SERUM LIPIDS IN THE YOUNG

AN EPIDEMIOLOGICAL VIEW OF EARLY ATHEROGENESIS

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AN EPIDEMIOLOGICAL VIEW OF EARLY ATHEROGENESIS

SERUM LIPIDEN BIJ JONGEREN

EEN EPIDEMIOLOGISCHE BENADERING VAN VROEGE ATHEROGENESE

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
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Aan mijn ouders
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Chapter 1
INTRODUCTION

1.1 Rationale

Cardiovascular diseases are the leading cause of mortality in the industrialized world, together accounting for about one-half of the total number of annual deaths. Coronary heart disease comprises about one-half of the cardiovascular mortality and cerebrovascular disease about one-quarter. Diseases of the circulation not only result in many lost years of life, but they also compromise the quality of life considerably.

The main cause of cardiovascular diseases is atherosclerosis. Many factors are implicated in atherogenesis, but serum lipids and especially serum cholesterol appear to play an essential role. In biochemical studies cholesterol was found to be the principal lipid ingredient of the atherosclerotic lesion.[1-4] In animal experiments it was shown that feeding cholesterol was necessary to produce major atherosclerotic lesions.[5,6] Clinical studies showed that patients with coronary heart disease had higher serum cholesterol concentrations than those free of this disease.[7,8] Epidemiological investigations revealed positive relations between serum cholesterol levels and the prevalence and incidence of coronary heart disease in both cross-sectional and longitudinal studies.[9-13]

Subsequently, various fractions of serum total cholesterol, carried in the blood by different lipoproteins,[14,15] were studied. New clinical and epidemiological studies were carried out to find the relevance of these lipid fractions as indicators or predictors of coronary heart disease. Once the positive relation between total cholesterol and coronary heart disease was established, emphasis was laid on high density lipoprotein (HDL) cholesterol as a possible protective factor. Although this protective relation had been suggested in the early fifties,[16] it was not until the mid seventies that by a publication of Miller and Miller,[17] the discussion revived. In the mean time low density lipoprotein (LDL) cholesterol was proposed as the main atherogenic factor.[18] Some investigators suggested that not LDL or HDL cholesterol, but rather their ratio, was the most important predictor of coronary heart disease.[19] More recently apolipoproteins, molecular protein structures on the surface of the lipoproteins, have been suggested to be even better predictors of coronary heart disease.[20]

Atherosclerosis does not only affect adults. Rather, evidence is accumulating that atherogenesis begins in childhood. Post mortem studies have shown aortic atherosclerotic lesions in children[21] and coronary lesions in young soldiers killed in Korea and Vietnam.[22,23] Aortic fatty streaks are present in virtually all children over the age of 3 years and by the age of 20 years many have coronary fatty streaks.[21,24] Although early atherosclerotic lesions are ubiquitous in children, the incidence of coronary heart disease is not the same all over the world.[25] The early fatty streaks may transform to fibrous plaques or progress into advanced atherosclerotic lesions.[26] Eventually, this may be brought about as one of the manifestations of coronary heart disease, notably angina pectoris, myocardial infarction or sudden death. As the process of atherosclerosis begins early in life, it may have merit to study its aetiology, pathogenesis and distribution in the young. An increase in knowledge about the aetiology and early determinants of cardiovascular diseases may provide more specific tools for early preventive activities. From studies in adults risk factors for coronary heart disease have been identified, notably blood pressure, serum lipids and cigarette smoking. The findings in a recently reported pediatric autopsy study suggest that childhood levels of risk factors for cardiovascular diseases may be related to even the earliest stages of atherosclerotic lesions in youth.[27] In this study, significant associations were observed between the percentage of fatty streak involvement of the aorta and previously measured total and LDL cholesterol, whereas VLDL cholesterol was related to coronary artery fatty streaks. Other studies of childhood risk factors and end-organ damage have supported the view that early in life risk factors are related to atherosclerosis, and that cardiovascular disease begin in childhood.[28,29] These observations provide the additional rationale for investigations of serum lipids in the young.

1.2 Study objectives

Several questions may be asked concerning serum lipids early in life. A first question is: what are the distributions of serum lipids in children and young adults and what determines the level? A question to follow naturally is whether there is any relation between serum lipid levels early in life and later on. A confirmative answer to this question is a prerequisite for prediction of future cholesterol levels and research into the aetiology of hypercholesterolemia, by

inquiries of patterns and determinants of change of lipid levels.

A next question is whether heart disease can be prevented by favourably modifying risk factor levels in children. Preventive actions should be based on a joint understanding of the aetiological concepts of chronic diseases and the theoretical aspects of prevention. A final question is whether it is possible to predict heart disease by measuring serum lipid levels early in life. This is not only important in finding high risk persons for preventive intervention, but it may also lead to new insights into early atherogenesis.

1.3 Chapter outline

The above mentioned questions led to study objectives that were investigated using several study designs and data analytical approaches. This is described in chapter 2, which also gives a description of the study population and measurements. In chapter 3, the distributions of total and HDL cholesterol in children and young adults are given (appendix 1). Determinants which are discussed include familial aggregation, anthropometrical variables, hormonal factors and life habits (appendices 1-4). The natural history of serum lipids is described in chapter 4. It includes prediction of future levels and patterns and determinants of change (appendix 5).

Chapter 5 gives a short introduction of lipid metabolism and atherosclerosis as a cellular proces. It continues with a discussion of the possiblities of prevention by introducing a hypothetical model of the relation between either level or change of serum cholesterol, and the risk of coronary heart disease. Such a model may serve as a rationale for either the whole-population or the high-risk approach of preventive actions. Finally, some aspects of the early prediction of coronary atherosclerosis are discussed (appendix 6).

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Chapter 2

METHODOLOGY

2.1 General

In epidemiology there are two major types of research: observational and experimental studies.[1] To establish cause-effect relationships, the experiment with randomization on the basic element in study design, serves as the 'gold standard'.[2] However, it is not always possible to perform experimental studies and therefore observational studies may have their own merits, provided that care has been taken to avoid bias in the design of the study or, when possible, to control bias in the analysis. The types of study described in this thesis are all observational in character and they include cross-sectional, follow-up and case-control studies. In table 2.1 the several study objectives of this thesis are summarized by type of study.

TABLE 2.1

Studies of serum lipids in young people reported in this thesis

Type	Study objectives	Chapter	Appendix
Cross-sectional	Distributions of serum lipids	3	1
	Determinants of serum lipids		
	familial aggregation	3	1
	anthropometrical variables	3	1
	hormonal factors	3	1,2,3
	life habits	3	1
Follow-up	Determinants of serum lipids		
	hormonal factors	3	2,3,4
	Natural history of serum lipids	4	5
Case-control	Early prediction of atherosclerosis	5	6

2.2 Study population

The study subjects described in this thesis actually derive from three different populations, including one general population and two hospital populations.

2.2.1 General population

In 1975 a large cross-sectional study on determinants of various chronic diseases including rheumatic disorders, chronic non specific lung disease, urinary tract infections and cardiovascular diseases was initiated by professor H.A. Val-

kenburg in Zoetermeer, a suburban town near The Hague (the EPOZ study, Epidemiologic Preventive Organisation Zoetermeer).[3] Two districts in Zoetermeer (which doubled in number of residents from about 40.000 in 1975 to about 80.000 in 1985) were chosen for this study, an urban and a rural one. From the 13.462 subjects eligible, 10.532 participated (78.2 %) in the baseline survey. In the group of 5-30 year olds, 5.367 subjects out of 6.672 eligible (80.4 %), were examined. Although it was cross-sectional in nature, the EPOZ study served as a starting point for follow-up studies.

A follow-up study on risk factors for cardiovascular diseases was initiated in those originally 5-19 years old. From the 4.649 (out of 5.670, 82.0 %) subjects in this age group who took part in the study, 1.615 were selected for yearly follow-up.[4] They were selected if they had a risk factor level that was above the upper decile of the age and sex specific distribution of blood pressure, serum cholesterol, body mass index or smoking habits. A control group was selected at random from the remainder of the 5-19 year olds.

From the 1.615 subjects selected for yearly follow-up a subsample could be constituted which represented a random sample of the original population. It comprised 596 yougsters (296 females) and it will be referred to in several analyses in this thesis.

Because the EPOZ study was designed as a family study, risk factor levels of the parents of the study children were also available.

As the initial examination took place between 1975 and 1979, the maximum follow-up period for an individual who entered the study in 1975, was 10 years and for those who entered in 1979, 6 years.

2.2.2 Hospital population

The studies described in appendices 4 and 6 are based on hospital populations from the Zuiderziekenhuis hospital in Rotterdam (Department of Cardiology), the Juliana Children's hospital in The Hague and the University hospital in Leiden (Department of Pediatrics).

Zuiderziekenhuis hospital All male patients under the age of 60, who were referred to the Zuiderziekenhuis hospital for a first coronary angiography to evaluate angina pectoris in the period from August 1983 to February 1985, were eligible for the study on early prediction of atherosclerosis (as described in

appendix 6). Two groups of patients were selected on the basis of an angiogram: (1) those with no coronary atherosclerosis (less than 20% stenosis), and (2) those with severe coronary atherosclerosis (at least 3 vessels with over 70% stenosis). Excluded from the study were patients who had no children, suffered from diabetes mellitus, hypertension (defined as at least one year of treatment with anti-hypertensive drugs), had lipid lowering medication, or were known to have cardiomyopathy or valvular defects. Twenty-nine men with no coronary sclerosis and 35 men with severe coronary sclerosis were selected for the study, and their families, including their youngest son and/or daughter, were invited to participate. The response rate was 92%. Twenty-seven families with 23 sons and 14 daughters, of whom the father was free of coronary atherosclerosis, and 32 families, with 29 sons and 14 daughters, of whom the father suffered from severe atherosclerosis, were examined. The age distribution in the two groups of children was very similar and ranged from 6-36 years, with a mean age of 21.2 years.

Juliana Children's hospital and University hospital Leiden In the outpatient clinics of these hospitals extremely tall prepubertal girls were treated with high doses of estrogens in an attempt to increase their rate of bone maturation and so to reduce their final adult height. In the period between november 1978 and februari 1980 there were seventeen girls, aged 10 to 14 years, who attended these clinics. Fasting blood samples were taken from them at the start and at the end of a period, that ranged from 4 to 17 months, to study the effect of large doses of ethinylestradiol on lipid levels. A reference group of age-matched girls was selected from the EPOZ study.

2.3 Measurements

For each yearly examination in the EPOZ study the subjects visited the study centre in Zoetermeer. Those 10 years and older completed a questionnaire about smoking habits, physical activity, and the use of alcohol and coffee. Data on smoking habits included a question on the average number of cigarettes smoked daily. A physical activity score was based on questions about regular sports activities, including membership in sports clubs, daily bicycling and walking. Questions were asked about the average number of cups of coffee consumed daily. The frequency of alcohol consumption was assessed in three categories: none, occasionally and daily consumption. As the question on alcohol intake

was introduced later in the study, we did not obtain data on alcohol consumption for all subjects from the start of the study. Females were also asked about their menstrual history, pregnancy and current use of oral contraceptives. Later on in the study this part of the questionnaire was extended with questions on number of pregnancies, brands of oral contraceptives used and the duration of their use. To refresh a womans memory a box with samples was used.

Blood pressure was measured with a random zero sphygmomanometer after at least 15 minutes of rest. Body height (in cm) and weight (in kg) were measured with the subjects wearing indoor clothes and no shoes. The Quetelet index, the ratio of body weight over height squared, was used as an index of body mass. Blood was taken by venipuncture from the nonfasting subjects. Before leaving the studycentre each respondent was seen by a medical doctor to be informed about the course of the study and to be motivated to participate. During this talk some notes were made, which sometimes appeared to be of use later on in the data analyses (e.g., duration of pregnancy, initiation of oral contraceptive use). Another method to increase the response rate over time was by making home visits to those who were not able to come to the studycentre. Participants who had moved outside the study area (i.e., the two districts of Zoetermeer) remained in principle in the study population and often received home visits.

A few weeks after their visit to the studycentre a letter was sent to the respondents with the results of their examination. Because the study was set up as an observational study and because at the start there was little known about harmful effects of high levels of risk factors in childhood, no intervening actions were undertaken. However, when a participant maintained a blood pressure or cholesterol level above the 95th percentile, he or she was advised to see the family doctor, who was informed about the study results.

The determinations of total and HDL cholesterol were carried out in the laboratory of the department of Epidemiology and checked regularly from 1978 onward by the WHO Regional Lipid Reference Centre in Prague (Dr. D.Grafnetter). The measurements were within limits throughout the entire study period. For total cholesterol the laboratory also participated from 1980 onward in a control program of a Dutch organisation, the KCA (Clinical Chemical Analysis), which led to certification in 1985. Serum total cholesterol was measured with an automated enzymatic method, utilizing the colour reactions according to Ka-

geyama.[5] HDL cholesterol was also measured using an automated enzymatic method with the Trinder colour reaction,[6] after precipitation with phosphotungstate-Mg^{2+}. This measurement was introduced later on in the study, by the end of 1979, and therefore fewer data are available on HDL cholesterol than on total cholesterol. All serum that was left over after chemical analyses was stored in well-closed tubes at -20°C. For the case-control study on the early predition of atherosclerosis new serum variables were determined in cooperation with the laboratory of the department of Biochemistry I (Dr. P.H.E. Groot). Triglycerides, LDL cholesterol, HDL_2 and HDL_3 cholesterol, apolipoprotein A-I and A-II were determined in the laboratory of the department of Epidemiology, whereas apolipoprotein B was measured in the laboratory of the department of Biochemistry I. Triglycerides and cholesterol subfractions were determined according to enzymatic methods (Boehringer Peridochrom and Monotest, respectively) after precipitation with polyvinylsulfate for LDL cholesterol and Mn^{2+}-heparin and dextransulfate for HDL_2 and HDL_3 cholesterol.[7] Apolipoprotein A-I and A-II were determined by radial immunodiffusion[8] against specific antiserum. Monospecific antisera against human apolipoprotein A-I and A-II were raised in goats (provided by Dr. L. Havekes, Gaubius Institute, Leiden) and sheep (Boehringer Mannheim), respectively. Total apolipoprotein B was measured by quantitative immunoelectrophoresis according to Laurell,[9] as described elsewhere.[10]

Questionnaires, biomedical and laboratory measurements were coded on optical reader forms and entered on file in the PDP11/44 computer of the department of Epidemiology.

2.4 Data analytic approach

Mean values of total and HDL cholesterol were calculated by gender and age in one year intervals. The method of 3-year moving averages was used for graphical presentation of the data. The cholesterol concentrations for the 5th, 50th and 95th percentiles of the distribution were assessed for males and females, in 5-year age categories.

For cross-sectional comparisons of lipid levels average values by comparison group were calculated and Student's t-test was used to assess the statistical significance of differences between the groups. When comparisons between groups were based on longitudinal data, average changes in lipid levels were assessed

by group over the follow-up period concerned, and statistical significance of the difference between these changes was assessed with Student's t-test, with the appropriate standard errors.

In the analyses frequently a multiple linear regression model was used, in which total or HDL cholesterol was the outcome variable, and indicator variables were used as the independent variables.[11,12] In this way average cholesterol levels could be computed by different categories of the independent variables. An independent variable could be divided in two categories, like users of oral contraceptives and non-users, or smokers and non-smokers, or in more than two categories. The latter was used when for example an independent variable was divided into categories according to distribution quartiles. In that case three indicator variables (x_1, x_2, x_3) were created and entered into the regression equation. The lowest quartile served as the reference category and was reflected in the model by the intercept. Indicator variables were set to 0 (not in this category) or 1 (in this category). Thus, the second quartile was reflected in x_1, the third in x_2 and the fourth in x_3. The model equation was:

$$y = a + b_1.x_1 + b_2.x_2 + b_3.x_3$$

Because each value of the independent variable belonged exclusively to one of the categories chosen, the equation for an individual with an independent variable in the second quartile of the distribution was: $y = a + b_1.x_1$, and for individuals with an independent variable in the third and fourth quartile it was: $y = a + b_2.x_2$ and $y = a + b_3.x_3$, respectively. In this way, the regression coefficients in this model served as the difference between the reference category (first quartile) and the category under study. An example will illustrate this.

To study the relation between body weight and serum cholesterol levels in 5-14 years old girls, the distribution of weight in this age group was divided according to quartiles:

quartile 1 = < 33 kg
quartile 2 = 33-50 kg
quartile 3 = 50-59 kg
quartile 4 = > 59 kg

The regression analysis yielded the following results: the intercept was 185 mg/100 ml and the regression coefficients were: b_1 = 2 mg/100 ml, b_2 = -2 mg/100 ml, and b_3 = -5 mg/100 ml. This resulted in the following average cholesterol

levels per quartile of body weight:
quartile 1 = 185 mg/100 ml
quartile 2 = 187 mg/100 ml
quartile 3 = 183 mg/100 ml
quartile 4 = 180 mg/100 ml

To adjust for possible confounding factors, e.g., age, the regression coefficient of this confounding factor was multiplied with the average level of the confounding factor, and this product was added up to the intercept, to result in the adjusted average level of cholesterol for the lowest quartile. The (adjusted) regression coefficients were then added up to the (adjusted) intercept to give adjusted average cholesterol levels per quartile. Significance of the difference in cholesterol level between the first and one of the other quartiles was assessed by the ratio of the regression coefficient and its standard error. This amounted to a Z-statistic with a standard normal distribution.

The methods used to describe and analyse the longitudinal data on the natural history of serum total and HDL cholesterol in early life are given separately in chapter 4. Distinction will be made between the question of prediction of future cholesterol levels and the question of the aetiology of high cholesterol levels and atherosclerosis. The issue of prediction is often referred to as 'tracking', i.e., does a child maintain its position in the distribution over time. Both tracking of the total population and tracking of extremes of the distribution will be discussed. Aetiological issues will be discussed in terms of the natural history of serum lipids, conceptualized as change of serum lipids over time. This concept will include patterns ('horse-racing', slopes) and determinants of change.

Most of the data presented in this thesis were analysed with the BMDP statistical program.[13]

2.5 Discussion

Observational studies give rise to various methodological questions and some of these will be discussed below.

First there is the question of the external validity of the study. Is it possible to generalize the results? The non-response rates varied from 13% in those 5-9 years of age, to 16% in those 10-14 years and 23% in those 15-19 years of age. There is little reason to think that the non-responders in these age groups would

have had different levels of the risk factors, than the participants or that they would have shown other relationships between the determinants and risk factors. Therefore, we think it is justified to extend the conclusions from our study to all young people.

A further concern is the internal validity of the study. Do our results deviate from reality, the true effect estimate? This question can be discussed in terms of the occurence of two types of bias: information bias and confounding bias.

Information bias can be present in the form of misclassification of subjects, leading to random error in the determinant. However, this bias will give an underestimation of the effect, rather than lead to a 'spurious' association.

Confounding bias in follow-up studies may result from loss to follow-up and from differences at baseline. In a longitudinal study loss to follow-up may produce confounding when the cohort attrition is not randomly distributed over the participants, but is selective conditional on the study objectives. There were, however, no significant differences in initial blood pressure or serum cholesterol levels between those who stayed and those who were lost to follow-up in the EPOZ study.

For assessing relationships between serum lipid levels and putative determinants, data obtained in cross-sectional studies are widely used. However, caution should be exercised, and when possible cross-sectional relationships should be verified with longitudinal ones. The study of the relation between the use of oral contraceptives and serum cholesterol and blood pressure gives an illustration of this (appendix 2). Cross-sectionally, no differences were observed in total cholesterol or blood pressure between users of oral contraceptives and non-users. However, when changes in cholesterol and blood pressure over the two years of follow-up were taken into account, it was observed that women who were going to use oral contraceptives showed a larger increase in cholesterol and blood pressure than non-users. If this analysis had been the result of an experimental study design (with random allocation of users and non-users) than the inference would have been much stronger.

Another issue, inherent to a longitudinal study of the natural history of a variable, is the possible change in that variable caused by the study itself. As stated above, some participants were advised to see their family doctor when they had repeatedly elevated levels of blood pressure or cholesterol. However,

such a referral occurred only rarely and most of the time it concerned elevated blood pressure.

The study design used to assess possible predicting factors for coronary heart disease by measuring serum lipids in children of patients who had had coronary angiography (appendix 6) is based on the following considerations. Measuring serum lipids in those who already have the disease (i.e., coronary atherosclerosis) and comparing them with serum lipd levels in those free of the disease, may result in differences, that mainly are an epiphenomenon of the disease. In children, however, the disease, if already present, is much less outspoken. So, differences observed between the two groups of children are less likely to be the result of the disease. On the other hand, the lipid found to be the best discriminator between the two groups does not need to be causally related to atherosclerosis, i.e., it does not need to play a role in the atherogenetic pathway. Rather, it might be a marker for a metabolic defect that indirectly is related to atherosclerosis.

A final methodologic issue concerns data-analysis. A linear regression model often was used to calculate average lipid levels by categories of the study determinant, while controlling for possible confounding factors. The regression coefficient in the model served as the adjusted difference between the reference category and the study category, and the standard error of the regression coefficient served as the standard error of this difference. In this way the statistical significance of the adjusted difference could be assessed, using a standard normal distribution. To determine the standard error of the mean of the calculated adjusted average levels of the categories the most conservative way was chosen and the standard error of the unadjusted level was taken as the measure of variance.

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Chapter 3

DISTRIBUTIONS AND DETERMINANTS OF SERUM LIPIDS IN YOUNG PEOPLE

3.1 General

A first step in assessing associations of determinants and a study variable is a description of the distribution of that variable by age and gender. Age and gender are important determinants of total and HDL cholesterol and variations in their distributions may provide clues about other possible determinants. Differences in the level and distributions of serum lipids between different populations may yield insight into the reasons of these variations. This chapter therefore will start with a review of investigations of the level and distributions of total and HDL cholesterol in children and young adults in various populations. Thereafter, the evidence concerning the determinants of serum lipids in early life will be discussed, notably familial aggregation, anthropometrical measurements, hormonal factors and life habits.

3.2 Distributions

Total cholesterol Many investigators have studied serum cholesterol levels in children and young adults. A number of reviews has been published, in particular by Rafstedt until 1955,[4] by Hickie until 1974[27] and lately by Berenson.[65] However, a complete collection of all studies on the distributions of serum cholesterol in childhood is not available. References 1-69 include practically all studies on this subject in chronological order. Table 3.1 gives an overview of most of these studies with regard to number of study subjects and the average total cholesterol levels by age. One should be cautious in comparing cholesterol levels between the different populations, especially with regard to the older studies, because different analytic methods have been used. However, within populations average levels can be compared between the different age and race groups. A recent international comparison of cardiovascular risk factors in children from 15 countries has been described by Wynder.[51] The data were restricted to 13 year old boys and average total cholesterol levels were compared between all but two countries. The highest

TABLE 3.1

Total cholesterol levels from studies in children and young adults

Population	Age groups (years)	Cholesterol (mg/100 ml) Males	Females	Reference
New York n=380	3-7,8-12,13-17 18-22,23-27	180,180,176 185,195	209,196,183 193,202	5
Evans County, white n=1,283	6,7,8, 9,10,11 12,13,14 15,16,17	158,163,170 172,172,176 175,184,185 192,193,185	156,164,169 168,170,171 177,182,189 191,194,179	9
, non-white n=466	6,7,8 9,10,11 12,13,14 15,16,17	137,151,157 173,173,184 175,175,185 185,189,183	137,154,159 173,177,178 187,177,196 196,189,204	
Iowa n=952	14-19 20-24, 25-29	160 164,180	173 161,177	10,15
Tecumseh n=3,242	4-9,10-14,15-19 20-24,25-29	177,172,166 195,214	178,176,173 189,194	11
New York, Caucasian n=635 , Chinese , Negro , Puerto Rican	10-13	160 149 155 154		14
Pretoria n=1,843	7-11,12-15	219,207		16
Burlington n=707	12,13,14 14,16,17	169,161,157 162,167,171	165,160,166 169,168,176	17
Mexico n=209	5-9,10-14	100,100		21
Wisconsin n=328	5-9,10-14	188,185		21
Denmark n=153	6-10,11-15,16-20	194,201,196	216,206,220	23
California n=59	8-12	179		25
Australia n=613	11-18	200		27
Muscatine n=4,829	6-18	182		30
Australia n=214	11-19	180		32
Arizona n=2,249	5-9,10-14 15-19,20-24	147,151 150,173	148,149 159,177	33
Denmark n=552	8-17	183	191	34

Bogalusa, white	5,6,7	161,168,161	159,161,163	35
n=3,446	8,9,10	162,166,163	168,167,162	
	11,12,13	164,162,157	166,163,165	
	14	151	158	
, black	5,6,7	170,170,170	166,179,170	
	8,9,10	165,177,169	179,173,172	
	11,12,13	171,173,160	174,164,168	
	14	165	167	
Cincinnati, white	6-11,12-17	162,156	163,157	37
n=6,775 , black	6-11,12	167,161	171,165	
Norway	13-14,15-16	175,170	179,184	39
n=404				
Rochester	6-10,11-14,15-18	159,156,153*	160,157,163*	40
n=2,421				
LRCPPS , white	0-19	155	159	48
n=13,655 , black	0-19	162	167	
Israel	17-18	134	151	49
n=2,565				
Finland , rural	13-15	205	201	52
n=967 , urban		193	189	
Germany	12,13,14	182,178,171	185,177,170	53
N=742	15,16	164,158	177,175	
Greece	10,11,12	153,155,151	155,157,151	54
n=935	13,14	147,143	149,149	
Norway	11,12,13	205,202,194	206,204,191	55
n=856	14	189	196	
Italy	11,12,13	154,147,149	155,155,151	56
n=386	14,15	146,142	149,150	
Thailand	10,11,12	164,159,159	169,170,165	57
n=1,090	13,14	167,163	177,176	
New York	12,13,14	159,155,148	162,158,157	58
n=3,662	15	147	157	
Netherlands	10,11,12	182,186,178	182,178,182	59
n=1,016	13,14	174,162	170,174	
Nottingham	13,14,15	178,176,174**	184,193,178**	61
n=625	16,17,18	167,170,170	189,189,203	
Switzerland, Swiss	8-9,14-15	189,170	192,181	62
n=803				
, Italian	8-9,14-15	178,170	185,177	62
Italy	11,12,13	155,150,146**	150,156,149**	63
n=644	14,15	144,143	155,156	
Israel, Israeli	7,11	153,148	156,150	64
n=808 , European		154,155	164,157	
, Asian		149,149	151,143	
, N African		139,146	147,145	
Norway	11,12,13	186,184,178**	186,189,186**	66
n=920	14,15,16	179,176,174	192,185,192	

Beaver County n=561	22, mean	185	179	68
Finland n=3,596	3,6,9,12 15,18	183,191,191 191,173,174	186,198,196 189,183,183	69

* median levels
** extrapolated from graphs

average values were observed in Finland, followed by other northern European countries, in particular, Norway, West-Germany and the Netherlands. Nigeria, Italy, Greece and Japan showed the lowest cholesterol levels. In a recent report of Knuiman and Katan,[70] serum cholesterol levels in the Netherlands were found to be considerably higher than those in the United States.

A striking finding when inspecting serum cholesterol distributions by age, is the "dip" in the average level from about age 9-10 years until the age of about 16 years. In many studies this "dip" has been observed,[5,12,18,19,27,35,37,38,40,41,48,53,59,61,66,69] although in some others no relation of cholesterol with age has been found.[20,21,26,30,32] Another finding, which has often been reported, is the somewhat higher cholesterol levels in girls as compared to boys.[17,23,37,39,40,48,61] In summary, serum total cholesterol values measured in the first two decades vary considerably between populations, and by gender and age. The age and gender differences were also observed in our data (appendix 1).

HDL cholesterol Data on HDL cholesterol early in life are sparser than those on total cholesterol, but they still include a considerable number of investigations.[36,40-47,49,50,56,58,60,61,63-66,68,69] Because international standardization programs for HDL cholesterol are much less developed than for total cholesterol, it is hard to compare average levels between different countries. Knuiman[47] has studied total and HDL cholesterol levels in school children from 16 countries. Total and HDL cholesterol concentrations were determined in one laboratory to reduce methodological variability. Average HDL cholesterol levels appeared to be lower in countries with lower total cholesterol levels (the less developed countries), and higher in countries with relatively high levels of total cholesterol (the more developed countries).

As with total cholesterol, in most studies differences by age and gender were observed in early adulthood. Until about the age of 16 years there is

little difference in HDL cholesterol between males and females. Males however, show a slight decrease from the age of 12 years, whereas females have a gradual increase with age, and this results in a marked difference between men and women in their early twenties. It has been suggested that this observation may partly explain the greater occurrence of coronary heart disease in middle aged men compared to women.[65]

In summary, HDL cholesterol levels show little variation between males and females until the age of about 16 years. Thereafter, females show markedly higher average levels than males. Our data (appendix 1) largely confirm these observations.

3.3 Determinants

The determinants of serum lipids that have been investigated may be categorized as familial aggregation, anthropometrical variables, hormonal factors and life habits.

3.3.1 Familial aggregation

The familial aggregation of serum lipid levels has been well recognized. Both parent-offspring and sibling studies have been conducted[4,11,20,40,71-78] and they have demonstrated consistently positive correlations for total cholesterol levels between different family members. However, this does not allow definite conclusions concerning the question whether familial aggregation is due to sharing the same genes or sharing the same environment. Hames and Greenberg[9] observed a correlation between blood group and serum total cholesterol levels in a preliminary analysis of children, aged 6-11 years. However, when they included children aged 12-17 years in the analysis, the correlation decreased in magnitude. They suggested that this might be due to the fact that when children grow older, environmental factors tend to dilute the mainly genetically determined differences observed at earlier ages. In our study (appendix 1) we found parental cholesterol levels to be associated with total cholesterol in both children and young adults.

3.3.2 Anthropometrical variables

Body weight and other indices of body mass, like Quetelet index and triceps

skinfold, have been investigated as determinants of both total and HDL cholesterol. Most of the observed positive associations of body mass with total cholesterol were restricted to older age groups or male subjects.[11,13,17,40,61,68,79] In younger age groups in general no association between anthropometrical variables and total cholesterol was observed.[1,9,10,11,27,30,30,79] In contrast with total cholesterol, HDL cholesterol was found to be negatively associated with indices of body mass,[40,42,44,45,60,79] especially in males. In our study (appendix 1), body weight and Quetelet index were positively related to total cholesterol in those 15 years and older, but not in the younger children. For HDL cholesterol body weight was a determinant in males older than 15 years of age.

3.3.3 Hormonal factors

Maturation has been implicated in the occurrence of the "dip" in total cholesterol between the ages of 10 and 16 years,[56,60,61,66,79,80,81] and hormonal changes have been suggested to account for differences in cholesterol and its subfractions. Laskarzewski et al. reported associations between endogenous sex hormones and plasma lipids in adolescent boys.[82] Others however, were unable to find a correlation between change in sex hormones and change in plasma lipids during puberty in boys.[83] As the stage of maturation seems to play such an important role in determining the levels of serum lipids, the absence of a dip in a population study may well be the result of different stages of maturity within the same age-group. Longitudinal studies may therefore be preferred.[84] Besides, it has been suggested to include measurements of maturity stage in epidemiological studies of serum lipids, rather than age.[66] In females, we did not observe a significant difference in total cholesterol levels between pre- and postmenarchal girls. However, we did find significantly higher HDL cholesterol levels in girls that had had menarche, than in those who had not (appendix 1).

The role of exogenous sex hormones, notably oral contraceptives, in determining serum lipid levels has also been studied in young women.[45,60,85-88] The results, however, are equivocal and they largely depend on the age of women and the dose of estrogens and progestogens they were taking.[89] In the cross-sectional part of our study the use of oral contraceptives was positively

associated with total cholesterol levels, but no association with HDL cholesterol was observed (appendix 1). A longitudinal analysis (appendix 2) confirmed that women who started the use of oral contraceptives showed a larger increase in serum total cholesterol, over a two-year period, than women who did not use oral contraceptives. In a separate longitudinal study the effect of large doses of ethinylestradiol on lipid levels in excessively tall prepubertal girls was investigated (appendix 4). Girls, who were treated with hormones showed a considerably larger rise in total cholesterol than the agematched reference subjects.

The increase in serum lipids during pregnancy is well recognized,[90-92] and hormonal alterations have been implicated in this rise. A decline in lipid levels after pregnancy has been reported from several studies, but there is only limited evidence concerning the question whether lipid concentrations completely decline to pre-pregnancy levels. The investigation of Darmady,[93] however, revealed a large individual variation in the time necessary to decline toward cholesterol and triglyceride levels before pregnancy. She found that mothers who breast-fed their children showed a more rapid fall in serum triglyceride, than mothers who bottle-fed their infants. In our study (appendix 3) we could confirm the increase in serum total and HDL cholesterol in pregnant women, as compared to a matched non-pregnant reference series. Also, women in the third trimester of pregnancy showed higher levels than those in the first or second trimester. However, when cholesterol levels of women were compared the year before and the year after pregnany, it was observed that HDL cholesterol levels were significantly lower, in the year after pregnancy.

3.3.4 Life habits

Life habits that have been studied in relation to serum lipid levels in young people, include physical activity, the use of alcoholic beverages and cigarette smoking. Hickie et al.[27] observed higher total cholesterol levels in boys who were in the lower quartile of physical fitness as compared to boys who were in the top quartile. Physical activity and the use of alcoholic beverages have been shown to be positively related to HDL cholesterol, especially in adults,[94-96] but also in adolescents.[61,68] Smoking in youngsters has been associated with

increased total cholesterol and decreased HDL cholesterol levels.[44,60,61,68] In our study we found that young men who consumed alcohol daily had higher total cholesterol levels than men who only drank alcohol occasionally. With regard to cigarette smoking we observed a weak association between this habit and total cholesterol in females (appendix 1).

3.4 Concluding remarks

Total and HDL cholesterol levels show marked differences by gender and age. Most pronounced is the 'dip' in total cholesterol for the ages 10-16 in both boys and girls. Another finding is the increase in total cholesterol from the age of 16 years onward, which is accompanied in boys with a decrease in HDL cholesterol. This difference early in life between males and females might partly explain the gender difference in coronary heart disease at later ages. From a preventive point of view it may be of importance that body weight in males is negatively associated with HDL cholesterol. The role of body weight as a determinant of total cholesterol is different for older and younger subjects. In those younger than 15 years other determinants are likely to be related to serum lipid levels. Investigations into factors that are related to physical maturation may yield other determinants of cholesterol concentrations early in life.

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Chapter 4

NATURAL HISTORY OF SERUM LIPIDS

4.1 General

Atherosclerosis is generally considered to be a life long process which begins early in life. Therefore, it may have merit to study the natural history of its risk factors in young people. The relationship between serum lipids and cardiovascular diseases is well established in adults. Thus, it is important to answer the question whether serum lipids in children relate to adult levels. This concept is often referred to as 'tracking'. Tracking may be defined as the ability to predict future lipid levels from earlier observations, or as the maintenance of the position of an individual in the distribution of lipids over time. In other words, if there is tracking of serum total cholesterol, it should be possible to detect future hypercholesterolemic subjects early in life.

From an aetiological point of view, investigations of change and determinants of change of serum lipids may yield new insights. In a longitudinal study of blood pressure it has been postulated that a person may be better characterized by the rate of change than by the actual level of a determinant.[1] And Hames et al.[2] suggested already in 1961 that the rate of increase of blood lipids might be the key factor in atherosclerosis. A pattern of change which appears particularly interesting is 'horse-racing'. This refers to the situation where those with the highest inital level of a characteristic also experience the highest rate of increase over time. Another way to investigate change is to determine the slope, level and variability of serum cholesterol over time, and to relate it to putative determinants of change.

4.2 Prediction of future levels

Whether or not there is 'tracking' of serum total and HDL cholesterol can be assessed in various ways. In particular, one may consider the total population or only those in the extremes of the distribution, and one may use a single remeasurement or multiple remeasurements. Because serum cholesterol values vary with age, it is preferable to transform the data to obtain age-adjusted values. Transformation into percentiles or z-scores, based upon the age- and

sex-specific distributions of total and HDL cholesterol, is easily performed. A z-score is determined by the following equation:
$z = (x - M) / sd$, where x is the individual value, M the age- and sex-specific mean value, and sd the age- and sex-specific standard deviation of this mean value.

4.2.1 Tracking of the total population

To assess the stability of a child's rank in the cholesterol distribution over time correlation coefficients between initial measurements and various follow-up examinations, expressed in either percentiles, or z-scores may be calculated in several age categories, separately for males and females. In this way, possible differences by age and gender are observed. In our study (appendix 5) we found that males showed higher correlation coefficients than females, for both total and HDL cholesterol. Overall, tracking coefficients for HDL cholesterol were somewhat lower than for total cholesterol.

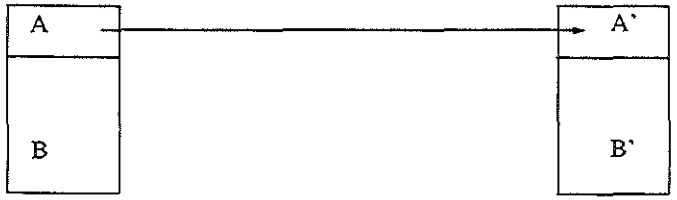
Other indices of tracking have been described by McMahan[3] and Foulkes,[4] using different statistical models.

4.2.2 Tracking of the extremes of the distribution

In clinical practice, the main concern is with those who might be at risk, which means that their total cholesterol is 'high' or their HDL cholesterol is 'low'. The interpretation of 'high' and 'low' depends on age- and sex-specific distributions of the serum lipids. It is more or less arbitrary to define those with a 'high' cholesterol level to be above the upper quintile, decile, or whatever cutoff point of the distribution and the same holds for a 'low' HDL cholesterol level. A key question is whether those in the extremes of the distribution keep their position over time. The concept of tracking applies equally to those in the extremes of the distribution as well as to the total population, but it may be useful to study the 'high-risk' and the 'total-population' approach separately. The methods that are described below may be divided in methods using a single remeasurement and methods using multiple remeasurements. If all values of total and HDL cholesterol are transformed into z-scores, it is possible to combine different age groups.

When only one single remeasurement is taken into account, the main issue

is to find a measure for the probability of having an abnormal lipid level several years (time x') after the initial measurement (time x, see figure).



There are several ways to look at this 'high level' tracking, depending on the null hypothesis (H_0).

Consider, H_0:

the position in the distribution at time x' has no relation with the position in the distribution at time x.

Example: suppose subjects in A are above the upper quintile of the distribution. Under the null hypothesis one fifth of those above the upper quintile of the distribution at time x (A) are to be expected above the upper fifth of the distribution at time x', so E(xpected)=1/5. If the observed proportion of A in A' is 1/5, then there is no relation with the place in the distribution, the observed/expected (O/E) ratio = 1. If the proportion of A in A' is, say 2/5, then the O/E ratio = 2.

The O/E ratio is a measure of high risk tracking, rather than a measure of the probability to keep a high rank order in the distribution over time. An O/E ratio of 4 means that there are 4 times more people in A', who originally were in A, than would have been expected if there had been no relation with the position in the distribution at time x. A disadvantage of the O/E ratio is that its scale depends on the cutoff point. If the cutoff point is a quartile, the maximum O/E ratio will be 4, if it is a decile, it will be 10.

As A' will be composed of people coming from A (high level) as well as people coming from B (normal level), it will be relevant to compare the proportion of A in A' with the proportion of B in A'. Therefore consider, H_0:

those with a high level at time x have the same probability of having a high level at time x', as those with normal levels at time x.

Example: the proportion of A in A' is 0.67 and of B in A' is 0.17. In other words, the probability for those at a high level at time A, to keep that high

level at time A' is 0.67 and they have a 3.9 times larger change to have a high level at time x', than those with normal levels at time x. This socalled relative probability (RP) does not have a maximum value, like the O/E ratio and there is a simple relationship between them:

$$RP = \frac{O(A)}{O(B)} = \frac{O/E \text{ for } A}{O/E \text{ for } B}$$

The relative probability of having a high level at time x', is the ratio of the O/E ratio for those having a high level at time x, and the O/E ratio for those with normal levels at time x.

TABLE 4.1

O/E ratio and RP for males and females related to the upper quintile of the distribution of total cholesterol

initial measurement		sixth year of follow-up proportion > 80%	O/E	RP
males				
> 80%	n= 44	0.568	2.84	5.16
< 80%	n=173	0.110	0.55	1
females				
> 80%	n= 37	0.459	2.30	3.45
< 80%	n=135	0.133	0.67	1

TABLE 4.2

O/E ratio and RP for males and females related to the lower quintile of the distribution of HDL cholesterol

initial measurement		fifth year of follow-up proportion < 20%	O/E	RP
males				
< 20%	n= 46	0.514	2.57	4.90
> 20%	n=143	0.105	0.53	1
females				
< 20%	n= 27	0.370	1.85	2.52
> 20%	n=116	0.147	0.74	1

In our data we observed that the relative probability of being in the high level category at follow-up for those who had initially a high level, compared to those who had not, was larger for males than for females. This is in accordance with the results concerning the total population. It seems that in males there is a somewhat larger correlation between initial and final measurement of total and HDL cholesterol than in females (see table 4.1 and 4.2).

When all remeasurements between the initial and final examination are to be taken into account, the following method may be used. The probability for having a 'high' cholesterol at the 'n'th follow-up examination for subjects never having had a 'high' cholesterol can be compared with the probability of those who have been 1, 2, ...n times above the upper quintile of the distribution. The relative probability for those who had a 'normal' cholesterol at each occasion is set to 1. The same approach, but then for low levels, may be applied to HDL cholesterol.

As expected, the relative probability of having a high level at the final examination increases with the number of times one has been in this high category of the distribution during previous measurements. For example, in our data, males who had 4 times (out of 6) total cholesterol levels that were above the upper

TABLE 4.3

Absolute and relative probability of being above the upper quintile of the distribution of total cholesterol at the sixth year of follow-up

	previous times above the upper quintile	n	probability of being above upper quintile at 6th year absolute	relative
males	0	85	0.059	1
	1	21	0.095	1.61
	2	12	0.333	5.64
	3	8	0.125	2.12
	4	5	0.600	10.17
	5	10	0.700	11.86
	6	9	0.889	15.07
females	0	58	0.034	1
	1	19	0.053	1.56
	2	13	0.308	9.06
	3	16	0.375	11.03
	4	7	0.714	21.00
	5	6	0.667	19.62
	6	3	1.000	29.41

TABLE 4.4

Absolute and relative probability of being below the lower quintile of the distribution of HDL cholesterol at the fifth year of follow-up

	previous times below the lower quintile	n	probability of being below lower quintile at 5th year absolute	relative
males	0	81	0.037	1
	1	24	0.167	4.51
	2	15	0.458	12.38
	3	14	0.469	12.68
	4	7	0.857	23.16
	5	3	1.000	27.03
females	0	57	0.053	1
	1	26	0.192	3.62
	2	14	0.357	6.74
	3	7	0.571	10.77
	4	2	0.500	9.43
	5	3	1.000	18.87

quintile of the distribution, had a 10 times larger probability to have a high cholesterol level at the final examination than those who never had a high total cholesterol level (table 4.3 and 4.4).

In summary, there seems to be a moderately high degree of 'tracking' for total and HDL cholesterol in young people. Overall, males seem to have somewhat larger coefficients, both in the total as in the extremes of the distribution. However, to detect people who are at risk in the future it may be fruitful to look at change, rather than level over time.

4.3 Patterns and determinants of change

It has been suggested that a person may be better characterized by his rate of change than by the actual level of the determinant.[1,2,5] In chapter 5 a hypothetical model will be proposed which relates change, rather than level of a determinant, to risk of disease. Here, three approaches to the study of change will be presented.

4.3.1 Horse-racing

The concept of 'horse-racing' stems from Fletcher et al.[6] in their study of pulmonary function. The model postulates that those with the highest initial level of a characteristic will experience the highest rate of increase in that

characteristic over time. To assess horse-racing for a characteristic it should be investigated whether there is a positive relation between change in serum lipids and initial lipid levels. The change in serum lipid levels in an individual can be obtained by linear regression of serum lipid levels on time, using the least-square approach. This method provides a slope, reflecting the change, and an intercept, which may serve as the estimated initial level. The relation between change and initial level may then be assessed by regressing the individual slopes on the individual intercepts. The regression coefficients yielded by this model, are affected by regression toward the mean, i.e. they are either less positive or more negative than the 'true' coefficient. To adjust for this phenomenon, the method of Blomqvist[7] can be adapted, which employs a λ coefficient. This λ coefficient is the ratio of the average error of the individual slopes and the error of the initial values. The smaller λ is, the smaller the adjustment for regression to the mean. In our data (appendix 5) there was no evidence for 'horse-racing', neither for total nor for HDL cholesterol. This finding does not necessarily imply that the study of change of lipid levels is irrelevant, it rather suggests that there is no relation between change and initial level of serum cholesterol.

4.3.2 Level, trend and variability

An excellent example of the study of change has been provided by Lauer et al.[8] For the longitudinal analysis of blood pressure data in children, they defined level, trend and variablity, as characteristics of interest. They also transformed all measured variables into age- and sex-specific percentile ranks for each survey year. For each subject the average of the percentile ranks of the measured variables was calculated and referred to as level, with percentile as unit. For each subject and each variable, a line describing the change of the study variable (in percentiles) over time was calculated by the method of least-squares. The slope of this line was referred to as trend, with percentiles per year as unit. The variation around this regression line is defined as the average squared deviation of the observed values from the predicted values. The square root of this quantity represents a measure of closeness of fit and was referred to as variability, with percentiles as unit. By defining ranges of level, trend and variability, special groups can be selected. For example, those with a high level,

a rising trend, and little variability, as those who are tracking toward future high values, or those with a low level, a flat trend and little variability, as those who consistently have a low level. Characteristics of these selected groups can be compared, in order to find determinants of change.

4.3.3 Determinants of change

Although the determinants of level of serum lipids have been extensively studied, much less attention has been paid to determinants of change. In principle, the same kind of determinants can be investigated, measured either at the start of the follow-up period or as a change over the time period under study. The use of a determinant measured at the start of the study to characterize the individual, may lead to an underestimation of the true relation of the determinant and the change of the study variable. For example, smoking is positively related to serum total cholesterol, and it may be related to the change in cholesterol. However, those who smoke at first measurement may stop, and those who do not smoke may take on this habit during follow-up. If smoking has an effect on change in cholesterol, this effect may be diminished by misclassification of the study subjects. Relating change in a determinant (if possible) to change in the study variable may therefore be favoured.

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Chapter 5

ATHEROSCLEROSIS, A VIEW ON PREVENTION AND PREDICTION

5.1 General

Central issues in the epidemiological approach to atherosclerosis are its prediction and prevention. Before discussing these aspects, a short exposé of the function and metabolism of lipoproteins will be given, with special reference to their role in atherogeesis. More comprehensive reviews on lipid metabolism and atherosclerosis can be found in references 1, 2, 12, 17 and 19.

5.2 Lipid metabolism

The major lipids of plasma or serum are unesterified cholesterol, cholesteryl esters, triglycerides, phospholipids and free fatty acids. Although in the present context much emphasis is given to the role of plasma lipoproteins in atherosclerosis, their physiological function is laid in transport of lipids between organs in the body. Triglycerides, for example, synthesized in gut and liver, serve as energy supply for muscles, and cholesterol not only is an essential component of all cell membranes, but also is a precursor of steroid hormones produced by the adrenal gland and the ovary. Under normal conditions, there is a balance between cholesterol input and excretion. The input consists of exogenous cholesterol (about 335 mg/day) from the diet and from de novo synthesis in the liver (about 800 mg/day). Cholesterol 'leaves' the body primarily by way of the bile (600 mg/day), bile acids (400 mg/day), and the skin (85 mg/day), or is transformed to steroid hormones (50 mg/day) and excreted by the urine.

Lipids are water-insoluble substances and apart from free fatty acids, which are bound to albumin, they are transported through the plasma in spherical lipid-protein multimolecular complexes, called lipoproteins. The surface of these lipoproteins is composed of amphipathic constituents and contains specific proteins (apolipoproteins), free cholesterol and phospholipids. Inside the core the hydrophobic triglycerides and cholesteryl esters are located. There are several different lipoproteins, categorized either by their density, as determined by ultracentrifugation, or by their electrophoretic mobility (table 5.1).

TABLE 5.1

Categories of lipoproteins by density and electroforetic migration

	density in g/ml	electroforetic migration
chylomicrons	< 0.95	-
VLDL	< 1.006	pre-β
IDL	1.006 - 1.019	-
LDL	1.019 - 1.063	β
HDL	1.063 - 1.21	α

VLDL=very low density lipoprotein, IDL=intermediate density lipoprotein, LDL=low density lipoprotein, HDL=high density lipoprotein.

Most authors prefer the classification according to density, which reflects the different ratios of lipids and proteins. However, in view of the functional interrelationships of these lipoproteins, one may consider other classifications. The function and metabolism of the different lipoproteins will first be given, followed by a description of the apolipoproteins.

5.2.1 Lipoproteins

Chylomicrons are formed exclusively in the duodenum and jejunum and consist mainly of triglycerides, taken up from the diet. After secretion by the enterocytes into the intestinal lymph they enter the general circulation through the thoracic duct. The principal apolipoproteins of chylomicrons are apo A (subclasses I, II, and IV) and apo B (more specifically B-48). Apo C's and E are incorporated in the chylomicrons after entry into the plasma compartiment by transfer from other plasma lipoproteins (mainly HDL). Apo C-II is an obligatory cofactor of lipoprotein lipase, the enzyme involved in the lipolysis of triglycerides. The half-life of chylomicrons is only a few minutes as their degradation to core and surface remnants is a very fast process. Core remnants are taken up by the liver through apolipoprotein E receptors, whereas surface remnants are, at least partially, transferred to the high density lipoprotein (HDL_3).

Very low density lipoproteins (VLDL) are synthesized in the liver to transport endogenous triglycerides in the blood. As a result of interaction with lipoprotein lipase they are degraded to intermediate density lipoproteins (IDL). A major fraction of IDL is converted in the plasma compartment into low density lipoprotein (LDL), in a not fully understood process, while the rest is taken up

by the liver via the apo B.E receptor. The major apolipoproteins of VLDL are apo B (B-100), apo C's and apo E. During the first step in VLDL catabolism (VLDL→IDL) apo C's are lost (transferred to HDL) while in the second step (IDL→LDL) apo E is lost and also transferred to HDL.

Low density lipoproteins (LDL) carry about 65-70% of the total cholesterol concentration of the blood and about 90-95% of the total amount of apo B. They transport cholesterol (derived from the liver and secreted in VLDL) and cholesterylesters (taken up from HDL by cholesterylester transfer) to the peripheral somatic cells for membrane synthesis. Their catabolism has been elucidated by the work of Goldstein and Brown.[3] They demonstrated the existence of LDL receptors in fibroblasts, smooth muscle cells and lymphocytes by which the intracellular cholesterol metabolism is regulated. The total number of receptors on a cell membrane is a reflection of the demand of the cell for cholesterol. The apo B component of LDL interacts with the receptor, enabling a receptor-mediated endocytosis. After internalization of LDL into the cell three mechanisms are activated: 1) the de novo synthesis of cholesterol in the cell is decreased, 2) the transformation of cholesterol to its ester form is stimulated, and 3) the synthesis of cholesterol receptors is inhibited. In this way an accumulation of cholesterol in the cell is avoided. When there is a defect in the LDL receptor (as is the case in familial hypercholesterolemia, see below) the serum cholesterol concentration increases dramatically as both LDL and its precursor IDL cannot be taken up in the cell, leading to an accumulation of (mainly) LDL in plasma.

The metabolism of high density lipoproteins (HDL) has not yet been clarified completely. Based on the density gradient analysis of plasma there are three subclasses (HDL_1, HDL_2, HDL_3). The function of HDL is to transport cholesterol from the peripheral tissues back to the liver. It is thought to be formed in the plasma compartment out of precursors, synthesized by liver and gut, and surface remnants from chylomicrons and VLDL by interaction with lecithin:cholesterol acyltransferase (LCAT) and lipid transfer proteins. During hydrolysis of chylomicrons and VLDL discoidal particles are formed which consist of a phospholipid bilayer, free cholesterol and apo A-I. Direct synthesis of these disc shaped particles by liver and gut has also been demonstrated. The discoidal form of HDL ('nascent' HDL) is rapidly transformed into a more global one by the uptake

and esterification of cholesterol. The enzyme LCAT plays an important role in the esterification and is also involved in the transformation of HDL_3 to HDL_2 and HDL_1. Several details of HDL metabolism are still unclear, for instance the transfer of cholesterylesters of HDL to the liver. This may be by a specific receptor-mediated process or by transfer of cholesterylesters to other lipoproteins. Also, little is known about the origin of apo A-II and the mechanisms responsible for the regulation of the HDL subfractions.

5.2.2 Apolipoproteins

The above identified lipoproteins contain a number of structurally and functionally different apolipoproteins. Up till now apo A, B, C, D, E and some subcategories, according to molecular weight, chemical composition and function have been identified. Table 5.2 gives an overview of the major apolipoproteins, their functional properties and the lipoproteins on which they are found.

TABLE 5.2

Categories of apolipoproteins, their lipoproteins and functions

Apolipoprotein	Lipoprotein	Function
A-I	chylomicron, HDL	co-factor of enzyme LCAT
A-II	HDL	phospholipid-binding properties
A-III (= D)	HDL	largely unknown (LCAT?)
A-IV	chylomicron	unknown
B-100	VLDL, IDL, LDL	interaction with apo B,E receptor
B-48	chylomicron	structural component chylomicrons
C-I	chylomicron, VLDL, HDL	unknown
C-II	chylomicron, VLDL, HDL	cofactor of lipoprotein lipase
C-III	chylomicron, VLDL, HDL	inhibitor of uptake of VLDL by apo B/E and apo E receptors
E	chylomicron, VLDL	interaction with apo E and apo B,E receptor

Apo A-I is an important structural component of HDL, like apo A-II, and also a cofactor of the enzyme LCAT. Both apo A-I and apo A-II are capable of forming complexes with phospholipids, which can bind cellular cholesterol. Apo A-III, which is often referred to as apo D, probably also plays a role in this enzymatic activity. The function of apo A-IV is unknown, but is has been found in chylomicrons. Two forms of apo B can be distinguished, namely, apo B-48

(apparent in chylomicrons) and apo B-100 (in LDL, IDL and VLDL). Apo B-48 is an obligatory structural component of chylomicrons as is apo B-100 for VLDL. Apo B-100 accounts for the receptor interaction with apolipoprotein B,E receptor cells. Apo C-II is a cofactor of the enzyme lipoprotein lipase and is mainly present in chylomicrons and VLDL. Apo C-III, which has three subclasses, is probably a modulator of the interaction of apo B containing lipoproteins with the apo B/E and apo E receptors. C-apolipoproteins are also able to form protein-lipid complexes with phospholipids and they are mainly present in chylomicrons, VLDL and HDL subfractions. Apo E, finally, has a function in chylomicrons and VLDL, in providing the receptor interaction with apo B,E receptor cells and with apo E receptors of hepatic cells.

5.2.3 Familial hypercholesterolemia

Abnormal lipid or lipoprotein concentrations can be the result of a variety of defects in the above described metabolism. Familial hypercholesterolemia,[4] probably one of the best known lipid disorders, is strongly related to atherosclerosis and can be recognized in childhood, or even prenatally.[5] It is an autosomal dominant inheritable disease, based on a gene defect leading to impaired LDL receptor function. Homozygotes are unable to make functionally active LDL receptors, which, as discussed above leads to an enormous increase in serum cholesterol concentrations (above 600 mg/100 ml) and premature development of severe atherosclerosis (even by the age of 6 years old). Heterozygotes have a 50% decreased capacity to synthesize active LDL receptors as compared to normal individuals, leading to cholesterol concentrations of over 300 mg/100 ml and an increased risk of premature heart disease. Apart from the receptor mediated pathway to remove LDL from the plasma, also a 'scavenger' pathway has been postulated, which is independent of apo B/E receptors. Cells involved in the scavenger pathway include macrophages and histiocytes of the reticuloendothelial system, which in normal individuals remove about 15% of the total plasma LDL pool. Recent studies have suggested that macrophages may play an important role in atherogenesis. They can absorp and degrade enormous amounts of lipoproteins and store the cholesterol portion and eventually transform to foam cells. Because subjects with familial hypercholesterolemia have such a large LDL pool, much more LDL is metabolized by this scavenger pathway, which may ac-

count for the increased amount of atherosclerotic lesions. Familial hypercholesterolemia is a clinically very impressive disease, with a frequency in the population of about 1-2/1000. It has also given rise to an outstanding model for lipid metabolism and its relation to atherosclerosis. However, for the prevention of coronary heart disease it is of limited importance, as only a small proportion of all heart disease patients has this inherited lipoprotein disorder (3-6% among survivers of myocardial infarction is known to have familial hypercholesterolemia[1]).

5.2.4 Concluding remarks

The role of cholesterol in the pathogenesis of coronary heart disease seems to be quite well established, although the mechanism by which it plays its harmful role still needs to be further elucidated. The finding that the atherosclerotic lesion contained lipids, especially cholesterol, together with the observation of a dose-response relationship of the serum cholesterol level and the risk of coronary heart disease in both cross-sectional and longitudinal studies, gave rise to a growing interest in serum lipids and their metabolism. The combination of biochemical studies and findings from clinical and epidemiological investigations led to the hypothesis that cholesterol measured in VLDL and LDL reflects the atherogenic property, whereas cholesterol measured in HDL might reflect a anti-atherogenic (or protective) property of serum lipids.[6] Most of the serum total cholesterol is present in the LDL fraction, which may be an explanation of the observed relationships between cardiovascular diseases and serum total cholesterol.[7] Lately, more emphasis has been put on apolipoproteins as markers for cardiovascular diseases. Like cholesterol, apolipoproteins are divided over the several lipoproteins with apo B being present in VLDL and LDL (the atherogenic lipid components) and apo A predominantly in HDL (the 'protective' agent). Because apolipoproteins are not only structural, but also functional components of lipoproteins, they might serve as better indicators or predictors of atherosclerosis and its clinical manifestations, than cholesterol fractions.[8]

5.3 Atherosclerosis

Atherosclerosis has been described on a macroscopic, microscopic and even cellular and molecular level. Regularly, with advances in methodology, new in-

sights occurred and new hypotheses were developed with regard to its pathogenesis. This paragraph will give a short overview of the description of the atherosclerotic lesion, its presence early in life and some theories concerning its development. Most of the studies reviewed are restricted to atherosclerotic lesions in the aorta and coronary arteries.

5.3.1 Macroscopic lesions

The earliest studies on the morphological structure of the atherosclerotic plaque were performed in autopsy material. Macroscopically, three stages of atherosclerotic lesions were distinguished.[9] They included fatty streaks, fibrous plaques and complicated lesions. Fatty streaks were described as intimal lesions that could be stained with Sudan IV (because of their lipid content) and that showed no other change in the vascular wall. Fibrous plaques were described as elevated intimal lesions, that could be partially or completely stained with Sudan IV. When the lesion showed hemorrhage, ulceration, thrombosis or necrosis, it was referred to as a complicated lesion.

These macroscopically visible lesions have been investigated in different populations, varying by age, gender, race and geographical location. It was noted that aortic fatty streaks were already present in children, in many of them below the age of three years and in all of them older than three years.[10] Coronary artery fatty streaks, on the contrary, were rare below the age of 10 years, but they rapidly increased in frequency during the next decade to become present in nearly all individuals of 20 years and older.[11]

Much work on the 'natural history' of atherosclerotic lesions in the aorta and coronary arteries has been performed as part of the International Atherosclerosis Project (IAP),[12] which studied autopsies from persons aged 10 years and older, in several geographic and ethnic groups according to a standard protocol. Some striking differences between the 'behaviour' of fatty streaks in on the one hand the aorta, and on the other hand the coronary arteries, were observed. The percentage of intimal surface with fatty streaks in the aorta showed an increase with age in most populations until about the age of 30 years, after which it leveled off or, more frequently, showed a decline.[13] This decline in percent intimal involvement might be a real one, suggesting the reversibility of fatty streaks. It might also be due to replacement of fatty streaks by fibrous

plaques (see below), or it might be due to the method of measuring fatty streaks as a percentage of the surface involved, as the aorta may have grown disproportionally rapidly. The percentage of the coronary artery intimal surface with fatty streaks was shown to increase evenly from age 10 until age 40 years, without a tendency to regress in any population studied.[13] The presence and extent of aortic fatty streaks did not differ greatly between the various race-location groups. This observation is in contrast with that of fatty streaks in the coronary arteries, which showed more variation among the different groups. And coronary artery fatty streaks in those 15-39 years of age were to some degree related to more advanced lesions in older persons of the same populations, whereas aortic fatty streaks did not show such a relation.[12] This suggested, at least for coronary lesions, that fatty streaks might be precursors of more advanced atherosclerotic lesions.[14] Although this transformation can never be proven from autopsy studies, it has also been suggested by several other observations. For example, the age sequence: in all studies fatty streaks appeared at earlier ages than fibrous plaques. Also, in one study[15] it was observed that the anatomical distribution of fatty streaks within the coronary arteries was similar to the distribution of fibrous plaques in coronary arteries of older persons of the same population. And, in another study[16] it was demonstrated that histologic characteristics of fatty streaks differed among populations with different dispositions to severe atherosclerosis, suggesting a gradual transition of fatty streaks into fibrous plaques.

5.3.2 Microscopic lesions

With advancing techniques more detailed aspects of early and advanced atherosclerotic lesions could be investigated. Also, the morphology of the normal vascular wall and its changing pattern with age became matters of interest. The roles of vascular endothelium, smooth muscle cells, macrophages and monocytes in the pathogenesis of atherosclerosis were and still are studied, discussed, revised and updated.[17,18,19]

The normal artery wall consists of three morphologically distinct layers. The intima, with a monolayer of endothelial cells adjacent to the arterial lumen, resting on a layer of elastic fibers, the internal elastic lamina. Between the endothelial cells and the elastic fibers a variable amount of extracellular connec-

tive-tissue matrix and isolated smooth-muscle cells are present. With increasing age multiplications of the internal elastic lamina and proliferations of the incidental smooth muscle cell occur in the coronary arteries, resulting in a so-called musculo-elastic layer.[20] This musculo-elastic layer is less pronounced in ethnic groups known to have a lower incidence of coronary heart disease[21] and its composition changes with age. In younger persons smooth-muscle cells and elastic fibers predominate, whereas in older persons this layer is mainly composed of collagen fibers. The media, or middle layer, consists of diagonally oriented smooth-muscle cells, surrounded by variable amounts of collagen, small elastic fibers and proteoglycans. The morphology of the media does not generally change with age. The adventitia, or outermost layer of the artery, consists principally of fibroblasts, some smooth-muscle cells and bundles of collagen, surrounded by proteoglycans.

The microscopical description of the above mentioned 'classical atherosclerotic lesions', especially the fatty streak and fibrous plaque, includes different cellular components, some of which are not visible at gross inspection. Stary investigated systematically, in several hundreds of children and young adults, a highly vulnerable site of the coronary artery system, the proximal portion of the left anterior descending artery.[22] He noted that in those less than 10 years of age, no fatty streaks were grossly visible, but that microscopically isolated monocytes and macrophage foam cells frequently were present in a thickened intima. In those older than 10 years, clusters of monocytes and macrophages were observed, together with lipid containing smooth muscle cells, whereas with the unarmed eye no fibrous plaques were visible. By the age of 15 years, foci of necrosis began to appear in the intima and after age 20, areas with necrotic foci associated with lipid loaden smoothmuscle cells and macrophage foam cells became larger and more frequent. Macroscopically, some of these lesions resembled uncomplicated fatty streaks, while others had the gross characteristics of fibrous plaques. However, the age sequence of the occurrence of microscopically visible changes in the intima of a coronary artery strongly supports the view that coronary atherosclerosis begins in childhood. It suggests that the process may begin with monocyte and macrophage infiltration, and that smooth muscle cell lipid accumulation and intimal necrosis are later developments. Although early atherosclerotic lesions in the aorta appear to be identical to those in the

coronary artery, it is more difficult to trace the evolution of fatty streaks to fibrous plaques. In the aorta there is no limited anatomic predilection site for atherosclerosis and furthermore the distribution of aortic fibrous plaques in adults according to sex, race and anatomical position, does not correlate with the distribution of aortic fatty streaks in younger persons. In summary, there is evidence that coronary atherosclerotic lesions begin in childhood and develop into adult life. Aortic atherosclerotic lesions presumably begin early in life as well, but it can not be concluded definitely that the early aortic lesions progress into more advanced atherosclerosis.

5.3.3 Hypotheses

The pathogenesis of atherosclerosis has been subject to many hypotheses arising from microscopical observations of atherosclerotic plaques, animal models or culture of cells implicated in atherosclerosis. In this paragraph an outline of the major theories will be presented. For more details, references 17, 19 and 20 are useful.

The *lipid theory* assumes an increased transport of plasma lipoproteins through the endothelium, as a consequence of high plasma LDL concentrations. The elevated lipid levels in the vessel wall give rise to an increased uptake by smooth muscle, endothelial or macrophage cells. When the capacity of the cells to take up lipids is exceeded, cholesterol and other lipids accumulate.

In the *response-to-injury hypothesis* the damage of the endothelial barrier is supposed to be the initiating event.[17] Factors such as hyperlipidemia, high blood pressure, hormone dysfunction or others may injure the endothelium. The underlying subendothelial connective tissue becomes exposed to platelets and other elements in the circulation. Microthrombi occur and platelets release the contents of their granules. Arterial smooth-muscle cells migrate from the media into the intima, proliferate and produce large amounts of connective tissue matrix. Lipids are deposited both within the cells and in the surrounding connective tissue matrix. Ultimately, restoration of the endothelial barrier occurs and the lesion regresses if the source of endothelial injury has disappeared. If however, the injury is continuous or repeated, further proliferation of smooth-muscle cells and accumulation of connective tissue and lipids occur. The balance between re-endothelialization and cell proliferation and necrosis may be disturbed, result-

ing in an atherosclerotic lesion. Risk factors such as hyperlipidemia, hypertension and smoking may influence this balance by interfering with the normal response-to-injury of tissue.

The *monoclonal hypothesis* proposes that each lesion of atherosclerosis is derived from a single smooth-muscle cell that serves as a progenitor for the remaining proliferative cells.[23] The atherosclerotic lesion may be seen as a benign neoplasm arising from one transformed cell, that may be regulated by other factors than the normal arterial smooth-muscle cell.

The *clonal-senescence hypothesis* is based on the age-related decline in replicative activity of cells in culture.[24] A paradoxical function of declining stem cell activity in the arterial media is suggested to be the underlying mechanism of atherosclerosis. The intima and media contain a relatively small amount of stem cells, which replicate to form smooth muscle cells, which then secrete 'chalones', that inhibit further replication of stem cells. With increasing age the concentration of chalones in the intima is decreased, as a function of decreased replication of smooth-muscle cells in the media and a diminished diffusion of chalones into the intima, resulting in smooth-muscle cell accumulation in atherosclerotic plaques. The endothelial stem cells may also play a role according to this hypothesis. Diminished activity of these cells might affect the endothelial barrier, resulting in the sequelae described above.

These proposed hypotheses are not necessarily mutually exclusive. It has been the merit of Steinberg[25] to combine the lipid infiltration hypothesis with the endothelial injury hypothesis. Others have gone in more detail in investigating the roles of endothelial, smooth muscle, macrophage or monocyte cells, trying to find factors that regulate their actions and lead to formation of atherosclerotic plaques. Recently, the role of monocytes as initiators of the atherosclerotic lesion, has been suggested.[19] Faggiotto et al.[26] observed in pigtail monkeys, who were fed a high-fat, high-cholesterol diet, clusters of monocytes on the endothelium, often in junctional areas, where they migrated subendothelially, accumulated lipids and took on the appearance of foam cells. These changes constituted the first stage of fatty streaks in these animals, similar to fatty streaks observed in humans. These fatty streaks enlarged by accumulation of smooth muscle cells migrating from the media into the intima and also accumulating lipid. It has been suggested that accumulation of monocytes on hyper-

cholesterolemic endothelium was due to changes in the monocyte as well as in endothelial cells or in the underlying smooth muscle cell.[27] After a few months of elevated LDL levels, the endothelium retracts over the fatty streak, principally at branches and bifurcations, leaving macrophages and connective tissue matrix exposed to the circulation. In this way platelets have the opportunity to adhere, aggregate and release their granule contents. Platelet derived growth factor (PDGF) may be of particular importance in atherosclerosis, because it is both chemotactic and mitogenic. It therefore can induce both smooth-muscle cell migration and proliferation.[28] A PDGF-like factor is also produced by the endothelium,[29] by macrophages[30] and by smooth-muscle cells themselves.

5.3.4 Concluding remarks

It is reasonable to assume that atherosclerosis has its origin in childhood and that the early atherosclerotic lesions develop into more complicated plaques, at least in coronary arteries. Endothelial cells, monocytes, macrophages, smooth-muscle cells, and platelets presumably all play a role in the atherogenic process. The intimal smooth-muscle cell proliferation may be the predominant feature, initiated by a response to injury mechanism. However, more has to be known about the factors that initiate and complicate the process.

5.4 Prevention

Preventive activities may be focussed on either the whole population, or on high risk groups. There has been much debate which strategy has to be preferred,[31,32] and what effect either of the strategies has in terms of prevented number of cases of disease. To answer the question of the relative merits of these approaches, a hypothetical model for the aetiology of chronic diseases, in particular cardiovascular diseases, may be considered. This hypothetical model has the following assumptions about the relation between the characteristic of interest (e.g., serum cholesterol or blood pressure) and the disease (e.g., coronary heart disease):

(1) it proposes that the 'genetic' level of the study characteristic (e.g., serum cholesterol) is not related to the probability of disease.

(2) it suggests that the rate of increase of the characteristic over time determines the disease probability.

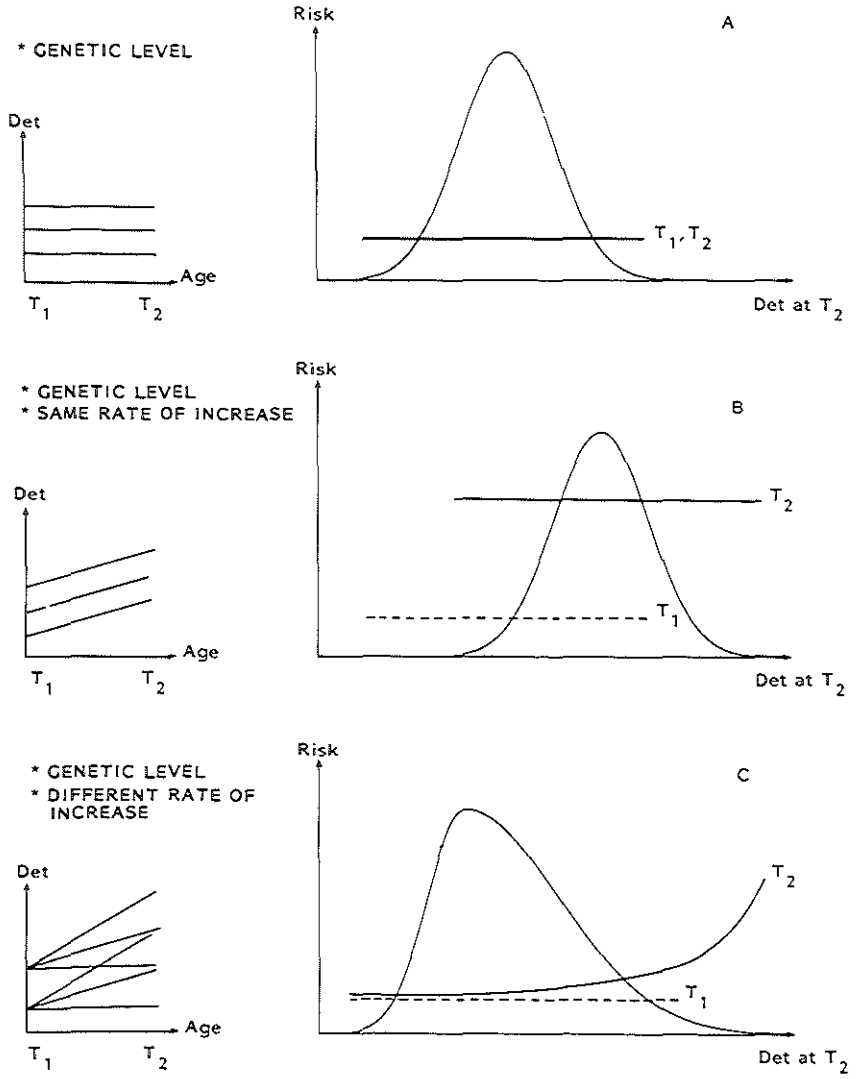

Figure 5.1

These two elements of the model are based on the thought that it is in general on a priori grounds unlikely that the level of a biological characteristic at birth is a risk factor for a certain disease. For instance, in the case of atherosclerosis, it may be reasonable to think that there is a state of equilibrium between the vascular endothelium and serum cholesterol or blood pressure. The

vascular endothelium may be resistant against the 'genetic background' level of these characteristics, whether or not they are high at birth. However, with a high rate of change of the characteristic, this equilibrium may be distorted. Hence, according to this reasoning, it is the rate of increase in the biological characteristic that determines the risk, and this may reflect 'genetic susceptibility' for the disease. It should be emphasized that genetic predisposition cannot be perceived without its interaction with environmental factors. Therefore, a person may inherit the susceptibility to a rise in a biological characteristic and thereby his disease risk, but the realization of this susceptibility, i.e., the rise in the characteristic, can only occur in a certain, 'riskful', environment.

Consider the following three situations, graphically presented in figure 5.1, in which the left side shows the relation between the determinant and age (from T_1 -> T_2) and the right side the distribution of the determinant at a certain age (T_2), and the relation between the disease risk and the level of the determinant at age T_1 and T_2.

Situation A

This situation is the basic one: all subjects have a constant genetic level of the characteristic over time. This model has the following elements:

* At T_2 the distribution of the determinant is the same as at T_1.
* The disease probability is invariant over the determinant.
* There is a certain background risk, because of other causes operating.

Situation B

In this situation all members of the population experience the same rate of increase of the characteristic. This has the following implications:

* At T_2 the whole distribution of the determinant is shifted to the right.
* The disease risk is similarly increased for each individual (dotted and solid horizontal line).
* Although the risk is increased compared to situation A, the characteristic under study is not a risk factor, because its level within this population is unrelated to the disease.

In the case of between-population comparisons the above described situations might occur. Population A, with an average low level of the characteristic also has a low level of risk, and population B, with an average higher level, has an increased risk. It should be noted, however, that the higher level of risk of

population B is based on the rate of increase of the characteristic, rather than on its level. A way to explore whether this is the case, is to compare childhood levels, or the rate of increase between childhood and adult levels of the characteristic, between populations. Because all members of the population in B are similarly susceptible to the disease, the best prevention strategy here would be the total population approach. This should aim at the prevention of increase of the characteristic and therefore intervene relatively early in life.

Situation C

This situation describes a different rate of increase of the characteristic for the members of the population. This has the following implications:

* The distribution of the determinant becomes skewed at T_2.
* The level of the determinant is related to the disease risk, because the highest category of the determinant is loaded with susceptibles, i.e., with subjects who had the largest increase.
* The largest proportion of preventable cases is present at the highest levels of the determinant.

This situation probably is the best reflection of true life. It might also provide part of the explanation for the fact that not all people with high risk factor levels experience the disease. The high level may in some individuals reflect a high 'genetic background' level, rather than a high rate of increase of the characteristic over life. The reverse holds for those with a relatively low level. In this situation the high-risk approach probably would be most effective for disease prevention, because especially later in life, the susceptibles are best indicated by their high risk factor level.

What are the implications of this reasoning? For *aetiologic research* it suggests that perhaps the increase over time rather than the level of blood pressure or cholesterol in relation to disease should be investigated. And that perhaps one should focus more on patterns of increase and try to find early markers for the rate of increase. For *prevention* this means that the population approach may be particularly suited early in life, to prevent the rise of a determinant, whereas the high-risk approach may be suited better later in life, to include the susceptibles and prevent them from further increasing their risk factors. In this way, the whole-population and the high-risk strategy go hand in hand in the prevention of chronic diseases. However, if one desires to use the high-risk

approach also at young ages, it becomes necessary to find a marker for the susceptibles, i.e., those who will show an increase in the characteristic over time. This will be discussed in the final section of this chapter.

5.5 Prediction

The study of the early prediction of atherosclerosis or coronary heart disease is important in two aspects: aetiology and prevention. If factors measured early in life, can be identified as predictors for heart disease at older ages, more insight into the early pathogenesis of atherosclerosis may be gained, and high-risk persons may be detected in an early stage. The question of aetiology may preferably be investigated in a study which has atherosclerosis as an endpoint, whereas the question of prevention may also be investigated in a study with clinical disease as endpoint. The design of the study therefore is of importance. This section starts with a discussion of possible study designs, before it will give the major findings from studies of the predictive value of serum lipids in childhood for coronary heart disease or atherosclerosis later in life.

5.5.1 Study design

The study designs that will be discussed here are based on one of the following characteristics: follow-up of children, assessment of clinical disease or atherosclerosis in parents, or assessment of atherosclerosis in children themselves.

Follow-up of children

The Framingham Heart Study[33] serves as the paradigm for epidemiological inquiries into the prediction of cardiovascular diseases in adult persons. In this study a cohort comprising 5,127 subjects, aged 30 to 62 at entry, has been regularly examined and followed for more than 30 years. If a cohort of children, in wich risk factors have been measured, has to be followed until the age that they may develop coronary heart disease, it would take even more years and more effort to avoid loss to follow-up. Therefore, although longterm observations would be preferable, for practical reasons studies of this type have not been conducted. However, a mid-way solution could be a retrospective follow-up study. Over the past decades many data on risk factors in children have been collected (chapter 3). In countries with an accurate civil registration, it may be possible

to trace those children over many years, and study their present health status. An advantage of this type of study probably is that the subjects would be less influenced in their behaviour by the study itself. Still, it would be a tremendous effort and other study designs may be favoured for being more efficient.

Parents with clinical disease

Familial aggregation of cardiovascular diseases has since long be recognized.[34,35,36] Although the major risk factors for heart disease also seem to cluster in families,[37,38,39] this familial aggregation appears to explain only a part of the familial aggregation of clinical disease. However, as children of parents with coronary heart disease are at far greater risk of developing atherosclerotic diseases than children of parents without heart disease,[40,41] the disease status in parents can be used to discriminate two groups of children. The next step is to compare putative risk factors of these children, who are prone to atherosclerosis based on their family history, with a group of children with a negative family history for heart disease. In the analysis, average levels of risk factor variables can be compared between the groups, but also a discriminant analysis can be performed to find the variable that best discriminates the two groups of children.

In this design the decision which of the clinical manifestations of cardiovascular disease has to be chosen and the assessment of the presence or absence of this disease are paramount. Clinical manifestations of atherosclerosis, like angina pectoris, myocardial infarction, cerebral stroke, or sudden death can be studied as one group of cardiovascular diseases, or they can be considered separately. When there is a strong indication for a different pathogenesis it may be better to study the clinical diseases separately. Misclassification may occur by absence of clinical symptoms in parents who already have a considerable amount of atherosclerosis, although they may form a special group. The age of the parents under study is of major importance, especially because most of the concern goes to the premature occurrence of heart diseases.

Finally, the outcome of studies with this design needs some comment. When in children whose parents have cardiovascular diseases elevated levels of risk factors are found, this may just reflect familial aggregation of risk factors. On the other hand, when abnormal lipid levels are already observed in childhood, it may be concluded either that those lipids are involved in the early pathoge-

nesis, or that they are just predictors or markers for altered metabolism. The presence of clinical disease in parents may also be used as a screening tool to find susceptibles, either those with elevated risk factor levels at young ages, or those who will experience a large increase (see section 5.4).

Parents with atherosclerosis

Following a saying of Dr. Lewis Kuller[42] that in epidemiological research 'sooner or later we must study coronary atherosclerosis rather than heart attacks', it may have additional merit to study the offspring of subjects who underwent coronary angiography. Although this method restricts the prediction of cardiovascular diseases to coronary heart disease, it may provide insight into the early determinants of the atherosclerotic process. The utility of coronary arteriography in the study of the natural history of coronary heart disease has been carefully reviewed by Pearson.[43] The major advantages of using coronary angiography over clinical criteria is the improved accuracy of definition of case and control groups. Besides, coronary angiography makes it possible to subdivide cases into categories with varying severity of disease. However, in studies based on coronary angiograms, selection bias may occur for several reasons. Only survivors of myocardial infarction can be selected for angiography and this may lead to an underrepresentation of severe atherosclerosis. Also, those who do not have clinical symptoms, like angina pectoris, may not have a coronary angiography and this may lead to an underrepresentation of those with minor atherosclerotic lesions. However, for the study of early predictors of atherosclerosis this is of less importance than the diagnostic suspicion bias,[44] which may operate if those with high risk factor levels are more likely to be subject to coronary angiography. Selection bias may also be related to the recruitment of the control series who may be patients with clinical symptoms but without evidence for atherosclerotic lesions. They conceivably may be patients who are very concerned about their health status, which may be reflected in lower than average levels of the risk factors. The design of studies of predictors of atherosclerosis can be the same as described for the study based on clinical disease in the parents.

Atherosclerosis in childhood

Although coronary angiography serves as the 'gold standard' in assessing the amount of atherosclerotic lesions, it does not allow to be applied randomly

to the population. This method may bring about some complications, involves exposure to radiation, is quite expensive and not comfortable for the patients undergoing it. In the near future non-invasive techniques may be developed that are as reliable as coronary angiography in assessing atherosclerosis, but that are without its disadvantages. Such a technique may then be used to measure early atherosclerotic lesions, like fatty streaks. Until now, there has been only one post-mortem study,[45] that has related early atherosclerotic lesions to risk factor levels in children and young adults. New non-invasive methods may not only relate the extent of fatty streaks to risk factor levels cross-sectionally, but they may also enable relating early atherosclerotic lesions to more advanced fibrous plaques longitudinally. In this way the natural history of atherosclerosis may be followed during life.

5.5.2 Study results

A number of investigators have studied the relation between parental cardiovascular disease and serum lipids in their children.[46-55] In general, elevated serum total cholesterol or triglyceride levels,[46-50,52-54] or decreased HDL cholesterol levels[51] were observed in children of parents with cardiovascular disease, notably myocardial infarction. Most of the studies aimed at finding high-risk subjects, rather than at finding markers or predictors of future disease. Lately, apolipoproteins have been suggested to be better predictors or markers of coronary heart disease.[56-60] Sniderman et al.[55] observed elevated levels of apo B in children of parents with myocardial infarction and hyperbetalipoproteinemia (elevated levels of apo B, in the presence of normal LDL cholesterol concentrations). They concluded that elevated levels of apo B in families with premature coronary heart disease occurred long before clinical symptoms emerged, and that therefore this metabolic disorder may be of importance in the pathogenesis of atherosclerosis. In our study (appendix 6) we also found higher levels of apo B in sons of fathers with coronary atherosclerosis, as determined by angiography, compared to sons of fathers without coronary atherosclerotic obstructions. Our findings still may reflect familial aggregation of apo B levels, although we did not select our patients on their (apo)lipoprotein levels. Moreover, a discriminant analysis in the fathers showed the ratio of HDL-cholesterol/LDL cholesterol to be the best discriminator, whereas in the sons the ratio

of apo B/apo A-I best discriminated between sons of fathers with and sons of fathers without coronary atherosclerosis. Whether apo B is implicated in the early pathogenesis of atherosclerosis, or just a marker for those susceptible for future disease, cannot be concluded from our study.

5.5.3 Concluding remarks

The relation of serum lipids and atherosclerosis early in life deserves further investigation, but fruitful epidemiological studies await the further development of non-invasive techniques to detect early atherosclerotic lesions. The level and determinants of cholesterol subfractions, apolipoproteins, the role of macrophages, and endothelial and smooth muscle cells need further inquiry, with emphasis on studies early in life. If possible, in aetiologic research the study of risk factors should be related to the underlying process, the atherosclerotic lesion, rather than to clinical manifestations of this process. For both aetiology and prevention the search for early markers or predictors of future cardiovascular diseases should be continued.

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Chapter 6

SUMMARY

The relationship between serum lipids and the occurrence of atherosclerosis and its clinical sequalae has been extensively investigated in adults. However, as evidence is increasing that atherosclerosis already starts in childhood, it may have merit to study its determinants early in life. The study of serum lipids in the young as described in this thesis comprises the following questions: what are the average levels and determinants of serum lipids? Is there a relation between lipid levels measured early in life and later on? And what are the possibilities early in life of prevention and prediction of future heart diseases? (chapter 1).

To answer these questions different types of study design and data-analytic method were used. Most of the data collected in this thesis, especially those on total and HDL cholesterol, are derived from a longitudinal investigation in 5-19 years old youngsters living in the Dutch town of Zoetermeer. Apart from this study population, two studies were carried out in young subjects selected from hospital populations (chapter 2).

The question related to the level and distribution of serum lipids at young ages has been investigated in different populations. Although considerable differences in levels of total cholesterol between these populations have been reported, in most studies a 'dip' in serum cholesterol concentrations between ages 10 and 16 years was observed. For HDL cholesterol levels a remarkable difference between males and females in their early twenties was observed in many studies. The findings in our study closely resembled these observations. Apart from age and gender, the following determinants were studied in their relation to serum lipid levels in children and young adults: familial history, body height and weight, hormonal factors (oral contraceptives, pregnancy) and life habits, like smoking, use of alcoholic beverages and physical activity. Age, parental cholesterol levels and body weight were the most important determinants of total cholesterol levels in youngsters. In accordance with other studies, a positive association between body weight and total cholesterol was observed in our study, however, only in those older than 15 years of age. This

finding suggests that early in life other variables (e.g., factors related to growth and maturation) may play a part in determining serum lipid levels. The most important determinants of HDL cholesterol were gender, age and body weight (the latter only in males older than 15 years). The relation between exogenous sex hormones and serum lipids was studied in two longitudinal investigations. Young women who started using oral contraceptives showed a larger increase in serum total cholesterol, than women who did not use oral contraceptives. Extremely tall prepubertal girls, who were treated with ethinylestradiol to inhibit further growth, showed a considerably larger increase in serum total cholesterol than an age matched reference group. Hormonal factors may also be involved in the increase in serum total and HDL cholesterol levels during pregnancy. When serum lipid levels of pregnant women were compared to the levels one year before and one year after pregnancy, it was observed that the year after pregnancy serum total cholesterol levels returned to pre-pregnancy levels, whereas HDL cholesterol levels dropped below pre-pregnancy concentrations (chapter 3).

The study of the natural history of serum lipids in the young raises two questions. First, there is a question of prediction: is there an association between cholesterol levels early in life and later on? This is often referred to as 'tracking'. Second, there is a question concerning the etiology of hypercholesterolemia: what are the patterns and determinants of change in serum lipid concentrations? There are several methods to assess the magnitude of tracking. In general, higher tracking coefficients were observed in males than in females, for both total and HDL cholesterol. The coefficients for total cholesterol were higher than for HDL cholesterol. With regard to patterns and determinants of change, a negative association was observed between initial level and subsequent change of total and HDL cholesterol, even after adjustment for regression to the mean. The change in total cholesterol was positively associated with cholesterol level in the mother (in males), initial weight (in males), initial smoking habit (in males), and negatively associated with initial use of oral contraceptives (in females). The change in HDL cholesterol was positively associated with initial weight (in females) and negatively associated with change in weight (chapter 4).

In the final chapter some background information is given on lipid

metabolism and atherosclerosis. Also, a hypothetical model on the aetiology of chronic diseases is presented and the implications for aetiological research and prevention are discussed. If the increase over time rather than the level of a determinant is of importance in relation to diseases, for preventive actions the population approach may be particularly suited early in life and the high-risk approach later in life. In the last part of this chapter some aspects of early prediction of atherosclerosis or cardiovascular diseases are discussed (chapter 5).

Chapter 7

SAMENVATTING

De relatie tussen serum lipiden en het optreden van hart- en vaatziekten of atherosclerose is met name bij volwassenen uitvoerig bestudeerd. Daar echter de opvatting dat atherosclerose op jonge leeftijd begint, steeds meer terrein wint, lijkt het zinvol de risikofaktoren reeds bij jeudigen te bestuderen. De vraagstellingen met betrekking tot serum lipiden bij jongeren die in dit proefschrift aan de orde komen zijn: wat zijn de gemiddelde waarden en determinanten van serum lipiden? Is er een verband tussen serum lipiden op jonge leeftijd en later in het leven? En wat zijn de mogelijkheden vroeg in het leven ten aanzien van preventie en prediktie van hart- en vaatziekten? (hoofdstuk 1).

De verschillende vraagstellingen met betrekking tot het onderzoek van serum lipiden bij jongeren leidden tot verschillende typen onderzoek en analytische modellen. Het merendeel van de gegevens over serum lipiden, met name totaal en HDL cholesterol, is afkomstig uit een longitudinaal onderzoek onder Zoetermeerse jongeren, die aanvankelijk 5-19 jaar oud waren. Daarnaast werden twee onderzoekingen verricht bij jongeren afkomstig uit een ziekenhuis populatie (hoofdstuk 2).

De vraag met betrekking tot de hoogte van het cholesterol op de jonge leeftijd, is in verschillende populaties onderzocht. Hoewel er aanzienlijke verschillen bestaan in de cholesterol spiegel tussen deze populaties, laten de meeste onderzoekingen bij jongeren een 'dip' zien in de totaal cholesterol koncentratie tussen de leeftijd van 10 en 16 jaar. Voor het nivo van HDL cholesterol tonen de meeste onderzoekingen een opmerkelijk verschil tussen mannen en vrouwen rond het twintigste levensjaar. Deze resultaten werden ook in onze studie gevonden. Naast leeftijd en geslacht werden de volgende faktoren onderzocht als mogelijke determinanten van het nivo van de serum lipiden bij kinderen en jong volwassenen: familiaire achtergrond, gewicht, hormonale faktoren (orale antikonceptiva, zwangerschap) en leefgewoonten, zoals roken, alcohol gebruik en lichamelijke inspanning. Leeftijd, het cholesterol nivo van de ouders en het gewicht waren de belangrijkste

determinanten van totaal cholesterol bij jongeren. In overeenstemming met diverse andere onderzoekingen vonden wij een positief verband tussen gewicht en totaal cholesterol, echter alleen in personen, ouder dan 15 jaar. Deze bevinding suggereert dat vroeg in het leven mogelijk andere faktoren van invloed zijn op het nivo van de serum lipiden, dan later in het leven. Faktoren als groei en rijping zouden op jongere leeftijd van belang kunnen zijn. De belangrijkste determinanten van HDL cholesterol waren: geslacht, leeftijd en gewicht (dit laatste alleen voor mannen ouder dan 15 jaar). De invloed van exogene hormonen op serum lipiden bleek met name uit twee longitudinale studies. Jonge vrouwen die de pil gingen gebruiken vertoonden een sterkere stijging in totaal cholesterol, dan vrouwen die niet de pil gebruikten. Extreem lange prepuberale meisjes, die werden behandeld met oestrogenen om verdere groei tegen te gaan, vertoonden een aanzienlijk grotere stijging in totaal cholesterol dan een voor leeftijd gematchte kontrole groep. Hormonale faktoren zijn mogelijk ook betrokken bij de stijging in totaal en HDL cholesterol gedurende de zwangerschap. Het jaar na de zwangerschap bleek totaal cholesterol weer op het nivo van voor de zwangerschap te zijn teruggekomen. Voor HDL cholesterol echter, vonden wij een daling in het nivo in het jaar na de zwangerschap ten opzichte van het jaar voorafgaand aan de zwangerschap (hoofdstuk 3).

Bij de bestudering van het natuurlijk beloop van serum lipiden op jonge leeftijd zijn twee vragen te onderscheiden. Ten eerste, de vraag van prediktie: is er een verband tussen cholesterol koncentraties op jonge leeftijd en later in het leven? Vaak wordt dit verband aangeduid met de term 'tracking'. Ten tweede, de vraag met betrekking tot de etiologie van hypercholesterolemie: wat zijn de patronen en determinanten van verandering in serum lipiden koncentraties? Er zijn verschillende methoden om de mate van tracking vast te stellen. Over het algemeen werden hogere tracking coefficienten voor jongens dan voor meisjes gevonden, zowel voor totaal als HDL cholesterol. De coefficienten voor totaal cholesterol bleken hoger dan voor HDL cholesterol. Wat betreft patronen en determinanten van verandering, werd er een negatief verband gevonden tussen initieel nivo en de mate van verandering in totaal en HDL cholesterol, zelfs na korrektie voor regressie naar het gemiddelde. De verandering van totaal cholesterol was positief geassocieerd met cholesterol

nivo van de moeder (bij jongens), initieel gewicht (bij jongens), initiele rookgewoonten (bij jongens), en negatief geassocieerd met initieel pilgebruik (bij meisjes). De verandering in HDL cholesterol was positief geassocieerd met initieel gewicht (bij meisjes) en negatief geassocieerd met verandering in gewicht (hoofdstuk 4).

In het laatste hoofdstuk wordt achtergrond informatie gegeven over lipiden metabolisme en atherosclerose. Vervolgens wordt een hypothetisch model aangaande de etiologie van chronische ziekten gepresenteerd met de implikaties voor etiologisch gericht onderzoek en preventie. Indien de verandering en niet het nivo van een determinant van belang is in relatie tot ziekten, dan verdient het voorkeur preventieve akties op jonge leeftijd op de hele bevolking te richten, terwijl op oudere leeftijd de benadering van groepen met een hoog risiko meer geschikt is. Tenslotte worden enige aspecten van vroege prediktie van atherosclerose of hart- en vaatziekten besproken (hoofdstuk 5).

Appendix 1

DISTRIBUTIONS AND DETERMINANTS OF TOTAL AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL IN DUTCH CHILDREN AND YOUNG ADULTS

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Summary

Serum total cholesterol and its putative determinants were measured in 5,089 subjects, ages 5-30 years, comprising 76% of the total population of two districts of the Dutch town of Zoetermeer. From this group 596 subjects, ages 5-19 years, were randomly selected, and distributions and determinants of high-density lipoproteins (HDL) cholesterol were studied in 458 of them. The variables studied included body weight; Quetelet index; menarche; parental cholesterol; physical activity; consumption of coffee; alcohol and tobacco; and use of oral contraceptives.

Mean total cholesterol levels showed a decrease in both boys and girls between the ages of 10 and 16 years. Mean HDL cholesterol levels remained the same until the age of 17, after which they showed an increase for females and a decrease for males. Total cholesterol was associated with age, body weight (in those older than 15 years), and parental cholesterol concentrations. For HDL cholesterol the most important determinants were gender, age and body weight (the latter only for males 15 years and older). These findings suggest that during growth and maturation the determinants of serum cholesterol are different from those later on in life.

INTRODUCTION

Many studies have shown that the serum cholesterol levels in adults predict the occurrence of cardiovascular diseases (CVD).[8,17,26] There is a consensus that serum total cholesterol and its fractions play a part in athero- and thrombogenesis.[20,28] Evidence is increasing that the roots of atherosclerosis may be traced to early life.[3] Therefore, investigations of the distribution, determinants and development of serum cholesterol in childhood may provide insight into the etiology as well as the prevention of CVD.

This report deals with the distribution and determinants of serum total cholesterol and high density lipoprotein (HDL) cholesterol in 5,089 subjects ages 5-30 years, of a general Dutch population.

SUBJECTS AND METHODS

Population

Between April 1975 and June 1978 all residents ages 5 years and over of two districts in the Dutch town of Zoetermeer, a suburban residential community near the Hague, of about 60,000 inhabitants, were invited to participate in a study of risk indicators for cardiovascular diseases (EPOZ study) as described previously.[14,16,29] Of 6,672 eligible persons, ages 5-30 years, 5,367 (2,673 males) participated in the study. Total cholesterol was measured in 5,089 of these (76% of those eligible). This group comprised 2,512 subjects ages 5-14 years (1,274 males) and 2,577 subjects ages 15-30 years (1,245 males).

From the 3,820 subjects ages 5-19 years who took part in the study, 596 were selected as a random sample for yearly follow-up examinations. During these examinations serum total cholesterol was measured. In 1980, HDL cholesterol was determined for the first time in 458 subjects (244 males) of the random sample (77% of those eligible).

Measurements

For the determination of both total and HDL cholesterol, blood was taken by venipuncture from non-fasting subjects. The determinations were carried out in the laboratory of our department. Serum total cholesterol was measured with

an automated enzymatic method, utilizing the color reactions according to Kageyama as discussed in Ref 30. HDL cholesterol was also measured using an automated enzymatic method with the Trinder color reaction,[30] after precipitation with phosphotungstate-Mg^{2+}.[5] The determinations for total and HDL cholesterol were checked regularly by the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter). Serum total cholesterol concentrations of the parents of the study children were determined at the initial examination. Parental cholesterol levels were available for 93% of the 5-14 year old subjects and for 60% of those 15-30 years of age.

Body height (in cm) and weight (in kg) were measured with the subjects wearing indoor clothes and no shoes. The Quetelet index, the ratio of body-weight over height squared, was used as an index of body mass.[18] Smoking habits, physical activity, and the use of coffee, alcohol, and oral contraceptives were assessed by questionnaire in children ages 10 years and over. Data on smoking habits included questions on the average amount of cigarettes smoked daily. A physical activity score was based on questions about regular sports activities, including membership in sports clubs and daily bicycling and walking. Questions were asked about the average number of cups of coffee consumed daily. The frequency of alcohol consumption was assessed by three categories: none, occasionally and daily consumption. As this question was introduced later in the study, we did not obtain data on alcohol consumption for all subjects. Females 10 years and older were asked about their menstrual history and about their current use of oral contraceptives.

Data analysis

The analysis of the distributions and determinants of serum total cholesterol and HDL cholesterol was based on different samples. For total cholesterol data on all 5,089 5 to 30-year-old subjects were used, whereas HDL cholesterol was analyzed for those 458 subjects of the random sample who participated in the study at the time HDL cholesterol was first measured.

Mean values of total and HDL cholesterol are presented graphically as 3-year moving averages (Figs. 1 and 3). The 5th, 50th and 95th percentiles of the distributions are shown by 5-year age groups (Figs. 2 and 4).

Determinants of total and HDL cholesterol levels were studied using multiple linear regression for males and females separately and in two age-groups (5-14

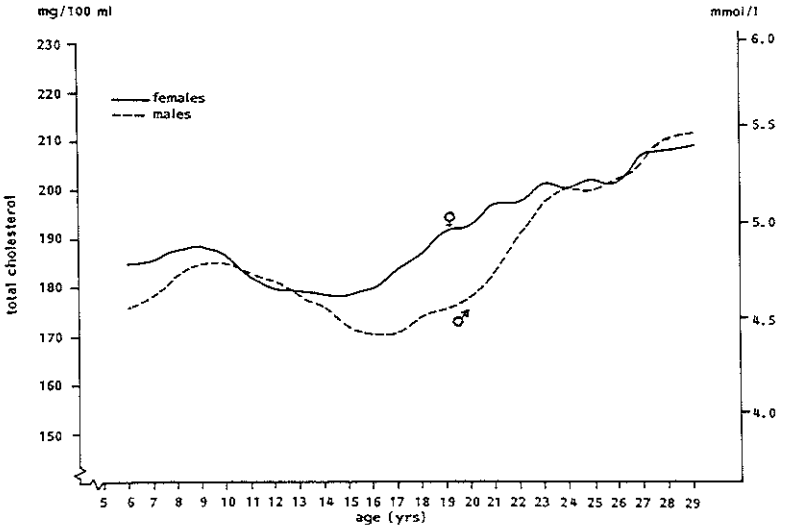

Fig. 1. Serum total cholesterol in subjects ages 5-30 years, 3-year moving averages (EPOZ 1975-1980).

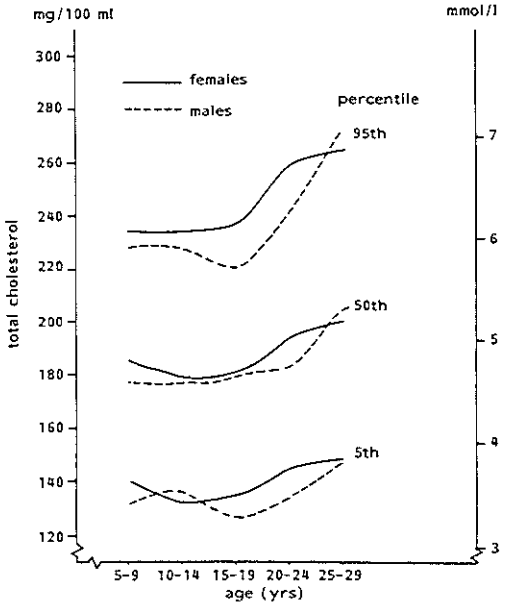

Fig. 2. The 5th, 50th and 95th percentiles of serum total cholesterol in subjects ages 5-30 years, in 5-year age groupings (EPOZ 1975-1980).

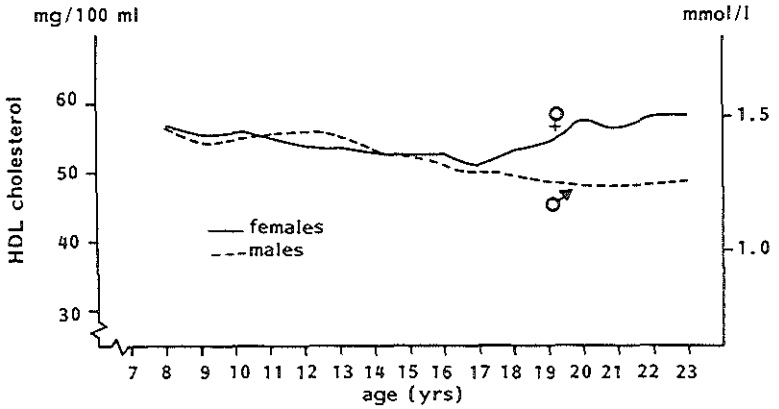

Fig. 3. Serum HDL cholesterol in subjects ages 8-23 years, 3-year moving averages (EPOZ 1975-1980).

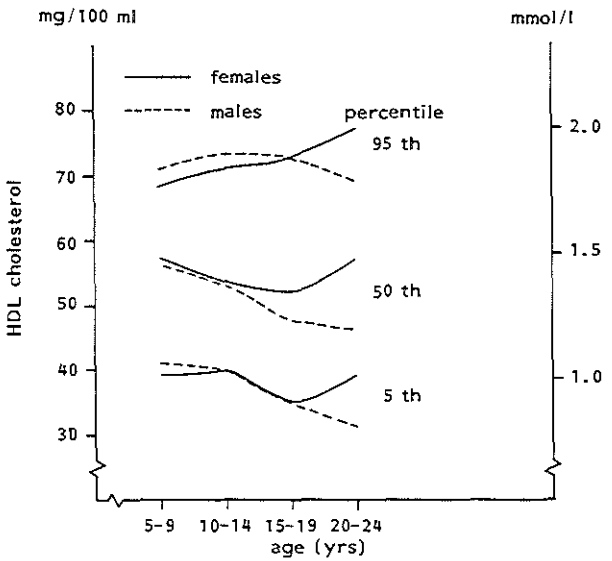

Fig. 4. The 5th, 50th and 95th percentiles of serum HDL cholesterol in subjects ages 5-24 years, in 5-year age groupings (EPOZ 1975-1980).

and 15-30 years). Total or HDL cholesterol was the outcome variable for all regression models. We used indicator variables to compute average cholesterol levels by different categories of the determinants. Body weight, Quetelet index, and parental cholesterol were devided into categories according to distribution quartiles. Each category of determinants was entered into the model as a separate indicator (0=not in this category, 1=belongs to this category). The regression coefficients yielded by this model served as the difference in total or HDL cholesterol between categories. An adjustment for different distributions of age and, if not already in the model, body weight was made for all analyses. In the tables and figures, P(2) refers to a two-tailed test of significance.

RESULTS

Mean values and distributions

Total cholesterol For both boys and girls, the average concentration of total cholesterol decreased between ages 10 and 16 (Fig. 1; detailed tables available upon request). The same pattern, although less distinct, was observed for the 5th, 50th and 95th percentiles (Fig. 2).

HDL cholesterol Mean serum HDL cholesterol levels decreased slightly for both boys and girls from 8 to 17 years of age (Fig. 3; detailed tables available upon request). Thereafter the decrease in boys continued, whereas in girls average HDL cholesterol levels showed an increase. As a result, at 20-24 years of age, average HDL cholesterol levels were considerably higher in women than in men. The percentile distribution of HDL cholesterol showed a similar phenomenon (Fig. 4).

Determinants

Total cholesterol For males and females of 15 years and over, a positive relationship between total cholesterol and body size was found. Those in the upper category (according to quartiles) of body weight and Quetelet index had significantly higher cholesterol levels than those in the lowest one. There was no positive relationship between body weight and total cholesterol in subjects under 15 years of age (Fig. 5).

Total cholesterol concentrations for both fathers and mothers were positively related to those of their children, after adjustments for age and body weight

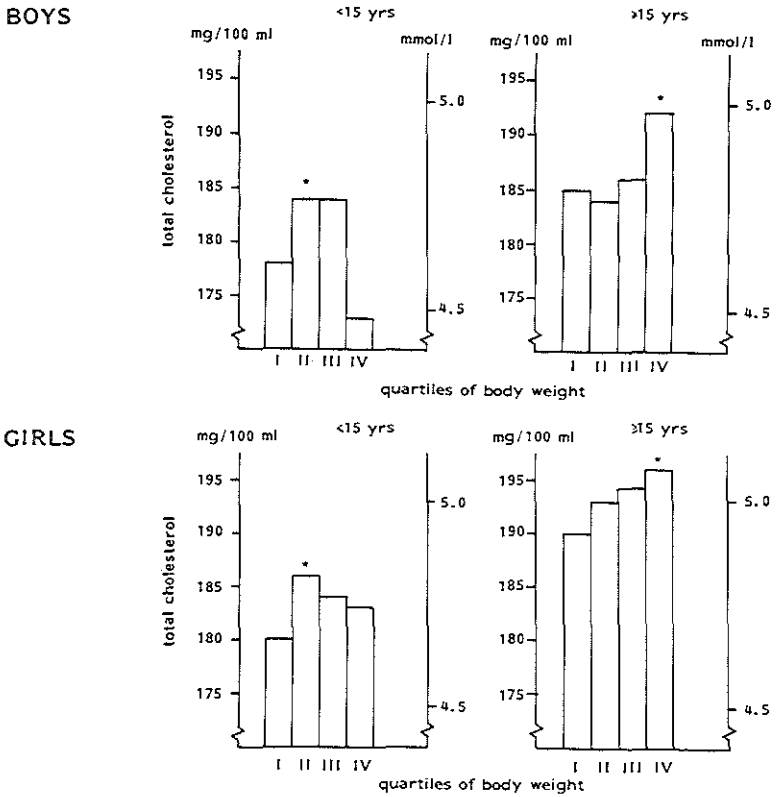

Fig. 5. Average total cholesterol levels, adjusted for age, by categories according to quartiles of body weight in boys and girls, in two age groups (EPOZ 1975-1980). * P(2) < 0.05 with category I as reference.

TABLE 1

Mean total cholesterol levels ± SEM (mg/100 ml), adjusted for age and body weight, by categories of alcohol consumption in males and females, 15 yrs and older

	Men			Women		
Alcohol Consumption	n	Mean	SEM	n	Mean	SEM
None	30	189	6.1	42	190	6.3
Occasionally	216	179(a)	2.0	254	193	2.1
Daily	67	194(b)	4.4	28	196	6.2

(a) P(2) < 0.10 with None as reference category
(b) P(2) < 0.001 with Occasionally as reference category

TABLE 2

Mean total cholesterol levels ± SEM (mg/100 ml), adjusted for age and body weight, by smoking habits in males and females, 15 yrs and older

	Men			Women		
Smoking habits	n	Mean	SEM	n	Mean	SEM
Never	351	188	1.8	340	193	2.0
Stopped	259	188	2.3	299	194	2.2
1- 9 cig/day	212	184	2.5	317	189(a)	1.7
10-19 cig/day	253	186	2.2	251	197(b)	2.3
> 20 cig/day	155	187	3.3	105	198	3.2

(a) P(2) < 0.10 with Never as reference category
(b) P(2) < 0.005 with 1-9 cig/day as reference category

TABLE 3

Mean total cholesterol levels ± SEM (mg/100 ml), adjusted for age and body weight, by use of oral contraceptives in women 15 years and older

Use of oral contraceptives	n	Mean	SEM
Yes	476	197	1.5
No	824	191(a)	1.3

(a) P(2) < 0.005

TABLE 4

Mean total cholesterol levels ± SEM (mg/100 ml), adjusted for age and body weight, by having reached menarche in girls ages 11-16 years

Menarche	n	Mean	SEM
No	312	181	1.6
Yes	360	178	1.7

TABLE 5

Mean HDL cholesterol levels ± SEM (mg/100 ml), adjusted for age and body weight, by having reached menarche in girls ages 11-16 years

Menarche	n	Mean	SEM
No	25	49	2.2
Yes	43	56(a)	1.4

(a) P(2) < 0.10

(Fig. 6).

Physical activity level and coffee consumption were unrelated to serum total cholesterol concentrations. The relationship between total cholesterol and alcohol consumption is given in Table 1. Men drinking daily had higher cholesterol levels than those drinking occasionally. Women who smoked 10 or more cigarettes a day had higher cholesterol levels than those who smoked less (Table 2).

Females using oral contraceptives had significantly higher cholesterol levels than those who did not, even after adjustment for age, body weight, and smoking habits (Table 3). Total cholesterol levels did not differ between girls who had reached menarche and those who had not (Table 4).

HDL cholesterol Body weight was negatively related to HDL cholesterol levels in males ages 15 and over, but this negative association did not reach statistical significance in the other study groups (Fig. 7). The Quetelet index showed a similar negative relation with HDL cholesterol levels.

No associations of physical activity, smoking habits, and use of coffee, alcohol or oral contraceptives with HDL cholesterol levels were found.

Girls ages 11-16 years who had had their first menstruation showed higher HDL cholesterol levels than those who had not yet reached menarche (Table 5).

DISCUSSION

Our observations confirm previous reports of a considerable decrease in total cholesterol between the ages of 9 and 16 years.[3,6,10,21] Biological maturation has been implicated in this, and it has been suggested that hormonal changes play a part in the occurrence of this 'dip'.[4,24] Although pre- and postmenarchal females have different sex hormone levels, we could not observe a significant difference between their total cholesterol levels. Likewise, Benion et al.[2] were unable to find a correlation between changes in sex hormone ratios and changes in plasma lipids during puberty in boys. Other investigators, however, have reported an association between endogenous sex hormones and plasma lipids in adults[12,13] and in adolescent boys.[19]

Body size, expressed in various ways, is a major determinant of both total and HDL cholesterol. Previous studies have shown a positive association between body size and total cholesterol and a negative one with HDL cholesterol levels,

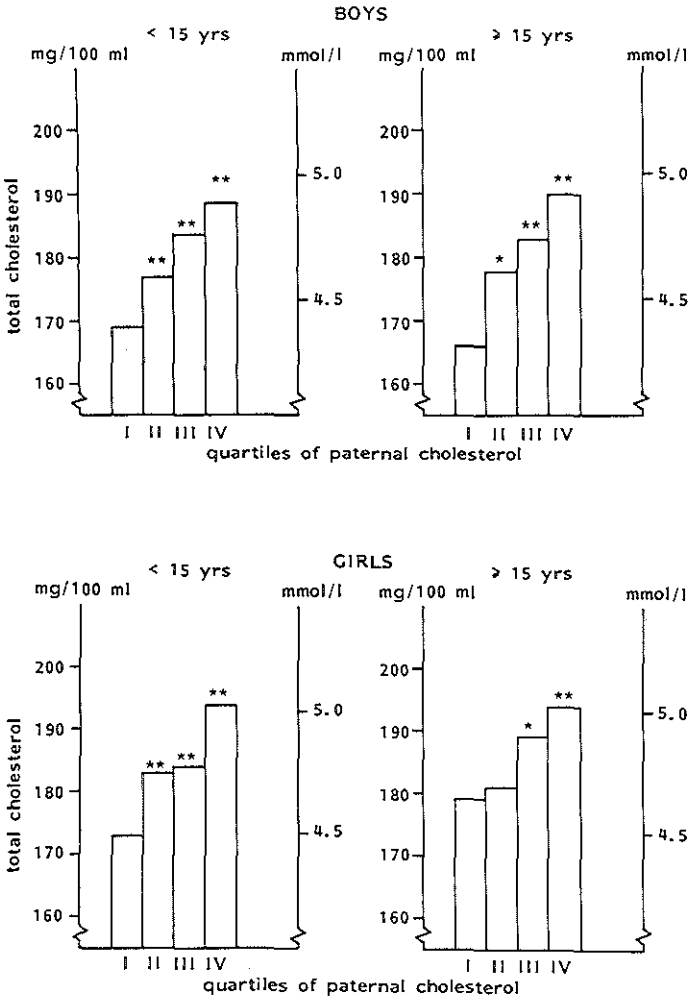

Fig. 6. Average total cholesterol levels, adjusted for age and body weight, by categories according to quartiles of paternal and maternal cholesterol in boys and girls, in two age groups (EPOZ 1975-1980). * P(2) < 0.005 with category I as reference. ** P(2) < 0.001 with category I as reference.

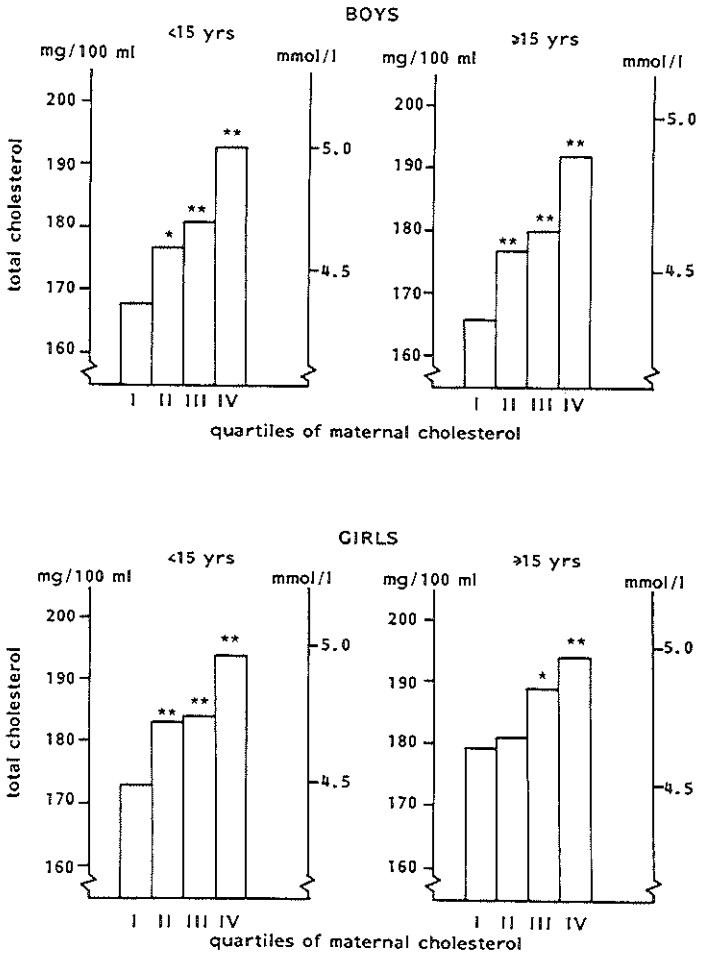

Fig. 6 - continued

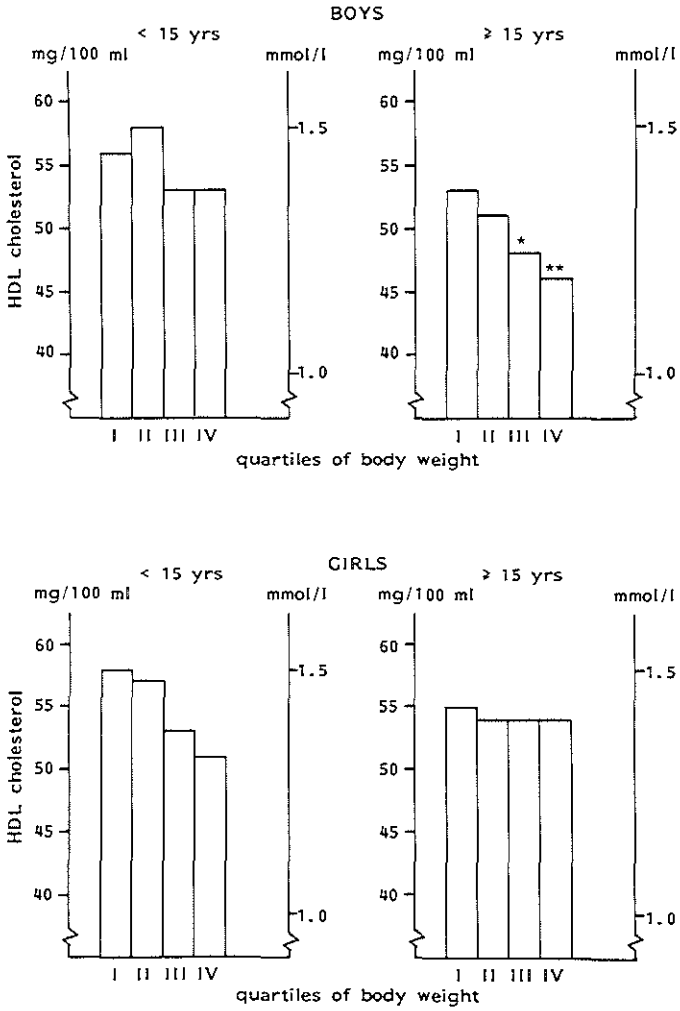

Fig. 7. Average HDL cholesterol levels, adjusted for age, by categories according to quartiles of body weight, in boys and girls in two age groups (EPOZ 1975-1980). * $P(2) < 0.05$ with category I as reference. ** $P(2) < 0.01$ with category I as reference.

especially in adults.[11,25,27] In our study, we found body weight to be a determinant of total cholesterol in the older age groups but not in the younger ones. This suggests that early in life determinants of total cholesterol may differ from those operating later in life, e.g., those related to physical maturation. Our observations indicate that this may also apply to HDL cholesterol. The only determinant that is common to children and young adults is parental cholesterol levels. This confirms a report by Morrison et al.,[22] who observed, both in children who lived with their parents and in those who did not, positive correlations between their cholesterol levels and those of their parents. In a separate analysis, we did not find evidence for a multiplicative effect of paternal and maternal cholesterol levels on those of their children.

An interesting finding is that after the age of about 17 years, average HDL cholesterol increases in women but decreases in men. This leads to a considerable gender difference in HDL cholesterol levels in early adulthood.[1] From a preventive point of view, it may be of importance that men showed a significant negative relationship between HDL cholesterol and body weight, as has been observed elsewhere.[11]

We cannot confirm previous findings of an association between physical activity and total or HDL cholesterol. This may be due to dilution of the 'true' relationship towards the null value, as a result of random error in the measurement of physical activity. In the case of HDL cholesterol, it may also be due to the relatively small numbers.

The evidence concerning the relationship between oral contraceptive use and cholesterol concentration is equivocal. Our finding of a relationship between such contraceptives and total cholesterol and total cholesterol and the absence of a relationship with HDL cholesterol confirms a report by Wallace et al.[31] However, this does not warrant a firm conclusion about the effects of oral contraceptives on cholesterol levels, as the different compositions of oral contraceptives were not taken into account.

We found no association between coffee consumption and cholesterol levels, and this confirms our previous report on adults from the same population.[16] Our observations on the relationship between cholesterol levels and either alcohol consumption or smoking habits are at variance with some other reports.[7,9,23,25] We did not observe a positive relationship between HDL chole-

sterol and alcohol consumption. This might be due to the fact that we only referred to the frequency of drinking and not to the amount consumed.

In summary, we found differences in HDL and total cholesterol levels by age and gender. The determinants of these levels seem to be different for children and adults. This underscores the important role of growth and maturation in determining lipid levels. Further investigation of the impact of factors related to physical maturation on lipid metabolism seems a promising way to elucidate the determinants of cholesterol levels in childhood.

ACKNOWLEDGMENTS

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Appendix 2

DO ORAL CONTRACEPTIVES INCREASE BLOOD PRESSURE AND SERUM TOTAL CHOLESTEROL IN YOUNG WOMEN?

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Summary

The effects of the use of oral contraceptives on serum lipids and blood pressure were studied in young women who participated in a longitudinal survey of risk factors for coronary heart disease in the Netherlands. During follow-up 53 women started to use oral contraceptives. The year before they started they were 14 to 24 years of age, and they continued using oral contraceptives for at least two subsequent years. From 53 age-matched control subjects, who did not use oral contraceptives, data were obtained for the same follow-up period. Women on oral contraceptives showed a significantly greater rise in serum total cholesterol levels than the reference subjects (14 mg/100 ml/2 year vs 4 mg/100 ml/2 year). They also showed a somewhat greater increase in systolic blood pressure (4.7 mmHg/2 year vs 2.1 mmHg/2 year), but this difference was not statistically significant. In a separate analysis oral contraceptive users were divided in those who used oral contraceptives with 50 μg of ethinylestradiol or more and those who used oral contraceptives with less than 50 μg . There were no significant differences in the increase in total cholesterol or blood pressure between these two groups of oral contraceptive users.

These findings suggest that the use of oral contraceptives may be associated with an enhanced rise in total cholesterol, and perhaps in blood pressure, during adolescence.

INTRODUCTION

Since the introduction of the oral contraceptive pill in the early sixties, repeatedly attention has been focussed on its possible harmful side-effects.[1,2] Especially, the relation between oral contraceptives and cardiovascular diseases has been studied.[2-6] Also the putative association between oral contraceptives and risk factors for heart disease, like blood pressure[7,8,9] and serum lipids[9-12] has been the subject of investigations. The results of these studies, however, have been equivocal, and appear to depend on the age of the study population, the hormonal composition of the contraceptive pill, and the place and time of the study. The study reported here differs from some former ones in that it deals with young women, who started the use of oral contraceptives during a follow-up study.

SUBJECTS AND METHODS

Population

From 1975 to 1978, all residents aged 5 year and older living in two districts of Zoetermeer, a suburban town near The Hague in the Netherlands, were asked to participate in a study for risk indicators of cardiovascular disease (EPOZ), as described earlier.[13] From the 4,649 subjects aged 5-19 years who took part in the study (82% of those eligable), a random sample of 596 children (296 females) was selected for yearly follow-up.

Measurements

During the yearly examinations body height (in cm) and weight (in kg) were measured with the study subjects wearing indoor clothes without shoes. Blood pressure was measured using a random zero sphygmomanometer, twice in sitting position, and blood was taken by venipuncture from the non-fasting subjects. Serum total cholesterol was measured with an automated enzymatic method, utilizing the color reactions according to Kageyama.[14] Serum HDL cholesterol was also measured using an automated enzymatic method with color reaction according to Trinder, after precipitation with phosphotungstate-Mg^{2+}.[15] As the measurement of HDL cholesterol was initated only in 1980, fewer data were available. The determinations of both total and HDL cholesterol were carried out in

the laboratory of our department, which participates in the lipid standardization program of the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter).

Each year the respondents completed a questionnaire on smoking habits, use of alcoholic beverages and use of coffee. Girls were also asked about their menstrual history, pregnancy and use of oral contraceptives.

Selection of persons

During follow-up 101 women indicated oral contraceptive use at least at one yearly examination. Of 53 women information on serum lipids and blood pressure was available both of the year before the start of use and two subsequent years of use. From the remainder of the cohort, i.e., those who never used oral contraceptives, 53 reference subjects (controls) were selected, matched for age. They were not pregnant and they participated in at least three consecutive annual examinations. In figure 1, the design of this study is graphically displayed. At year 0, which may be any chronological year during follow-up, the "cases" were not yet using oral contraceptives and they were not pregnant either. At year 1, the cases used oral contraceptives for the first time and they still used them at year 2. Control women did not use oral contraceptives, nor were they pregnant at year 0, 1 or 2. In table 1, baseline characteristics of future users of oral contraceptives and reference women are given.

TABLE 1

Baseline characteristics (with standard deviations) of users and non-users of oral contraceptives

	Users (n=53)	Non-users (n=53)
Age (years)	17.8 (2.3)	17.8 (2.3)
Body weight (kg)	58.2 (10.8)	59.5 (9.5)
Quetelet index (kg/m2)	20.6 (3.5)	20.9 (2.6)
Cigarette smoking (%)	45	23
Total cholesterol (mg/100 ml)	180.2 (27.7)	188.8 (26.8)
HDL cholesterol (mg/100 ml) *	50.4 (13.1)	51.7 (11.5)
Systolic blood pressure (mmHg)	110.1 (11.7)	114.1 (12.6)
Diastolic blood pressure (mmHg)	67.3 (8.5)	67.3 (9.1)

* based on 25 users and 39 non-users

Data analysis

Our data analytic approach was two-fold. Firstly, average total and HDL cholesterol concentrations and blood pressure values were compared between users and non-users at year 0, 1 and 2. Secondly, the change in cholesterol and blood pressure over the two years of follow-up was compared between users and non-users. An adjustment for different distributions of age and body weight was made in all analyses. For this purpose we used multiple linear regression, in which serum lipids or blood pressure (or the change in these characteristics) were the dependent variables. Indicator variables were used to compute average lipid or blood pressure levels (or the average change in these variables) by the comparison groups.[16] The regression coefficients yielded by this model served as the adjusted difference in cholesterol or blood pressure level (or in change of cholesterol and blood pressure) between the comparison groups.

Year	0	1	2
Cases (n=53)	OC −	OC +	OC +
Controls (n=53)	OC −	OC −	OC −

Fig. 1. Design of the study.

RESULTS

Average levels

The average levels of total and HDL cholesterol in year 0, 1 and 2 for both users of oral contraceptives and non-users are given in fig 2. No significant differences were observed in either total or HDL cholesterol in any year. Also, systolic and diastolic blood pressure levels during year 0, 1 and 2, were not significantly different between users and non-users (Fig. 3).

Change in level

The changes in serum total and HDL cholesterol, and in systolic and diastolic blood pressure in the two years of follow-up for users and non-users of oral contraceptives are given in table 2. The difference in the change of total cholesterol between users and non-users was 9.8 mg/100 ml (95% confidence

interval: 0.1 - 19.6). For HDL cholesterol this difference was -2.7 mg/100 ml (95% CI: -8.6 - 3.3). The increase in systolic blood pressure was 2.6 mmHg larger (95% CI: -1.8 - 6.9) in users of oral contraceptives than in non-users, while the change in diastolic pressure did not differ between users (0.1 mmHg) and non-users (0.0 mmHg). A separate analysis in which women who used oral contraceptives with an oestrogen dose of $\geqslant 50\,\mu g$ were compared with women who used $< 50\,\mu g$, showed non-significant differences in the change in total cholesterol and systolic blood pressure (table 3).

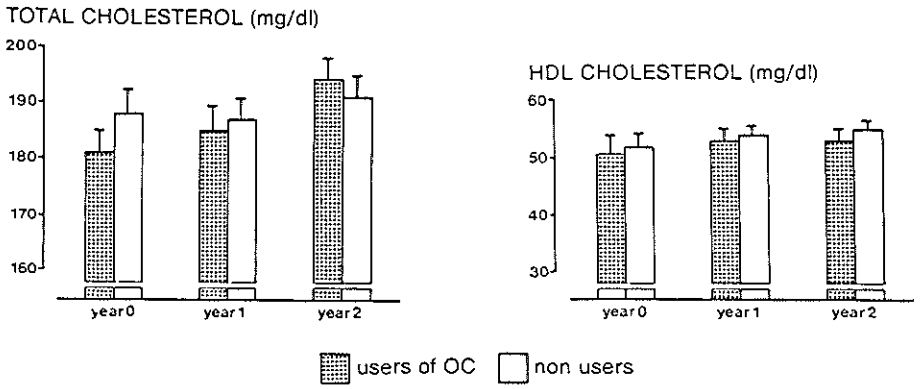

Fig. 2. Average levels of total and HDL cholesterol in mg/100 ml (with S.E.M.) in users of oral contraceptives and non users, during year 0, 1 and 2.

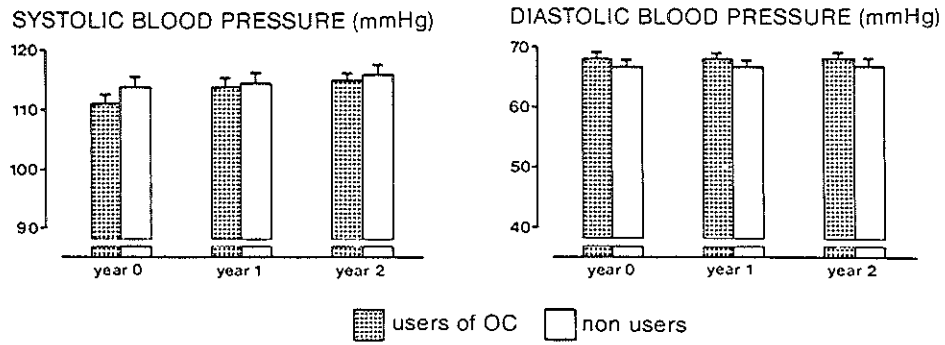

Fig. 3. Average levels of systolic and diastolic blood pressure (with S.E.M.) in users of oral contraceptives and non users, during year 0, 1 and 2.

TABLE 2

Average change * in total and HDL cholesterol and systolic and diastolic blood pressure in two years of follow up, for users and non-users of oral contraceptives

	Users (n=53)	Non-users (n=53)	P(2)
Total cholesterol (mg/100 ml)	14.0	4.2	0.05
HDL cholesterol (mg/100 ml)	0.3	3.0	0.38
Systolic blood pressure (mmHg)	4.7	2.1	0.25
Diastolic blood pressure (mmHg)	0.1	0.0	0.99

* Adjusted for age and body weight

TABLE 3

Average change * (with S.E.M.) in total cholesterol and blood pressure for users of oral contraceptives, with either $\geq$ 50 μg or < 50 μg ethinylestradiol

	$\geq$ 50 μg (n=16)	< 50 μg (n=22)
Total cholesterol (mg/100 ml)	14.4 (6.9)	15.5 (5.2)
Systolic blood pressure (mmHg)	5.8 (2.5)	3.1 (2.5)

* Adjusted for age and body weight

DISCUSSION

This study comprises both a cross-sectional and a longitudinal comparison of serum lipids and blood pressure between users of oral contraceptives and non-users. Before we base our inference on these findings, we need to comment on two observations; namely, (1) there were initial differences between users and non-users and (2) the results of the cross-sectional and the longitudinal analysis were different.

Although the initial differences in serum total cholesterol and systolic blood pressure between users and non-users were not statistically significant, they might point to a limited comparability of users and non-users of oral contraceptives at baseline. Women who are going to use oral contraceptives may be

different in aspects relevant for serum lipids and blood pressure, from women who do not. An explanation for the initial differences may be that young women who are going to use oral contraceptives are biologically more mature than those who are not. We looked at menarchal age, as a measure for maturity, but the average age at menarche was the same for both users and control women. The frequency of cigarette smoking was somewhat higher among the future users of oral contraceptives smokers, than among the control women (table 1). This is in agreement with previous findings of a higher prevalence of smokers among oral contraceptive users.[5] However, as smoking was positively associated with total cholesterol values in our data, this would have caused a difference in the opposite direction. Selection bias could have occurred if the control women were excluded from oral contraceptives because of elevated cholesterol or blood pressure levels. However, all initial blood pressure and cholesterol levels were within normal ranges in both groups. Moreover, any bias introduced by exclusion of subjects with elevated blood pressure for oral contraceptive use would again have acted against a difference in blood pressure between the groups. And although blood pressure is regularly measured by general practioners in the Netherlands, serum cholesterol concentrations hardly ever are. Therefore, most probably the observed initial differences are due to chance, but they de obscure possible differences at year 1 and 2. We are therefore inclined to favor the longitudinal analyses for inference, and to adjust for possible confounding factors that influence change in the risk factors. Possible confounders that were taken into account were change in weight and change in smoking behavior. There were no differences between the two groups of women in change of body weight or smoking status. Although this is not an experimental study and therefore bias still has to be considered, we feel confident to conclude that young women who start using oral contraceptives may experience a larger rise in serum total cholesterol, and perhaps also in blood pressure, than young women who do not.

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Appendix 3

SERUM LIPIDS IN YOUNG WOMEN BEFORE, DURING AND AFTER PREGNANCY

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Summary

The associations between pregnancy and serum lipids were investigated in a cohort of 831 Dutch women, initially aged 5-19 years. These women were examined yearly for an average period of 6 years, as part of a longitudinal survey of risk factors for coronary heart disease. During this period 62 women became pregnant and their serum total and HDL cholesterol levels were compared to that of an age-matched reference series of non-pregnant women, derived from the same cohort. Pregnant women showed higher total cholesterol levels (235 $\pm$ 7.4 mg/100 ml) than non-pregnant women (205 $\pm$ 2.7 mg/100 ml). Pregnant women also had higher levels of HDL cholesterol (66 $\pm$ 2.1 mg/100 ml) than their referents (57 $\pm$ 1.0 mg/100 ml). Total and HDL cholesterol increased with duration of pregnancy. When serum lipid levels of pregnant women were compared to the levels one year before and one year after pregnancy, it was observed that the year after pregnancy HDL cholesterol levels dropped below pre-pregnancy concentrations. At the final examination, women who ever had been pregnant were compared with women who never had been pregnant. Those who ever had been pregnant showed lower HDL cholesterol levels than those who never had been pregnant. The difference was most marked in users of oral contraceptives. These observations suggest that serum total and HDL cholesterol are elevated during pregnancy, probably due to hormonal changes. They furthermore point to a possibly lowering effect of parity on HDL cholesterol. These findings may help to explain the reported positive association between parity and the occurrence of cardiovascular diseases.

INTRODUCTION

There appears to be growing agreement with the view that atherosclerosis has its onset early in life[1] and that preventive action should be focussed on youngsters.[2] In adults serum lipids have been found to be highly associated with coronary atherosclerosis.[3] Recently, postmortem studies in youngsters revealed evidence for an association of serum lipids and atherosclerosis early in life.[4] Inquiries into the determinants of serum lipid levels in young people may therefore yield insight into the early pathogenesis of atherosclerosis.

In women, hormonal status has been implicated in both serum lipids and cardiovascular disease. Several studies have shown an increase of serum lipids,[5-7] as well as a strong increase in the occurrence of cardiovascular disease[6-10] after menopause. Studies of the association between parity and cardiovascular disease have shown equivocal results. In some investigations an increased cardiovascular risk in multiparous women was observed,[7,10,11] whereas in other studies parity was not associated with cardiovascular disease.[12,13] Beard et al. observed an increased cardiovascular risk for women who had their first pregnancy at an early age.[14] Pregnancy has been suggested to increase phospholipids, triglycerides, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol.[15-20] However, there is only limited evidence available from prospective follow-up studies with measurements of serum lipids both before and after pregnancy.[21]

To investigate the relation between pregnancy and serum lipids, we studied 62 women who became pregnant during a follow-up study of cardiovascular risk factors in young people.

SUBJECTS AND METHODS

Population

From 1975 to 1978, all 5,670 residents aged 5 - 19 years and living in two districts of Zoetermeer, a suburban town near the Hague in the Netherlands, were asked to participate in a study for risk indicators of cardiovascular disease (EPOZ), as described earlier.[22] From the 4,649 subjects who took part in the study (i.e., 82 per cent of those eligible), those who had levels of one or more

of the cardiovascular risk indicators (blood pressure, serum total cholesterol, Quetelet index, cigarettes smoking) in the upper ten percent of the distribution at the initial examination and those who served as a reference group taken at random from the remaining part of the initially examined children were selected for yearly follow-up. A total of 1,597 youngsters, among whom were 831 women, were re-examined each year and the average follow-up period was 6 years.

Measurements

During the yearly examinations body height (in cm) and weight (in kg) were measured with the subjects wearing indoor clothes without shoes, blood pressure was measured with a random-zero device, and blood was taken by venipuncture from the non-fasting subjects. Serum total cholesterol was measured with an automated enzymatic method, utilizing the colour reactions according to Kageyama.[23] Serum HDL cholesterol was also measured using an automated enzymatic method with colour reaction according to Trinder, after precipitation with phosphotungstate-Mg^{2+}.[24] Measurements of HDL cholesterol were started only in 1980. The determinations of both total and HDL cholesterol were carried out in the laboratory of our department and were within the limits of the standardisation program of the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter), throughout the entire study period. Every year the respondents filled in a questionnaire on smoking habits, use of alcoholic bevarages and use of coffee. Girls were also asked about their menstrual history, pregnancy and use of oral contraceptives.

Comparison groups

For this paper different analyses were made based on the following comparisons:

Currently pregnant versus non pregnant women During the follow-up period 62 women had their first pregnancy at the time of the annual examination. Their ages ranged from 18-28 years (mean 23 years, SD 2.5 years). For each pregnant woman three non-pregnant reference women matched for age were selected. Serum total cholesterol levels were available for 58 pregnant women and 165 reference women. Serum HDL cholesterol levels were determined in 45 pregnant and 155 reference women. In a separate analysis, a subdivision of the pregnant women in three groups was made according to the duration of their pregnancy.

Before, during and after pregnancy Depending on the moment of their preg-

nancy during the follow-up, some women had also had an annual examination the year before and/or the year after their pregnancy. In this analysis, only women who had lipid measurements before, during and after their pregnancy were included. This applied to 30 women for serum total cholesterol and to 22 women for serum HDL cholesterol.

Ever versus never pregnant women At the last examination during the follow-up 58 women stated that they ever had been pregnant, but were not pregnant now. Their ages ranged from 21 to 30 years at the last examination. There were 187 women of the same ages, who never had been pregnant. Serum total and HDL cholesterol were measured at the last examination in 53 women who ever had been pregnant, and in 179 women who never had been pregnant. Separate comparisons were made for current users versus non-users of oral contraceptives.

Data analysis

Average values of total and HDL cholesterol were compared between the groups. An adjustment for different distributions of age and body weight was made in all comparisons. For this purpose a multiple linear regression model was used, in which serum total or HDL cholesterol was the outcome variable and an indicator variable by comparison group was added as the independent variable. The regression coefficients yielded by this model served as the adjusted difference in total or HDL cholesterol between comparison groups. In the tables and figures p-values refer to a two-tailed test of significance.

RESULTS

Currently pregnant versus non pregnant women

In table 1 average total and HDL cholesterol levels, and the ratio of HDL/total cholesterol, for pregnant women and their matched controls are given. Pregnant women had significantly higher levels of both total and HDL cholesterol. However, the ratio of HDL/total cholesterol was similar for pregnant women and their reference subjects. Separate comparisons with control women who used or did not use oral contraceptives did not materially change the results.

In figure 1 total and HDL cholesterol levels, and their ratio, adjusted for

TABLE 1

Average total and HDL cholesterol (mg/100 ml) and the ratio of HDL/total cholesterol (%), in pregnant women and matched controls (EPOZ study, 1975-1985)

	Pregnant women			Non pregnant controls			
	N	Mean	SEM	N	Mean	SEM	p*
Total cholesterol	57	235	7.4	165	205	2.7	< 0.001
HDL cholesterol	45	66	2.1	155	57	1.0	< 0.001
HDL/total cholesterol	45	29	0.9	155	28	0.5	0.46

* Adjusted for differences in age and body weight

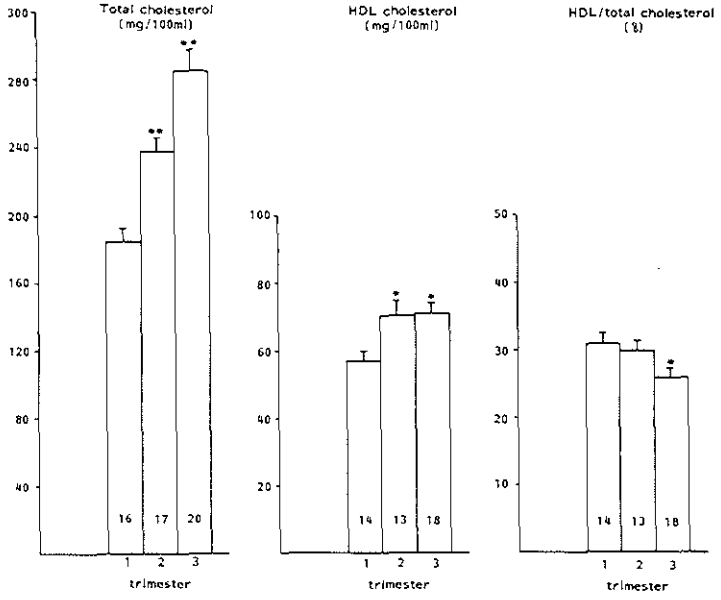

Fig. 1. Total and HDL cholesterol, and the ratio of HDL/total cholesterol (with SEM) in pregnant women during their first, second and third trimester. EPOZ study, 1975-1985. * $p < 0.01$, ** $p < 0.001$, with first trimester as reference category.

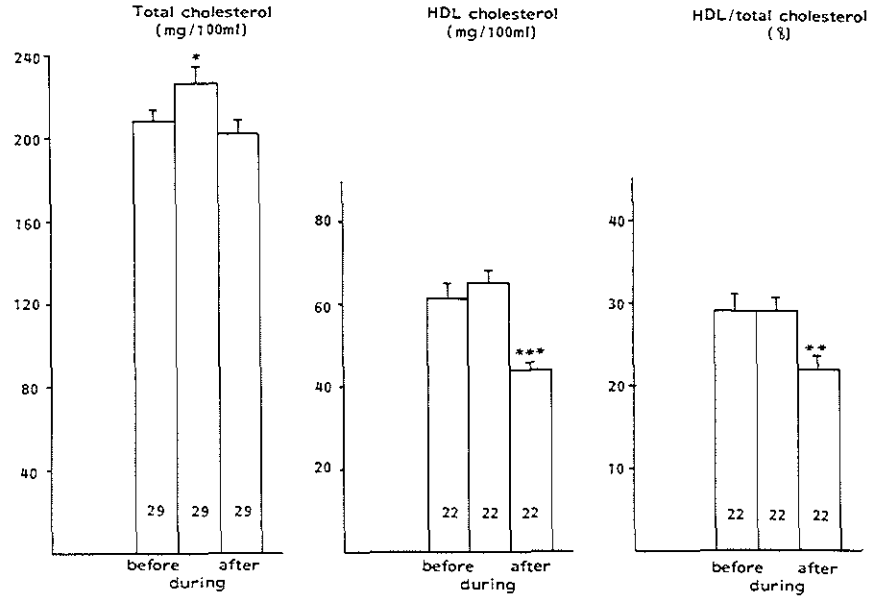

Fig. 2. Total and HDL cholesterol, and the ratio of HDL/total cholesterol (with SEM) in women before, during and after their pregnancy. EPOZ study, 1975-1985. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, with before as reference category.

TABLE 2

Average total and HDL cholesterol (mg/100 ml) and the ratio of HDL/total cholesterol (%) in women who were ever and who were never pregnant, by use of oral contraceptives (EPOZ study, 1975-1985)

	Ever pregnant			Never pregnant			
	N	Mean	SEM	N	Mean	SEM	p*
Non-users							
Total cholesterol	31	212	7.3	70	197	4.6	0.16
HDL cholesterol	31	51	2.3	70	56	1.3	0.10
HDL/total cholesterol	31	25	1.3	70	29	0.8	0.008
OC-users							
Total cholesterol	22	218	8.3	109	211	3.3	0.44
HDL cholesterol	22	46	2.2	109	58	1.3	< 0.001
HDL/total cholesterol	22	22	1.5	109	28	0.6	< 0.001

* Adjusted for differences in age and body weight

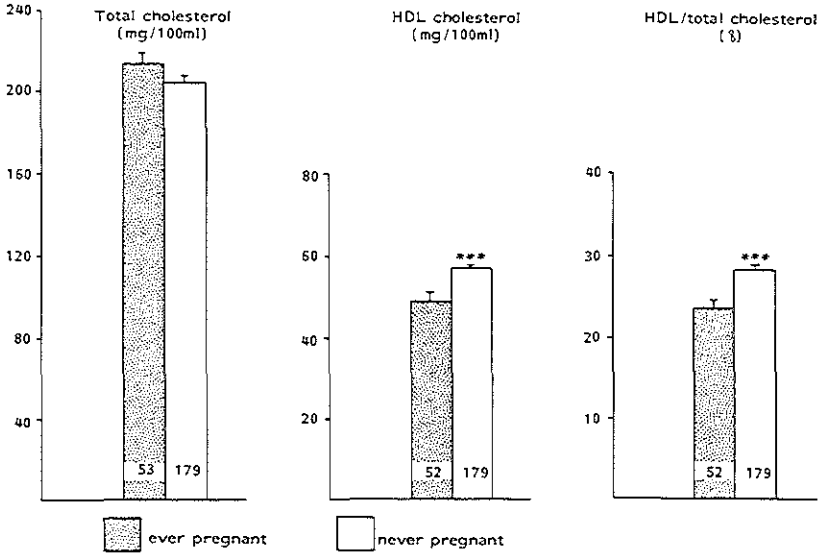

Fig. 3. Total and HDL cholesterol, and the ratio of HDL/total cholesterol (with SEM) in women who ever but not currently were pregnant, and in women who never were pregnant. EPOZ study, 1975-1985. *** $p < 0.001$.

age and body weight, are given for women in their first, second and third trimester of pregnancy. Both total and HDL cholesterol showed an increase with the duration of pregnancy. The ratio of HDL/total cholesterol showed a decline during pregnancy.

Before, during and after pregnancy

In figure 2 serum total and HDL cholesterol levels are given for 30 and 22 women, respectively, of whom lipid measurements were available both the year before and the year after the examination during which they were pregnant. Serum total cholesterol rose significantly during pregnancy and dropped to pre-pregnancy values again. Serum HDL cholesterol showed a small rise during pregnancy, but post-pregnancy levels fell below the levels observed before pregnancy. The ratio of HDL/total cholesterol, also shown in figure 2, was significantly lower the year after pregnancy as compared to concentrations before pregnancy.

Ever versus never pregnant women

Figure 3 shows serum total and HDL cholesterol at the final examination, adjusted for age and body weight, in women who were ever and those who were

never pregnant. No significant differences were observed in total cholesterol levels between those who ever and those who never were pregnant. HDL cholesterol was significantly lower in those who ever were pregnant. A separate analysis showed that the differences were mainly observed in those who currently used oral contraceptives (table 2). The ratio of HDL/total cholesterol, also shown in figure 3, was significantly lower for those who ever were pregnant, whether or not they currently used oral contraceptives (table 2).

DISCUSSION

This study gives both cross-sectional and longitudinal data of the association of pregnancy with serum total and HDL cholesterol in a population of young women. Although many studies have investigated the effect of pregnancy on serum lipids,[15-21] only one study[21] included measurements of total cholesterol before, during and after pregnancy, whereas HDL cholesterol was not measured.

Higher levels of total cholesterol in pregnant women, as compared to post-pregnancy levels or non-pregnant women, have been described before.[16-21] The increase of total cholesterol with the duration of pregnancy suggests that hormonal alterations may be implicated. Punnonen[25] found a significant correlation between the changes in serum total cholesterol and oestradiol levels in pregnant women. Some investigators observed an increase in serum HDL cholesterol during pregnancy,[18,20] whereas others did not.[19] In our study there was no overall difference in the ratio of HDL/total cholesterol between pregnant and non-pregnant women. However, during the last pregnancy trimester this ratio appears to be decreased. Oliver and Boyd[16] and Watson[17] also noted a change in the alpha-/beta lipoprotein ratio (HDL/LDL) in pregnant women. With increasing duration of pregnancy, this ratio decreased.

A new and unexpected finding is the decreased HDL cholesterol the year after pregnancy. This was not due to changes in laboratory methods over time, as we discussed on the basis of a preliminary report elsewhere.[26] Differences in age and body weight also could not explain the decrease, as adjusting for these characteristics did not alter the results. The proportion of users of oral contraceptives was unequal, the year before and after pregnancy. When controlling for this, HDL cholesterol was lower the year after pregnancy, compared to

the year before. The percentage smokers was the same the year before and the year after pregnancy. Both smokers and non-smokers showed a decrease in HDL cholesterol the year after pregnancy. The analysis in which we compared women who had ever been pregnant with women who had never been pregnant, confirmed the negative association between HDL and pregnancy. Especially women on oral contraceptives, who ever were pregnant, showed lower HDL cholesterol levels than women who never were pregnant and also used oral contraceptives. The ratio of HDL/total cholesterol was significantly lower in all women who ever were pregnant. Several studies have shown a relation between parity and coronary heart disease.[7,10,11] However, little research has been done on intermediate factors, such as lipid levels. This study suggests that women who experienced pregnancy have lower HDL cholesterol levels and may therefore be at higher risk of coronary heart disease, than women who never were pregnant. It deserves further investigation to elucidate the relation between parity, HDL cholesterol and coronary heart disease.

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Appendix 4

THE EFFECT OF LARGE DOSES OF ETHINYLESTRADIOL ON APOLIPOPROTEIN LEVELS IN EXCESSIVELY TALL PREPUBERTAL GIRLS

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[Metabolism, in press]

Summary

Seventeen constitutionally tall prepubertal girls, aged 10 to 14 years, were treated with large doses of ethinyl estradiol (EE) to reduce their final height. The serum concentration of cholesterol, triglyceride, and apolipoproteins before and after four to 17 months of treatment were compared with the same variables in a reference group, initially matched for bone age and height. In the patients, cholesterol rose by 24% (1.1 $\pm$ 0.8 mmol/L), triglyceriden by 105% (0.97 $\pm$ 0.70 mmol/L), LDL apo B by 48% (27 $\pm$ 19 mg/dL), apo A-I by 45% (62 $\pm$ 17 mg/dL), and apo A-II by 21% (12 $\pm$ 11 mg/dL). In the reference group, none of these variables changed significantly. The ratio of LDL apo B/apo A-I remained constant in both groups.

INTRODUCTION

Excessively tall girls may be treated with high doses of estrogens in an attempt to increase their rate of bone maturation and so to reduce their final adult height. Possible side effects of the administration of estrogens could be harmful or beneficial. Small quantities of estrogens given to postmenopausal women may give some protection against cardiovascular death.[1]

Protection by estrogens against vascular disease has been attributed to beneficial effects on circulating lipoproteins, both Low Density Lipoproteins (LDL)[2] and High Density Lipoproteins (HDL).[3] Recently, attention has been focused on apolipoproteins as predictors of atherosclerotic disease.[4]

We investigated the effect of EE, given in a dose of 0.2 mg per day to constitutionally tall girls on the apolipoproteins of HDL (apo A-I and apo A-II) and of LDL (apo B) and on total serum cholesterol and triglyceride. We compared changes in lipoprotein levels in these girls with those from another group of tall girls that did not receive estrogens.

SUBJECTS AND METHODS

Seventeen apparently healthy, tall prepubertal girls, aged 10 to 14 years, were studied. They received 0.2 mg per day EE per os for about 2 years. During 5 days each month they also took 10 mg per day medroxyprogesteronacetate (MPA). Postabsorptive blood samples were taken at the start and at the end of a period varying from 4-17 months.

Seventeen reference subjects were participants of a longitudinal study of risk factors of cardiovascular disease in 1.600 youngsters, initially aged 5-19 years and selected from an open population.[5] Each year they underwent an examination in which their body weight and height were measured and a venous blood sample was taken to measure serum lipoproteins. Out of all female participants, controls were individually matched with the patients for chronological age ($\pm$ 9 months), and bone age ($\pm$ 9 months). From each age-category that matched the patient, the tallest girl was included. Skeletal age in all subjects was estimated according to Tanner.[6]

Serum concentrations of cholesterol were determined according to Abell et

al.[7] of triglyceride according to Giegel et al.[8] and of apolipoproteins by means of radial immunodiffusion against monospecific antibodies according to Cheung and Albers (for apo A-I and apo A-II)[9] and according to Havekes et al. (for LDL-apo B).[10]

RESULTS

Table 1 shows mean initial and final values of the clinical and biochemical measurements in the two groups. It appears that the two groups were incompletely matched with respect to tallness. There were also differences in the initial values of the apolipoproteins, although the serum cholesterol levels were similar. All lipid and apolipoprotein levels increased considerably in the experimental group, but not in the reference group. In order to compare the changes in the two groups, we performed multiple linear regression analysis with initial height, weight, chronological age, and level of lipid fractions as possible confounders in the model. Table 2 shows average values of changes in total cholesterol and apolipoproteins in patients and a reference group controlled for these variables. The differences between patients and reference group in changes of total cholesterol, apolipoprotein A-I, A-II and B were highly significant. Variations in the length of the observation period had no significant influence on the outcome, neither had the relation of sampling time to the pseudomenstrual cycle.

DISCUSSION

The increase in lipid and apolipoprotein levels in the experimental group was far in excess of the changes in the reference subjects. It was difficult to obtain full matching for height, and this is reflected in the different average body height at baseline between patients and controls. However, controlling for body height in the analysis did not materially influence the results. The findings suggest that high doses of exogenous estrogens strongly increase serum total cholesterol, triglycerides, and apolipoproteins. The magnitude of the effect, also compared to changes reported in longitudinal studies of lipoprotein cholesterol in random samples of peripubertal children,[11] supports the view that the relationship is a causal one.

TABLE 1

Clinical and laboratory data on patients and controls at the first and second visit

	First Visit	
	Patients	Reference group
Age (yr)	11.9 ± 1.4	11.9 ± 1.0
Height (cm)	168.7 ± 7.5	161.9 ± 6.3#
Weight (kg)	49.4 ± 7.0	49.7 ± 7.9
Quetelet (kg/m^2)*	17.3 ± 1.8	18.9 ± 2.2@
Bone age (Tanner RUS) (yr)	11.9 ± 1.0	12.6 ± 1.2
Triglycerides (mmol/L)	0.92 ± 0.43	1.25 ± 0.66
Total cholesterol (mmol/L)	4.53 ± 0.48	4.64 ± 0.76
Apo B (mg/dL)	55.2 ± 13.7	42.6 ± 9.7&
Apo A-I (mg/dL)	136.5 ± 23.4	119.9 ± 13.1&

	Second Visit	
	Patients	Reference group
Age (yr)	12.5 + 1.4	12.9 + 1.1
Height (cm)	172.0 + 5.8	167.2 + 5.3
Weight (kg)	56.6 + 5.2	55.5 + 10.8
Quetelet (kg/m^2)*	19.1 + 1.3	19.8 + 3.2
Bone age (Tanner RUS) (yr)	-	13.6 + 1.0
Triglycerides (mmol/L)	1.89 + 0.78	0.84 + 0.31
Total cholesterol (mmol/L)	5.63 + 0.93	4.55 + 0.75
Apo B (mg/dL)	81.9 + 12.4	42.6 + 12.7
Apo A-I (mg/dL)	197.9 + 20.3	124.4 + 19.9
Apo A-II (mg/dL)	68.3 + 9.6	48.8 + 6.4

Values are given as mean ± SD
* Quetelet index = $weight/height^2$
# Significantly different from patients at first visit ($p < 0.01$)
@ Significantly different from patients at first visit ($p < 0.05$)
& Significantly different from patients at first visit ($p < 0.001$)

Student's T-test for unpaired observations

TABLE 2

Average increase between two measurements in total and cholesterol apolipoprotein levels in patients and controls

	Patients	Controls	p*
Total cholesterol	0.99 ± 0.2	0.01 ± 0.1	0.0007
Triglyceride	0.8 ± 0.2	-0.1 ± 0.2	0.009
Apolipoprotein A-I	59.3 ± 5.0	7.5 ± 4.2	<0.00001
Apolipoprotein A-II	12.0 ± 3.4	-0.3 ± 1.3	0.005
Apolipoprotein B	33.1 ± 5.8	-4.2 ± 2.6	<0.00001

Values are given as mean ± SEM and are adjusted for differences between patients and controls in initial height, body weight, age, and lipid fractions.
* Two-tailed p-value.

The changes of the apo A-I levels and apo A-II levels are in the same direction as those reported earlier in other groups of women.[9,12,13] In one study,[14] apo A-II was not affected. The change in apo B is opposite to what has been reported with other types of estrogen and/or smaller dosages. Determination of apo B is designed to exclude VLDL-particles,[10] but a certain contribution of particles with an intermediate size between VLDL and LDL cannot be excluded. Estradiol, given orally, reduces LDL levels[13] and has even been recommended as a therapeutic for postmenopausal hypercholesterolemia.[2] EE given in a dosage of 0.06 mg per day reduced LDL cholesterol levels in a postmenopausal female subject.[15]

Estrogens have a number of metabolic actions, which may oppose each other in their ultimate effect on LDL levels. On the one hand, there is a stimulation of the LDL receptor activity[16] and this would speed up the removal of LDL and apo B from the circulation. EE also stimulates the triglyceride synthesis in the liver[17] and this may be accompanied by an increased influx of apo B (as the major apoprotein of Very Low Density Lipoproteins) in the circulation.[14] Our data would point to a dominance of this second effect when large doses of EE are used.

The main clinical relevance of changes in circulating lipids and apolipoproteins is their relation to cardiovascular risk. LDL and apo B are associated with

an increase in risk whereas HDL and apolipoproteins A-I and A-II are associated with a decrease. The ratio apo A-I/apo B has been reported as a good risk discriminator.[4] In the present investigation this ratio remained almost constant (2.49 before EE and 2.41 on EE) and thus the beneficial and harmful effects of large doses of estrogens given to young women may be in balance.

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Appendix 5

DETERMINANTS OF CHANGE IN SERUM TOTAL AND HDL CHOLESTEROL IN EARLY LIFE

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Summary

The change in serum total and high density lipoprotein (HDL) cholesterol and its determinants were investigated in 596 Dutch children, initially aged 5-19 years. The children were randomly selected from residents of two districts of the town of Zoetermeer. This paper is based on 398 children (217 boys) who had at least three annual measurements of total cholesterol between 1975 and 1985. The stability of a child's position in the distribution of serum lipids over time ('tracking') was studied by correlating the initial level with subsequent levels of serum lipids. Tracking correlations for total cholesterol after six years amounted to about 0.7 in boys and 0.5 in girls. For HDL cholesterol these figures were 0.5 in boys and 0.4 in girls. To investigate the determinants of change in serum lipids, the rate of change in total and HDL cholesterol was obtained by least-squares regression of serum lipids on time for each subject. Initial levels of total and HDL cholesterol were negatively associated with subsequent change in these serum lipids, even after adjustment for regression toward the mean. Parental history of heart disease was unrelated to the change in serum lipids in their children. Serum cholesterol in the mother was positively associated with the change in cholesterol in boys. Initial body mass index was positively related to change in serum total cholesterol in boys and to change in HDL cholesterol in girls. The change in weight, height and body mass index was negatively related to the change in HDL cholesterol in boys as well as in girls. Use of oral contraceptives was negatively associated with change in serum total cholesterol. These observations suggest that there is tracking of a moderate magnitude of serum lipids in childhood. They furthermore suggest that there is no 'horse-racing' of serum lipids early in life.

INTRODUCTION

The determinants of the level of serum lipids in childhood have been investigated quite extensively in recent years.[1-11] By contrast, the determinants of change of lipids in early life have only received limited attention.

This paper reports a study of the natural history of serum lipids in childhood and of the determinants of change in total and HDL cholesterol in early life. We studied 596 Dutch children, initially aged 5-19 years, who were randomly selected from a general population and who had at least three annual measurements of serum total cholesterol.

SUBJECTS AND METHODS

Population

All residents aged 5-19 years of two districts of Zoetermeer, a suburban residential area of about 55.000 inhibitants in 1980, located near the Hague in the Netherlands, were invited to take part in a study of risk indicators for cardiovascular disease between 1975 and 1979, as described elsewhere.[12,13] Out of 5.670 eligible subjects, 4.649 (82 per cent) participated. A random sample of 596 children (300 boys) was taken from those examined initially and these children were invited to yearly follow-up examinations. This paper is based on 398 of them (67 per cent) in whom at least three annual measurements of serum total cholesterol were obtained. The children who were not taken into the present analysis had essentially the same average levels of serum total cholesterol as those on which this report is based. The attrition of the cohort was largely due to migration. No intervention measures were taken, and all children received the same advice about life habits.

Measurements

During the yearly examinations body height (in cm) and weight (in kg) were measured with the subjects wearing indoor clothes without shoes. Serum total cholesterol was measured with an automated enzymatic method, utilizing the colour reactions according to Kageyama.[14] Serum HDL cholesterol was also measured using an automated enzymatic method with colour reaction according to Trinder, after precipitation with phosphotungstate-Mg^{2+}.[15] As measurement of

HDL cholesterol was initated only in 1980, fewer data were available for this variable. The determinations of both total and HDL cholesterol were carried out in the laboratory of our department, which participates in the lipids standardisation program of the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter).

Each year the respondents completed a questionnaire on smoking habits, use of alcoholic beverages, and use of coffee. Girls were also asked about their menstrual history, pregnancy and use of oral contraceptives.

Data analysis

The analysis of the data comprised two elements. First, the stability of a child's position in the distribution of serum lipids over time (sometimes referred to as 'tracking') was studied by correlating the initial lipid levels with subsequent levels. Second, the determinants of change in serum lipids were investigated by performing multiple linear regression of the change in serum lipids on the determinants measured at the first examination. The change in serum lipids was estimated by least-squares linear regression of total or HDL cholesterol on time for each individual. This yielded a slope and an intercept, which served as measures of the individual rate of change and the estimated initial level of lipids, respectively. In the regression models of putative determinants on the rate of change in lipids, the initial level of the lipids was included and therefore the coefficients represent the effect of a determinant on the rate of change conditional on initial level. All analyses were based on values of serum lipids transformed to per centile values.[16] In a seperate analysis attention was focussed on the relation between the initial level of lipids and their subsequent change. A positive relation between initial level and change has been referred to as 'horse-racing'. In this analysis the individual slopes were regressed on the intercepts. This yielded a regression coefficient, which was corrected for regression toward the mean using the approach of Blomqvist.[17] In this approach the ratio of the average residual variance of the estimated initial values on time and the variance of the individual regressions of serum lipids on time are used to obtain a maximum likelihood estimate of the true coefficient of regression of slope on initial level. The regression coefficients are presented with their standard errors for six categories of age and gender for total serum cholesterol, and four categories for HDL cholesterol. The analysis was performed on children with at least

three to a maximum of ten annual lipid measurements. An analysis restricted to those who had at least five annual measurements yielded essentially the same findings.

RESULTS

Stability of position

Correlation coefficients of total serum cholesterol at the first examination and of various annual follow-up examinations are given in table 1. The coefficients after six years ranged from 0.68 to 0.75 in boys, and from 0.33 to 0.51 in girls. In table 2 the coefficients are presented for HDL cholesterol. After five years the coefficients were 0.42 to 0.56 in boys and 0.38 to 0.41 in girls.

Change in serum lipids

The average per centile rate of change in serum total and in HDL cholesterol are given in tables 3 and 4. In boys aged 5-14 years initially an average decrease in total cholesterol was observed, whereas in 15-19 year old boys and in girls aged 5-19 years an average rise in cholesterol was found. In all age and gender categories there was an average fall in HDL cholesterol.

Determinants of change

Initial level The observed coefficients of regression of the individual slopes on the intercepts were negative for both total and HDL cholesterol in all age-categories. This applied to boys as well as to girls. After adjustment for regression to the mean, the coefficients remained negative (tables 5 and 6).

Parental characteristics A history of coronary heart disease in the parents was not associated with the rate of change of serum total and HDL cholesterol (tables 7 and 8). The level of serum cholesterol of the mother, measured at the occasion of the initial examination of the child, was positively related to change in cholesterol in boys.

Anthropometrical characteristics The initial level of body mass index was positively related to the rate of change of total cholesterol in boys (table 7). Initial body mass index was positively associated with HDL cholesterol in girls (table 8). The change in body weight and body mass index was unrelated to the change in total cholesterol (table 7). The change in body weight, height and body mass index was negatively related to the change in HDL cholesterol

TABLE 1

Correlation coefficients of initial total cholesterol and one to six years of follow-up for males and females in three categories of initial age

Years of follow-up	5-9 years	10-14 years	15-19 years
Males	n=54	n=75	n=87
1	0.63	0.66	0.73
2	0.64	0.66	0.71
3	0.66	0.57	0.71
4	0.61	0.65	0.67
5	0.54	0.69	0.67
6	0.68	0.65	0.75
Females	n=49	n=71	n=52
1	0.65	0.58	0.24
2	0.40	0.61	0.30
3	0.38	0.58	0.37
4	0.37	0.54	0.34
5	0.27	0.62	0.52
6	0.33	0.59	0.51

TABLE 2

Correlation coefficients of initial HDL cholesterol and one to five years of follow-up for males and females in two age categories

Years of follow-up	5-14 years	15-19 years
Males	n=72	n=117
1	0.47	0.56
2	0.21	0.54
3	0.41	0.61
4	0.39	0.56
5	0.42	0.56
Females	n=69	n=74
1	0.39	0.47
2	0.45	0.35
3	0.46	0.33
4	0.41	0.35
5	0.41	0.38

in both boys and girls (table 8).

Other characteristics Smoking habits measured at the first examination were positively related to the rate of change in total and HDL cholesterol in boys, but not in girls (tables 7 and 8). The use of oral contraceptives at the first examination was associated negatively with serum total cholesterol (table 7), but not with HDL cholesterol (table 8).

TABLE 3

Average change in total cholesterol (per cent/year) for males and females in three groups of initial age

	5–9 years		10–14 years		15–19 years	
	Mean	SE	Mean	SE	Mean	SE
Males	−0.26	0.48	−0.23	0.45	0.89	0.40
Females	0.60	0.65	1.25	0.46	1.98	0.57

TABLE 4

Average change in HDL cholesterol (per cent/year) for males and females in two groups of initial age

	5–14 years		15–19 years	
	Mean	SE	Mean	SE
Males	−2.57	0.58	−1.98	0.48
Females	−0.07	0.66	−1.56	0.66

TABLE 5

Adjusted# coefficients of linear regression of rate of change of total cholesterol (in per cent/year) on initial level of total cholesterol (per cent)

Age in years	Males		Females	
	b	SE	b	SE
5–9	−0.02	0.02	−0.07	0.02
10–14	−0.05	0.01	−0.04	0.01
15–19	−0.02	0.01	−0.05	0.02
Total	−0.03	0.01	−0.05	0.01

according to Blomqvist (17)

TABLE 6

Adjusted# coefficients of linear regression of rate of change of HDL cholesterol (in percent/year) on initial level of HDL cholesterol (in per cent)

Age in years	Males		Females	
	b	SE	b	SE
5-14	-0.09	0.01	-0.10	0.01
15-19	-0.08	0.01	-0.10	0.01
Total	-0.08	0.01	-0.09	0.01

according to Blomqvist (17)

TABLE 7

Coefficients# of linear regression of rate of change of serum total cholesterol (per cent/year) on several determinants

	Males		Females	
Determinant	Mean	SE	Mean	SE
Heart disease parents	1.692	1.191	-1.443	2.711
Cholesterol father	-0.003	0.006	-0.005	0.008
Cholesterol mother	0.014**	0.006	-0.001	0.008
Initial weight (%)	0.014*	0.008	0.011	0.011
Initial height (%)	0.003	0.008	-0.001	0.010
Initial QI (%)	0.028***	0.008	0.013	0.010
Weight slope (%/yr)	-0.006	0.073	-0.124	0.087
Height slope (%/yr)	-0.195**	0.076	-0.074	0.097
QI slope (%/yr)	0.016	0.070	-0.119	0.087
Initial smoking	1.168*	0.640	1.169	0.763
Initial OCC			-6.044***	1.511

adjusted for inital total cholesterol levels
* $p < 0.10$ ** $p < 0.05$ *** $p < 0.01$

TABLE 8

Coefficients# of linear regression of rate of change of HDL cholesterol (per cent/year) on several determinants

	Males		Females	
Determinant	Mean	SE	Mean	SE
Heart disease parents	-0.489	1.676	-2.182	2.912
Initial weight (%)	0.003	0.012	0.027*	0.014
Initial height (%)	0.008	0.011	0.021	0.014
Initial QI (%)	-0.005	0.012	0.028**	0.014
Weight slope (%/yr)	-0.230***	0.089	-0.189*	0.108
Height slope (%/yr)	-0.179*	0.114	-0.246*	0.139
QI slope (%/yr)	-0.181**	0.086	-0.190*	0.106
Initial smoking	1.438*	0.804	-0.981	0.980
Initial OCC			-1.751	1.232

adjusted for inital HDL cholesterol levels
* $p < 0.10$ ** $p < 0.05$ *** $p < 0.01$

DISCUSSION

The determinants of the level of serum lipids in children have been studied extensively. Familial aggregation of serum total cholesterol has been reported.[1-3] Anthropometrical characteristics[1,4-8] as well as hormonal factors[8-11] and life habits[5,6,9,11] have been suggested to be associated with serum lipids in early life.

This study presents evidence concerning the determinants of the change in serum lipids in children and young adults. We did not find a positive relation between initial level of serum lipids and their subsequent change, even after adjustment for regression toward the mean. This observation renders it unlikely that there is 'horse-racing' of serum total and HDL cholesterol in the first decades of life. The magnitude of the 'tracking' coefficients for total cholesterol in our study is relatively large, and this agrees well with other reports.[18-23]

The main correlates of change in total cholesterol in boys appear to be maternal cholesterol, initial body mass index and initial smoking habits. It remains to be clarified to change in total cholesterol in girls. A main finding is that the change in body weight, height and body mass is negatively related to the change in HDL cholesterol in girls.

In summary, these observations suggest that there is moderate tracking of serum lipids in early life. There is no evidence for horse-racing of serum lipids in childhood. Level and change in anthropometrical variables appear to be related to the change in serum total and HDL cholesterol.

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Appendix 6

[Preliminary Note]

IS THE RATIO OF APO B/APO A-I AN EARLY PREDICTOR OF CORONARY ATHEROSCLEROSIS?

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[Atherosclerosis, in press]

Summary

It is unknown which lipoprotein in childhood is the best predictor of atherosclerosis later on in life. We measured serum triglycerides, total cholesterol, its subfractions (LDL, HDL, HDL_2, HDL_3) and apoproteins (A-I, A-II, B) in two groups of children. They were offspring of fathers who had severe coronary atherosclerosis or no coronary sclerosis, as determined by coronary angiography. Fasting blood lipids were measured in 49 children of fathers with severe sclerosis, and in 37 children of fathers without sclerosis. Sons of fathers with severe coronary atherosclerosis had higher levels of apo B and of the ratio apo B/apo A-I than sons of fathers free of atherosclerosis. No differences in lipid levels in daughters were observed. These observations suggest that apolipoproteins play a part in early atherogenesis. They further indicate that it may be possible to detect children who have a high probability of developing severe coronary atherosclerosis later in life.

INTRODUCTION

Serum lipids are strongly associated with manifest atherosclerosis in adults, but little is known about their role in early atherogenesis. In particular, it is not clear which serum lipid parameter is the best predictor in childhood of atherosclerosis later on in life, although apolipoproteins have been suggested[1] [Freedman, unpublished report]. As children of parents with coronary heart disease are at far greater risk of developing atherosclerotic diseases than children of parents without heart disease,[2] we investigated whether children of men with angiographically defined severe coronary atherosclerosis differ in serum lipid and (apo)lipoprotein concentrations from offspring of men without coronary atherosclerosis.

SUBJECTS AND METHODS

All male patients under the age of 60, who were referred to the Cardiology department of the Zuiderziekenhuis hospital in Rotterdam for a first coronary angiography to evaluate angina pectoris from August 1983 to February 1985, were eligible for the study (n=360). The angiograms were clinically assessed[3] by one observer (HACMK) who had no knowledge of serum lipid profiles. Two groups of patients were selected: (1) those with no coronary atherosclerosis (less than 20% stenosis), and (2) those with severe coronary sclerosis (at least 3 vessels with over 70% stenosis). Excluded from the study were patients who had no children, suffered from diabetes mellitus, hypertension (defined as at least 1 year treatment with anti-hypertensive drugs), had lipid lowering medication, or were known to have cardiomyopathy or valvular defects. This left 29 men with no coronary sclerosis and 35 with severe atherosclerosis. Their families were invited with their youngest son and/or daughter and 59 (92%) participated in the study. Twenty-seven families (with 23 sons and 14 daughters) of whom the father was free of coronary atherosclerosis and 32 families (with 29 sons and 20 daughters) of whom the father suffered from severe atherosclerosis were examined. The mean ages of the fathers were 54 and 50 years, for those with severe and no atherosclerosis, respectively. They did not differ in average weight, smoking habits, use of alcoholic beverages, or regular physical exercise.

Of the fathers with severe atherosclerosis 55% used beta-blocking medication, as compared to 15% in the fathers without sclerosis. The age distribution was very similar in both groups of children (range 6-36 years, mean 21.2 years). After an overnight fast all participants underwent an examination which included antecubital venipuncture of 30 ml blood, measurements of height and body-weight, and a questionnaire on smoking habits, alcohol consumption, physical exercise and current medication. Triglycerides, total cholesterol and its subfractions were determined according to enzymatic methods (Boehringer Peridochrom and Monotest, respectively), after precipitation with polyvinylsulfate for LDL cholesterol, Mg^{2+}-phosphotungstate for HDL cholesterol and Mn^{2+}-heparin and dextransulfate for HDL cholesterol subfractions.[4] Apolipoprotein A-I and A-II were determined by radial immunodiffusion[5] against specific antiserum. Monospecific antisera against human apolipoprotein A-I and A-II were respectively raised in goats (provided by Dr. L. Havekes, Gaubius Institute, Leiden) and sheep (Boehringer Mannheim). Total apolipoprotein B was measured by quantitative immunoelectrophoresis according to Laurell,[6] as described earlier.[7]

Average lipid levels of the two groups of fathers, sons and daughters were compared. Students t-test for unpaired observations was used to assess statistical significance.

RESULTS

The table shows average concentrations of serum lipids and apolipoproteins in fathers with and without coronary atherosclerosis, and in their sons and daughters. Levels of triglycerides, total and LDL cholesterol and apo B were significantly higher in fathers with severe atherosclerosis than in fathers without sclerosis, whereas levels of HDL, HDL_2 and HDL_3 cholesterol, and levels of apoproteins A-I and A-II were significantly lower. In the sons, significant differences between the two groups were observed in levels of apo B ($p=0.02$) and the apo B/apo A-I ratio ($p=0.02$). In fathers, the largest differences were observed in levels of triglycerides (a 65% difference) and in the ratio of apo B/apo A-I (51% difference). In the sons the ratio of apo B/apo A-I was 25% higher in the offspring of fathers with severe atherosclerosis. A separate analysis which adjusted for the small differences in age distribution did not materially affect the obser-

TABLE

Lipid levels (in mg/100 ml) in fathers with coronary atherosclerosis (CAS+) and without coronary atherosclerosis (CAS-), and in their sons and daughters

	Fathers		Sons		Daughters	
	CAS+	CAS-	CAS+	CAS-	CAS+	CAS-
Number of subjects	29	27	29	23	20	14
Age ± SD	54±6	50±9	22±6	20±7	23±6	20±6
Triglyceride	226*** (18)	137 (12)	113 (11)	100 (12)	103 (10)	110 (11)
Total cholesterol	282*** (7)	245 (8)	217 (9)	197 (7)	209 (8)	205 (11)
LDL cholesterol	215*** (7)	175 (7)	146 (9)	128 (8)	140 (8)	135 (12)
HDL cholesterol	38*** (1)	48 (2)	47 (1)	49 (2)	52 (2)	53 (3)
HDL_2 cholesterol	5** (1)	9 (1)	9 (1)	10 (1)	13 (1)	14 (2)
HDL_3 cholesterol	33** (1)	38 (1)	38 (1)	39 (1)	39 (1)	38 (2)
Apoprotein A-I	136*** (4)	158 (5)	151 (4)	152 (4)	164 (8)	176 (12)
Apoprotein A-II	43* (2)	48 (2)	46 (1)	45 (1)	48 (2)	48 (2)
Apoprotein B	157*** (5)	121 (5)	102* (6)	84 (4)	97 (6)	97 (9)
Apo B/apo A-I	1.18*** (0.05)	0.78 (0.04)	0.70* (0.06)	0.56 (0.03)	0.63 (0.06)	0.59 (0.07)

Values are means (+ S.E.M.)
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

ved differences. A discriminant analysis, in which the outcome variable was coronary atherosclerosis in fathers and the independent variables included all lipid fractions, confirmed that in sons the apolipoprotein ratio was the best predictor of atherosclerosis in their fathers. In fathers themselves the best discriminator was the ratio of HDL/LDL cholesterol.

DISCUSSION

These observations indicate that young men who are at high risk for coronary atherosclerosis may have increased ratios of serum apo B/apo A-I. The power of this ratio to discriminate between survivors of myocardial infarction and a control population has been demonstrated by Avogaro et al.[8] However, Schmidt et al.[9] found that the ratio of HDL/total cholesterol correlated better with angiographic findings in male patients. In our study, the best discriminator in the fathers was the ratio of HDL/LDL cholesterol, whereas in their sons it was the apo B/apo A-I ratio. This might point to an early predictive, but not necessarily aetiological role for apolipoproteins, whereas HDL and LDL cholesterol levels might mainly be affected later on in life. In children, an early predictive role for apo B in coronary disease has been suggested by Sniderman et al.[1] In their study the parents were selected when they had a myocardial infarction combined with elevated apo B levels. The higher levels of apo B, as observed in their offspring compared to control children, may suggest early prediction of coronary artery disease, but may also be due to familial aggregation of apolipoproteins. In contrast to the study of Sniderman et al., our patients were not selected on their levels of plasma lipoproteins. Our findings closely agree with those of Freedman et al. [unpublished data], who showed higher apo B/apo A-I ratios in offspring of fathers with myocardial infarction compared to children without paternal myocardial infarction. Apo B increases with age, but because of the similar age distributions this did not affect our findings, as was borne out by a separate multivariate analysis.

The absence of an association in daughters is remarkable, but hard to explain. This finding agrees with Schmidt et al.[9] who observed no difference in plasma lipoprotein levels between women with and without coronary atherosclerosis. In women atherosclerosis becomes evident later in life and its pathogenesis may

be different from that in men. We also did not observe differences in HDL cholesterol levels between the two groups of sons or daughters. This is in contrast with findings of Pometta et al.,[10,11] who observed familial aggregation of HDL cholesterol levels in patients with myocardial infarction.

It has been suggested that selection bias may occur in studies of angiophically defined coronary atherosclerosis.[3] We applied very strict inclusion criteria, and it is difficult to see how selection bias would have affected apolipoproteins only. However, we cannot fully rule out this possibility and therefore consider our findings preliminary. Our observations imply that persons at high coronary risk may perhaps be identified early in life, and they also suggest that apolipoproteins may be implicated in early atherogenesis. If these preliminary results are confirmed, intervention studies to alter the apo B/apo A-I ratio in early life may be initiated.

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EPILOOG

Het schrijven van een proefschrift is meer dan louter het maken van een verslag van een onderzoek. Voor mij is het, naast een stuk opleiding tot epidemioloog, ook een weerslag van de samenwerking met en de steun van velen. Dit nawoord wil ik graag gebruiken om hen te bedanken. Allereerst mijn ouders, die mij in alle vrijheid hebben laten studeren en mij alle kans hebben gegeven om datgene te worden wat ik wilde. Dit boekje draag ik daarom graag aan hen op.

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About the author

Willy-Anne van Stiphout was born on November 21, 1956 in Breda, the Netherlands. After she graduated from secondary school at the Mencia de Mendoza Lyceum in 1975, she attended medical school at the Catholic University in Nijmegen. During the last year of her medical training she was initiated in epidemiology by Prof. F. Sturmans (former head of the department of Social Medicine) at the same university. She obtained her medical degree in October 1982. In February 1983 she started an in service training in epidemiology as a scientific research worker, at the department of Epidemiology (head: Prof. H.A. Valkenburg), of the Erasmus University in Rotterdam. Here she worked on the material that is set out in this thesis. She is a fellow of the Council on Epidemiology and Prevention of the International Society and Federation of Cardiology. She is a member of the European Society of Cardiology, Working Group on Epidemiology and Prevention.