 cigarette = 1 gram, 1 package of own-rolled tobacco = 40 gram, 1 cigar/cigarillo = 3 gram, 1 package of pipe tobacco = 50 gram). Subjects were counted as smokers if they smoked minimally 1 gram of tobacco (i.e. 1 cigarette) per day. Each measurement year, subjects were categorized as non-smokers (< 1 gram tobacco per day), light smokers (1 ≤ gram tobacco per day < 10) or moderate to heavy smokers (≥ 10 gram tobacco per day). Categorizing tobacco consumption into smoking groups will enable us to investigate the linearity of the associations of interest and will yield easy interpretable results. Since the literature provides no clinically relevant cut-off value for tobacco consumption, we decided to use 10 g of tobacco per day as a cut-off value. This cut-off value has also been used by others[15] and yields (more or less) equal smoking groups in the present study. At the age of 36, subjects filled out an extensive questionnaire about tobacco smoking in the past in order to obtain a rough indication of lifetime tobacco smoking. Lifetime tobacco smoking was expressed in pack-years by multiplying smoking duration (starting at first onset) with daily tobacco consumption. One pack-year is defined as one packet, or 20 g tobacco, smoked each day over a course of one year.[16] At the age of 36, ex-smokers were defined as subjects smoking less than 1 gram of tobacco per day with pack-years > 0. We discriminated between short-term ex-smokers and long-term ex-smokers based on the reported years of abstinence. Ex-smokers who had quit smoking for more than four years were defined as long-term ex-smokers, whereas ex-smokers who had quit for four years or less were defined as short-term ex-smokers. The cut-off value of 4 years was chosen because there were four years between the measurements at age 32 and the measurements at age 36. Never smokers at the age of 36, were defined as subjects who had never smoked at least 7 gram of tobacco per day.

Pre-test smoking condition

All participants were requested no to smoke on the measurement day. Since all participants were not left unattended during the hour preceding the treadmill running, and none of the participants reported using a nicotine patch on the measurement day, we were able to rule out short-term effects of smoking.

Cardiovascular fitness heart rate response to exercise

Three different measures of cardiovascular fitness were used. The first of these measures was absolute maximal oxygen uptake ($VO2_{max}$), expressed in $l.min^{-1}$, which was measured directly with the Ergoanalyzer (Jaeger, Bunnik, The Netherlands) during a maximal treadmill running test.[17] This measurement of gas exchange was validated against the classical method of collecting expired air in Douglas bags and the Scholander technique to analyse the carbon dioxide and oxygen content of the expired air.[17] Absolute $VO2_{max}$ is considered as a valid estimate of true cardiovascular fitness, since it is highly correlated with cardiac output.[18,19] The slope of the treadmill was increased every two minutes until exhaustion while the running speed was kept constant at 8 $km.h^{-1}$. During the treadmill running test, the electrocardiogram (ECG) was monitored telemetrically and continuously and recorded at the end of each minute from the final 15 R-R intervals. The second measure of cardiovascular fitness was the maximum slope reached during the treadmill running test ($Slope_{max}$). The third measure of cardiovascular fitness was resting heart rate (HR_{rest}), measured in a seated position prior to the treadmill running test.

Heart rate response to exercise (i.e. chronotropic response) was measured during the treadmill running test by: 1) heart rate at a treadmill slope of 5% (HR_{submax}), 2) maximum heart rate (HR_{max}) and 3) heart rate reserve (HRR), the change in heart rate from rest to maximal exercise calculated by HR_{max}-HR_{rest}..

Statistical Analyses

To study smoking in relation to $VO2_{max}$, $Slope_{max}$, HR_{rest}, HR_{submax}, HR_{max} and HRR longitudinally, Generalized Estimating Equation (GEE) analyses[20] were carried out with the Statistical Package for Interactive Data Analysis (SPIDA).[21] In all the longitudinal analyses, light smokers and moderate to heavy smokers were compared with non-smokers. With GEE analysis, the relation between two longitudinally measured variables can be studied using all longitudinal data simultaneously and correcting for differences in time interval between measurement periods and for within person correlations caused by the repeated measurement on each subject.[22] To investigate whether or not the relationships between smoking and cardiovascular fitness and heart rate response to exercise were time dependent, we studied the interaction between smoking and time of measurement. Furthermore, with data from

all time points included in the longitudinal model, the difference in cardiovascular fitness between smoking groups at age 36 was predicted. This predicted difference was based on the longitudinal model to which interactions with time were added (assuming a linear interaction). The longitudinal analysis to predict differences between smoking groups at age 36 differs from a cross-sectional analysis, because data of all time points were used instead of only at one time point.
A simplification of the longitudinal model is presented below:

$$\text{Outcome variable}_t = \beta_0 + \beta_1 \times \text{smoking1}_t + \beta_2 \times \text{smoking2}_t + \beta_3 \times \text{time} + (\beta_4 \times \text{smoking1}_t \times \text{time}) + (\beta_5 \times \text{smoking2}_t \times \text{time})$$

$_t$ indicates that both the outcome variable and smoking status were time dependent
β_0 = intercept
β_1 = difference in outcome variable between light smokers and non-smokers at t= 0
β_2 = difference in outcome variable between moderate/heavy smokers and non-smokers at t=0
β_3 = change in outcome variable per year
β_4 = change in the difference in outcome variable between light smokers and non-smokers per year
β_5 = change in the difference in outcome variable between moderate to heavy smokers and non-smokers per year
smoking1 = light tobacco smoking, smoking2 = moderate to heavy tobacco smoking
time = time (or year) of measurement and varies between t = 0 (age 13 - 1) and t = 24 (age 36)

The expected difference in $VO2_{max}$ between light smokers and non-smokers at the age of 36 (i.e. t = 24), was calculated by $\beta_1 + (24 \times \beta_4)$. Similarly, the expected difference in $VO2_{max}$ between moderate to heavy smokers and non-smokers was calculated by $\beta_2 + (24 \times \beta_5)$.

To investigate the reversibility of smoking effects, linear regression analyses were used in which smoking status was categorized into never smokers, short-term ex-smokers, long-term ex-smokers or current smokers, and related to cardiovascular fitness and heart rate response to exercise in 36-year-old men and women. Never smokers were used as the reference group.

Covariables
All relationships were studied using an adjusted and unadjusted model. In the unadjusted model (model 1) we only included time of measurement. In the adjusted model (model 2) we corrected for body height, physical activity and body weight.

We expected physical activity to be an important confounder in the association between smoking and cardiovascular fitness and heart rate response to exercise, since physical activity tends to be associated with both smoking behaviour and physical fitness. In the relationship between smoking and HR_{submax}, $Slope_{max}$ was added to the covariables of the adjusted model, in order to take differences in exercise capacity into account. Daily physical activity was estimated by a structured physical activity interview that was specially developed for the AGAHLS and has been shown to yield quite valid estimates of daily physical activity.[23] Activities included organized sports activities, unorganised sports activities, leisure time activities, activities at school, work or during active transportation. Physical activities with a duration shorter than five minutes and activities with a metabolic intensity of less than four times resting metabolic rate (< 4 METs) were not taken into account. All other physical activities were categorized into moderate physical activity (4-7 METs), heavy physical activity (7-10 METs) and very heavy physical activity (>10 METs) and expressed in minutes per week. An average total weighted (metabolic) activity score was calculated and expressed as METs per week by combining duration, frequency and intensity (number of METs) of all physical activities in the previous three months.[24] The activity interview that was used at age 36 differed slightly from the interview that was used in earlier years in that it covered more activities.

Results

Table 1 presents the number of subjects that participated in the AGAHLS and the prevalence of smoking at each measurement year. Table 2 presents the characteristics of the participants at age 13 and 36. At the age of 36, 85 males and 89 females reported to have ever smoked tobacco (pack-years > 0). In these ever-smokers the median number of pack-years and range was 7.43 [0.02-45.00] in males and 5.71 [0.01-37.70] in females.

Longitudinal relationships

Smoking and cardiovascular fitness

$VO2_{max}$. In both men and women, a negative relationship was found between moderate to heavy smoking and $VO2_{max}$, which was stronger in men than in women (Table 3). The β regression coefficient in males from the adjusted longitudinal model indicates that $VO2_{max}$ of moderate to heavy smokers was overall (i.e. when data from all ages were included) 0.19 $l.min^{-1}$ lower than in non-smokers. $VO2_{max}$ of moderate to heavy smoking females was overall 0.08 $l.min^{-1}$ lower than in non-smokers. In addition, the significant negative interaction between smoking and time of measurement in men (Table 3), indicates that the negative association between smoking and $VO2_{max}$ became stronger with increasing age.

Table 1. Mean age, number of participants, and the prevalence of light tobacco smoking (L) and moderate to heavy tobacco smoking (M/H) at each measurement year.

		Males			Females		
			Smoking (%)			Smoking (%)	
Year	Mean age ± SD	N	L	M/H	N	L	M/H
1977	13.0 ± 0.6	195	0.5	0.5	215	0.9	0.0
1978	14.1 ± 0.7	148	4.1	1.4	172	1.7	1.1
1979	15.0 ± 0.6	152	9.2	2.0	168	9.5	3.6
1980	16.1 ± 0.7	140	6.4	6.4	172	16.5	3.5
1985	21.1 ± 0.8	93	8.7	23.9	107	11.3	23.6
1991	27.1 ± 0.8	84	10.7	23.8	98	10.2	13.3
1993	29.1 ± 0.7	77	11.7	20.8	88	9.1	10.2
1996	32.4 ± 0.9	197	10.9	11.5	230	5.7	11.4
2000	36.0 ± 0.7	178	8.5	12.5	200	8.5	9.0

SD = standard deviation

Table 2. Characteristics of participants at age 13 and age 36

	Males		Females	
	13	36	13	36
Body height (cm)	161.5 ± 8.6	183.8 ± 6.5	161.8 ± 7.4	170.4 ± 6.3
Body weight (kg)	45.6 ± 8.0	83.8 ± 10.7	48.3 ± 8.3	68.0 ± 10.2
Maximum oxygen uptake ($l.min^{-1}$)	2.67 ± 0.4	4.24 ± 0.61	2.44 ± 0.32	2.75 ± 0.43
Maximum treadmill slope (°)	13.2 ± 2.4	11.1 ± 3.3	10.3 ± 1.9	6.4 ± 2.6
HR_{rest} (bpm)	80.5 ± 14.3	68.5 ± 10.4	86.3 ± 14.0	73.4 ± 11.0
Heart rate at sub maximal exercise (bpm)	180.5 ± 11.9	163.5 ± 15.2	192.6 ± 9.9	176.3 ± 11.4
Heart rate reserve (bpm)	121.0 ± 7.3	116.4 ± 12.2	115.5 ± 14.8	109.8 ± 10.9
HR_{max} (bpm)	201.5 ± 7.3	184.9 ± 9.3	201.7 ± 8.0	182.9 ± 8.9
Physical activity (METs/week)	4730 ± 1996	4394 ± 2739	3766 ± 1677 [a]	5380 ± 3688 [a]

Data are mean ± SD.

bpm = beats per minute, HR_{rest} = resting heart rate, HR_{max} = maximum heart rate

[a] At the age of 36, the activity interview covered more activities than in earlier years, which explains the relatively high METS score in females at age 36.

Slope$_{max}$. In men, moderate to heavy smoking was negatively related to Slope$_{max}$ (Table 3). The β regression coefficient in men from the adjusted longitudinal model indicates that moderate to heavy smokers reached an overall Slope$_{max}$ that was 0.87% lower than in non-smokers. Furthermore, a significant negative interaction between smoking and time of measurement was found in both light and moderate to heavy smokers. This means that the negative association between smoking and Slope$_{max}$ became stronger with increasing age in all male smokers. In contrast to the results in men, smoking was not related to Slope$_{max}$ in women (Table 3).

HR$_{rest}$. Only in men, a negative relationship was found between light smoking and HR$_{rest}$, whereas no relationship was found between moderate to heavy smoking and HR$_{rest}$ (Table 3). Light tobacco smoking males reached an overall HR$_{rest}$ that was 2.73 beats per minute lower than in non-smokers. No significant interaction between smoking and time was found.

Smoking and heart rate response to exercise

HR$_{submax}$. In both men and women, smoking was negatively related to HR$_{submax}$, which was more pronounced in moderate to heavy smokers than in light smokers and more pronounced in men than in women (Table 4). In men, light smokers reached an overall HR$_{submax}$ that was 3.36 beats per minute lower than in non-smokers, whereas moderate to heavy smokers reached an overall HR$_{submax}$ that was 6.04 beats per minute lower than in non-smokers. No significant interaction between smoking and time was found.

Table 3. Regression coefficients (β) and 95% confidence intervals (C.I.) regarding the longitudinal relationship between smoking and maximum oxygen uptake ($VO2_{max}$), maximal treadmill slope ($Slope_{max}$) and resting heart rate (HR_{rest}). Light smokers (< 10 g of tobacco/day) and moderate to heavy smokers (≥ 10 g of tobacco/day) are compared with non-smokers (< 1 g of tobacco/day).

	Model 1		Model 2		Interaction with time [a]	Expected difference
	β	95% C.I.	β	95% C.I.		At age 36 [b]
$VO2_{max}$ (l.min^{-1})						
Males						
Light	0.10	-0.03, 0.22	-0.06	-0.17, 0.05	- *	-0.24 (5.7%)
Moderate/heavy	-0.01	-0.13, 0.12	-0.19*	-0.29, -0.10	- *	-0.37 (8.7%)
Females						
Light	0.06	-0.01, 0.13	0.02	-0.04, 0.07	+ *	+0.12 (4.4%)
Moderate/heavy	-0.08*	-0.15, -0.00	-0.08*	-0.14, -0.01	+	-0.03 (1.2%)
$Slope_{max}$ (%)						
Males						
Light	-0.01	-0.51, 0.49	-0.20	-0.63, 0.22	- *	-1.53 (13.7%)
Moderate/heavy	-0.58*	-1.11, -0.04	-0.87*	-1.30, -0.45	- *	-1.72 (15.4%)
Females						
Light	0.09	-0.22, 0.40	0.11	-0.19, 0.40	+	+0.27 (4.3%)
Moderate/heavy	-0.39	-0.85, 0.06	-0.25	-0.67, 0.17	+	+0.09 (1.3%)
HR_{rest} (bpm)						
Males						
Light	-2.83*	-5.42, -0.23	-2.73*	-5.27, -0.20	+	-2.11 (3.0%)
Moderate/heavy	-1.75	-4.42, 0.91	-1.44	-4.10, 1.21	+	-0.18 (0.0%)
Females						
Light	-1.44	-3.55, 0.66	-1.21	-3.31, 0.88	-	-1.90 (2.7%)
Moderate/heavy	-0.84	-3.42, 1.74	-1.27	-3.77, 1.23	-	-2.95 (4.3%)

* $p < 0.05$, bpm = beats per minute

Model 1: crude model with correction for time of measurement

Model 2: Corrections for body height, physical activity and body weight

[a] Interaction smoking × time of measurement, expressed as positive (+) or negative (-).

[b] Calculated from model 2 + interaction smoking × time of measurement. Expressed as the absolute difference and the percentage of the mean (between brackets).

Table 4. Regression coefficients (β) and 95% confidence intervals (C.I.) regarding the longitudinal relationship between smoking and heart rate response to sub maximal exercise (HR_{submax}). Light smokers (< 10 g of tobacco/day) and moderate to heavy smokers (≥ 10 g of tobacco/day) are compared with non-smokers (< 1 g of tobacco/day).

	Model 1		Model 2		Interaction with time [a]	Expected difference at age 36 [b]
	β	95% C.I.	β	95% C.I.		
HR_{submax} (bpm)						
Males						
Light	-3.37*	-6.29, -0.46	-3.36*	-5.75, -0.97	-	-5.76 (3.5%)
Moderate/heavy	-4.76*	-7.46, -2.05	-6.04*	-8.35, -3.73	-	-7.92 (4.9%)
Females						
Light	-1.65*	-3.20, -0.10	-1.77*	-3.04, -0.51	-	-2.97 (1.7%)
Moderate/heavy	-3.01*	-4.94, -1.08	-4.55*	-6.28, -2.82	-	-5.66 (3.2%)

* $p<0.05$, bpm = beats per minute

Model 1: Crude model with corrections for time of measurement

Model 2: model 1 with corrections for body height, physical activity, body weight and maximum slope.

[a] Interaction smoking × time of measurement. Expressed as positive (+) or negative (-).

[b] Calculated from model 2 + interaction smoking × time of measurement. Expressed as the absolute difference and the percentage of the mean (between brackets).

HR_{max}. A negative longitudinal relationship was found between moderate to heavy smoking and HR_{max}, which was quite similar in men and women. The overall HR_{max} in moderate to heavy smoking men was 2.47 beats per minute lower than in non-smokers versus 2.07 beats per minute in moderate to heavy smoking females. In men, a negative interaction between moderate to heavy smoking and time of measurement was found (Table 5), indicating that the negative association between moderate to heavy smoking and HR_{max} in males became stronger with increasing age.

HRR. In both sexes, no significant longitudinal association was found between smoking and HRR (Table 5). The association between smoking and HRR was similar at all ages, since no interaction was found between smoking and time of measurement.

Table 5. Regression coefficients (β) and 95% confidence intervals (C.I.) regarding the longitudinal relationship between smoking and maximum heart rate (HR_{max}) and heart rate reserve (HRR). Light smokers (< 10 g of tobacco/day) and moderate to heavy smokers (≥ 10 g of tobacco/day) are compared with non-smokers (< 1 g of tobacco/day).

	Model 1		Model 2		Interaction with time [a]	Expected difference at age 36 [b]
	β	95% C.I.	β	95% C.I.		
HR_{max} (bpm)						
Males						
Light	-1.48	-3.05, 0.10	-1.34	-2.93, 0.25	-	-1.38 (0.7%)
Moderate/heavy	-2.65*	-4.08, 1.22	-2.47*	-3.92, -1.02	- *	-4.35 (2.4%)
Females						
Light	-0.79	-1.83, 0.26	-0.47	-1.51, 0.58	+	+0.23 (0.1%)
Moderate/heavy	-2.15*	-3.46, -0.83	-2.07*	-3.42, -0.72	+	-1.76 (1.0%)
HRR (bpm)						
Males						
Light	1.07	-1.54, 3.69	1.16	-1.43, 3.76	-	+0.42 (0.4%)
Moderate/heavy	-1.00	-3.64, 1.64	-0.98	-3.62, 1.66	-	-4.15 (3.6%)
Females						
Light	0.21	-2.03, 2.44	0.27	-1.98, 2.53	+	+1.77 (1.6%)
Moderate/heavy	-1.48	-4.49, 1.53	-1.00	-3.95, 1.95	+	+0.78 (0.7%)

* $p < 0.05$, bpm = beats per minute

Model 1: Crude model with corrections for time of measurement

Model 2: model 1 with corrections for body height and physical activity

[a] Interaction smoking × time of measurement. Expressed as positive (+) or negative (-).

[b] Calculated from model 2 + interaction smoking × time of measurement. Expressed as the absolute difference and the percentage of the mean (between brackets).

To investigate the single contribution of physical activity in model 2, we re-analysed the data without correction for physical activity. When physical activity was not included in model 2, the previously described results remained largely the same (data not shown).

Predicted differences at age 36

The predicted difference between smokers and non-smokers in cardiovascular fitness at the age of 36, is presented in Table 3. The expected difference between smokers and non-smokers in heart rate response to exercise at the age of 36 is presented in tables 4 and 5.

Most striking are the expected reductions in $VO2_{max}$ (i.e. 5.7 % and 8.7 %) and $Slope_{max}$ (13.7 % and 15.4 %) in male smokers as compared to male non-smokers.

Cross-sectional relationships

Reversibility of smoking effects

Table 6 presents the results from the cross-sectional linear regression analyses between smoking status (i.e. long-term ex-smokers, short-term ex-smokers, and current smokers versus never smokers) and cardiovascular fitness and heart rate response to exercise at age 36. In 36-year-old males, current smokers (N=37) reached a lower $VO2_{max}$, $Slope_{max}$, HR_{submax}, HR_{max}, and HRR than never smokers. Both short-term ex-smokers (N=25) and long-term ex-smokers (N=23), on the contrary, reached a $VO2_{max}$, $Slope_{max}$, and HRR similar as in never smokers. However, with regard to HR_{max} and HR_{submax}, short-term ex-smokers mimicked the results found in current smokers, whereas long-term ex-smokers mimicked the results of never smokers. In 36-year-old females, no relationship was found between smoking status and cardiovascular fitness and heart rate response to exercise, except for HR_{submax}. Current smokers (N=35) reached a lower HR_{submax} than never smokers, while both short-term ex-smokers (N=20) and long-term ex-smokers (N=34) did not differ from non-smokers (Table 6).

Table 6. Reversibility of smoking effects. Regression coefficients (β) and 95% confidence intervals (95% C.I.) regarding the relationship between smoking status (never smokers, long-term ex-smokers, short-term ex-smokers, and current smokers) and cardiovascular fitness and heart rate response to exercise in males and females. Never smokers were used as the reference group

	Males						Females					
	Long-term ex-smokers		Short-term ex-smokers		Current smokers		Long-term ex-smokers		Short-term ex-smokers		Current smokers	
	β	95% C.I.	β	95% C.I.	β	95% C.I.	β	95% C.I.	β	95% C.I.	β	95% C.I.
$VO2_{max}$ ($l.min^{-1}$)†	0.01	-0.24, 0.26	-0.12	-0.35, 0.12	-0.45*	-0.65, -0.25	-0.11	-0.26, 0.04	-0.09	-2.28, 0.09	-0.03	-0.17, 0.11
$Slope_{max}$ (%)†	0.25	-1.12, 1.63	0.45	-0.85, 1.75	-1.96*	-3.07, -0.85	-0.29	-1.30, 0.71	0.33	-0.92, 1.58	-0.08	-1.06, 0.90
HR_{rest} (bpm) †	-4.34	-8.92, 0.25	-1.91	-6.65, 2.83	-1.67	-5.61, 2.28	1.52	-2.78, 5.82	2.74	-2.66, 8.14	-2.02	-6.21, 2.16
HR_{max} (bpm)†	-0.01	-4.14, 4.12	-5.03*	-8.93, -1.13	-7.30*	-10.81, -3.80	-0.80	-4.47, 2.86	0.52	-4.25, 5.30	-2.71	-6.28, 0.86
HRR (bpm)†	2.03	-3.53, 7.60	-0.59	-5.85, 4.67	-5.20*	-9.70, -0.68	-2.45	-6.91, 2.02	-0.11	-5.93, 5.71	-0.70	-5.05, 3.65
HR_{submax} (bpm)‡	2.31	-2.97, 7.58	-6.55*	-11.54, -1.58	-10.63*	-15.23, -6.03	3.58	-1.28, 8.43	-0.35	-5.62, 4.93	-6.30*	-10.52, -2.07

* $p<0.05$, bpm=beats per minute

$VO2_{max}$ = maximum oxygen uptake, $Slope_{max}$ = maximal treadmill slope, HR_{rest} = resting heart rate, HR_{max} = maximum heart rate, HRR = heart rate reserve, HR_{submax} = heart rate response to sub maximal exercise.

†Corrections were made for body height, physical activity and body weight

‡Corrections were made for body height, physical activity, body weight and maximum slope.

Discussion

The results from our longitudinal analyses indicate that smoking is negatively related to cardiovascular fitness and heart rate response to exercise in men and women between age 13 and 36. The strongest associations were found with $VO2_{max}$ and $Slope_{max}$, as indicators of cardiovascular fitness and with HR_{submax}, as an indictor of heart rate response to exercise. The associations were stronger in moderate to heavy smokers than in light smokers and stronger in males than in females. In line with the expectations, the negative relationship between smoking and $VO2_{max}$, $Slope_{max}$ and HR_{max} became stronger over time in males. Cross-sectional analyses suggest that the adverse effects of smoking were reversible in 36-year-old males.

Smoking and cardiovascular fitness

When we only consider $VO2_{max}$ and $Slope_{max}$ as indicators of cardiovascular fitness, we found a negative (longitudinal) relationship between smoking and cardiovascular fitness, which is in line with prior studies.[12,25-28] The maximum level of exhaustion was similar in smokers and non-smokers, since no differences were found in respiratory gas exchange ratio (RER) at the end of the treadmill running test (i.e. mean RER in smokers was 111.4 SD 8.52 and in non-smokers 112.4 SD 9.19). In contrast to prior studies,[4,6,29] HR_{rest} was lower in light smoking males than in non-smoking males. An explanation might be that only long-term effects of smoking were studied in the present study, since the short-term effects could be ruled out.

Although it is commonly known that HR_{rest} rises directly after smoking due to increased sympathetic drive and reduced vagal modulation,[29-32] less is known about the long-term effects of smoking on HR_{rest}. Hayano *et al.*[30] found a blunted heart rate response to postural change in heavy smokers. In the present study, HR_{rest} was measured prior to the maximal exercise test in a sitting position. The sitting position and psychological stress due to the coming treadmill running test, might have increased HR_{rest} in non-smokers to a higher extent than in light smokers, due to a blunted heart rate response in light smokers. In other studies where HR_{rest} was measured prior to the maximal exercise test, the results were contradictive. Several studies found no association between long-term smoking and HR_{rest},[10,25,27,31] whereas others found a positive[6,4] or a negative association.[3,5] Unfortunately, this cannot explain why the lower HR_{rest} was not found in moderate to heavy smokers.

Smoking and heart rate response to exercise

Our study shows that the reduced heart rate response to exercise in smokers found by others,[5,6,4] was already present in young and healthy men and women. Although the lower HR_{rest} in smokers can explain part of the lower HR_{submax}, the differences in HR_{rest} between smokers and non-smokers were smaller than in HR_{submax}. This

indicates that the lower heart rate response to exercise cannot be fully explained by the lower HR_{rest}, especially not in moderate to heavy smokers.

Reversibility of smoking effects

In accordance with our findings, Sandvik *et al.*[3] found the adverse effects of smoking on physical fitness to be reversible in men. Hirsch *et al.*[33] even found abstinence from smoking for one day, to increase $VO2_{max}$. Hashizume *et al.*[34] on the other hand, found a prolonged exercise time after six or seven days of abstinence from smoking, but no change in $VO2_{max}$.

Gender differences

Very few studies have described the association between smoking and cardiovascular fitness in women.[11] In the present study, the association between smoking and cardiovascular fitness in women was weaker than in men, similar as in Boreham *et al.*[11] Prior studies on smoking and HR_{rest} in women are contradictive. Whereas Sidney *et al.*[6] found no difference in HR_{rest} between female smokers and non-smokers, Lauer *et al.*[5] found female current smokers to reach a lower HR_{rest} than female never smokers. The differences between men and women in the present study, regarding the association between smoking and fitness parameters, might be explained by the lower median number of pack-years in women. Nevertheless, only very small differences were found between men and women in the association between smoking and heart rate response to exercise, which is in line with other studies.[5,6,9] In contrast to the males, no reversibility of smoking effects was found in females with the exception of HR_{submax}. Additional reversibility analyses with and without correction for physical activity showed similar results, suggesting that differences in physical activity level between males and females could not explain differences in reversibility. Furthermore, no significant interaction was found between smoking status and physical activity level, suggesting that the reversibility of smoking effects was not dependent on physical activity level. Other explanations, such as gender differences in metabolism or weight management do not seem likely. Although several studies reported weight gain after smoking cessation, most studies reported no differences between males and females.[35] The most plausible explanation for the observed gender differences seems to be that the associations between smoking and cardiovascular fitness parameters in females were simply not strong enough to show any reversibility.

Mechanism smoking and heart rate response to exercise

There is no agreement about the mechanisms by which smoking reduces heart rate response to exercise. Smoking increases the sympathetic drive to the heart and

muscle blood vessels[36] and reduces vagal cardiac control.[30] Narkiewicz *et al.*[36] found that baroreflexes, which respond to increasing blood pressure as a result of smoking, play an important role in reducing the sympathetic nerve traffic and limiting the increase in heart rate in young healthy subjects. However, when baroreflex function is impaired, smoking increases sympathetic tone, which might lead to a reduced heart rate response to exercise via down regulation of β-adrenergic receptors.[37]

Limitations of the study

Due to the limitations of cross-sectional analyses, the results on the reversibility of smoking effects, should be interpreted with caution. The results from the cross-sectional analyses do not prove that the effects of smoking are reversible but only suggest its reversibility. However, the finding that the results in short-term ex-smokers were mostly in between the results of long-term ex-smokers and current smokers, further strengthens the idea of reversibility. Caution should also be taken when interpreting the expected differences at age 36, which are based on the interaction between smoking and time of measurement and the initial differences in cardiovascular fitness and heart rate response to exercise between smoking groups. The most important reasons are that the initial differences between smoking groups are based on a few subjects and on the assumption that the interaction between smoking and time of measurement is linear. Finally, converting tobacco consumption from a continuous variable (grams per day) into a discrete measure (i.e. non-smokers, light smokers, moderate/heavy smokers) brings about some disadvantages. The most important disadvantage is that smokers consuming 1 g of tobacco per day are considered to be similar to those consuming 9 g of tobacco per day, whereas smokers consuming 9 g of tobacco per day are considered to be different from those who consume 10 g of tobacco per day. However, by using a discrete measure for tobacco consumption, we were able to show that same of the associations between smoking and cardiovascular fitness were non-linear.

Conclusion

Even in young healthy men and women, smoking is negatively related to cardiovascular fitness and heart rate response to exercise, which are both predictors of all-cause mortality. In men, the negative relationship between smoking and cardiovascular fitness and heart rate response to exercise becomes stronger over time.

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Chapter 6

How are changes in tobacco consumption related to changes in biological risk factors for cardiovascular disease in 21 to 36-year-old males and females?

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Submitted

Abstract

Objectives. The aim of this study was to investigate the relationship between changes in tobacco consumption and changes in biological risk factors for cardiovascular disease in relatively healthy and young adults during a follow-up period of four to six years.

Methods. All subjects (165 men and 195 women) were participants of the Amsterdam Growth and Health Longitudinal Study (AGAHLS) and followed-up four times between the age of 21 and 36. From these data, three follow-up periods of four to six years (i.e. age 21-27, 27-32, and 32-36), were considered for analyses on changes in tobacco consumption. Data on changes in tobacco consumption and changes in biological risk factors for cardiovascular disease came from one of these three follow-up periods. We used multiple linear regression analyses with corrections for age and changes in other lifestyles.

Results. In both sexes we found trends for a reduction in blood pressure, high-density lipoprotein cholesterol (HDL-C), body weight and waist-to-hip ratio (WHR), and a rise in the ratio between total serum cholesterol (TC) and HDL-C (TC/HDL-C) with increasing tobacco consumption. Opposite trends were found with reducing tobacco consumption. In females, body weight, WHR and waist circumference reduced significantly and independently with increasing tobacco consumption and increased significantly with decreasing tobacco consumption.

Conclusions. These results suggest that voluntary changes in tobacco consumption go together with both 'healthy' and 'unhealthy' changes in biological risk factors for cardiovascular disease.

Introduction

Smoking is one of the most important risk factors for mortality from cardiovascular disease (CVD) in elderly Europeans.[1] Fatty streaks and fibrous plaque in the aorta and coronary arteries, however, are already detectable in smoking children and smoking young adults.[2] Fortunately, the effect of tobacco smoking on the cardiovascular system seems to be reversible. Reported beneficial effects of smoking cessation are reductions in fibrinogen and white blood cell count,[3] and increases in high-density lipoprotein cholesterol (HDL-C).[4-6] High levels of HDL-C are associated with a low risk of CVD, especially when the ratio between total serum cholesterol (TC) and HDL-C is low.[7]

Although many studies have investigated the effects of smoking[8-10] and smoking cessation[11-14] on cardiovascular risk, little is known on the effect of voluntary changes in tobacco consumption in a young and free-living population. Most prior studies have restricted changes in tobacco consumption to smoking cessation,[4-6,11,13-18] and/or have used relatively short follow-up periods (i.e. 4-17 weeks) under controlled experimental conditions[3,4,6,15,18-20] and/or have used nicotine replacement therapy to facilitate smoking reduction.[3,6,20] The question rises whether or not the results of these studies are comparable to the effects of voluntary changes in tobacco consumption (both increasing and decreasing) in free-living subjects during several years.

The aim of this study was to investigate the relationship between changes in tobacco consumption and changes in biological risk factors for CVD in relatively healthy and young adults during a follow-up period of four to six years.

Methods

Study population

All subjects were participants of the Amsterdam Growth and Health Longitudinal Study (AGAHLS) that started in 1977 with 298 13-year-old boys and 334 13-year-old girls from the first and second grade of two secondary schools in Amsterdam, The Netherlands.[21,22] Between the age of 21 and 36, the participants were followed up four times. From these data, three follow-up periods of four to six years (i.e. age 21-27, 27-32, and 32-36), were considered for analyses on changes in tobacco consumption. The participants included in the analyses were 165 males and 195 females. All of these participants were measured at least during one of the three follow-up periods and did not differ in biological risk factors for CVD from dropouts (i.e. participants who dropped out of the AGAHLS before reaching the age of 21). The AGAHLS was approved by the Medical Ethical Committee of the VU University medical center and subjects provided written informed consent.

Exclusion criteria

Data from women who were pregnant during the time of the measurements were excluded from the analyses

Data inclusion criteria

The latest reported change in tobacco consumption of each participant was included in the analyses. Changes in other lifestyles and changes in biological risk factors for cardiovascular disease were derived from the same follow-up period. For participants reporting no change in tobacco consumption in the most recent follow-up period (i.e. age 32-36), for example, changes reported in the five-year follow-up period between the age of 27 and 32 were included. When no change in tobacco consumption was reported during any of the three follow-up periods (e.g. in never smokers), we included the absence of change from the last follow-up period.

Tobacco consumption

Current tobacco consumption was assessed with help of a smoking questionnaire at all measurements and expressed as total gram of tobacco smoked per week (1 cigarette=1 gram, 1 package of own-rolled tobacco=40 gram, 1 cigar/cigarillo=3 gram, 1 package of pipe tobacco=50 gram).[23]

Biological risk factors for CVD

Body weight. Body weight was measured with a spring balance (Van Vucht, Amsterdam, The Netherlands).

Fat distribution. Waist-to-hip ratio (WHR) and waist circumference were used as proxy measures of fat distribution. Waist and hip circumference were measured with a flexible steel tape to the nearest 0.1 cm.

Serum cholesterol levels. Blood serum was obtained from a sample of approximately 10 ml of venous blood, taken from the vena antecubitis in a non-fasting state. Both blood sampling and serum preparations were done between 08:00 and 12:00 a.m. Total serum cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured in serum with standard methods. External quality control took place with target samples from a World Health Organization (WHO) reference laboratory (Lipid Standardization Laboratory, Atlanta, Georgia). Furthermore, the ratio between TC and HDL-C was also used as a risk factor of CVD.

Blood pressure. Blood pressure was measured in a seated position prior to a treadmill running test, using an indirect method. A standard pressure cuff (12 cm) was placed around the left upper arm. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in the brachial artery using a sphygmomanometer (Speidl-Keller No. 2010; Franken & Itallie, The Netherlands).

The lowest SBP and DBP were used in the analyses and expressed in mm Hg.

Other lifestyles

Daily physical activity was assessed by a standardized activity interview.[22,24] This interview covered the previous three months and concerned the total time spent on physical activities in relationship to school, work and other activities. In combination with the intensity of the different activities a total weighted (metabolic) activity score (expressed in METs.week^{-1}) was calculated. Dietary intake was measured by a modification of the cross-check dietary history, which was specially developed for the AGAHLS.[25] The method was used to assess the food intake in the previous month. Total energy intake (Kjoule), total fat intake (energy percentage), saturated fat intake (energy percentage) and alcohol consumption (gram.week^{-1}) were calculated based on the Dutch Food Composition Table 1996.[26]

Statistical analyses

To study changes in tobacco consumption in relationship to changes in biological risk factors for CVD, multiple linear regression analyses were carried out with the Statistical Package for Social Sciences (SPSS for Windows version 9.0, 1999, SPSS Inc., Chicago, Illinois). Changes in tobacco consumption were expressed as a continuous variable. All relationships were studied with correction for age at baseline. In addition to the age-adjusted model, a second model adjusted additionally for changes in other lifestyles (i.e. physical activity, energy intake, fat intake, intake of saturated fats, and alcohol consumption). In the relationship between changes in tobacco consumption and changes in blood pressure, we additionally corrected for changes in body weight.

Linearity

By using changes in tobacco consumption as a continuous variable, we assumed that the associations between increasing tobacco consumption (with 70 g/week) and changes in cardiovascular risk, were equal but opposite to the associations between reducing tobacco consumption (with 70 g/week) and changes in cardiovascular risk. To verify this assumption, we studied linearity of the associations by including a quadratic term for changes in tobacco consumption into the linear regression analyses. Linearity was assumed when the p-value of the quadratic term was ≥ 0.2, and non-linearity was assumed when the p-value was < 0.2. In case of non-linearity, linear regression analyses with dummy variables were performed. Dummy variables were constructed by dividing changes in tobacco consumption into the following four groups, based on an arbitrary cut-off value of 70 g of tobacco per week: 1. strong tobacco reduction (≥ 70 g/week), 2. light tobacco reduction (< 70 g/week), 3. light

tobacco increase (< 70 g/week) and 4. strong tobacco increase (≥ 70 g/week). These dummy variables allowed for a comparison in cardiovascular risk between these four groups and the reference group (i.e. non-changers). The reference group consisted of 242 never smokers and 10 steady smokers. All analyses were stratified for gender.

Results

The smokers in the AGAHLS reported to smoke mainly cigarettes and own-rolled tobacco, whereas the use of cigars and pipe tobacco was negligible (e.g. 2.0% and 0.2% respectively at the age of 36). Of the 360 participants, 53 males and 55 females voluntarily changed their tobacco consumption during at least one of the follow-up periods. Of the male changers, 25 males decreased (13 due to cessation) and 28 males increased tobacco consumption. Of the female changers, 32 females decreased (19 due to cessation) and 23 females increased tobacco consumption.

Table 1 presents the baseline characteristics of tobacco reducers, non-changers, and tobacco increasers. Tobacco reducers were on average younger and were followed-up slightly longer than non-changers and tobacco increasers. Males who reduced tobacco consumption during follow-up scored lowest on SBP and highest on TC/HDL-C. These differences between smoking groups were significant. In females, smoking groups differed significantly in SBP, HDL-C and TC/HDL-C before follow-up. Females who reduced tobacco consumption scored lowest on SBP, HDL-C and highest on TC/HDL-C. Table 2 presents the results from the multiple linear regression analyses between changes in tobacco consumption and changes in cardiovascular risk factors in males and females.

Results from the linear regression analyses

Blood pressure

In both sexes, there was a trend for a negative association between changes in tobacco consumption and changes in blood pressure (Table 2). All of these associations were linear. Figure 1 illustrates the associations between changes in tobacco consumption and changes in DBP and SBP in males that are based the regression coefficients presented in Table 2 (model 2). With each *increase* in tobacco consumption of 70 g/week, DBP reduced with 1.01 mm Hg and SBP with 0.82 mm Hg, whereas DBP increased with 1.01 mm Hg and SBP with 0.82 mm Hg with each *reduction* in tobacco consumption of 70 g/week. In females, the negative associations between changes in tobacco consumption and changes in SBP were significant and independent of age, but lost significance after correction for changes in other lifestyles.

Table 1. Baseline characteristics of male and female tobacco reducers, non-changers and tobacco increasers.

	Males				Females			
	Reducers (n=25)	Non-changers (n=112)	Increasers (n=28)	p-value[a]	Reducers (n=32)	Non-changers (n=140)	Increasers (n=23)	p-value[a]
Tobacco use (gram/week)[b]	70.0 [14.9-126.0]	0.0 [0.0-0.0]	14.0 [0.0-89.3]	-	75.0 [33.3-153.5]	0.0 [0.0-0.0]	0.0 [0.0-70.0]	-
Age (years)	28.6 (4.1)	31.0 (3.2)	29.7 (4.0)	0.01	26.8 (4.9)	31.5 (2.0)	30.8 (3.3)	0.00
Follow-up (years)	4.64 (0.8)	4.19 (0.6)	4.43 (0.7)	0.00	4.97 (0.9)	4.09 (0.4)	4.22 (0.6)	0.00
DBP (mm Hg)	80.6 (9.3)	86.0 (8.7)	82.9 (7.4)	0.01	79.8 (10.0)	83.9 (9.0)	80.2 (8.3)	0.03
SBP (mm Hg)	133.8 (10.8)	136.4 (12.9)	131.3 (9.3)	0.12	122.5 (10.2)	125.7 (9.8)	122.8 (7.8)	0.14
TC (mmol/l)	5.13 (0.96)	4.90 (0.90)	4.99 (0.94)	0.50	4.87 (0.65)	4.95 (0.80)	4.86 (0.78)	0.78
HDL-C (mmol/l)	1.13 (0.23)	1.22 (0.27)	1.23 (0.24)	0.22	1.40 (0.33)	1.62 (0.33)	1.58 (0.42)	0.01
TC/HDL-C	4.78 (1.53)	4.18 (1.08)	4.17 (0.96)	0.05	3.66 (0.97)	3.16 (0.73)	3.27 (0.93)	0.01
Body weight (kg)	79.5 (16.0)	80.5 (9.6)	76.9 (9.0)	0.29	62.7 (10.1)	66.2 (9.4)	65.4 (9.2)	0.17
WHR	0.92 (0.05)	0.92 (0.05)	0.93 (0.06)	0.84	0.79 (0.04)	0.79 (0.04)	0.79 (0.05)	0.78
Waist circumference (mm)	832.1 (100.9)	827.2 (72.0)	819.5 (77.7)	0.86	696.3 (80.9)	711.8 (67.0)	684.7 (44.6)	0.17

Values are presented as mean and standard deviations

[a] p-value regarding difference in cardiovascular risk factor between tobacco reducers, non-changers and tobacco increasers

[b] values are presented as median + inter quartile range

DBP: diastolic blood pressure, SBP: systolic blood pressure, TC: total serum cholesterol, HDL-C: High-density lipoprotein cholesterol, WHR: Waist-to-hip ratio.

Table 2. Associations between changes in tobacco consumption (for an increase of 70 g/week) and changes in cardiovascular risk factors in males and females separately. Results from linear regression analyses.

	Males		Females	
	β	95% C.I.	β	95% C.I.
Diastolic blood pressure (mmHg)				
Model 1	-1.42	-3.47, 0.62	-1.22	-3.29, 0.85
Model 2[a]	-1.01	-3.05, 1.03	-0.64	-2.76, 1.47
Systolic blood pressure (mm Hg)				
Model 1	-1.12	-3.56, 1.32	-2.53*	-5.05, -0.01
Model 2[a]	-0.82	-3.30, 1.65	-1.56	-4.14, 1.02
Total Cholesterol (TC) (mmol/l)				
Model 1	0.008	-0.133, 0.149	-0.007	-0.159, 0.145
Model 2	0.013	-0.130, 0.157	-0.014	-0.167, 0.145
High-density lipoprotein cholesterol (HDL-C) (mmol/l)				
Model 1	-0.041	-0.086, 0.004	-0.047	-0.111, 0.017
Model 2	-0.042	-0.087, 0.004	-0.047	-0.110, 0.017
TC/HDL-C				
Model 1	0.188	-0.002, 0.378	0.112	-0.026, 0.251
Model 2	0.188	-0.003, 0.380	0.101	-0.035, 0.237
Body weight (kg)				
Model 1	-0.60	-1.46, 0.26	-1.77*	-2.76, -0.77
Model 2	-0.73	-1.61, 0.14	-1.75*	-2.76, -0.75
Waist-to-hip ratio				
Model 1	-0.009	-0.027, 0.008	-0.024*	-0.045, -0.004
Model 2	-0.010	-0.028, 0.008	-0.021*	-0.042, -0.001
Waist circumference (mm)				
Model 1	-3.59	-16.78, 9.59	-19.61*	-33.66, -5.56
Model 2	-5.20	-18.50, 8.10	-18.04*	-32.10, -3.98

* $p < 0.05$,

[a] additional correction for changes in body weight

Model 1: age at baseline

Model 2: Model 1+ changes in physical activity, energy intake, fat intake, intake of saturated fats, and alcohol consumption

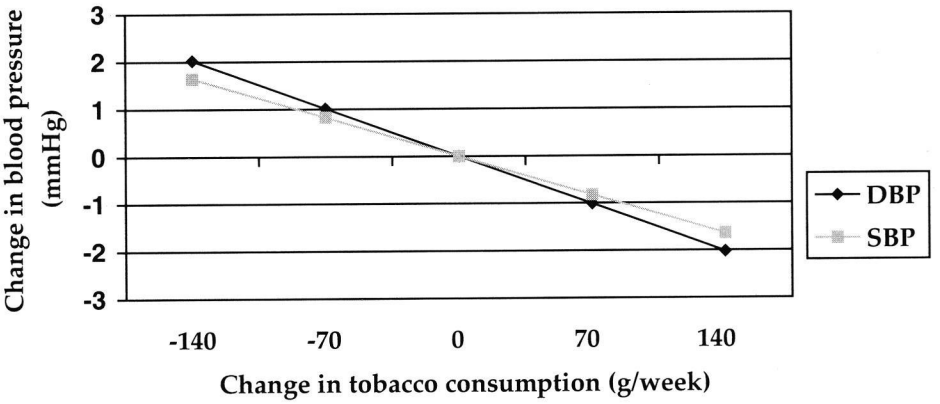

Figure 1. Relationship between changes in tobacco consumption and changes in blood pressure parameters in males, based on the results from linear regression analyses

Blood lipids

In both sexes, there was a clear trend for a negative association between changes in tobacco consumption and changes in HDL-C, indicating a reduction in HDL-C with tobacco increase and a rise in HDL-C with tobacco reduction. The association between changes in tobacco consumption and changes in HDL-C was linear in both males and females, whereas the associations between changes in tobacco consumption and changes in TC and TC/HDL-C were linear in females and non-linear in males. In females, there was a trend for a positive association between changes in tobacco consumption and changes in TC/HDL-C, indicating a rise in TC/HDL-C with tobacco increase and a reduction with decreasing tobacco consumption. There was no association between changes in tobacco consumption and changes in TC in females. Correcting for changes in other lifestyles (model 2) had no effect on the associations.

Body composition

In males, there was a trend for a negative association between changes in tobacco consumption and changes in body weight and WHR, indicating a reduction in body weight and WHR with tobacco increase and a rise with decreasing tobacco consumption. The association between changes in tobacco consumption and changes

in waist circumference was non-linear. In females, on the other hand, changes in tobacco consumption were significantly and negatively associated with changes in body weight, WHR and waist circumference. All of the associations in females were linear and independent of age (model 1) and changes in other lifestyles (model 2).

Linearity

Figure 2 presents the results from dummy variable linear regression analyses comparing strong tobacco reducers (≥ 70 g/week), light tobacco reducers (< 70 g/week), light tobacco increasers (< 70 g/week) and strong tobacco increasers (≥ 70 g/week) with non-changers in tobacco consumption.

The associations between changes in tobacco consumption and changes in TC (fig. 2A), TC/HDL-C (fig. 2B) and waist circumference (fig. 2C) were U-shaped in males. Light tobacco reducers, for instance, showed a significant decline in TC of -0.47 mmol/l (95% C.I. [-0.88, -0.06]) as compared to non-changers, whereas strong tobacco reducers did not (fig. 2A). Furthermore, strong tobacco increasers showed a significant rise in TC/HDL-C of 0.55 (95% C.I. [0.00, 1.10]), whereas other changers in tobacco consumption did not (fig. 2B). In addition, not only strong tobacco increasers, but also strong tobacco reducers showed a rise in waist circumference (fig. 2C). Only the rise in strong tobacco increasers, however, was significantly different from non-changers.

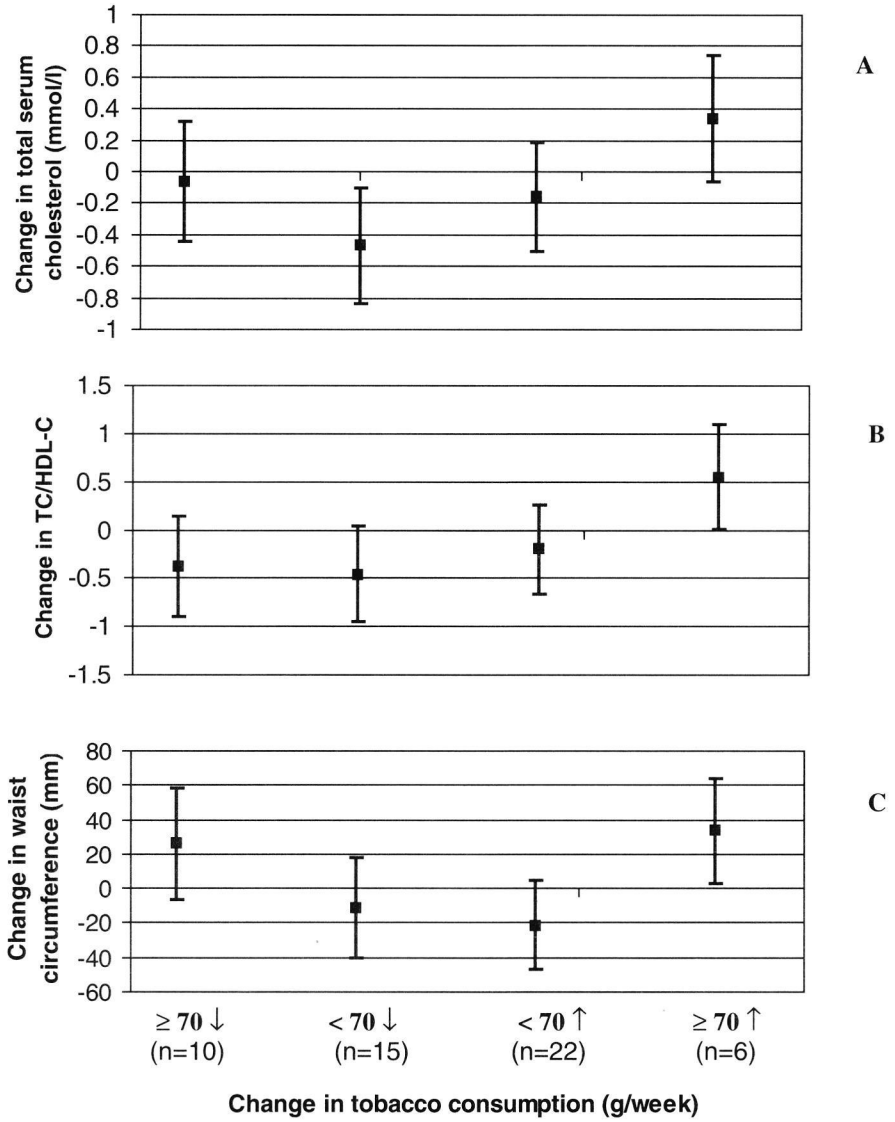

Figure 2. Results from the dummy variable linear regression analyses between changes in tobacco consumption and changes in serum total cholesterol (TC) (A), the ratio between total serum cholesterol and high-density serum cholesterol (TC/HDL-C) (B) and waist circumference (C) in males. Non changers in tobacco consumption were used as the reference. Results are presented as mean and 95% confidence intervals. All associations are corrected for age at baseline, changes in physical activity, energy intake, fat intake, intake of saturated fats, and alcohol consumption.

Discussion

We found no significant associations between changes in tobacco consumption and changes in any of the biological risk factors for CVD in males, but we did find a significant reduction in body weight, WHR and waist circumference with increasing tobacco consumption and a significant rise in these risk factors with reducing tobacco consumption in females. However, since our results are based on a small study sample, trends might be important to consider as well. With increasing tobacco consumption, we found trends for a reduction in blood pressure, HDL-C, body weight and WHR and a rise in TC/HDL-C. Opposite changes were found with reducing tobacco consumption.

Since SBP, HDL-C and TC/HDL-C differed significantly between reducers, non-changers and increasers at baseline, part of our results on changes in tobacco consumption might be due to a regression to the mean. However, the observed differences in biological risk factors for CVD at baseline were probably not due to chance, but mainly due to differences in tobacco consumption at baseline. In other words, the baseline differences were expected based on known associations between smoking and biological risk factors for CVD. Correcting for biological risk factors for CVD at baseline might therefore 'over-adjust' the associations.

Blood pressure

Smoking cessation studies reported contradictive results on changes in blood pressure.[5,6,14,15,19] Some studies reported a rise in blood pressure after smoking cessation,[6,14] whereas others reported no change.[5,15,19] Time since smoking cessation might have been too short to show changes in blood pressure in some of these studies.[15,19] The observed trend for a rise in blood pressure after reducing tobacco consumption, might be a normal compensatory reaction of the nervous system. Whereas long-term smokers show a decrease in ß-adrenergic receptor density due to increased sympathetic tone,[27] the opposite might happen in tobacco reducers, resulting in increased sympathetic stimulation and concomitant increases in blood pressure.

Body composition

Similar as in smoking cessation studies,[5,11,13,16-18] we found a rise in body weight after reducing tobacco. The increase in central fat distribution in females with tobacco reduction seems to contradict with the frequently reported higher central fat distribution in smokers as compared to non-smokers,[28-31] but is in agreement with a study that showed a significant increase in WHR after 12 months of smoking cessation,[17] and with a recent study that showed increased adipose tissue lipoprotein lipase activity in the abdominal region after four weeks of smoking cessation.[18]

It does contradict, however, with the results of Lissner *et al.* who reported a stronger increase in WHR in female continuous smokers than in female quitters after six-years of follow-up.[32] The rise in central body fat distribution with tobacco reduction might be explained by the same mechanism that was previously described to explain the rise in blood pressure with tobacco reduction.[27] Another explanatory mechanism might be through increased psychological stress (due to reduced nicotine intake), that increases the sensitivity of the hypothalamus pituitary adrenal (HPA)-axis and subsequently adreno-corticotropin hormone secretion, cortisol release and central storage of body fat.[33]

Blood lipids

Similar as in most smoking cessation studies,[5,12,16] TC did not decline in tobacco reducing females and males who reduced tobacco consumption with at least 70 g/week. Male light tobacco reducers (i.e. <70 g/week), however, showed a significant drop in TC as compared to non-changers, which is in line with a smoking cessation study reporting a smaller increase in TC in quitters than in continuing smokers.[13] Our trend for a rise in HDL-C with tobacco reduction is in accordance with smoking cessation studies,[3,5,6] although this rise in HDL-C was only temporary in one of the smoking cessation studies.[6] Despite a significant increase in HDL-C/LDL-C after eight weeks of smoking reduction, reported by Eliasson *et al.*,[3] we have found no study that reported on changes in tobacco consumption and changes in TC/HDL-C.

An important limitation of our study is the small number of participants that changed tobacco consumption, especially since the detection of non-linearity is heavily based on power. By using a relatively high cut-off value for the p-value of the quadratic term (i.e. 0.2), however, we expect to have detected all relevant non-linear associations. Nevertheless, the results from the dummy variable linear regression analyses should be interpreted with caution. Furthermore, a potential limitation may come from the fact that our non-changers consisted also of a few steady smokers. Excluding these steady smokers from the linear regression analyses, however, did not change our results. A similar limitation comes from the fact that our tobacco reducers consisted of 'quitters' and 'reducers without quitting'. Running separate analyses on these groups would have yielded interesting information if the number of participants in both groups had been larger. A final potential limitation is that tobacco reducers might have compensated for their reduction in tobacco intake by increasing the number of puffs or the inhalation depth.

Although we found 'unhealthy' changes in blood pressure, body weight and central body fat distribution with tobacco reduction and 'healthy' changes with tobacco increase, it seems unwise to encourage smokers to increase tobacco consumption or discourage them to reduce tobacco consumption. As was previously

discussed, the rise in blood pressure might be a normal compensatory reaction, and small increases in body weight and body fat distribution have a much smaller deleterious effect on the cardiovascular system and on general health than smoking.

Conclusions

In females, increasing tobacco consumption was significantly associated with a reduction in body weight, WHR and waist circumference, whereas decreasing tobacco consumption was associated with a rise in body weight, WHR and waist circumference. Furthermore, we found trends for a reduction in blood pressure, a deterioration in blood lipids and a reduction in body weight and WHR with increasing tobacco consumption in both sexes. Trends for opposite associations were found with tobacco reduction. Our results on blood pressure, body weight and central body fat distribution should not be used to encourage smokers to increase tobacco consumption or to prevent them from tobacco reduction.

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Chapter 7

Smoking and quantitative ultrasound parameters in the heel bone in 36-year-old men and women

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Submitted

Abstract

Objectives. Little is known on the association between smoking and quantitative ultrasound (QUS) parameters in men and women below the age of 40. Broadband Ultrasound Attenuation (BUA) and Speed of Sound (SOS), are believed to provide information on bone quality besides information on bone mineral density (BMD). The aim of this study was to investigate: 1. Current tobacco smoking; 2. Lifetime tobacco smoking; and 3. Years since smoking cessation, in relationship to QUS and BMD parameters in 36-year-old men and women.

Methods. Data came from the ninth measurement of the Amsterdam Growth and Health Longitudinal Study (AGAHLS), in which 174 men and 187 women participated with an average age of 36 years (SD 0.7). BUA (dB/MHz) and SOS (m/s) of the heel bone were assessed by using the CUBA Clinical instrument. BMD of the lumbar spine (L1-L4), total hip and total body were measured with dual-energy X-ray absorptiometry (DXA). We used multiple linear regression analyses with correction for body weight, physical activity, calcium intake and alcohol consumption.

Results. We found no significant associations between smoking and any of the BMD parameters in 36-year-old men and women. However, both current and lifetime tobacco smoking were significantly and negatively associated with BUA in women. Lifetime tobacco smoking was significantly and negatively associated with SOS in both sexes. The latter association was independent of body weight, calcium intake, physical activity and alcohol consumption in women, but not in men.

Conclusions. Our results suggest that both current and lifetime tobacco smoking are associated with a deterioration in bone quality but not with a reduction in BMD. However, since BMD parameters and QUS parameters were not measured at the same sites, our results may also simply suggest that the heel bone is affected by smoking at an earlier stage than the lumbar spine, hip and total body.

Introduction

Osteoporosis mainly occurs in elderly people, but many of its risk factors are already present in adolescence and young adulthood. One of these risk factors is smoking.[1] The deleterious effects of smoking on bone mineral density (BMD), however, have mainly been found in postmenopausal women, and only sporadically in young women below the age of 40.[1] A recently published meta-analysis shows that the association between smoking and BMD is stronger in men than in women and is independent of physical activity, body weight and calcium intake.[1]

Very few studies have investigated the relationship between smoking and bone strength parameters measured with quantitative ultrasound (QUS).[2-5] QUS offers two parameters that are believed to provide information on bone structure and material properties (bone quality), independently of BMD: broadband ultrasound attenuation (BUA) and speed of sound (SOS). BUA is believed to reflect bone mass and bone structure, whereas SOS has often been used as an indicator of bone elasticity.[6] A reduction in BUA is the result of reduced scattering of the ultrasound signal due to the breakdown and disappearance of 'trabeculae'.[7] QUS parameters are most often measured at the heel bone, which mainly consists of trabecular bone and is suffering more rapidly from bone loss than cortical bone.[8] Although smoking is most often not associated with BMD in young and healthy subjects, it might be associated with a decrease in SOS and BUA measured in the heel bone.

The present study was conducted to investigate current tobacco smoking, lifetime tobacco smoking, and years since smoking cessation, in relationship to QUS parameters of the heel bone and BMD parameters of the lumbar spine (L1-L4), total hip and total body in 36-year-old healthy men and women.

Methods

Study population

All subjects were participants of the Amsterdam Growth and Health Longitudinal Study (AGAHLS) that started in 1977 with 298 13-year-old boys and 334 13-year-old girls from the first and second grade of two secondary schools in Amsterdam and Purmerend, The Netherlands.[9] All data came from the ninth measurement of the AGAHLS, that took place in the year 2000 when 174 males and 187 females participated at an average age of 36 years (SD 0.7). At prior measurements of the AGAHLS, QUS parameters were not obtained. Almost all subjects were Caucasians (96.8%). The AGAHLS was approved by the Medical Ethical Committee of the VU University Medical Center and participants provided written informed consent.

Exclusion criteria

Female participants were excluded from the BMD measurements when they were pregnant or possibly pregnant at the time of the measurements.

Tobacco consumption

1. Current tobacco smoking.

Current tobacco smoking was defined as a tobacco consumption of at least 7 gram per week (i.e. 1 cigarette per day) at the age of 36. It was assessed with the help of a smoking questionnaire and expressed as the total gram of tobacco smoked per week (1 cigarette = 1 gram, 1 package of own-rolled tobacco = 40 gram, 1 cigar/cigarillo = 3 gram, 1 package of pipe tobacco = 50 gram).

2. Lifetime tobacco smoking.

Lifetime tobacco smoking was expressed as pack-years and calculated retrospectively with the help of an extensive questionnaire about current tobacco smoking, smoking in the past and quitting attempts.[10] Pack-years covered the period between smoking onset and the age at the ninth measurement of the AGAHLS. One pack-year was defined as one packet, or 20 gram of tobacco per day, smoked over a course of one year.

3. Years since smoking cessation.

Years since smoking cessation were assessed in ex-smokers using the previously described extensive questionnaire,[10] by subtracting age at the last smoking cessation attempt from the age at the ninth measurement.

Bone strength

1. Quantitative ultrasound (QUS) parameters: BUA and SOS.

Quantitative ultrasound measurements were made to assess BUA (dB/MHz) and SOS (m/s) of the heel bone, using the CUBA Clinical instrument (McCue Ultrasonics Limited, Winchester, UK). We used a calcaneal fixed single point transmission system with coupling by means of ultrasonic gel. Both the left and right heel bone were measured twice. When both measures on one side differed more than 10% in BUA, a third measurement was performed. Mean values at either side (from two or three measurements) were used in the analyses. Before measuring, the posterior part of the heel was wiped with alcohol. Ultrasonic gel was placed at the lateral and medial side of the posterior heel and also at the transducers. The participants were seated in a chair with their knee and ankle placed in a fixed position. Each day before the first measurement, a phantom measurement was performed for calibration and standardisation.

2. Bone mineral density (BMD) parameters.
BMD (grams/cm^2) of the lumbar spine (L1-L4) and total hip were measured with a regional body scan by using Dual-energy X-ray absorptiometry (DXA) (software version V5.67A; Hologic, QDR 2000, Hologic, Inc., Waltham, MA, USA). The hip was scanned at the non-dominant hand side. BMD of the total body was measured with a total body scan with DXA.

Covariates

Body weight. Body weight was measured with a spring balance (Van Vucht, Amsterdam, The Netherlands).
Calcium and alcohol intake. Dietary intake was measured by a computerised modification of the cross-check dietary history (Dutch Dishes),[11] covering the previous month. Calcium and alcohol intake were calculated based on the Dutch Food Composition Table (1996).[12]
Physical activity level. Physical activity level was estimated by a computerised modification of a standardised activity interview.[13] This interview covered the previous three months and concerned the total time spent on physical activities in relationship to school, work and other activities. In combination with the intensity of the different activities a total weighted (metabolic) activity score (expressed in METs) was calculated.[9, 14]

Statistical analyses

To study current tobacco smoking, lifetime tobacco smoking and years since smoking cessation in relationship to bone strength parameters at the age of 36, multiple linear regression analyses were carried out with the Statistical Package for Social Sciences (SPSS for Windows version 9.0, 1999, SPSS Inc., Chicago Illinois). For each relationship two analyses were carried out: 1. An unadjusted analysis in which no corrections were made for covariates, and 2. An adjusted analysis in which corrections were made for variables known to be associated with BMD (i.e. body weight, physical activity, calcium intake and alcohol consumption). In the analysis between years since smoking cessation and bone strength parameters, we additionally adjusted for pack-years.

Results

Of the initial 361 participants, complete data were available of 343 participants (165 men and 178 women). Table 1 presents the characteristics of smokers (36 men and 33 women) and non-smokers (129 men, 145 women) with regard to current and lifetime tobacco consumption, physical activity, alcohol consumption, body weight, current

calcium intake. The prevalence of contraceptive use in women was similar in smokers and non-smokers (39.4% versus 40.7%).

Comparison between smokers and non-smokers at the age of 36

Table 2 presents the mean values and standard deviations of all bone strength parameters, for men and women separately. The differences in bone strength parameters between smokers and non-smokers showed a similar trend in men and women. With the exception of lumbar BMD (L1-L4), all bone strength parameters were lower in smokers than in non-smokers. In women, the difference in BUA was significant ($p=0.04$) and the difference in SOS borderline significant ($p=0.06$).

Table 1. Descriptives of 36-year-old smokers and non-smokers. Stratified for gender.

	Men		Women	
	Smokers (n=36)	Non-smokers (n=129)	Smokers (n=33)	Non-smokers (n=145)
Current tobacco smoking (g/week)[1]	100.0 [7.0-280.0]	0.0 [0.0-3.6]	70.0 [14.0-210.0]	0.0 [0.0-4.9]
Lifetime tobacco smoking (pack-years)[1]	12.9 [0.56-27.0]	0.0 [0.0, 45.0]	9.7 [0.57-37.7]	0.0 [0.0-37.4]
Activity level (METs/week)[1]	3056 [731-13368]	4139 [613-15155]	3971 [1181-20057]	4565 [508-21712]
Alcohol consumption (g/day)[1]	17.6 [0.0-142.4]	12.9 [0.0-70.3]	11.8 [0.0-69.8]	4.3 [0.0-75.7]
Body weight (kg)[2]	84.6 (12.9)	83.3 (9.9)	67.6 (11.9)	68.1 (9.9)
Calcium intake (mg/day)[2]	1429 (787)	1431 (551)	1337 (541)	1230 (369)

[1]median + range

[2]mean + standard deviation

Table 2. Comparison between bone strength parameters in smokers and non-smokers at a mean age of 36.

	Men			Women		
	Smokers (n=36)	Non-smokers (n=129)	p-value	Smokers (n=33)	Non-smokers (n=145)	p-value
Mean BUA (dB/MHz)	94.39 (21.06)	98.43 (17.02)	0.24	81.76 (15.67)	88.06 (15.34)*	0.04
Mean SOS (m/s)	1645.78 (47.93)	1655.75 (33.27)	0.15	1640.59 (37.86)	1653.44 (34.34)	0.06
BMD L1-L4 (g/cm^2)	1.07 (0.14)	1.10 (0.15)	0.39	1.07 (0.10)	1.07 (0.12)	0.68
BMD total hip (g/cm^2)	1.06 (0.11)	1.08 (0.14)	0.42	0.95 (0.12)	0.96 (0.14)	0.52
BMD total body (g/cm^2)	1.18 (0.11)	1.20 (0.10)	0.42	1.08 (0.07)	1.10 (0.08)	0.17

BUA= Broadband ultrasound attenuation, SOS= speed of sound, BMD= bone mineral density

* $p < 0.05$ (independent samples T-test)

Current tobacco smoking and bone strength

Table 3 presents the results from the linear regression analyses between current tobacco smoking and both QUS and BMD parameters.

Table 3. Results from the linear regression analyses between current tobacco smoking (per 70 gram of tobacco per week), QUS parameters and BMD parameters (stratified for gender).

	Men		Women	
	β	95% C.I.	β	95% C.I.
SOS (m/s)				
Crude model	-6.981	-14.962, 1.000	-7.899	-17.326, 1.528
Adjusted model	-3.063	-10.928, 4.803	-8.329	-18.280, 1.622
BUA (dB/MHz)				
Crude model	-2.130	-6.028, 1.768	-3.924	-8.073, 0.225
Adjusted model	-1.140	-5.123, 2.842	-4.180*	-8.311, -0.048
BMD Total hip (g/cm^2)				
Crude model	-0.0082	-0.038, 0.022	-0.0054	-0.042, 0.031
Adjusted model	-0.0096	-0.039, 0.020	-0.0050	-0.042, 0.032
BMD L1-L4 (g/cm^2)				
Crude model	-0.0146	-0.047, 0.017	-0.0035	-0.035, 0.028
Adjusted model	-0.0121	-0.044, 0.020	-0.0156	-0.047, 0.016
BMD Total Body (g/cm^2)				
Crude model	-0.0097	-0.032, 0.013	-0.0089	-0.029, 0.011
Adjusted model	-0.0089	-0.031, 0.013	-0.0141	-0.035, 0.006

Crude model: no corrections

Adjusted model: Corrected for current calcium intake, body weight, activity level and alcohol consumption.

BUA= Broadband ultrasound attenuation, SOS= speed of sound, BMD= bone mineral density

* $p<0.05$

QUS parameters

Speed of sound. We found a negative association between current tobacco smoking and SOS in men (p=0.09). However, after correction for covariables, the strength of the association became weaker, mainly by the correction for current alcohol consumption. In women, the association between current tobacco smoking and SOS was slightly more negative than in men, but non-significant (p=0.10).

Broadband ultrasound attenuation. In men, current tobacco smoking was not associated with BUA. In women, however, we found a significant negative association between current tobacco smoking and BUA, but only after correction for current calcium

intake, body weight, activity level and alcohol consumption.

BMD parameters

In both sexes, current tobacco smoking was not associated with BMD of the lumbar spine (L1-L4), total hip or total body.

Table 4. Results from the linear regression analyses between lifetime tobacco smoking (pack-years), QUS parameters and BMD parameters (stratified for gender).

	Men		Women	
	β	95% C.I.	β	95% C.I.
SOS (m/s)				
Crude model	-0.890*	-1.621, -0.159	-0.810*	-1.589, -0.031
Adjusted model	-0.562	-1.290, 0.165	-0.840*	-1.665, -0.016
BUA (dB/MHz)				
Crude model	-0.194	-0.554, 0.166	-0.316	-0.660, 0.028
Adjusted model	-0.123	-0.493, 0.247	-0.398*	-0.740, -0.055
BMD L1-L4 (g/cm^2)				
Crude model	-0.0019	-0.005, 0.001	0.0010	-0.002, 0.004
Adjusted model	-0.0020	-0.005, 0.001	-0.0004	-0.003, 0.002
BMD Total hip (g/cm^2)				
Crude model	0.0009	-0.002, 0.004	-0.0005	-0.004, 0.002
Adjusted model	0.0005	-0.002, 0.003	-0.0012	-0.004, 0.002
BMD Total Body (g/cm^2)				
Crude model	-0.0007	-0.003, 0.001	-0.0003	-0.002, 0.001
Adjusted model	-0.0010	-0.003, 0.001	-0.0009	-0.003, 0.001

Crude model: no corrections

Adjusted model: Corrected for current calcium intake, body weight, activity level and alcohol consumption.

BUA= Broadband ultrasound attenuation, SOS= speed of sound, BMD= bone mineral density

* $p<0.05$

Lifetime tobacco smoking and bone strength

Table 4 presents the results from the linear regression analyses between lifetime tobacco smoking and both QUS and BMD parameters

QUS parameters

Speed of sound. In both sexes, lifetime tobacco smoking was significantly and negatively associated with SOS. In women, the association was independent of current calcium intake, body weight, physical activity and alcohol consumption,

whereas in men the association lost significance after adjustment for these covariates.
Broadband ultrasound attenuation. Lifetime tobacco smoking was not associated with BUA in men. In women, lifetime tobacco smoking was negatively and significantly associated with BUA, but only after correcting for current calcium intake, body weight, physical activity and alcohol consumption.

BMD parameters
In both sexes, lifetime tobacco smoking was not associated with BMD of the lumbar spine (L1-L4), total hip and total body.

Smoking cessation and bone strength
Among the 36-year-old ex-smokers (n=89), 35 men and 40 women reported the number of years since they had quit smoking. The median number of years since smoking cessation was 3.0 years in men (inter-quartile range: 1.0-10.0) and 7.0 years in women (inter-quartile range: 3.0-14.0). In male ex-smokers, years since smoking cessation were positively associated with SOS. However, this significant association disappeared after correction for pack-years, current calcium intake, body weight, activity level and alcohol consumption. Years since smoking cessation were not associated with BUA or BMD parameters. In women, years since smoking cessation were not associated with any of the bone strength parameters.

Discussion
The present study was performed to investigate current and lifetime tobacco smoking and years since smoking cessation, in relationship to QUS parameters of the heel bone, and BMD parameters of the lumbar spine, total hip and total body in 36-year-old healthy men and women. Our hypothesis that smoking might be associated with a decrease in SOS and BUA of the heel bone in a young and healthy population was partly confirmed. Smoking was not associated with BMD parameters measured with DXA, whereas both current and lifetime tobacco smoking were negatively and significantly associated with BUA in women after correction for covariates. Lifetime tobacco smoking was negatively and significantly associated with SOS in both sexes, which was independent of calcium intake, physical activity, body weight and alcohol consumption in women, but not in men. Finally, SOS increased significantly with years since smoking cessation in men, but only when no corrections were made for covariables.

Very few other studies have investigated the relationship between smoking and QUS parameters in subjects below the age of 40 years.[3, 5] One study found a negative relationship,[3] whereas another study did not.[5] The results of both studies, however, were not only based on premenopausal women but also on

postmenopausal women. Studies that included only postmenopausal women found contradictory results as well. Gregg *et al.*[4] found no association between smoking and QUS parameters, whereas Kim *et al.*[15] did. In the latter study,[15] however, smoking was no longer associated with QUS parameters, after correcting for age and years since menopause. The absence of a significant negative association between smoking and QUS parameters in the elderly might be due to the fact that factors other than smoking are stronger associated with BUA and SOS.

Similar to most prior studies[1] we found no association between smoking and BMD parameters in our 36-year-old healthy population. We did, however, find some significant and negative associations between smoking and QUS parameters in the heel bone. This can be explained in at least two ways. The first explanation is that smoking in 36-year-old men and women is associated with a deterioration in bone quality (i.e. breakdown of 'trabeculae' and/or loss of elasticity) but not in BMD. This explanation, however, does not take into account that QUS parameters and BMD parameters were measured at different sites. It is known that QUS parameters of the heel bone correlate less strongly with BMD of the lumbar spine and the hip, than with BMD of the heel bone.[16] The second explanation is that the heel bone is affected by smoking prior to other bone sites. This explanation seems plausible since bone loss is more rapid from sites with high trabecular content[8] and the heel bone consists almost exclusively of trabecular bone.

Our finding that years since smoking cessation were not independently associated with bone strength parameters, is in contrast to the results from smoking cessation studies that reported a positive dose-response relationship between years since smoking cessation and BMD at several hip sites in elderly men and women,[17] and a decrease in hip fracture risk by time since quitting in male ex-smokers.[18] Our study population, however, was much younger than in prior studies.[17,18] Since the effects of smoking on bone strength parameters are relatively small in subjects younger than 40 years,[1] strong effects of smoking cessation are not to be expected at the age of 36. To our knowledge, this is the first study that investigated smoking cessation in relationship to QUS parameters.

When interpreting the results of this study, it is important to know whether or not the observed reductions in QUS parameters are clinically relevant. It is known that premenopausal women lose approximately 0.3% of their skeleton per year, whereas postmenopausal women lose about 2% each year.[19] BUA and SOS seem to remain stable in pre menopausal women.[20] Results from the linear regression analyses in our study, show that women with a current tobacco consumption of 70 gram of tobacco per week (i.e. 10 cigarettes per day) scored, on average, 4.180 dB/MHz lower on BUA than non-smoking women. This difference is 4.8% of the mean BUA in women. Furthermore, women with a lifetime tobacco consumption of

one pack-year scored, on average, 0.398 dB/MHz lower on BUA than women who had never smoked (i.e. 0.46% of the mean). Assuming a linear relationship, this means that women with a lifetime tobacco consumption of 30 pack-years will show a mean difference in BUA of 11.94 dB/MHz, as compared to never smokers. In other words, women who start smoking one packet of cigarettes per day at the age of 20 and continue doing so until they reach the age of 50, will be at a much higher risk of fractures than women who have never smoked.

To conclude, our findings suggest that both current and lifetime tobacco smoking are associated with a deterioration in bone structure and elasticity, but not with a reduction in bone density. However, since BMD parameters and QUS parameters were not measured at the same sites, our findings may also simply suggest that the heel bone is affected by smoking at an earlier stage than the hip, lumbar spine and total body.

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Chapter 8

General discussion

Objective of this thesis

Most people consider the harmful effects of smoking as a fact and are surprised to hear that research in this field is still being performed. Although there is indeed considerable evidence for the harmful effects of smoking, there is still much to investigate. Relatively few studies have focused on the effects of smoking in young and healthy people who, on average, have a much smaller history of (lifetime) tobacco smoking (i.e. number of pack-years) than older people. Furthermore, few studies have investigated the *longitudinal* dose-response relationship between smoking and health parameters in adolescents and young adults, or the effects of *voluntary* changes in tobacco consumption in a *free-living* population. In other words, despite strong evidence for the deleterious effects of smoking, little is known on the extent to which deleterious effects of smoking are already present and measurable in adolescence and young adulthood and how such effects develop over time to become or not to become a serious threat to health. This thesis focused on the cross-sectional and longitudinal association between smoking and health parameters in young and relatively healthy males and females between the age of 13 and 36 years. In this chapter, the main results and conclusions are summarized and discussed.

Main results and conclusions

Self-report versus a dipstick method (NicCheck 1®)

Tobacco consumption can be assessed by self-report or with the help of biochemical methods. In epidemiological studies, self-report is the most frequently used method since biochemical methods are often too expensive or time consuming. Self-reported tobacco consumption, however, is only a moderate indicator of nicotine intake as a result of inter-individual differences in puffing and inhalation behaviour and cigarette yield.[1] Nicotine is believed to contribute to cardiovascular disease via activation of the sympathetic nervous system[2] and is highly correlated to "tar" intake.[3] The best way to measure nicotine intake is to assess nicotine and its metabolites, but the available methods to do so are often very expensive. NicCheck 1® (Dynagen Inc. Cambridge, MA, USA), on the other hand, is a quick and inexpensive colorimetric dipstick method to assess nicotine intake in the previous 20 hours that reacts with nicotine and all its metabolites in urine. It is a promising instrument to be used in large epidemiological studies but cannot be considered as a "gold standard". *Chapter 2* describes the agreement between self-reported tobacco consumption and NicCheck 1® with regard to smoking status and nicotine intake. The agreement between self-report and NicCheck 1® was high regarding smoking status, but contradictive regarding nicotine intake. Although self-report and NicCheck 1® agreed well on average, there was a large overlap in self-reported tobacco consumption between NicCheck 1® levels. In other words, some subjects who

scored low on NicCheck 1® reported a higher tobacco consumption than subjects who scored high on NicCheck 1®. This wide inter-individual variation in nicotine absorption has also been reported in other studies.[4] Nevertheless, the interpretation of this finding is hampered as long as it is unknown whether self-report or NicCheck 1® should be considered as the best indicator of nicotine intake. We tried to validate self-report and NicCheck 1® by associating them with blood lipid parameters that are known to be associated with smoking. The results of these analyses suggest that self-report does equally well as NicCheck 1®, in assessing nicotine intake.

Lifetime tobacco consumption

Lifetime tobacco consumption is often expressed in pack-years and calculated retrospectively with the help of questionnaires or smoking interviews. Despite its popularity, little is known about its validity. In *Chapter 3* retrospectively calculated pack-years (py-retro) were compared with prospectively calculated pack-years (py-pro) in order to gain insight into the (relative) validity of py-retro. Py-pro can only be calculated in prospective studies by taking into account longitudinal data. In *Chapter 3* we assumed py-pro to be a better indicator of lifetime tobacco consumption than py-retro, since it relies to a lesser extent on the memory of subjects. The results section of *Chapter 3* describes a moderate agreement between py-retro and py-pro and a reduction of this agreement with increasing number of pack-years. *Chapter 3* ends with the conclusion that future researchers in the field of smoking should be aware of the moderate (relative) validity of py-retro. In addition, it describes the option of categorizing py-retro into smoking groups. This results in a misclassification error that is smaller than the quantitative error in continuous py-retro, but of course, goes together with a loss of information.

Smoking and biological maturation during adolescence

Skeletal age, years from peak height velocity (PHV) and years from menarche are considered to be more precise estimates of biological maturation than calendar age. The figures in *Chapter 4* show a gradual rise in smoking prevalence with all four estimates of biological maturation, and suggest that skeletal age, years from peak height velocity (PHV) and years from menarche are no better predictors of smoking status during adolescence than calendar age. In addition, timing of biological maturation was expressed as skeletal age *minus* calendar age, and used as an indicator of early or late maturation. Only at calendar age 13, a higher maturation rate was associated with a higher chance of smoking (OR=3.34 [1.58-7.07]). Timing of biological maturation during adolescence did not predict smoking status in adulthood. It should be noted, however that the number of smokers at calendar 13 was small (n=10), and that not all these early maturers were measured in adulthood (i.e. at the age of 36 years).

Smoking and health parameters

Chapter 5 describes the longitudinal analyses on smoking in relationship to cardiovascular fitness and heart rate response to exercise between the age of 13 and 36 years. The results suggest that both cardiovascular fitness and heart rate response to exercise are already reduced in young healthy smokers. The strongest associations were found with maximum oxygen uptake ($VO2_{max}$) and maximum slope during treadmill running ($Slope_{max}$) as indicators of cardiovascular fitness, and with heart rate at a treadmill slope of 5% (HR_{submax}) as an indictor of heart rate response to exercise. The associations were stronger in moderate to heavy smokers (i.e. smoking at least 10 cigarettes per day) than in light smokers (i.e. smoking less than 10 cigarettes per day) and stronger in males than in females. In men, we found a significant positive interaction with time, meaning that the adverse effects of smoking become stronger over time.

In *Chapter 6*, increasing tobacco consumption was associated with 'unhealthy' changes in blood lipids on the one hand, and 'healthy' changes in blood pressure and body composition parameters on the other hand. Decreasing tobacco consumption was associated with 'healthy' changes in blood lipids and 'unhealthy' changes in blood pressure and body composition parameters. Most conclusions drawn in *Chapter 6* are based on trends instead of on significant associations, due to the relatively small number of 'changers' included in the study. This, however, this doesn't hold for the association between changes in tobacco consumption and changes in body composition parameters in females. Body weight, waist-to-hip ratio and waist circumference were significantly reduced with increasing tobacco consumption in females, and significantly increased with decreasing tobacco consumption, independent of age and changes in other lifestyles.

In both sexes, smoking was not associated with bone mineral density (BMD) parameters measured with dual-energy x-ray absorptiometry (DXA) at the age of 36 years (*Chapter 7*). However, smoking was significantly and negatively associated with quantitative ultrasound (QUS) parameters assessed with the Cuba Clinical instrument in both men and women. Broadband ultrasound attenuation (BUA) and speed of sound (SOS) are both QUS parameters. Besides bone strength, BUA is believed to provide information on bone structure, whereas SOS is believed to provide information on bone elasticity.[5] Current and lifetime tobacco smoking were significantly and negatively associated with BUA in females, whereas lifetime tobacco smoking was significantly and negatively associated with SOS in both sexes. The latter association was independent of body weight, calcium intake, physical activity and alcohol consumption in females, but not in males.

Our results suggest that both current and lifetime tobacco smoking are associated with a deterioration in bone quality, but not with a reduction in BMD. However, since BMD parameters and QUS parameters were not measured at the same sites, our results may also simply suggest that the heel bone is affected by smoking at an earlier stage than the lumbar spine and the hip.

Reversibility of smoking effects

In *Chapter 5* we suggested that the effects of smoking on cardiovascular fitness and heart rate response to exercise are reversible in 36-year-old men. This conclusion, however, is based on a cross-sectional study in which short-term ex-smokers, long-term ex-smokers and current smokers were compared with never smokers. Due to the limitations of cross-sectional analyses, the results on the reversibility of smoking effects, should be interpreted with caution. However, the finding that short-term ex-smokers were mostly in between long-term ex-smokers and current smokers with regard to cardiovascular fitness and heart rate response to exercise, strengthens the idea of reversibility. Our results on heart rate response to exercise are more or less comparable to results from the Framingham Heart Study, in which current smokers were less likely to reach their "target heart rate" during exercise, than were never smokers and ex-smokers.[6] Furthermore, our findings on reversibility of smoking effects are in agreement with results from the British Doctors' study, in which doctors who stopped smoking before the age of 35 had life expectancies similar to never smokers.[7]

Methodological considerations

All studies described in this thesis are based on data from the AGAHLS. The AGAHLS is an ongoing cohort study that provides unique data on the longitudinal development of lifestyle behaviours and numerous health outcomes between the age of 13 and 36 years. The following paragraphs discuss some methodological considerations that should be taken into account when interpreting the results described in this thesis.

External validity

The AGAHLS study population is a selective sample of the average Dutch population, which limits the generalization of our results to other populations. At entrance, all participants of the AGAHLS were pupils from two secondary schools with education levels above the average (i.e. HAVO/VWO *in Dutch*), located in two cities in the northwestern part of the Netherlands (i.e. Amsterdam and Purmerend). Only few participants had a non-Caucasian background. Nevertheless, even if the AGAHLS had been a non-selective sample of the average Dutch population, the

translation of results to other populations, living in other countries and at different times, would still have been limited. The most important reason for this limitation is that results from studies on smoking are largely dependent on characteristics of the study population, such as gender, age, educational level and country of residence. Children who attend schools with a relatively low educational level are more inclined to start smoking than children who attend schools with relatively high educational levels.[8] In the year 2000, the prevalence of smoking in the AGAHLS was far lower than in the average Dutch population (i.e. 19.1% versus 33%). The prevalence of smoking in the average Dutch population, on the other hand, was higher than in most other European countries in the year 2000.[9,10] Countries with a higher smoking prevalence were Germany (38% in 1997), Greece (38% in 1994) and Spain (34% in 1997), which was mainly the result of a relatively high smoking prevalence among men. In many other countries, however, different definitions for "smoker" and other inclusion criteria for age have been used than in The Netherlands. Furthermore, countries do not only differ in smoking prevalence, but also with regard to most popular type of tobacco. Commercial cigarettes and own rolled tobacco are popular tobacco products in the Netherlands, whereas 'snus' (i.e. a smokeless tobacco product that is placed under the lip) is popular is Sweden and Norway and 'bidis' (i.e. finely ground, sun dried tobacco, rolled in a brown tendu leaf) is popular in India. Finally, prevalence of smoking and type of tobacco vary through time as well. In the Netherlands, changes in tax policy have played an important role in this variations.[11]

Internal validity: selection bias

Internal validity reflects the extent to which conclusions of a study can be generalized to the 'source population'. The source population of the AGAHLS is the cohort that was started with in 1977. Various types of bias can distort the internal validity: selection bias, confounding and information bias. Loss to follow-up is the major cause of selection bias in observational cohort studies.[12] In the AGAHLS, all pupils from the first and second grade of two secondary schools gave permission to participate in the AGAHLS. During the first four years of the study, participants did not dropout for any reason other than for changing schools. Unfortunately, after the fourth measurement, when participants left secondary school, participants started to drop out due to several reasons. During the 23-years of follow-up, about one in three participants of the AGAHLS dropped out. As a consequence, we may have underestimated the smoking prevalence within the AGAHLS if smokers dropped out more frequently than non-smokers. Results presented in *Chapter 4*, however, suggest that this is rather unlikely since smokers and non-smokers showed similar probabilities of dropping out in four out of five measurement periods.

Another possibility is that we underestimated the strength of the associations between smoking and health parameters. This may have happened if relatively unhealthy smokers dropped out more frequently than relatively healthy smokers. In order to investigate this 'selective dropout effect' we compared smokers who dropped out at the ninth measurement with smokers who did not. Fortunately, dropouts performed equally well as non-dropouts on the treadmill running test at the eighth measurement with regard to $VO2_{max}$ and $Slope_{max}$.

Internal validity: confounding

Confounding is 'confusion of effects'. It occurs when the effect of the variable of interest (in this case smoking) is distorted because the effect of an extraneous factor is mistaken for or mixed with the actual exposure effect.[13] The most obvious (possible) confounders in the association between smoking and health outcomes are lifestyles that tend to go hand in hand with smoking (e.g. alcohol consumption, physical inactivity). In the AGAHLS, many lifestyles other than smoking (i.e. physical activity, nutritional intake and alcohol consumption) have been measured longitudinally. As a consequence, in all the studies described in this thesis we corrected for the most obvious confounders.

Internal validity: information bias

Information bias can occur whenever there are errors in the measurement of subjects.[13] Data on smoking status and current and lifetime tobacco consumption used in this thesis, were mainly obtained with the help of questionnaires or interviews. As a consequence, underreport of tobacco consumption may have overestimated the strength of the associations between smoking and health parameters, if all smokers underreported their tobacco consumption (expressed as a continuous variable) to the same extent. However, a scenario of strictly underreport seems rather unlikely. Instead, it seems more likely to assume that part of our smokers under-reported, while others over-reported their tobacco consumption, resulting in *random measurement error*. Results described in *Chapter 3* strengthen the idea that random measurement error occurred. Py-retro did not under- or overestimate lifetime tobacco smoking, but provided random measurement error, that became larger with higher number of pack-years. When *random measurement error* occurs, the strength of the associations can either be overestimated or underestimated. Results described in *Chapter 2* suggest that self-report does equally well as NicCheck 1® in assessing nicotine intake in the previous 24 hours, but this doesn't rule out measurement error.

Population size

Although the AGAHLS started in 1977 with around 600 pupils, the relatively low number of smokers has limited the interpretation of the results of this thesis to a certain extent. A small population sample goes together with a large random error (i.e. low precision), which reduces the chance of rejection of test hypotheses. In *Chapter 6,* for instance, the hypothesis that changes in tobacco consumption are not associated with changes in cardiovascular risk parameters, was tested. This hypothesis was not rejected in males (based on p-values above 0.05), despite strong indications for a reduction in blood pressure, a deterioration in blood lipid profile, and a reduction in body weight and central body fat distribution with increasing tobacco consumption. A larger number of 'tobacco changers' would probably have resulted in significant associations.

Implications

Assessing current tobacco smoking

The results of *Chapter 2* suggest that the agreement between self-report and NicCheck 1® is high with regard to smoking status but moderate with regard to nicotine intake, because of the large overlap in self-reported tobacco consumption between NicCheck 1® levels. As a consequence, it seems that there is no need for NicCheck 1® when smoking status is being assessed in healthy adults. It even seems that self-report is better than NicCheck 1® in smokers who do not smoke regularly or only one or two cigarettes per day. Unfortunately, our results cannot be generalised to other populations such as adolescents or subjects in intervention studies of which smoking cessation is expected. Subjects from these populations are more inclined to deny smoking or underreport tobacco consumption.[14] A weakness of the study described in *Chapter* 2, is that self-reported tobacco consumption was only compared with NicCheck 1® at the ninth measurement of the AGAHLS, and not during adolescence when the validity of self-reported tobacco smoking was probably lower than in adulthood.[14] Whether self-report or NicCheck 1® is better in assessing nicotine intake is still unknown. It is clear that both estimates provide different results but a 'gold standard' is needed to answer this question.

Assessing lifetime tobacco smoking

We found a moderate agreement between pack-years calculated retrospectively based on questionnaires or interviews (py-retro) and pack-years calculated prospectively based on longitudinal data (py-pro) (*Chapter 3*). Since most researchers have no access to longitudinal data, they have no other option than to use py-retro as an estimate of lifetime tobacco smoking. It is important that researchers in the field of smoking are aware of the moderate (relative) validity of py-retro when interpreting

the results of their studies. Researchers who do have access to longitudinal data or those who are planning to start a longitudinal study, should be aware of the importance of frequent measurements. During adolescence, smoking behaviour should be assessed at least once a year,[15] whereas smoking behaviour seems to be relatively stable during adulthood,[16] which allows measurements with longer time intervals.

Prevention of smoking initiation

The results in *Chapter 4* of this thesis suggest that skeletal age, years from peak height velocity and years from menarche (in girls) were no better predictors of smoking status than calendar age. In addition, early maturers had a higher chance to be a smoker at calendar age 13 than normal or late maturers, but this 'early maturation effect' disappeared at higher calendar ages. Moreover, early maturation was not associated with smoking status in adulthood. This suggests that campaigns aiming at the prevention of smoking initiation among children do not have to use different programs for children who mature relatively early, nor do they have to define 'high risk groups' based on maturation rate.

Smoking policy

The results described in this thesis do not argue against the current smoking policy of the Dutch government and the European Union that both aim to reduce the prevalence of smoking and to prevent youngsters from smoking initiation. In all countries of the European Union, for example, larger warnings about the damaging effects of smoking have been placed, or will be placed, on packets of cigarettes. In the Netherlands, these warnings have been placed on all packages since May 1 2002 (e.g. 'Smoking is lethal', 'smoking can harm sperm and reduces fertility'). In addition, advertisements in newspapers and magazines have been prohibited in The Netherlands since January 1 2003, similar as the sale of tobacco products to youngsters below the age of 16. Although the results described in *Chapter 5* suggest that detrimental effects of smoking on cardiovascular fitness and heart rate response to exercise are weak or even absent in relatively young light tobacco smokers (i.e. smoking less than 10 cigarettes per day), and that some of the detrimental effects of smoking are reversible in 36-year-old men (*Chapter 5*), these results should not be used as an argument against the current smoking policy. It is well known that even light tobacco smoking (i.e. defined as less than five or less than ten cigarettes per day) increases the risk of lung cancer[17-19] and that light tobacco smoking (i.e. less than six per day) is unstable and can easily change into moderate to heavy smoking.[20] Smoking itself, however, is very stable. Participants of the AGAHLS who smoked

during adolescence were ten times as likely to smoke at the age of 21 than participants who did not, and seven times as likely at the age of 27.[21]

Aided smoking cessation

The results presented in *Chapter 6* suggest that decreasing tobacco consumption goes together with unhealthy changes in body weight, central body fat distribution and blood pressure. Although the effects of smoking cessation on body weight seem temporary,[22,23] quitters who fail frequently will show a high variability in body weight, which might have negative health consequences.[24] In a recent prospective study among 40- to 59-year-old men, recent ex-smokers who showed weight fluctuation were at increased risk of mortality compared to recent ex-smokers who did not show any weight change.[25] Therefore, lots of effort should be spent on the prevention of relapse in recent ex-smokers. Two third of the Dutch smokers do 'unaided' quitting attempts, although the risk of failure in 'unaided' quitters is very high.[26] Unsuccessful quitting attempts reduce the chance of future successful quitting attempts by reduced feelings of self-efficacy. Therefore, one might argue that 'unaided' smoking cessation attempts do more harm than good. The Dutch Organisation for tobacco control offers free information about all available possibilities for smokers to get help with smoking cessation (e.g. tailored self-help materials, personal advice and support by telephone, group meetings with other smokers, nicotine replacement therapy etc). None of these smoking cessation aids or programs seem to pay much attention to changes in body weight, although the increase in body weight after smoking cessation can grow largely out of proportion. Williamson *et al.*[23] reported that 9.8% of the male sustained quitters and 13.4% of the female sustained quitters gained more than 13 kg of weight after smoking cessation. Smokers in a smoking cessation program should at least be informed about possible weight gain, fluctuations in body weight and the possibilities to prevent this.

Future research

The effect of smoking on mortality and morbidity has been studied extensively and the damaging effects of smoking are largely recognized. However, since both smoking behaviour and the prevalence of disease vary geographically and over time, associations between smoking, mortality and morbidity may also differ between countries and through time. Most studies on smoking have been performed in the western world and most of these studies only considered the use of manufactured cigarettes. As a consequence, more studies are needed on smoking and health in countries outside the western world and on tobacco products other than manufactured cigarettes.

Future research should also focus on the validity of current and lifetime tobacco smoking. Several biochemical methods are available to estimate tobacco consumption in the previous hours, but most of these methods are costly and time consuming. Moreover, none of these biochemical methods can be considered as the 'gold standard'. NicCheck 1® seems to be an inexpensive and quick alternative to be used in epidemiological studies. However, NicCheck 1® should first be compared with (other) biochemical methods to assess nicotine intake in a large population consisting of non-smokers, light smokers, moderate smokers and heavy smokers. From *Chapter 3,* it becomes clear that more studies are needed to draw more definite conclusions on the validity of py-retro. Prospective studies in which smoking behaviour is measured more frequently than in the AGAHLS are needed to validate py-retro against py-pro. Furthermore, consensus is needed on how to model different aspects of smoking history. For instance, there are no uniform definitions for current smoker, former smoker, light smoker, moderate smoker, etc. There is also no consensus on whether to use pack-years, smoking duration, smoking intensity or age at initiation when studying the relationship between lifetime tobacco smoking and health outcomes, although pack-years are most frequently used.[27]

More studies are needed to investigate how long changes in cardiovascular risk factors last after changes in tobacco consumption have occurred. Is the rise in body weight after decreasing tobacco consumption temporary or transient? Two smoking cessation studies[22,23] reported temporary changes in weight gain after smoking cessation. However, whereas Chen *et al.*[21] reported a temporary rise of two years, Williamson *et al.*[23] reported a 'temporary' rise that was still present after seven to twelve years. Furthermore, it needs to be investigated whether or not changes in cardiovascular risk factors differ between tobacco reducers and quitters. A recently published large prospective study showed that smoking reduction does not reduce mortality risk from tobacco related diseases.[28] The results of this study were adjusted for age.

Finally, studies on smoking have focused almost exclusively on the detrimental effects of smoking, although smoking can have some beneficial effects as well. For instance, smoking may offer comfort and strength to deal with the difficulties in life. In addition, smoking may help to relax in situations of undefined fear, and help to concentrate better under certain conditions.[29] One could argue that everyone should be free to decide whether or not to smoke. After all, smoking can offer pleasure, similar as taking an alcoholic beverage or eating food products that are considered unhealthy. Pleasure is a positive emotion that makes people more resistant to illness.[28] Future research should focus not only on the negative aspects of smoking but also investigate the extent to which smoking raises quality of life. It is without doubt, that the beneficial effects of smoking will never outweigh the

detrimental effects of smoking in moderate and heavy smokers. However, in light smokers and occasional smokers, beneficial effects of smoking may compensate for detrimental effects.

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Summary

Smoking and health from adolescence into adulthood.

Results from the Amsterdam Growth and Health Longitudinal Study

Summary

This thesis describes the cross-sectional and longitudinal relationship between smoking and biological health parameters in young and relatively healthy males and females between the age of 13 and 36 years, in order to investigate the extent to which unfavourable effects of smoking are already present and measurable in adolescence and young adulthood and how such effects develop over time. All data described in this thesis come from the Amsterdam Growth and Health Longitudinal Study (AGAHLS). In the AGAHLS ± 400 boys and girls from the first and second grade of two secondary schools in Amsterdam and Purmerend have been measured maximally nine times over a period of 23 years.

Contents of this thesis

The first part of this thesis focuses on the validity of *current* tobacco consumption and *lifetime* tobacco consumption (i.e. pack-years). The second part describes the development of tobacco smoking from adolescence into adulthood and its relationship with biological maturation. The third part describes the relationship between smoking and biological risk factors for cardiovascular disease, whereas the fourth part reports on the relationship between smoking and bone strength parameters at the age of 36.

1. Assessing tobacco consumption

Validity of current tobacco consumption

Chapter 2 compares self-reported tobacco consumption in the previous 24 hours with results from a dipstick method called NicCheck 1®. NicCheck 1® changes colour in the presence of nicotine and its metabolites after it has been dipped in urine. The results of this study indicate a high agreement between self-report and NicCheck 1® with regard to smoking status (i.e. smoking or non-smoking). The agreement was highest in moderate to heavy smokers and lowest in light tobacco smokers (≤ two cigarettes in the previous 24 hours). Despite the high correlation coefficient between self-report and NicCheck 1® in smokers, there was a large overlap in self-reported tobacco consumption between NicCheck 1® levels. This indicates the individual differences in the association between self-report and NicCheck 1®. To estimate the validity of both methods, smoking groups based on self-report and smoking groups based on NicCheck 1® were compared on blood lipid parameters that are known to be associated with smoking. This latter analysis suggested that self-report does not do worse than NicCheck 1® in assessing current tobacco consumption.

Validity of lifetime tobacco smoking

In the AGAHLS, lifetime tobacco smoking was assessed with help of an extensive

questionnaire on current tobacco smoking and smoking (and quitting) behaviour in the past. This 'retrospective' method to assess lifetime tobacco smoking was compared with a 'prospective' method in which all longitudinal data were taken into account. The prospective method was regarded as the better method, since it relies on the memory of the participants to a much lesser extent in comparison to the retrospective method. The results showed that the retrospective method did not under or overestimate lifetime tobacco smoking assessed with the prospective method. However, the agreement between both methods was moderate and dependent on the amount of tobacco consumption (i.e. the number of pack-years). The higher the number of pack-years, the lower the agreement. From these results, we concluded that researchers should be aware of the moderate (relative) validity of lifetime tobacco smoking assessed with the retrospective method. However, we do not advice researchers to use the prospective method only since many researchers do not have longitudinal data at their disposal. Furthermore, the validity of lifetime tobacco assessed with the prospective method is largely dependent on the frequency of the measurements.

2. Biological maturation and the development of tobacco smoking

Chapter 4 describes the development of smoking behaviour in boys and girls during adolescence and its relationship with four different estimates of biological maturation: 1. calendar age, 2. skeletal age, 3. years from peak height velocity (PHV) and 4. years from menarche (in girls). The expectation that calendar age would be worse than the other three estimates of biological maturation in predicting smoking during adolescence, was not confirmed. Prevalence of smoking rose rapidly but gradually with the increase in all four estimates of biological maturation. Early maturers (skeletal age > calendar age) had a higher chance to smoke than normal (skeletal age = calendar age) and late maturers (skeletal age < calendar age) at calendar age 13. At calendar age 14, 15, and 16, the higher prevalence of smoking among early maturers was no longer present. Moreover, early maturation did not predict smoking in adulthood.

3. Smoking and risk of cardiovascular disease

Chapter 5 describes the longitudinal relationship between smoking and cardiovascular fitness and heart rate response to exercise, which are predictors of all causes mortality. The results suggest that moderate to heavy tobacco consumption (≥ 10 cigarettes per day) is associated with reduced cardiovascular fitness and heart rate response to exercise. The associations were stronger in moderate to heavy tobacco smokers than in light tobacco smokers (< 10 cigarettes per day) and stronger in men than in women. Furthermore, the previous described negative relationships became

stronger with increasing age in men, but not in women. Finally, results from the cross-sectional analyses at the age of 36 suggest that the adverse effects of smoking are reversible in men but not in women.

The results described in *Chapter* 6 suggest that a rise in tobacco consumption is associated with unfavourable changes in blood cholesterol parameters but also with favourable changes in blood pressure, body weight, and (central) body fat distribution. A reduction in tobacco consumption was associated with the opposite effects.

4. Smoking and bone strength

This thesis ends with the relationship between smoking and bone strength at the age of 36. Bone strength was measured with dual-energy X-ray absorptiometry (DXA) in the lumbar spine (L1-L4), hip and total body, but also with quantitative ultra sound (QUS) in the heel bone, using the CUBA Clinical instrument. DXA measures bone mineral density (BMD), whereas QUS offers two parameters (i.e. BUA and SOS) that are believed to provide information on bone structure and material properties (bone quality), independently of BMD. The results suggest that both current and lifetime tobacco smoking are associated with a deterioration in BUA and SOS but not with a reduction in BMD. It is the negative relationship between pack-years and QUS that seems clinically relevant in women. Smoking one packet of cigarettes each day during 30 years goes together with a considerable deterioration in bone quality. Since BMD parameters were not measured at the same sites, our results may also simply suggest that the heel bone is affected by smoking at an earlier stage than the lumbar spine, hip and total body.

Conclusions

1. Self-report seems to be as good as NicCheck 1® in assessing current tobacco consumption in 36-year-old (relatively) health men and women. However, since a gold standard to measure current tobacco consumption is missing, we should be careful with our conclusions on the validity of self-reported tobacco consumption.

2. Although pack-years based on retrospective data (py-retro) do not seem to over or under estimate pack-years based on prospective data (py-retro), future researchers should be aware of large individual differences between py-retro and py-pro that may occur in individuals with a large smoking history.

3. Skeletal age, years from peak height velocity and years from menarche are no better predictors of smoking during adolescence than calendar age. Early maturation

was a predictor of smoking at calendar age 13, but not at calendar age 14, 15, 16 and 36.

4. Lower cardiovascular fitness and heart rate response to exercise were already present in 13- to 36-year-old smokers compared to non-smokers of similar age. These differences were larger in moderate to heavy smokers than in light tobacco smokers and larger in men than in women. In men but not in women, negative associations between smoking and cardiovascular fitness and heart rate response to exercise became larger with increasing age.

5. A reduction in tobacco consumption seems to be associated with favourable changes in blood lipids, but also with unfavourable changes in blood pressure, body weight and central body fat distribution.

6. Although we found no relationship between smoking and BMD in the lumbar spine and hip measured with DXA, our results suggest that smoking is associated with a reduced bone quality in the heel bone assessed with QUS.

Samenvatting

Roken en gezondheid van adolescentie naar volwassenheid.

Resultaten uit het Amsterdams Groei en Gezondheidsonderzoek

Samenvatting

Dit proefschrift probeert een antwoord te geven op de vraag of roken al op jonge leeftijd samengaat met biologische risicofactoren voor hart en vaatziekten en botsterkte. Tevens beschrijft het hoe de relatie tussen roken en gezondheid zich ontwikkelt tussen het 13e en 36e levensjaar. De resultaten die beschreven staan in dit proefschrift berusten op gegevens die verzameld zijn binnen het Amsterdams Groei- en Gezondheidsonderzoek (AGGO). Binnen het AGGO zijn ± 400 jongens en meisjes herhaald gemeten (maximaal 9 keer) tussen hun 13e en 36e levensjaar.

Opbouw

Het eerste deel van het proefschrift gaat over het meten van tabaksconsumptie en of zelfgerapporteerde tabaksconsumptie wel een juist beeld geeft van de werkelijke blootstelling aan (schadelijke) bestanddelen van tabak. Het tweede deel gaat over de ontwikkeling van rookgedrag tijdens de adolescentie en de samenhang met biologische ontwikkeling. Het derde deel gaat over roken in relatie tot biologische risicofactoren voor hart- en vaatzieken, en het vierde deel over roken en botsterkte.

1. Meten van tabaksconsumptie

Validiteit huidige tabaksconsumptie

In hoofdstuk 2 wordt zelfgerapporteerde tabaksconsumptie in de afgelopen 24 uur vergeleken met een 'dipstick' methode waarbij een strookje met chemicaliën (NicCheck 1®) in urine gedoopt wordt en van kleur verandert bij de aanwezigheid van nicotine en haar metabolieten. De mate van verkleuring is een maat voor de hoeveelheid nicotine en metabolieten in de urine. De overeenstemming tussen beide methoden was hoog voor wat betreft rookstatus (wel of niet roken), zeker bij de matig tot zware rokers. NicCheck 1® leek echter minder gevoelig bij lage tabaksconsumptie (≤ twee sigaretten in de afgelopen 24 uur). Ondanks de hoge correlatie tussen zelfgerapporteerde tabaksconsumptie en de score op NicCheck 1®, was er grote overlap in de NicCheck 1® score per zelfgerapporteerde tabaksconsumptie. Dit suggereert dat er grote individuele verschillen zijn in de relatie tussen zelfgerapporteerde tabaksconsumptie en de score op NicCheck 1®. Tot slot werd onderzocht welk van de twee methoden de beste is om huidige tabaksconsumptie te bepalen. Hiervoor werden rokersgroepen, bepaald m.b.v. zelfrapportage enerzijds en m.b.v. NicCheck 1® anderzijds, met elkaar vergeleken op twee cholesterol parameters. Geconcludeerd werd dat zelfrapportage even goed was als NicCheck 1® voor het bepalen van huidige tabaksconsumptie.

Validiteit levenslange tabaksconsumptie
Levenslange tabaksconsumptie wordt vaak uitgedrukt in pakjaren en bepaald met behulp van vragen over roken (en stoppen met roken) in het verleden. Het nadeel van deze retrospectieve methode is dat er een groot beroep wordt gedaan op het geheugen doordat deelnemers terug moeten denken over een lange periode (vanaf het moment dat zij voor het eerst gingen roken). In hoofdstuk 3 van dit proefschrift wordt deze 'retrospectieve methode' vergeleken met een 'prospectieve methode' waarin gebruik wordt gemaakt van alle longitudinale data om levenslange tabaksconsumptie te bepalen. De longitudinale dataset van het AGGO maakte dit mogelijk omdat vrijwel alle deelnemers pas begonnen met roken na de start van de studie. Het retrospectief bepaald aantal pakjaren (py-retro) gaf geen structurele overschatting of onderschatting van het prospectief bepaald aantal pakjaren (py-pro). Er waren echter wel grote verschillen tussen individuen in de overeenstemming tussen py-retro en py-pro. Deze verschillen werden groter naarmate het aantal pakjaren toenam. Geconcludeerd werd dat onderzoekers zich bewust moeten zijn van de matige validiteit van py-retro. Echter, het gebruik van levenslange tabaksconsumptie verkregen met de retrospectieve methode werd niet afgeraden. Veel onderzoekers beschikken immers niet over longitudinale gegevens. Bovendien is de validiteit van levenslange tabaksconsumptie verkregen met de prospectieve methode grotendeels afhankelijk van de frequentie van meten.

2. Biologische ontwikkeling en rookgedrag
In hoofdstuk 4 van dit proefschrift werd de prevalentie van roken uitgezet tegen vier verschillende maten voor biologische ontwikkeling: 1. kalenderleeftijd, 2. skeletleeftijd, 3. het aantal jaren vanaf de groeispurt, en 4. het aantal jaren vanaf de eerste menstruatie (bij meisjes). Omdat kalenderleeftijd slechts een grove maat is voor biologische ontwikkeling, was de verwachting dat deze een minder goede voorspeller zou zijn voor roken tijdens de adolescentie dan de overige drie maten voor biologische ontwikkeling. Dit bleek echter niet het geval te zijn. De prevalentie van roken steeg snel maar geleidelijk met het toenemen van alle vier de maten voor biologische ontwikkeling, zowel bij jongens als bij meisjes. Vroegrijpers (skeletleeftijd > kalenderleeftijd) hadden op 13-jarige leeftijd een grotere kans om te roken dan laatrijpers (skeletleeftijd < kalenderleeftijd) en gemiddelde rijpers (skeletleeftijd = kalenderleeftijd). Echter, op 14, 15 en 16-jarige leeftijd was dit niet meer het geval. Een vroege biologische ontwikkeling bleek bovendien geen voorspeller te zijn voor roken op 36-jarige leeftijd.

3. Roken en risicofactoren voor hart- en vaatziekten
Hoofdstuk 5 beschrijft de longitudinale relatie tussen roken en fitheid van het hart-

en vaatstelsel en hartslagrespons tijdens inspanning, die beiden voorspellers zijn voor algemene sterfte. Uit de resultaten blijkt dat matige tot zware tabaksconsumptie (≥10 sigaretten per dag) samengaat met een verlaagde fitheid en hartslagrespons tijdens inspanning. De gevonden relaties waren sterker bij matig tot zware rokers dan bij lichte rokers (< 10 sigaretten per dag) en sterker bij mannen dan bij vrouwen. Deze negatieve relaties werden bij mannen bovendien sterker met het toenemen van de leeftijd. Bij vrouwen was dit niet het geval. Resultaten uit de dwarsdoorsnede analyses suggereerden tevens dat de negatieve effecten van roken omkeerbaar waren bij mannen die voor hun 36e levensjaar gestopt waren met roken. Bij vrouwen werd dit effect van stoppen met roken niet gevonden, mogelijk omdat de effecten van roken bij vrouwen minder sterk waren. De verschillen tussen mannen en vrouwen konden niet verklaard worden door verschillen in tabaksconsumptie.

Hoofdstuk 6 beschrijft de relatie tussen (vrijwillige) veranderingen in tabaksconsumptie (zowel minderen als meerderen) en veranderingen in biologische risicofactoren voor hart- en vaatziekten. Een toename in tabaksconsumptie ging samen met ongunstige veranderingen in cholesterol parameters. Daarnaast ging het echter ook samen met een verlaging van de bloeddruk, een verlaging van het lichaamsgewicht en een minder centrale vetverdeling. Een afname in tabaksconsumptie ging samen met de tegenovergestelde effecten.

4. Roken en botsterkte

Tot slot wordt het proefschrift afgesloten met een studie naar de relatie tussen roken en botsterkte op 36 jarige leeftijd. Botsterkte werd gemeten met twee verschillende methoden. Ten eerste, met behulp van een scan waarbij gebruik wordt gemaakt van röntgen (dual-energy X-ray absorptiometry = DXA) en ten tweede met behulp van het CUBA Clinical instrument dat gebruik maakt van ultrageluidsmetingen. Het CUBA Clinical instrument meet zowel "Broadband Ultrasound attenuation" (BUA) als "Speed of Sound" (SOS) in het hielbot. Verondersteld wordt dat ultrageluidsmetingen informatie geven over botkwaliteit (bijvoorbeeld botstructuur of materiaaleigenschappen) en dat deze informatie onafhankelijk is van de botminerale dichtheid (BMD) die gemeten wordt met de DXA scan. De resultaten van dit onderzoek suggereren dat roken niet samengaat met BMD gemeten met DXA in de lumbale wervelkolom en de heup maar wel met botkwaliteit gemeten met de ultrageluidparameters in het hielbot. Met name de relatie tussen pakjaren en ultrageluidparameters lijkt klinisch relevant bij vrouwen. Het roken van één pakje sigaretten per dag over een periode van 30 jaar lijkt samen te gaan met een aanzienlijke verslechtering van de botkwaliteit. Bij de interpretatie van deze resultaten uit hoofdstuk 7 is echter voorzichtigheid geboden. Daar waar het Cuba

Clinical Instrument botsterkte mat in het hielbot, mat DXA botsterkte in de lumbale wervelkolom (L1-L4) en de heup. Een andere interpretatie van deze resultaten zou kunnen zijn dat de gevolgen van roken eerder meetbaar zijn in het hielbot dan in de lumbale wervelkolom en de heup.

Conclusies

1. Zelfrapportage lijkt bij 36-jarige (relatief) gezonde mannen en vrouwen een even goede methode ter bepaling van huidige tabaksconsumptie te zijn als NicCheck 1®. Echter, harde uitspraken over de validiteit van zelfgerapporteerde huidige tabaksconsumptie kunnen niet worden gedaan wegens gebrek aan een echte gouden standaard.

2. Het retrospectief bepaald aantal pakjaren (py-retro) gaf geen structurele overschatting of onderschatting van het prospectief bepaald aantal pakjaren (py-pro). Echter, onderzoekers dienen zich ervan bewust te zijn dat py-retro sterk kan afwijken van py-pro, met name bij deelnemers met een lange rookgeschiedenis.

3. Biologische maten voor ontwikkelingsleeftijd zoals skeletleeftijd en het aantal jaren voor of na de groeispurt of eerste menstruatie, zijn niet beter in het voorspellen van rookgedrag bij adolescenten dan kalenderleeftijd. Vroegrijpers hebben ten opzichte van andere tieners een grotere kans om te roken op 13-jarige leeftijd maar niet meer op latere leeftijd.

4. Roken lijkt al op jonge leeftijd samen te gaan met een verlaagde cardiovasculaire fitheid en hartslag respons tijdens inspanning. De bestudeerde relaties tussen roken en cardiovasculaire fitheid en hartslag respons bij inspanning waren over het algemeen sterker bij matig tot zware rokers dan bij lichte rokers en sterker bij mannen dan bij vrouwen. Met het toenemen van de leeftijd werden de negatieve relaties tussen roken, fitheid, en hartslagrespons sterker bij mannen maar niet bij vrouwen.

5. Het verminderen van tabaksconsumptie lijkt niet alleen samen te gaan met gunstige veranderingen maar ook met ongunstige veranderingen zoals een toename in bloeddruk, lichaamsgewicht, en centrale vetverdeling.

6. Roken is op 36-jarige leeftijd niet gerelateerd aan botminerale dichtheid gemeten in de lumbale wervelkolom en de heup met een DXA scan, maar ultrageluidsmetingen in het hielbot suggereren dat roken wel samen gaat met een slechtere botkwaliteit.

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About the Author

Claire Monique Bernaards was born on December 8th, 1974 in The Hague, The Netherlands. In 1993 she obtained her secondary school diploma at the Sint Laurens College in Rotterdam and started her study at the Faculty of Human Movement Sciences at the Vrije Universiteit in Amsterdam. In 1998, she graduated with a major in Health Science in relation to human movement and minors in Psychology in relation to human movement and Ergonomics and work. In the same year, she started working as a PhD student at the Institute for Research in Extramural Medicine (EMGO Institute) in Amsterdam, which resulted in the present thesis. During her work as a PhD student she attended statistical and methodological courses organised by the Postgraduate Epidemiology Programme of the EMGO Institute. In 1999, she attended the New England Epidemiology Summer Program at Bentley College in Waltham (MA, USA). She is currently working as a post-doctoral research fellow at the department of Social Medicine of the EMGO Institute. Her new research project is part of Body@Work TNO-VU, a Research Center for Physical activity, Work and Health and concerns a randomized controlled trial on the effectiveness of individual counseling on physical activity to reduce complaints in the upper extremities of VDU workers. In addition, she is employed as a researcher at TNO Work and Employment.