LIPIDS AND ATHEROSCLEROSIS

IN FAMILIES

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Lipiden en atherosclerose in families

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CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Rationale

In most industrialized countries, age-standardized cardiovascular disease mortality rates have shown a decreasing trend since the mid seventies (1). However, cardiovascular diseases are still the most important cause of morbidity and mortality in these countries. About one-half of the cardiovascular disease mortality results from coronary heart disease and about one-quarter from cerebrovascular disease. The main underlying process in these diseases is atherosclerosis. Symptomatic atherosclerotic cardiovascular disease is predominantly a disease of the adult population. However, there is increasing evidence to suggest that the atherosclerotic process begins in childhood. Enos et al. (2) showed that a majority of young soldiers autopsied during the Korean war had significant atherosclerotic lesions in their coronary arteries. Subsequently, Holman et al. (3) showed that lesions are present in the aorta of children. McNamara et al. (4) autopsied American soldiers killed in action in Vietnam and reported that 45% of them had atherosclerotic lesions, and as much as 5% had severe coronary atherosclerosis. These and more recent observations (5-10) from autopsy studies clearly showed the onset of atherosclerotic coronary artery disease by young adulthood. Similarly, autopsy studies suggested the presence of potential precursors of atherosclerotic lesions such as fatty streaks and fibrous plaques, or raised lesions, in the coronary arteries as early as the second decade of life (3, 11-13). In a recent report from the Bogalusa Heart Study (15), extensive intimal surface involvement with fatty streaks of the aorta and the coronary arteries was found in young adults, aged 6 to 30 years. Fibrous plaques were found in both the aorta and the coronaries. In the coronary arteries higher correlations of fatty streaks with raised fibrous plaques lesions were found than in the aorta. Particularly in the coronary arteries, the fatty streak lesion is a likely precursor of fibrous plaques (15). The above-mentioned studies reporting on vessel lesions in children and young adults made use of autopsies for their assessment. Recently, standardized noninvasive techniques for examining the carotid arteries of children and young adults have been developed (16), which potentially extends the possibilities for research on vessel wall characteristics to a more general population of children and young adults. Ultrasonic examinations in children have indicated that differences in the elastic properties of carotid arteries in children and young adults are detectable and relate to various risk factors for atherosclerotic cardiovascular disease and also to parental heart disease (17).

Cholesterol and cholesterol esters have since long been recognized to be a constituent of

atherosclerotic lesions (18-21). This has led to the assumption that plasma lipids, and more in particular plasma low density lipoprotein cholesterol, play an essential role in the process of atherosclerosis. In non-human primates, cholesterol feeding was shown to be a prerequisite for the development of major atherosclerosis (22,23), and later studies demonstrated that chronic diet induced hypercholesterolemia led to fatty streak formation (24,25) similar to the fatty streaks observed in humans. Epidemiologic and laboratory studies have identified several risk factors contributing to the high prevalence and incidence of atherosclerotic coronary artery disease in industrialized countries. Among these risk factors, hypertension, hypercholesterolemia and smoking were disclosed as conditions independently linked to atherosclerotic coronary artery disease. The positive relation of serum total cholesterol, as well as the inverse association of one of its subfractions, high density lipoprotein cholesterol with atherosclerosis is now well established. Similarly, in particular high levels of another subfraction, low density lipoprotein cholesterol are associated with coronary artery disease (26-32). In children and young adults, particularly males, aorta fatty streaks were found to be strongly related to antemortem levels of total cholesterol and low density lipoprotein-cholesterol and coronary artery fatty streaks with levels of triglycerides and very low density lipoprotein-cholesterol as well as with systolic and diastolic blood pressure (14).

Apolipoproteins are the protein components of lipoproteins, which are involved in the structure, receptor binding and enzymatic metabolism of these lipoproteins. These apolipoproteins have been studied in patients with ischaemic heart disease (33,34) as well as in their offspring (35), but their relative merit as risk factors for coronary artery disease still has to be elucidated.

It is since 1979 that more attention has been drawn to the possible association between atherosclerosis and triglyceride-rich lipoproteins that occur in plasma primarily in the postprandial or fed state. (36) However, hypotheses about such an association date back much earlier. Moreton (37) proposed in 1947 that it was because of investigating only casual lipid concentrations, and primarily in the postabsorptive (fasting) state, that the clue to the underlying etiology of atherosclerosis had been missed. Much later it was reported that the remnants of chylomicrons, the principal carriers of dietary (exogenous) cholesterol and fat, are atherogenic in human adults (36, 38), thus giving support to this theory. There is no insight yet into the role of triglyceride-rich lipoproteins in the young. The finding of a strong association between fatty streak involvement in the coronary arteries of young males and antemortem levels of triglyceride and very low density lipoprotein-cholesterol (14) may be an indication that triglyceride-rich lipoproteins do play a role.

In view of the process of atherosclerosis beginning in young adulthood it is worthwhile to

study this process in relation to serum lipids, (apo)lipoproteins and postprandial lipid metabolism in children and young adults as a means of gaining insight into how these factors may have contributed to the occurrence of coronary atherosclerosis in their parents. Eventually, this may not only lead to a better understanding of the etiology and the determinants of atherosclerotic cardiovascular diseases, but also provide a means for the prevention of these diseases.

These considerations form the rationale for investigating lipids, (apo)lipoproteins and lipid metabolism in the young in relation to disease in their parents, with emphasis on fat intake and postprandial changes in lipid profiles. In the studies presented in this thesis lipids, (apo)lipoproteins and postprandial lipids and lipoproteins were investigated in offspring of parents suffering from clinically manifest and angiographically proven coronary atherosclerosis. The natural history of lipids and lipoproteins was studied in children and young adults in relation to parental total serum cholesterol levels.

1.2 Study objectives

A general question that may be asked is whether and how efforts to prevent cardiovascular diseases should start with children and young adults. Prevention of a chronic disease such as cardiovascular disease implies knowledge about the relevant risk factors in the young and their role in the etiology of cardiovascular disease. The objectives of the studies presented in this thesis were to assess, within the realm of familial cardiovascular disease, the causal relations between coronary artery disease as measured in adults and (apo)lipoprotein levels as measured in their offspring as a means of studying their role in the occurrence of parental disease. A further objective was to evaluate if cardiovascular risk factor levels show aggregation in families over time by relating parental risk factor levels and offspring risk factor levels. The latter should provide evidence for the idea that high risk parents may have offspring with high risk factor levels, which is important in the concept of such risk factors being causally related to the life-long process ultimately leading to cardiovascular disease. Familial aggregation of risk factors over time is also an important premise for studies relating offspring risk factor levels to parental disease. Thus, in view of the knowledge on both the etiology and ultimately the prevention of atherogenesis it is important to assess whether levels of plasma lipids and (apo)lipoproteins measured at a young age are related to heart disease. Similarly, studying postprandial lipid metabolism may provide new insights into atherogenesis and shed light on

predictors of heart disease.

1.3 Chapter outline

Chapter 2 describes a patient-offspring study of postprandial lipid metabolism and vitamin A metabolism and their relation with coronary atherosclerosis. In chapter 3 a patient-offspring study is presented on the relation between lipids and (apo)lipoproteins in young people and coronary artery disease in their parents. Chapter 4 presents the natural history of serum total cholesterol and lipoprotein-cholesterol levels in a follow-up study of young people in relation to total cholesterol levels in their parents. General discussion I in chapter 5 focuses on specific design aspects and the interpretation of the patient-offspring study. General discussion II in chapter 6 places the findings of the studies as presented in this thesis in the context of the considerations in general discussion I.

Appendix

In this thesis the emphasis is on lipids, (apo)lipoproteins and postprandial lipoproteins as risk factors for coronary artery disease. In the following, a short overview of lipid metabolism and in particular lipid transport through plasma will be given.

The lipids that are predominantly found in human plasma are free cholesterol and cholesterol esters, triglycerides, free fatty acids and phospholipids. Free cholesterol is the principal form present in most body tissues, in particular as a structural component of cell membranes. It is a precursor of steroid hormones produced by the adrenal gland and the ovary. Cholesterol ester is the storage form of cholesterol found in tissues and also the predominant form found in plasma. Cholesterol is produced by many tissues in the body but predominantly by the liver and the intestine (newly formed) and is cleared from the body via the faeces (free cholesterol and bile acids), cell loss (skin) and urine (steroid hormones). Normally there is a balance between production including dietary intake and loss. Triglycerides are synthesized from glycerol and free fatty acids in adipose tissue, the liver and the intestine (from dietary intake). Their main role is to serve as energy supply for muscles. They are transported through plasma as triglycerides in the core of lipoproteins. Free fatty acids can also be transported through plasma mostly bound to albumin. Plasma phospholipids are mainly derived from the liver and small intestine. They are important components of cell membranes. In plasma lipoproteins they play a major role in keeping water-insoluble lipids a soluble state.

Plasma lipoproteins are the principal carriers for the transport of lipid molecules. As triglycerides and cholesterol esters are water-insoluble, the spherical shaped lipoproteins contain these molecules in their core which is surrounded by a monomolecular surface layer consisting of the amphipathic phospholipids and free cholesterol as well as proteins. Of these molecules, the hydrophobic part is directed to the lipid core and the hydrophillic part to the water phase (plasma), thus making the lipoprotein a water-soluble particle.

Lipoproteins are commonly, and in this thesis, classified according to their density. This density classification defines to a large extent differences in structure, composition and metabolism. According to density the following lipoproteins can be distinguished by increasing density and decreasing size: chylomicrons; VLDL = very low density lipoproteins; IDL = intermediate density lipoproteins (also: VLDL remnants); LDL = low density lipoproteins; HDL = high density lipoproteins; VHDL = very high density lipoproteins (also: pre-β HDL).

Apoproteins are the structural protein components of lipoproteins. They play an important

role in lipid and lipoprotein metabolism, mainly through their interaction with enzymes and receptors. They are, like phospholipids, important for the solubility of lipoproteins in water phases. Specific properties of apoproteins are listed in table 1.1.

Table 1.1. Characteristics and functions of apolipoproteins.

Apoprotein	Chromosome	Lipoprotein	Function
A-1	11	chylomicron, HDL	Structural protein of HDL, activates LCAT
A-2	1	HDL	Structural protein of HDL
A-4	11	chylomicron, VHDL	Reverse cholesterol transport?
B-100	2	VLDL, IDL, LDL	Structural protein of VLDL and HDL, ligand for LDL receptor
B-48	2	chylomicron	Essential for chylomicron formation
C-1	19	VLDL, HDL	Activator of LCAT?
C-2	19	chylomicrons, VLDL, HDL	Cofactor of lipoprotein lipase
C-3	11	VLDL, HDL	Regulator of lipoprotein lipase
D	3	HDL	?
E	19	chylomicron, VLDL, IDL, HDL	Ligand for remnant and LDL receptors

Lipid transport by lipoproteins can be subdivided into three major pathways: transport of exogenous (dietary) lipids; transport of endogenous lipids; and reverse cholesterol transport. In the following these pathways and some of their interrelations will be discussed. Transport through the plasma of exogenous (dietary-derived fat) is graphically depicted in figure 1.1.

After its digestion in the intestinal lumen, dietary fat is taken up by mucosal cells in the duodenum and proximal jejunum (figure 1.1). In the enterocytes dietary free fatty acids and monoglycerides are reesterified to form triglycerides. Phospholipids are synthesized as well. Part of the dietary cholesterol is esterified to cholesterol ester. Subsequently, these lipids are combined into chylomicrons. Chylomicrons are the largest and lightest of lipoproteins. Newly

formed chylomicrons contain apoproteins A-I, A-IV and B48, large quantities of triglyceride and smaller amounts of phospholipid and cholesterol ester. They are transported from the intestine through the lymphatic system and they enter the circulation via the thoracic duct, where they acquire the apoproteins C-II and E from HDL.

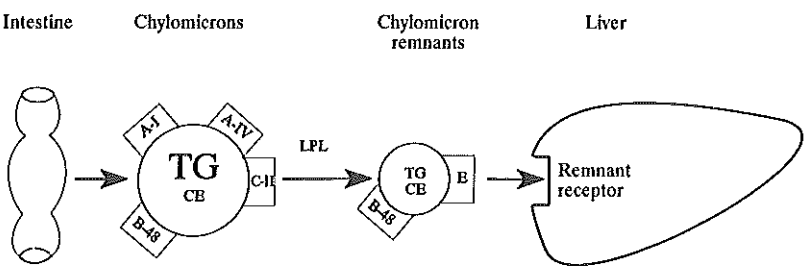

Figure 1.1. Exogenous lipid transport. TG = triglyceride; CE = cholesterol ester; A-I, A-IV, B-48, C-II and E are apoproteins.

Through the enzyme lipoprotein lipase (LPL), which is found on the luminal surface of endothelial cells in almost all tissues, and which is activated by the apoprotein C-II, triglycerides are hydrolized and taken up by the tissues. The remaining chylomicron remnants are then taken up by the liver via receptors that recognize apoprotein E. Apoprotein E enters the plasma mainly from the liver. There are three major isoforms namely E2, E3, E4. These isoforms are coded for by three different alleles that determine six apoprotein E phenotypes: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, E4/E4. Lipoprotein remnant clearance from the plasma is sensitive for apoprotein E polymorphism and is reported to be slower in subjects with E2/E2 phenotypes. Isoforms E3 and E4 interact readily with the LDL receptor while E2 does not. An LDL receptor related protein in the liver may also bind apoprotein E rich lipoproteins such as chylomicrons and their remnants. During the delipidation process the apoproteins A and C, phospholipids and free cholesterol are transferred from chylomicrons to HDL. All cholesterol esters end up in the chylomicron remnants.

The endogenous pathway (figure 1.2.) encompasses the transport of triglycerides and cholesterol as synthesized by the liver. These lipids are transported through the plasma by

liver-derived VLDL to sites of storage and utilization.

Newly formed VLDL contains apoproteins B-100, E and C-II, the latter also being transferred from HDL. Delipidation of VLDL may be compared with that of chylomicrons. During delipidation the VLDL lose apoprotein C-II. Most of the VLDL forms IDL (VLDL remnants). IDL may subsequently be taken up by the liver through liver receptors recognizing apoprotein E; or it may lose more triglycerides via the enzyme hepatic triglyceride lipase (HTGL) and apoprotein E to HDL and be converted into LDL. LDL contains free cholesterol and cholesterol ester (60 - 70% of total plasma cholesterol) and apoprotein B-100 as its only constituent apoprotein. LDL is cleared from the plasma mainly by the liver through the well established LDL-receptors which recognize apoprotein B-100 as well as apoprotein E.

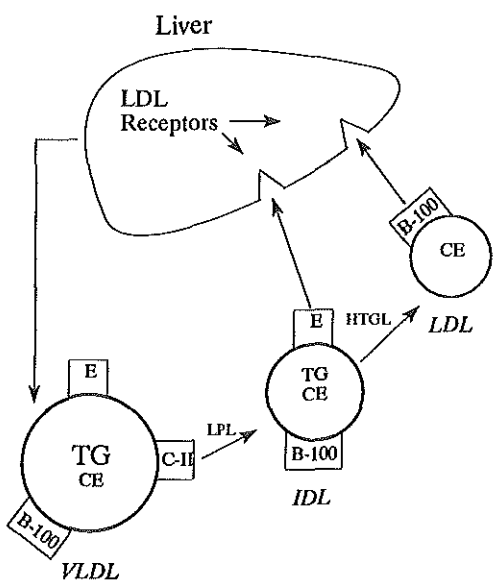

Figure 1.2. Endogenous lipid transport. TG = triglyceride; CE = cholesterol ester; B-100, C-II and E are apoproteins. VLDL = very low density lipoprotein; IDL = intermediate density lipoprotein; LDL = low density lipoprotein; LPL = lipoprotein lipase; HTGL = hepatic triglyceride lipase.

This receptor plays an important role in cholesterol homeostasis. Whenever cells have excess supply via the receptor of free cholesterol molecules they down-regulate the synthesis of LDL

receptor protein as well as the synthesis and activity of enzymes (HMG-CoA-reductase) involved in de-novo synthesis of cholesterol.

A surplus of cholesterol in extrahepatic cells can be removed by a process called reverse cholesterol transport for which a putative scheme is shown in figure 1.3.

HDL plays a key role in reverse cholesterol transport. Newly formed HDL's are precursor lipoproteins with a discoidal shape, containing phospholipids, free cholesterol and predominantly apoproteins A-I and A-II, but also apoprotein E. There are two commonly described sources of HDL. There are nascent HDL of liver and intestinal origin and there are discoidal lipoproteins (surface material) which are formed during lipolysis of chylomicrons and VLDL. An additional third putative source is shown in figure 1.3. It is proposed to be

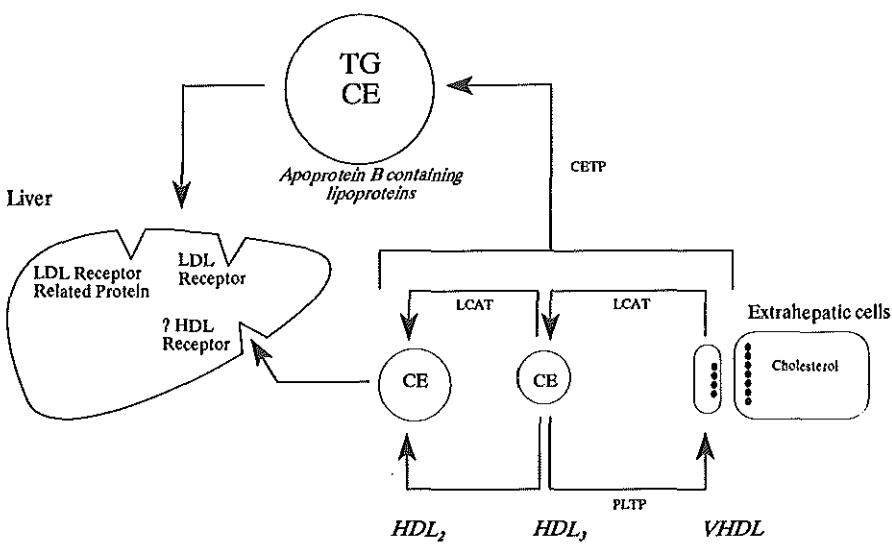

Figure 1.3. Reverse cholesterol transport. TG = triglyceride; CE = cholesterol ester; HDL = high density lipoprotein; VHDL = very high density lipoprotein; LCAT = lecithin cholesteryl acyl transferase; PLTP = phospholipid transfer protein; CETP = cholesterol ester transfer protein.

formed after conversion of HDL3 particles through phospholipid transfer protein (PLTP). Irrespective of the source of discoidal HDL it is capable of taking up free cholesterol from extrahepatic cells. This cholesterol is subsequently esterified by the enzyme lecithin cholesterol acyl transferase (LCAT) which is activated by apoprotein A-I. Through the uptake

of cholesterol ester the discoidal HDL becomes the spherical HDL3 which may subsequently be converted into the bigger HDL2 which is more enriched with cholesterol ester. Through cholesterol ester transfer protein (CETP), the cholesterol esters can then be transferred to apoprotein B containing lipoproteins and through that route be taken up by the liver. It is not exactly known which proportion of HDL is subsequently degraded as a particle. The presence of specific cellular binding sites has been proposed (HDL receptor).

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CHAPTER 2

POSTPRANDIAL TRIGLYCERIDE RESPONSE IN YOUNG ADULT MEN AND FAMILIAL RISK FOR CORONARY ATHEROSCLEROSIS

2. Postprandial Triglyceride Response in Young Adult Men and Familial Risk for Coronary Atherosclerosis

Abstract

In this cross-sectional study, the postprandial handling of a standardized oral lipid load (mean 397.6 grams), labelled with retinyl palmitate as a marker for chylomicrons and chylomicron remnants, was studied in 80 sons (mean age, 24.8 years) of patients with clinically manifest and angiographically proven severe coronary artery disease and in 55 sons (mean age 23.2 years) of angiographically negative controls. The objective was to determine whether an increased risk of coronary artery disease in young adults is related to changes in postprandial lipoprotein metabolism. Patients and controls were obtained from coronary angiography departments of four central general hospitals in the Netherlands. In both group of offspring postprandial levels of serum triglycerides, retinyl palmitate, and total cholesterol were measured during a 12 hour period. Both groups showed a marked rise in serum triglyceride and retinyl palmitate concentrations after the lipid load, reaching a maximum 4 to 6 hours postprandially. No changes in postprandial total cholesterol concentrations were observed in either group. In sons of patients with coronary artery disease postprandial hypertriglyceridemia was prolonged as compared to the sons of controls. Differences between groups mainly occurred between 6 and 12 hours after the lipid load. Similar results, although smaller and not statistically significant, were obtained with regard to postprandial retinyl palmitate, also showing differences mainly after 6 hours. It is concluded that healthy young sons of fathers with established coronary artery disease have a prolonged postprandial hypertriglyceridemia. These findings support the hypothesis that postprandial lipoprotein metabolism plays a role in atherogenesis.

Introduction

Atherosclerosis starts early in life (1, 2). Postprandial lipoprotein metabolism is proposed to be involved in this process (3). Cholesteryl ester-rich remnants of triglyceride-rich lipoproteins may directly promote accumulation of cholesterol esters in the arterial wall (4 - 6). Further, it has been reported (7, 8) that the protective effect of increased levels of high density lipoprotein (HDL) cholesterol levels on the risk for coronary artery disease may not only be explained by its role in reverse cholesterol transport but also by the relationship

between HDL and triglyceride metabolism (9). Plasma triglycerides have an effect on HDL composition and HDL-cholesterol levels (10); an inverse relationship between HDL_2 levels and postprandial triglyceride levels has been shown (11). Levels and composition of HDL could thus be a reflection of the effectiveness of triglyceride-rich lipoprotein catabolism. A deranged metabolism of triglyceride-rich lipoproteins in plasma has been reported in familial dysbetalipoproteinemia (12, 13), a disease associated with premature coronary atherosclerosis.

Studies comparing patients with coronary artery disease and persons without the disease have shown that differences with respect to postprandial hypertriglyceridemia (14 - 16) and postprandial retinyl palmitate concentrations (a marker of chylomicrons and their remnants) are detectable after an oral lipid load (14, 15). These results suggest a delayed clearance of these lipoproteins in patients with coronary artery disease. Thus, accumulating evidence indicates that postprandial lipemia plays an important role in causing coronary artery disease, and the implications with respect to treatment and primary prevention are increasingly being recognized (17).

Postprandial lipoprotein metabolism has not yet been studied in children and young adults with an increased risk for coronary atherosclerosis. In our study, male offspring of men with clinical manifestations of angiographically proven coronary atherosclerosis were compared with the male offspring of men who did not have coronary atherosclerosis (negative results after angiography). This approach enabled us to study a group of healthy young adults at a high familial risk for developing clinical manifestations of coronary artery disease later in life.

We assessed whether changes in the triglyceride response to a standardized oral lipid load, as reported in patients with coronary artery disease, can also be measured in healthy young male offspring of such patients.

Methods

Participants

Men with very severe coronary artery disease (patients), defined as more than 70% occlusion in at least three major coronary vessels were selected from coronary angiography databases of cardiology departments of the Zuiderziekenhuis Rotterdam (1988 - 1991), the University Hospital Rotterdam (1988 - 1989), the Refaja Ziekenhuis in Dordrecht (1990 - 1991), and the Antonius Ziekenhuis in Nieuwegein (1992), all hospitals situated in the Netherlands. Simultaneously, a reference group of men (controls) was selected who at

coronary angiography had no or, at most, only minor lesions, defined as 20% stenoses or less in all coronary vessels. Further, participants were selected according to the following additional criteria: 1) age between 45 and 65 years, 2) blood pressure not exceeding 160/100 mm Hg, 3) absence of liver disease, diabetes mellitus, thyroid disease, and renal disease, 4) first coronary angiography within two years before examination for our study, 5) first consultation of a physician for cardiac symptoms within 5 years before the examination for our study.

Eligible participants were sent a letter asking whether they had a son 15 to 30 years of age and, if so, whether the son was willing to participate in the study. These sons (identified by their fathers) received a separate letter inviting them to have an oral lipid loading test. Participants also received a short questionnaire about smoking habits, alcohol intake, physical activity, and fat intake.

We screened medical files of 629 patients whose coronary angiographic data met the criteria. Of these patients, 19 had died, 46 had diabetes mellitus, 6 had renal disease, 2 had thyroid disease, 183 had either no son or sons outside the required age range, 58 could not be contacted, 17 had no contact with their children, 78 had a cardiac history exceeding 5 years, and 63 could not be invited for other reasons (hospitalization, other serious diseases). Of 157 families (fathers and sons) who met all the criteria, 55 (either father or son) refused to participate in the study (response, 65%), leaving 102 fathers and 139 sons. The latter had oral lipid loading tests. The study protocol was approved by the medical ethics committees of the Zuiderziekenhuis Rotterdam and the University Hospital Rotterdam. Informed consent forms were obtained from all participants in the study.

Baseline measurements

Fathers were asked to visit the hospital at 9:00 a.m. after fasting for at least 12 hours. Fathers responded to a questionnaire about the number of first-degree relatives who had had myocardial infarctions and about the medication use at the time of the examination (medication was taken to the hospital where the examination took place). Systolic blood pressure and diastolic blood pressure were measured using a random zero sphyg-momanometer (Hawksley, Lancing, United Kingdom). Fasting serum blood samples were drawn by antecubital venipuncture for measurement of levels of triglycerides, total cholesterol, low density lipoprotein (LDL) cholesterol, and HDL cholesterol (and its subfractions HDL_2 and HDL_3). Height and weight were measured without shoes and without heavy clothing.

The sons were invited to come to the hospital, after the same period of fasting, on a separate day to have an oral lipid loading test. For sons, questionnaires were used to obtain data about use of medication, fat intake, alcohol intake, and smoking habits, referring to a 1-month period before the examination for this study. Daily total fat intake was calculated from an 81 item semiquantitative food frequency questionnaire by using a computerized food-composition table (17). In the sons, blood samples were taken by antecubital venipuncture for measurement of baseline levels of serum triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol (and its subfractions HDL_2 and HDL_3), apoprotein A-1, apoprotein A-2, apoprotein B, and retinyl palmitate concentrations. This baseline measurement of lipid levels was taken as the starting point (t_0) for the oral lipid loading test. In all of the sons, apolipoprotein E was phenotyped.

Oral lipid loading test

Sons of patients and sons of controls came to the hospital at 7:45 a.m. after an overnight fasting of 12 hours. Height and weight were measured first to calculate body surface area. Five minutes after the venipuncture for obtaining baseline lipid levels (at t_0), all participants received a liquid lipid load, which consisted of a mixture of dairy cream (40% fat), egg yolk, milk powder, and retinyl palmitate (in aqueous solution) (15). Participants received the lipid load in a dose based on their individual body surface area (77.5 g fat, 0.5 g cholesterol, and 27.000 IU of retinyl palmitate per square meter of body surface area). The mixture was consumed within 15 minutes.

The participants received an antecubital venous catheter (Venflon, Viggo AB, Helsingborg, Sweden), which was kept open during the test period by means of dispensable obturators (Venflon). Through this catheter, blood samples were drawn at 2 (t_2), 4 (t_4), 5 (t_5), 6 (t_6), 7 (t_7), 8 (t_8), 10 (t_{10}), and 12 (t_{12}) hours after starting consumption of the oral lipid load. Total cholesterol, triglyceride, and retinyl palmitate concentrations were determined in serum isolated from these samples. During the 12-hour period, other sources of calories were withheld from the participants. Because postprandial exercise has been reported to decrease postprandial lipemia (19), participants stayed in the hospital and were asked to refrain from heavy physical activity during the test period.

Laboratory analyses

Serum total cholesterol levels were measured with an automated enzymatic method (Boehringer Mannheim, Mannheim, Germany) CHOD-PAP reagent kit (20). Levels of HDL cholesterol and LDL cholesterol were measured by the same method after precipitation. For HDL cholesterol, the phosphotungstate method according to Burstein (21), with a minor modification as described by Grove (22), was used. For LDL cholesterol, precipitation was carried out with polyvinylsulphate (Boehringer Mannheim). Throughout the entire study period, results of total cholesterol and HDL cholesterol determinations were within limits of the quality control program of the World Health Organization Regional Lipid Reference Centre (Prague, Czechoslovakia). Levels of apoprotein A-1 and B were assayed using an automated immunoturbidimetric method (Kone Diagnostics, Espoo, Finland). Levels of apoprotein A-2 was determined by radial immunodiffusion against specific antiserum (Boehringer Mannheim, Germany) according to Cheung and Albers (23), with slight modifications. All automated analyses were carried out on the Kone Specific Analyzer (Kone Instruments) using frozen (-20°C) serum samples. High-density lipoprotein$_2$ and HDL$_3$ subfractions in serum were assayed as described by Gidez and colleagues (24), with slight modifications. High-density lipoprotein$_2$ and HDL$_3$ subfractions were separated using stepwise precipitation of apoprotein B containing lipoproteins with heparine/Mn^{2+} and HDL$_2$ with dextran sulphate. Apolipoprotein E phenotyping was done by isoelectric focusing of delipidated serum followed by immunoblotting, using apolipoprotein E antiserum as first antibodies (25). Retinyl palmitate analyses were performed as described previously by Groot and colleagues (15).

Statistical analysis

Mean ± SDs were calculated for baseline characteristics of all family members. The total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios were calculated for fathers and sons, and the apoprotein A-1/apoprotein B ratio was calculated for sons. Baseline differences between patients and controls, as well as their respective sons were evaluated using the two group *t*-test, or the Mann-Whitney U test when appropriate. Differences in postprandial responses between sons of patients and sons of controls with regard to triglycerides and retinyl palmitate were evaluated with age-adjusted repeated-measures analysis to determine if group differences at each postprandial measurement time differed significantly from group differences at any other measurement time. Subsequently, linear regression analysis was used to compare mean group postprandial responses. Adjustments were made for

age, HDL_2 cholesterol, HDL_3 cholesterol, apolipoprotein E phenotype, and daily total fat intake. This was done at each postprandial measurement time (t_2 to t_{12}) and for areas under the curve for several time ($\geq$ 2 hour) intervals. In the analyses both individual postprandial measurements and areas under the curve were evaluated after subtracting the initial individual values (t_0) for triglycerides and retinyl palmitate from all respective postprandial measurements, yielding the net postprandial change.

Results

Two sons of patients did not tolerate the oral lipid load because of gastrointestinal problems and were excluded from the study. All other participants tolerated the fat load very well and did not have gastrointestinal symptoms or steatorrhea during or after the test.
Two (other than already excluded) participants had baseline triglyceride values of 2.52 and 2.45 mmol/L, respectively. The first participant had six measurements of postprandial retinyl palmitate measurements that were more than 3 to 5 standard deviations above the mean, and the latter participant had five measurements of postprandial triglyceride measurements that were more than 3 to 5 standard deviations above the mean. Because of the marked effect of these outliers on the precision of the results obtained in the group as a whole, these two participants were excluded from analyses.

Baseline characteristics are given for both groups of fathers and sons in table 2.1. No statistically significant differences were noted between patients and controls. Sons of patients were slightly older and had a lower total fat intake. All patients (n=59) had had coronary angiography for evaluation of symptoms of chest pain. For controls, the indications for coronary angiography were symptoms of chest pain (n=30), evaluation of cardiac valves (n=7), suspected cardiomyopathy (n=5), and ventricular septum defect (n=1). At the time of coronary angiography, 19 (32.2%) patients and 11 (25.6%) controls were treated for hypertension. The average time elapsed between the date of the coronary angiography and date of the examination was 13.2 ± 0.9 months (mean ± SE) for patients and 13.2 ± 1.1 months for the controls.

Baseline serum measurements after an overnight fast are given for both groups in table 2.2. As expected, patients had higher serum levels of total cholesterol, LDL cholesterol, and triglycerides and had higher total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios than did controls. Levels of HDL cholesterol and its subfractions tended to be slightly lower in patients than in controls (Table 2.2). Compared with sons of controls,

Table 2.1. Baseline characteristics of fathers and sons.

Characteristic	Fathers			Sons of		
	Patients (n = 59)	Controls (n = 43)	P value	Patients (n=80)	Controls (n=55)	P value
Age (y)	55.5 ± 0.8	53.9 ± 1.2	>0.2	24.8 ± 0.6	23.2 ± 0.6	0.049
Height (cm)	176.4 ± 0.7	178.3 ± 1.2	0.15	183.2 ± 0.7	183.4 ± 0.9	>0.2
Weight (kg)	79.4 ± 1.2	79.8 ± 1.6	>0.2	78.0 ± 1.2	75.3 ± 1.3	0.19
Quetelet-index (kg/m^2)	25.4 ± 0.3	25.1 ± 0.4	>0.2	23.2 ± 0.03	22.4 ± 0.03	0.12
Systolic blood pressure (mm Hg)	125.4 ± 1.5	128.5 ± 2.4	>0.2	120.9 ± 1.4	118.1 ± 2.2	0.19
Diastolic blood pressure (mm Hg)	82.6 ± 1.5	82.3 ± 1.7	>0.2	77.6 ± 1.0	75.0 ± 1.8	0.10
Hypertension treatment (%)	32.2	25.6	>0.2	-	-	
Smoking (%)	15.3	27.9	0.19	28.8	31.6	>0.2
Alcohol use (%)	-	-		86.3	78.9	>0.2
Alcohol intake (g/d)	-	-		11.4 ± 1.7	9.6 ± 1.5	>0.2
Total fat intake (g/d)	-	-		133.6 ± 9.0	169.8 ± 13.9	0.012

Values are mean ± SD, where appropriate. P values compare patients and controls.

Table 2.2. Baseline serum measurements in fathers and sons.

Variable	Fathers			Sons of		
	Patients (n = 59)	Controls (n = 43)	P value	Patients (n = 80)	Controls (n = 55)	P value
Total cholesterol (mg/dL)	263.3 ± 5.0	241.2 ± 5.9	0.006	200.0 ± 3.7	195.8 ± 4.5	>0.2
LDL cholesterol (mg/dL)	193.4 ± 4.7	169.6 ± 5.5	0.002	137.4 ± 3.8	130.8 ± 4.5	>0.2
HDL cholesterol (mg/dL)	42.8 ± 1.4	45.4 ± 1.6	>0.2	43.4 ± 1.0	45.7 ± 1.3	0.15
HDL_2 cholesterol (mg/dL)	7.3 ± 0.7	8.6 ± 0.9	>0.2	7.7 ± 0.5	6.7 ± 0.7	>0.2
HDL_3 cholesterol (mg/dL)	36.0 ± 0.9	36.6 ± 1.1	>0.2	35.7 ± 0.8	38.9 ± 1.0	0.016
Triglycerides (mmol/L)	2.01 ± 0.12	1.53 ± 0.15	0.015	1.09 ± 0.06	1.15 ± 0.08	>0.2
Total cholesterol/HDL cholesterol	6.6 ± 0.3	5.6 ± 0.3	0.015	4.8 ± 0.1	4.4 ± 0.2	0.09
LDL cholesterol/HDL cholesterol	4.8 ± 0.2	3.9 ± 0.3	0.001	3.3 ± 0.1	3.0 ± 0.2	0.10
Apoprotein A-1 (mg/dL)				115.7 ± 2.4	120.1 ± 3.0	>0.2
Apoprotein A-2 (mg/dL)				43.1 ± 0.9	48.2 ± 1.1	0.001
Apoprotein B (mg/dL)				85.3 ± 2.3	85.6 ± 2.8	>0.2
Apoprotein A-1/Apoprotein B				1.45 ± 0.05	1.45 ± 0.06	>0.2
Retinyl palmitate (μg/dL)				2.8 ± 0.5	2.4 ± 0.6	>0.2

Values are mean ± SE, adjusted for age. LDL = Low-density lipoprotein; HDL = High-density lipoprotein. P values compare patients and controls.

sons of patients had a somewhat less favorable lipoprotein and apoprotein profiles (not statistically significant). Sons of patients had significantly decreased levels of HDL_3 cholesterol (p=0.016) and apoprotein A-2 (p=0.001) when compared with controls. The frequencies of apolipoprotein E phenotypes did not differ between sons of patients and sons of controls. Apoprotein E2 alleles were present in 8 sons of controls and 6 sons of patients, 1 of whom was of the E2/E2 phenotype. This participant was not an outlier with regard either to baseline lipid profile or postprandial data.

The mean fat load ingested by sons of patients was 399.4 ± 3.2 g (mean ± SE) by and by sons of controls was 395.1 ± 3.8 g. None of the sons in both groups had previous or current diseases or received medication known to have an effect on lipoprotein metabolism.

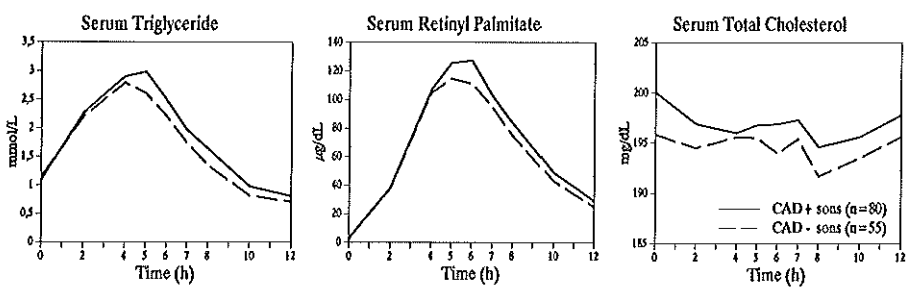

Figure 2.1. Serum lipid levels in young adult men after an oral fat load. Levels of postprandial serum triglyceride (left), retinyl palmitate (middle), and total cholesterol (right) are mean values adjusted for age. To convert cholesterol levels to mmol/L, multiply by 0.02586. CAD = coronary artery disease; CAD+ and CAD- refer to sons whose fathers do and do not have coronary artery disease, respectively.

Figure 2.1 shows age-adjusted mean postprandial curves for levels of triglyceride, retinyl palmitate, and total cholesterol. Figure 2.1 (left panel) shows mean postprandial triglyceride curves for sons of patients and sons of controls. In both groups, oral lipid loading led to markedly similar increases in triglyceride concentrations, maximum levels appeared between 4 to 6 hours postprandially. After 12 hours, triglyceride concentrations had decreased below initial levels. The age-adjusted repeated-measures analysis showed an interaction effect between group and time (χ^2=19.2; p=0.014), indicating that the two curves were significantly different from each other.

In Figure 2.1 (middle panel) the mean postprandial retinyl palmitate curves are shown. Retinyl palmitate concentrations increased from virtually zero in both groups to reach maximum concentrations between 5 and 6 hours postprandially. Thereafter, retinyl palmitate concentrations decreased to levels at t_{12} that were approximately tenfold the initial levels at t_0. The repeated-measures analysis for these curves indicated no significant difference (χ^2=6.42; p=0.60).

In Figure 2.1. (right panel) curves are shown for postprandial total cholesterol responses. Sons of patients had higher mean total cholesterol levels at t_0 than did sons of controls, and this difference remained remarkably constant during the lipid loading test, showing no significant response to the intervention. The age-adjusted difference in mean cholesterol levels (mean of all cholesterol measurements) was 2.3 ± 5.87 mg/dL (mean ± SE, p=0.70).

A slight difference in age was noted between sons of patients and sons of controls. Age has been shown to have an effect on postprandial lipoprotein changes (26) and postprandial retinyl ester response (27), and therefore the differences in postprandial response between sons of patients and sons of controls were adjusted for age.

Table 2.3 shows the age-adjusted differences between net postprandial changes in triglyceride and retinyl palmitate responses between sons of patients and sons of controls. Triglyceride response differences showed a maximum at t_5, and reached significance at times t_8, t_{10}, and t_{12}. With respect to retinyl palmitate, sons of patients and sons of controls showed a slight difference in response, increasing to a maximum at t_5 to t_6. None of the retinyl palmitate differences was found to be statistically significant.

In Figure 2.2.A, age-adjusted areas under the curves for 2-hour time intervals are given for postprandial triglyceride responses in sons of patients and sons of controls. Peak areas for both groups were found in interval t_4 to t_6. Differences were mainly found in intervals t_6 to t_8, t_8 to t_{10}, and t_{10} to t_{12}. In the latter interval, the triglyceride response of sons of controls decreased below t_0 (baseline) levels to a greater extent than in sons of patients. Figure 2.2.B shows total area under the curves for 10-, 8-, 6-, and 4-hour time intervals up to t_{12}. For all intervals, areas under the curve differed significantly between the groups. Figures 2.2.C and 2.2.D show similar data for retinyl palmitate responses in the groups. For 2-hour intervals and larger intervals, areas under the curves for sons of patients tended to be higher, although differences did not reach statistical significance.

The results for postprandial retinyl palmitate as well as triglycerides did not materially change after adjustment for baseline HDL_2 cholesterol levels, HDL_3 cholesterol levels, and apolipoprotein E phenotype. Total fat intake was decreased in sons of patients compared with sons of controls (p=0.012; Table 2.1). Adjustment for this difference in postprandial analyses

Table 2.3. Postprandial triglyceride and retinyl palmitate responses in sons of patients with coronary artery disease and sons of controls.

Time (h)	Triglycerides (mmol/l)			Retinyl palmitate (μg/dL)		
	Sons of patients (n=80)	Sons of controls (n=55)	Difference (95% CI)	Sons of patients (n=80)	Sons of controls (n=55)	Difference (95% CI)
2	1.17 ± 0.08	1.04 ± 0.09	0.13 (-0.11 to 0.37)	35.3 ± 3.0	35.3 ± 3.6	0.03 (-9.1 to 9.2)
4	1.81 ± 0.13	1.64 ± 0.16	0.17 (-0.24 to 0.58)	103.9 ± 6.0	101.7 ± 7.3	2.3 (-16.3 to 20.8)
5	1.89 ± 0.15	1.46 ± 0.18	0.43 (-0.02 to 0.88)	122.8 ± 7.4	110.8 ± 9.0	12.0 (-11.0 to 34.9)
6	1.40 ± 0.12	1.06 ± 0.15	0.34 (-0.03 to 0.72)	124.6 ± 8.1	106.6 ± 9.8	17.9 (-7.2 to 43.0)
7	0.87 ± 0.10	0.58 ± 0.13	0.29 (-0.03 to 0.61)	100.0 ± 7.2	90.4 ± 8.7	9.6 (-12.6 to 31.8)
8	0.54 ± 0.09	0.19 ± 0.11	0.35 (0.07 to 0.62)	80.7 ± 6.9	70.6 ± 8.4	10.0 (-11.4 to 31.5)
10	-0.12 ± 0.05	-0.33 ± 0.06	0.21 (0.06 to 0.36)	46.0 ± 4.6	40.0 ± 5.5	6.0 (-8.1 to 20.1)
12	-0.29 ± 0.04	-0.42 ± 0.05	0.13 (0.01 to 0.26)	26.2 ± 2.3	22.2 ± 2.7	4.0 (-3.1 to 11.1)

Triglyceride and retinyl palmitate values are mean ± SE, adjusted for age. Age-adjusted mean differences (values of sons of patients minus values of sons of controls) are given with their corresponding 95% CIs.

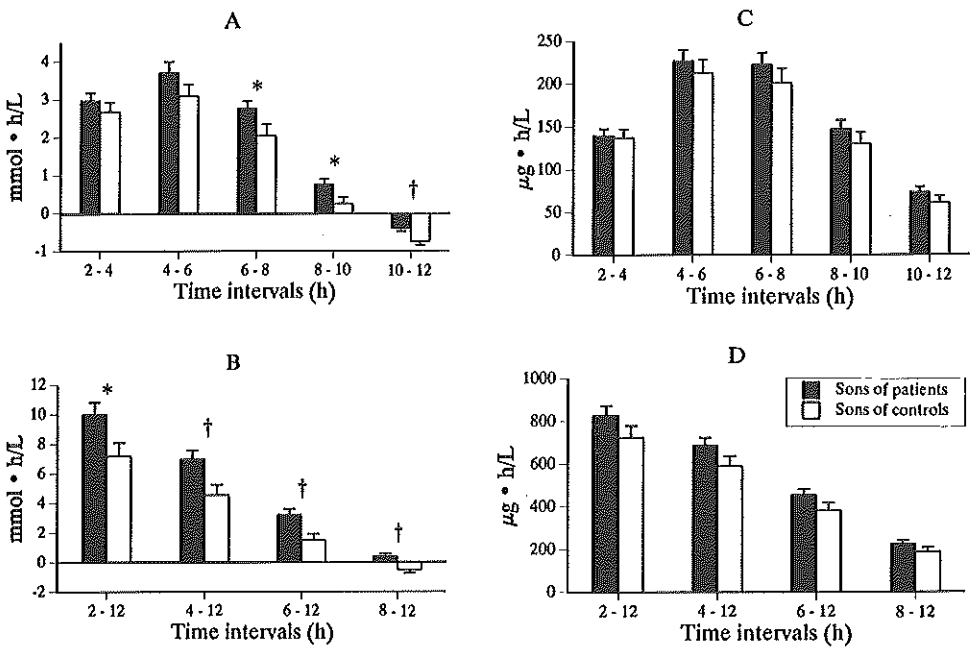

Figure 2.2. Age adjusted areas under the curve for levels of postprandial triglyceride and retinyl palmitate. A. Areas under the curve for postprandial triglyceride levels for 2-hour time intervals. B. Areas under the curve for larger time intervals. C and D. Areas under the curve for postprandial retinyl palmitate. Closed bars = sons of patients with coronary artery disease; open bars = sons of controls (those without coronary artery disease). * = $p<0.05$; † = $p<0.01$.

did not change the results, with adjusted group differences in triglyceride levels at t_8 of 0.32 mmol/L [95% CI, 0.04 to 0.59], at t_{10} of 0.2 mmol/L [95% CI, 0.047 to 0.36], and at t_{12} of 0.1 mmol/L [95% CI, 0.002 to 0.26]. All sons were asked whether they had changed their lifestyles with regard to smoking habits, physical activity, and fat intake since their fathers had had cardiac examination. None of the group differences was statistically significant. Also, a similar analysis of sons who reported to live separately from their fathers at the time of examination (45 sons of patients [56%] and 24 sons of controls [42%] yielded no significant group differences with respect to lifestyle changes. An analysis of postprandial data in these groups showed similar results, with sons of patients having higher triglyceride levels at t_8 (difference, 0.47 mmol/L; 95% CI, -0.018 to 0.95) and t_{10} (difference, 0.29 mmol/L; 95% CI,

0.015 to 0.57). When analyses were done for participants with normal levels of triglycerides only (serum triglyceride $\leq$ 2.28 mmol/L), thereby excluding 13 participants, no major changes in the results were found.

Discussion

Familial aggregation of risk factors for coronary artery disease is well established, and the offspring of patients with very severe coronary artery disease are at increased risk for this disease (28, 29). We assessed whether this increased familial risk was related to postprandial metabolic handling of an oral fat load in healthy young male adults whose fathers had severe coronary artery disease. Baseline measurements showed that sons of patients and sons of controls had similar levels of lipids and apolipoproteins, only levels of HDL_3 cholesterol and apoprotein A-2 were significantly decreased in sons of patients.

After oral lipid loading, however, prolonged postprandial hypertriglyceridemia was observed in the sons of patients, amounting to statistically significant group differences of 0.35 mmol/L after 8 hours, 0.21 mmol/L after 10 hours, and 0.13 mmol/L after 12 hours. Differences in postprandial triglyceride levels between the groups were mainly observed in the declining part of the curves, which suggests a delayed clearance of triglyceride from plasma. Data on postprandial retinyl palmitate responses also suggest similar differences for postprandial triglyceride levels with regard to the direction of the difference and the postprandial period in which differences appeared. However, these latter differences were not statistically significant.

A marked contrast between patients with coronary artery disease and controls with regard to angiographic findings was chosen to minimize the chance of misclassification of patients and also because of the anticipated subtle differences between both groups of offspring. Considering the efforts asked on a strictly voluntary basis from fathers, and particularly their offspring, the response rate of 65% in this study is satisfactory. However, it is possible that postprandial lipoprotein metabolism in these young healthy participants was in some way differentially related to the response in both groups and, although unlikely, it is possible that selection mechanisms have affected the findings. Because contraceptive steroids have an effect on postprandial lipid metabolism in young health women (30), we restricted the study to sons.

Chylomicron metabolism is thought to occur essentially in two successive steps. First, these particles interact with lipoprotein lipase, an enzyme which is located on capillary endothelial surfaces of various tissues, including heart, skeletal muscle, and adipose tissue (31). This lipase catalyses the hydrolysis of triglycerides, and this results in uptake of free fatty acids by tissues

(32). Thereafter, resulting chylomicron remnant particles are taken up relatively quickly and efficiently (33) by parenchymal cells in the liver. The importance of this process, with regard to atherogenesis, is that any hampering in this course of events might lead to higher plasma levels of atherogenic triglyceride-rich lipiproteins and their remnants.

In principle, the oral lipid loading test cannot distinguish between mechanisms involved in chylomicron entry into and clearance of chylomicrons and their remnants from the circulation. In our study, no attempt was made to assess differences between intestinal lipid absorption in the two groups of young adult men. In a study of postprandial lipoprotein metabolism, Weintraub and colleagues (13) showed that gastrointestinal transit time was similar in normal participants and participants with different types of hyperlipoproteinemias. Previous studies (14 - 16) showed that mean triglyceride and mean retinyl palmitate response curves are similar during the first 4 hours postprandially when comparing patients and controls, which we also found in our study in sons of such patients. This suggests that differences in fat absorption or in intestinal secretion, if any, are not likely to explain group differences during the second half of the test period.

Retinyl palmitate is a useful marker of the metabolism of intestinally derived triglyceride-rich lipoproteins and their remnants because it is incorporated into the core of these lipoproteins (34) and retained within them until their irreversible clearance from plasma by the liver (35). Although a considerable postprandial exchange between lipoproteins of total retinyl esters has been reported in man (36), little exchange of retinyl palmitate between intestinally derived lipoproteins and other lipoproteins has been shown (37, 38). In our study, retinyl palmitate responses showed considerable interindividual variation in both groups, which may explain why differences between group responses did not reach statistical significance. An alternative explanation might be that the postprandial retinyl ester response is greater in older persons than in younger persons (27), thus making it more difficult to detect group differences in the young. Further, postprandial hypertriglyceridemia may partly be induced by endogenous very-low-density lipoprotein and its remnants (39), which do not contain retinyl palmitate. Finally, significant group differences in retinyl palmitate may emerge after the 12-hour measuring period used in the present study and, thus, may have been missed (14).

In our study, data were analyzed after subtracting individual fasting triglyceride levels and retinyl palmitate levels from respective subsequent measurements. Differences in body height and weight were considered by standardizing the oral lipid load to body surface area as a function of body height and weight. Possible confounding effects of age, HDL_2 cholesterol levels, HDL_3 cholesterol levels, and apolipoprotein E phenotype were adjusted for in the analysis. Apolipoprotein E polymorphism has recently been shown to have an effect on

postprandial retinyl palmitate concentrations (40). Adjustment for these factors did not materially change results with regard to group differences.

The hypothesis that postprandial lipoprotein metabolism plays an essential role in atherogenesis was first postulated by Zilversmit (3). This hypothesis was adressed in recent studies in humans (14 - 16) in which patients with coronary artery disease were compared with controls with respect to postprandial lipemia; the study found that patients had higher postprandial triglyceride and retinyl palmitate responses. In another study, Simons and colleagues (41) found that 4 hours postprandially the apoprotein B48/apoprotein B100 ratio, used as a relative measure of chylomicrons and chylomicron remnants in plasma, was higher in patients with coronary artery disease than in controls. In addition, a positive correlation between the postprandial triglyceride response to an oral lipid load and carotid artery wall thickness has been reported (42). These studies suggest that prolonged presence of triglyceride-rich lipoproteins in plasma may indeed play a role in atherogenesis.

The design of our investigation enabled us to examine a group of young adult men at high familial risk for developing clinical manifestations of coronary artery disease much later in life. One advantage of the design of this study is that sons of these patients are not exposed to treatments such as medication that could alter triglyceride metabolism, as might occur when patients themselves are studied. However, if patients have been given advice about lifestyle, this may in turn have led to lifestyle changes in their sons. In both groups of sons, most reported not to have changed their levels of physical activity, smoking behavior, and fat intake since their fathers were examined for cardiac symptoms; approximately equal proportions reported to eat less fat. Additionally, an analysis of sons living separately from their fathers, who are assumed to be less influenced by lifestyle advice given to their fathers, yielded similar results with statistically significant differences in the declining part of the triglyceride curves, despite the considerably decreased number of participants. Although no major differences were noted with regard to reported fat intake, daily total fat intake as calculated from the food frequency questionnaire was significantly decreased in sons of patients compared with sons of controls. Long- and short-term effects of fat intake on postprandial lipoprotein levels have been reported (43), but in our study, adjustment for daily total fat intake did not change postprandial results. Thus, we believe that changes in physical activity, smoking behavior, and fat intake are not likely to explain the postprandial differences found between both groups of sons.

Our data show that healthy young sons whose fathers have established coronary artery disease have prolonged postprandial hypertriglyceridemia. This finding suggests that changes in postprandial lipid metabolism are associated with familial risk for coronary atherosclerosis.

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In an editorial accompanying the publication of the study described in chapter 2 (1), the possible explanations for postprandial offspring group differences found in this study were discussed. These included mechanisms by which the differences could be acquired or genetic, the latter being either monogenic of polygenic. It was proposed that a monogenic defect may exist that affects triglyceride metabolism. In that case the postprandial group differences might be explained by much more marked responses in a few sons who inherited this defect rather than a general shift of the distribution of responses for the group as a whole. In the reaction described below this possibility was analyzed.

Triglyceride levels in sons of patients with coronary artery disease (Re)

To the editor: We thank Dr. Grundy (1) for his supportive and useful discussion of our report (2), in which he considers various explanations for the prolonged postprandial hypertriglyceridemic response in the sons of patients with coronary artery disease. Dr. Grundy suggests that we examine the possible bimodality of responses in the offspring of patients, a bimodality that would theoretically emerge if group differences could be attributed to a heterozygous monogenic defect of triglyceride metabolism in patients. Given this monogenic hypothesis, a subgroup of patients having the gene would transmit it to 50% of their offspring. Consequently, a subgroup of sons would have marked postprandial responses, and Dr. Grundy suggests that these responses may explain the effect that we found.

A classic debate between Platt and Pickering (reviewed in reference 3) on the mode of transmission of primary hypertension on the basis of the shapes of blood pressure distribution curves has shown that bimodality, as distinct from left-skewing, in such curves is not easily proved. Further, a familially acquired abnormality in only a subgroup of families could theoretically lead to a similar bimodality. This and the relatively small sample size of our study initially led us not to engage in such an analysis. An alternative approach in unravelling putative modes of inheritance is segregation analysis, but such an analysis would not be overly informative in this case because of the limited number of siblings in our study. Nevertheless, we have attempted to address Dr. Grundy's suggestion. Figure 2.3 shows the frequency distribution of areas under the curve of the triglyceride response for sons of patients 6 to 12 hours after lipid loading. Although not a normal distribution, the number of observations does not allow definite conclusions on modality. Moreover, if those with a response above the arrow (n=7) are exluded from the analysis, the difference between offspring groups remains

of borderline significance (difference, 0.78 mmol x h/L; 95% CI, -0.015 to 1.574). Thus, it seems that offspring group differences cannot be fully explained by a subgroup of sons of patients with markedly high postprandial responses. Although this analysis must be evaluated with some caution, the monogenic mechanism as the sole explanation for prolonged postprandial hypertriglyceridemia does not appear to be supported by our data.

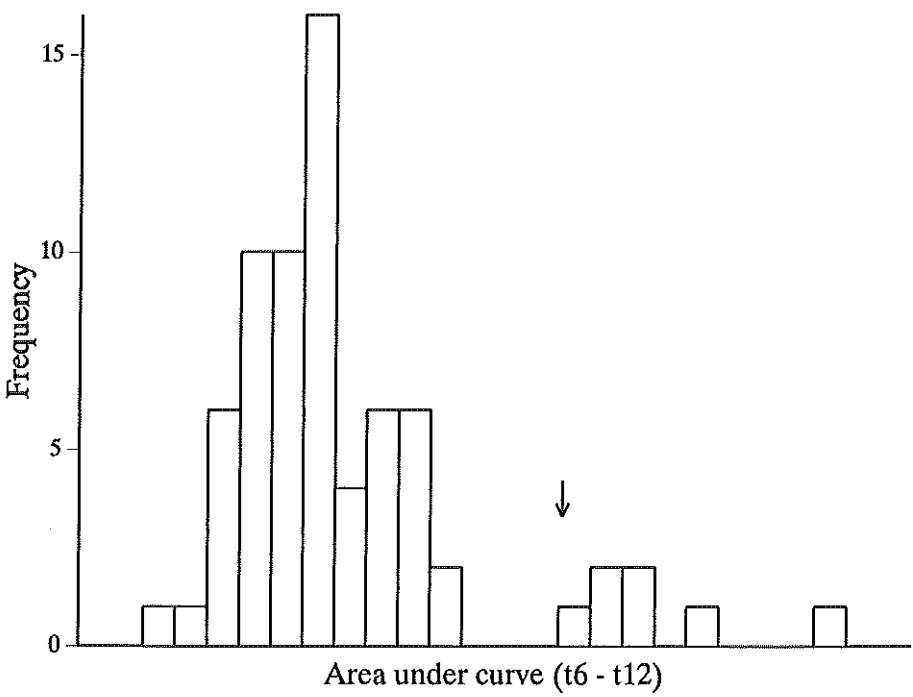

Figure 2.3. Frequency distribution of area under the curve (1 mmol x h/L intervals) for sons of patients with coronary artery disease. t6-t12 = triglyceride responses 6 to 12 hours after lipid loading. Patients with responses higher than that indicated by the arrow were excluded from the analysis.

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CHAPTER 3

LIPOPROTEINS AND APOLIPOPROTEINS IN YOUNG ADULT MEN AND WOMEN AND RISK FOR CORONARY ATHEROSCLEROSIS

3. Lipoproteins and Apolipoproteins in Young Adult Men and Women and Risk for Coronary Atherosclerosis

Abstract

Atherosclerotic lesions develop early in life. The role of lipoproteins and apolipoproteins in the atherosclerotic process at a young age is not completely elucidated. The aim of the present study was to assess lipoprotein and apolipoprotein levels in young adults in relation to coronary atherosclerosis in their parents.

We studied the offspring of patients who were admitted for diagnostic coronary angiography in several general hospitals in the Netherlands. From these patients we selected a group of male patients (n=90), who had severe coronary atherosclerosis at angiography, and a reference group of male patients (n=62), who had no coronary atherosclerosis at angiography. Lipoprotein and apolipoprotein levels were determined in 115 sons and 73 daughters of the patients with severe coronary atherosclerosis. These were compared to levels in 68 sons and 47 daughters of men from the reference group.

Lower levels of HDL_3 cholesterol and apolipoprotein A2 were found in sons of patients suffering from severe coronary atherosclerosis. Similar differences were observed in daughters of such patients without, however, achieving statistical significance.

Our findings add to the growing evidence that changes in lipoprotein and apolipoprotein levels can be detected relatively early in life. It is concluded that the low levels of HDL_3 cholesterol and apolipoprotein A2 found in sons of patients with severe coronary sclerosis may play a role in the development of coronary atherosclerosis.

Introduction

Cholesterol levels and other risk factors in the young are related to atherosclerotic lesions which may develop early in life (1-4). Total serum cholesterol levels measured in adolescent men have also been shown to be associated to the occurrence of manifest cardiovascular disease later in life (5). Less is known, however, about the role of lipoproteins and apolipoproteins at a young age. One way to study the relation between lipoproteins and apolipoproteins in relation to coronary artery disease is to examine the offspring of parents who suffer from coronary atherosclerosis. Coronary heart disease and its risk factors tend to aggregate in families (6-9). Recent prospective studies have shown that a parental history of

myocardial infarction is a risk factor for coronary artery disease, which is independent from classical cardiovascular risk factors (10,11). Thus, there may be other as yet unknown genetic or acquired risk factor for coronary artery disease.

In the present study lipoproteins and apolipoproteins in 188 children of fathers who suffered from severe clinically manifest and angiographically proven coronary atherosclerosis were compared to levels measured in 115 children of fathers who did not have coronary atherosclerosis at diagnostic coronary angiography.

Subjects and methods

From 1987 to 1992 two groups of male patients were selected from the coronary angiography databases of the cardiology departments of the Zuiderziekenhuis in Rotterdam (1987 - 1991), the Academic Hospital Dijkzigt in Rotterdam (1988 - 1989), the Refaja Ziekenhuis in Dordrecht (1990 - 1991), and the Antonius Hospital in Nieuwegein (1992), the Netherlands. The index group had severe coronary atherosclerosis, defined as more than 70% stenosis in at least three major coronary vessels at angiography (CAD+ fathers). A reference group had no or only minor angiographic lesions, defined as 20% stenosis or less in all coronary vessels (CAD- fathers). Additional criteria for both groups of men were: (1) age between 45 and 65 years, (2) blood pressure not exceeding 160/100 mmHg, (3) absence of liver disease, diabetes mellitus, thyroid disease, and renal disease, (4) coronary angiography within two years preceding examination, (5) first consultation of a physician for cardiac complaints within five years preceding examination for this study. Eligible subjects were sent a letter in which they, their children and the mothers of their children were invited to participate in the study. The average time elapsed since catheterisation to the present examination was 12.6 ± 6.0 months (mean ± SD) for CAD+ fathers and 13.6 ± 6.4 for CAD- fathers.

Measurements

The fathers, mothers, sons and daughters were asked to come to the hospital at 9.00 a.m. after fasting for at least 12 hours. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a random zero sphygmomanometer (Hawksley). Fasting serum blood samples were drawn by antecubital venipuncture for measurement of triglycerides, total

cholesterol, low density lipoprotein cholesterol (LDL cholesterol), high density lipoprotein cholesterol (HDL cholesterol), its subfractions HDL_2 cholesterol and HDL_3 cholesterol, apolipoprotein A1, apolipoprotein A2, and apolipoprotein B. Height and weight were measured without shoes and heavy clothing. The Quetelet-index was calculated as weight (kg) divided by the square of height (m^2).

The fathers answered a questionnaire with questions on the number of first degree relatives who had suffered from myocardial infarctions and on the use of medication at the time of examination (medication was taken to the hospital). Parents and children answered a questionnaire referring to a one month period prior to the examination, with questions on use of medication, habitual alcohol intake, coffee intake, and smoking habits. In addition, mothers and daughters were asked about the use of oral contraceptives, menstrual cycle and pregnancies. Sons and daughters were also asked about changes in lifestyle (physical activity, smoking behavior, and fat intake) since their fathers had first been examined by a physician for cardiac complaints. In the first 50 families selected for this study all members were invited for baseline measurements only. In these families the response rate was 92%. In the next 102 families the sons (80 sons of CAD+ fathers and 57 sons of CAD- fathers) were invited to come to the hospital on a separate day because of their simultaneous involvement in another study for which the criteria and conditions were similar to those in the present study. The sons as well as the daughters from these 102 families underwent the same measurements under the same conditions as the offspring of the first 50 families. The protocol of the extended study required sons from these 102 families to stay in the hospital for 12 hours, and the response rate was lower (65%) in this group of sons.

Laboratory analysis

Serum total cholesterol was measured with an automated enzymatic method, using the Boehringer Mannheim (FRG) CHOD-PAP reagent kit (12). HDL and LDL cholesterol were measured by the same method after precipitation. For HDL cholesterol the phosphotungstate method according to Burstein (13), with a minor modification as described by Grove (14) was used. For LDL cholesterol precipitation was carried out with polyvinylsulphate (Boehringer Mannheim, FRG). Throughout the entire study period the results of both total cholesterol and HDL cholesterol were within limits of the quality control programme of the WHO Regional Lipid Reference Centre (Prague, Czechoslovakia). Apolipoproteins A1 and B were assayed by an automated immunoturbidimetric method (Kone Diagnostics, Espoo, Finland).

Apolipoprotein A2 was determined by radial immunodiffusion against specific antiserum (Boehringer Mannheim, FRG) according to Cheung and Albers, with slight modifications (15). All automated analyses were carried out on the Kone Specific Analyzer (Kone Instruments, Espoo, Finland) using frozen (-20°C) serum samples. HDL_2 and HDL_3 cholesterol subfractions in serum were assayed as described by Gidez et al. (16) with slight modifications. HDL_2 and HDL_3 cholesterol subfractions were separated using stepwize precipitation of apolipoprotein B containing lipoproteins with heparin/Mn^{2+} in two steps and HDL_2 with dextran-sulphate.

Statistical methods

Mean values and standard deviations were calculated for all characteristics of the two groups of fathers, mothers, sons and daughters, separately. Mean group differences in baseline characteristics were tested with the t-test or Mann-Whitney U test when appropriate, and the χ^2 test for proportional data. All further analyses were adjusted for age. Adjusted mean values for lipoproteins levels in parents and offspring were obtained using linear regression analysis with the (apo)lipoprotein level as the dependent and a group indicator and age as the independent variables. The associations between (apo)lipoprotein levels in family members were assessed by linear regression analysis. This analysis included fathers and mothers and the first sons or daughters taking part in the study. Because of the dependency of measurements between brothers and between sisters within families, group differences between lipoprotein values in the offspring were analyzed using a repeated measures analysis of variance where fathers were considered the sampling units (BMDP, 1990). In this analysis, lipoprotein values in sons and daughters were considered repeated measures within fathers. In the repeated measures analysis mean lipoprotein levels were adjusted for age, as well as for a combination of age, height, weight, systolic blood pressure, diastolic blood pressure, smoking, alcohol intake and in daughters the use of oral contraceptives.

Results

Table 3.1 shows the characteristics of the CAD+ patients and the CAD- reference group, as well as those of their family members. On average CAD+ patients and their family members were older. Significant differences were present in a number of characteristics. Fathers of the CAD- group were taller, the proportion of smoking mothers was higher in the CAD- group,

systolic and diastolic blood pressure were on average higher in sons of CAD+ and the proportions of smokers and users of coffee as well as the Quetelet-index were higher in daughters of CAD+. After adjustment for age only the group differences in smoking in mothers and diastolic blood pressure in sons remained significant.

In table 3.2 the mean (apo)lipoprotein levels are given for fathers and mothers from both groups. There were considerable differences with regard to lipids and apolipoproteins between the two groups of fathers. CAD+ fathers had higher levels of total and LDL cholesterol, of triglycerides and of apolipoprotein B and lower levels of HDL cholesterol than CAD- fathers. Further adjustment for other risk factors did not materially change these results (data not shown). In the mothers the only difference was a higher triglyceride level in the wives of CAD+ and a higher apolipoprotein A1 after adjustment for other risk factors.

Table 3.3 shows the mean age adjusted lipoprotein and apolipoprotein values for the sons and the daughters. Sons and daughters of CAD+ fathers on average had adverse lipoprotein and apolipoprotein levels compared with sons and daughters of CAD- fathers.

Table 3.4 gives the mean differences between the groups of sons and daughters with regard to lipoprotein and apolipoprotein levels, as analyzed with repeated measures analysis of variance. Sons of CAD+ fathers had significantly lower levels of HDL_3 cholesterol and significantly lower levels of apolipoprotein A2. None of the other group differences was statistically significant. HDL_3 cholesterol and apolipoprotein A2 levels were also somewhat lower in daughters of CAD+ fathers, but none of these as well as other differences were statistically significant. An additional repeated measures analysis in which all families were excluded in which either one or both parents and one or more offspring had total cholesterol levels exceeding 300 mg/dl yielded similar results. When all families were excluded in which one of the parents had a total cholesterol level exceeding 300 mg/dl similar results were observed with significantly lower levels of HDL_3 cholesterol and apolipoprotein A2 in sons of CAD+ fathers.

For total cholesterol there were significant associations between levels in fathers and sons (regression coefficient b=0.38, 95% confidence interval 0.21,0.56), mothers and sons (b=0.47, 95% CI 0.24,0.70), fathers and daughters (b=0.32, 95% CI 0.13,0,51), and mothers and daughters (b=0.39, 95% CI 0.15,0.63). For LDL cholesterol and apolipoprotein B similar associations were observed. The strongest associations were found for apolipoprotein A1 between fathers and offspring and in particular sons (b=0.65, 95% CI 0.50,0.80), mothers and sons (b=0.78, 95% CI 0.60,0.95) and mothers and daughters (b=0.57, 95% CI 0.37,0.76). Apolipoprotein A1 also showed the strongest association between levels in sons and daughters

Table 3.1. Characteristics of CAD+ and CAD- fathers and mothers and offspring.

Variable	Fathers		Mothers		Sons		Daughters	
	CAD+ (n=90)	CAD- (n=62)	CAD+ (n=91)	CAD- (n=61)	CAD+ (n=115)	CAD- (n=68)	CAD+ (n=73)	CAD- (n=47)
Age (yrs)	56.4 ± 7.6	52.2 ± 8.1 †	52.5 ± 9.4	49.2 ± 7.9 *	25.5 ± 6.4	22.5 ± 5.6 †	25.4 ± 8.3	21.4 ± 7.7 †
Height (cm)	175.6 ± 6.0	178.6 ± 7.9 *	166.3 ± 6.1	166.2 ± 6.4	180.8 ± 9.7	181.3 ± 11.3	167.0 ± 7.5	167.6 ± 9.9
Weight (kg)	78.5 ± 8.7	79.8 ± 11.0	72.4 ± 12.5	71.4 ± 10.3	75.8 ± 12.9	73.8 ± 13.3	63.7 ± 11.5	60.1 ± 14.0
Quetelet-index (kg/m^2)	25.4 ± 2.2	25.0 ± 2.9	26.2 ± 4.5	25.9 ± 3.9	23.0 ± 2.9	22.3 ± 3.1	22.9 ± 4.0	21.2 ± 3.8 *
SBP (mmHg)	127.2 ± 12.8	127.5 ± 16.9	127.0 ± 16.7	125.3 ± 14.8	122.9 ± 13.4	118.2 ± 14.5*	113.5 ± 14.5	116.0 ± 11.2
DBP (mmHg)	84.1 ± 11.1	82.1 ± 10.3	83.6 ± 11.1	81.0 ± 8.3	79.4 ± 10.1	73.9 ± 12.7†	74.3 ± 11.0	72.5 ± 8.4
Smoking (%)	18.9	21.0	18.7	36.1 *	33.9	29.4	37.0	19.1 *
Alcohol intake (g/day)	13.8 ± 13.6	14.1 ± 11.4	6.4 ± 7.7	6.7 ± 8.0	11.6 ± 12.9	9.3 ± 9.6	4.8 ± 6.0	5.6 ± 6.4
Coffee drinking (%)	98.9	93.5	97.8	98.4	82.6	73.5	75.3	51.1 †
Post menarche (%)	-	-	-	-	-	-	92.8	84.1
Pregnant at examination (%)	-	-	-	-	-	-	2.9	7.1
Use of oral contraceptives (%)	-	-	-	-	-	-	43.5	45.2

Values are means ± SD, unless stated otherwise. SBP = systolic blood pressure, DBP = diastolic blood pressure.
* = $p<0.05$; † = $p<0.01$ for differences between CAD+ and CAD-.

Table 3.2. Lipoproteins and apolipoproteins of CAD+ and CAD- fathers and the respective mothers in both groups.

Variable	Fathers		Mothers	
	CAD+ (n=90)	CAD- (n=62)	CAD+ (n=91)	CAD- (n=61)
Total cholesterol (mmol/l)	6.9 ± 0.11	6.3 ± 0.14 ‡	6.6 ± 0.2	6.6 ± 0.18
LDL cholesterol (mmol/l)	5.1 ± 0.11	4.5 ± 0.13 ‡	4.6 ± 0.15	4.8 ± 0.19
HDL cholesterol (mmol/l)	1.1 ± 0.03	1.2 ± 0.04 *	1.4 ± 0.03	1.3 ± 0.03
HDL_2 cholesterol (mmol/l)	0.2 ± 0.01	0.2 ± 0.02	0.4 ± 0.03	0.3 ± 0.02
HDL_3 cholesterol (mmol/l)	0.9 ± 0.02	0.9 ± 0.02	1.1 ± 0.03	1.0 ± 0.02
Triglycerides (mmol/l)	2.09 ± 0.09	1.50 ± 0.12 ‡	1.48 ± 0.08	1.25 ± 0.06 *
Apolipoprotein A1 (mg/dl)	133.5 ± 2.5	135.6 ± 3.8	155.0 ± 3.5	145.2 ± 3.8
Apolipoprotein A2 (mg/dl)	45.3 ± 0.8	46.6 ± 0.8	47.3 ± 0.7	47.1 ± 0.9
Apolipoprotein B (mg/dl)	131.4 ± 3.3	110.2 ± 3.7 ‡	111.0 ± 3.6	106.0 ± 3.5

Values are means ± SEM, adjusted for age. LDL = low density lipoprotein, HDL = high density lipoprotein. * = $p<0.05$; ‡ = $p<0.001$ for differences between CAD+ and CAD-.

(b=0.55, 95% CI 0.35,0.74). Between fathers and mothers associations were observed for HDL_3 cholesterol (b=0.25, 95% CI 0.12,0.39) and apolipoprotein A1 (b=0.43, 95% CI 0.32,0.55). Much weaker or no associations between family members were observed for the other lipoproteins.

We have attempted to assess the effects of possible changes in lifestyle, possibly advised to fathers, which may have been passed on to the children. There were no differences between the two groups of sons with regard to changes in reported smoking behavior, alcohol intake and fat intake after their fathers had been first examined for cardiac complaints. However, since this first examination, 39% of daughters of CAD+ fathers reported to have diminished their fat intake, as opposed to 17% of daughters of CAD- fathers (Yates corrected χ^2 5.1,

Table 3.3. Lipoproteins and apolipoproteins of the offspring of CAD+ and CAD- fathers.

Variable	Sons		Daughters	
	CAD+ (n=115)	CAD- (n=68)	CAD+ (n=47)	CAD- (n=73)
Total cholesterol (mmol/l)	5.4 ± 0.11	5.0 ± 0.10	5.6 ± 0.14	5.0 ± 0.15
LDL cholesterol (mmol/l)	3.7 ± 0.11	3.3 ± 0.10	3.6 ± 0.14	3.1 ± 0.15
HDL cholesterol (mmol/l)	1.2 ± 0.02	1.2 ± 0.03	1.5 ± 0.04	1.5 ± 0.04
HDL_2 cholesterol (mmol/l)	0.2 ± 0.01	0.2 ± 0.02	0.4 ± 0.03	0.4 ± 0.03
HDL_3 cholesterol (mmol/l)	0.9 ± 0.02	1.0 ± 0.02	1.1 ± 0.02	1.1 ± 0.03
Triglycerides (mmol/l)	1.16 ± 0.06	1.13 ± 0.07	1.14 ± 6.0	1.04 ± 8.0
Apolipoprotein A1 (mg/dl)	126.6 ± 2.5	123.8 ± 2.8	159.6 ± 3.7	151.6 ± 4.3
Apolipoprotein A2 (mg/dl)	44.1 ± 0.6	47.5 ± 1.3	46.6 ± 1.0	48.0 ± 1.0
Apolipoprotein B (mg/dl)	89.4 ± 2.5	83.9 ± 2.5	86.9 ± 2.6	77.8 ± 3.6
Apolipoprotein A1/B	1.6 ± 0.04	1.5 ± 0.06	1.9 ± 0.07	2.1 ± 0.09

Values are means ± SEM, adjusted for age.

p=0.02). A repeated measures analysis was performed in which an adjustment was made for the offspring living either with their parents or on their own. This analysis showed lower levels of HDL_3 cholesterol (difference -2.6 mg/dl, 95% CI -5.3,0.2) and apolipoprotein A2 (-5.5 mg/dl, 95% CI -8.4,-2.5) in sons of CAD+ fathers. Similar results were observed in daughters of CAD+ fathers who had lower levels of HDL_3 cholesterol (-4.5 mg/dl, 95% CI -8.2,-0.9) and apolipoprotein A2 (-4.5 mg/dl, 95% CI -8.3,-0.7).

Table 3.4. Differences in lipoproteins and apolipoproteins of the offspring of CAD+ and CAD- fathers.

Variable		Sons		Daughters	
	Adjusted for	Difference	95 % C.I.	Difference	95% C.I.
Total cholesterol (mmol/l)	age	0.11	[-0.18, 0.41]	0.19	[-0.29, 0.68]
	age/risk factors*	0.0005	[-0.31, 0.32]	0.28	[-0.22, 0.78]
LDL cholesterol (mmol/l)	age	0.14	[-0.16, 0.44]	0.16	[-0.34, 0.66]
	age/risk factors	0.09	[-0.23, 0.42]	0.24	[-0.26, 0.74]
HDL cholesterol (mmol/l)	age	-0.04	[-0.13, 0.05]	-0.005	[-0.13, 0.12]
	age/risk factors	-0.07	[-0.16, 0.02]	-0.04	[-0.15, 0.06]
HDL_2 cholesterol (mmol/l)	age	0.02	[-0.03, 0.08]	0.05	[-0.03, 0.14]
	age/ risk factors	0.005	[-0.05, 0.06]	0.06	[-0.01, 0.12]
HDL_3 cholesterol (mmol/l)	age	-0.07	[-0.13,-0.0005]	-0.05	[-0.13, 0.04]
	age/risk factors	-0.09	[-0.16,-0.03]	-0.07	[-0.15, 0.02]
Triglycerides (mmol/l)	age	-0.03	[-0.22,0.16]	0.09	[-0.32, 0.15]
	age/risk factors	-0.11	[-0.32,0.09]	0.13	[-0.09, 0.36]
Apolipoprotein A1 (mg/dl)	age	3.1	[-6.3,12.5]	10.6	[-2.0, 23.3]
	age/risk factors	-2.8	[-11.7,6.1]	11.9	[-0.7, 24.6]
Apolipoprotein A2 (mg/dl)	age	-5.1	[-7.9,-2.3]	-2.7	[-5.9, 0.6]
	age/risk factors	-4.4	[-7.0,-1.9]	-2.1	[-5.3, 1.2]
Apolipoprotein B (mg/dl)	age	2.1	[-5.1,9.2]	5.7	[-4.5, 15.9]
	age/risk factors	-0.6	[-7.9,6.7]	8.1	[-1.3, 17.5]
Apolipoprotein A1/B	age	0.07	[-0.09,0.22]	-0.05	[-0.30,0.20]
	age/risk factors	0.007	[-0.14,0.16]	-0.08	[-0.31,0.14]

Values are mean differences (values offspring CAD+ fathers - values offspring CAD- fathers) as calculated with repeated measures analysis of variance; 95% C.I.= 95% confidence interval; LDL = low density lipoprotein, HDL = high density lipoprotein; * = adjusted for risk factors: height, weight, systolic blood pressure, diastolic blood pressure, smoking, alcohol intake, and (daughters) use of oral contraceptives.

Discussion

In this study we observed differences in lipoproteins and apolipoproteins between the offspring of fathers with and without coronary atherosclerosis. Sons and daughters of CAD+ fathers had a less favorable (apo)lipoprotein profile. Sons of patients with coronary artery disease had lower HDL_3 cholesterol and apolipoprotein A2 levels. Daughters of such patients tended to have lower levels of HDL_3 cholesterol and apolipoprotein A2 as well, but these differences reached only borderline significance.

Before interpreting these findings some methodologic aspects of the study need to be discussed. We have assessed lipoprotein and apolipoprotein levels in young healthy adults in relation to coronary atherosclerosis in their parents. The selection of patients was based on coronary angiography, which enabled us to relate lipoprotein and apolipoprotein levels directly to risk of atherosclerosis. Coronary angiographic criteria were severe three-vessel disease in CAD+ fathers and virtually no coronary sclerosis in CAD- fathers in order to maximize the atherosclerotic contrast between these groups. This contrast is reflected in the differences found between the two groups of fathers with regard to lipoproteins and apolipoproteins, CAD+ fathers having a less favorable (apo)lipoprotein profile.

Although there may be differences between the two groups of fathers with regard to lifestyle and medication, important differences, e.g. medication, will not be present in the offspring. A higher proportion of daughters of CAD+ fathers reported to have changed fat intake after their fathers had first been examined for cardiac complaints, but this was not accompanied by a change in other aspects of lifestyle, in particular smoking. Thus, the possibility that differential group changes of lifestyle could explain group differences in lipoproteins and apolipoproteins, cannot be completely ruled out. However, as it is not likely that changes to healthier lifestyles affect lipoprotein levels unfavorably, this, if present, results in an underestimation of the true differences. Support for this was found in the analysis in which we adjusted for possible differences between offspring living with or separately from their parents. The latter group is supposedly less affected by lifestyle advise given to the fathers. This analysis did not materially change the results.

The findings of the present study are generally in agreement with those in other studies (17) in that the offspring of patients suffering from coronary artery disease have less favorable lipid profiles. However, some studies have indicated that apolipoproteins, and particularly the ratio apolipoprotein A1/apolipoprotein B, measured in offspring of CAD patients are more strongly related to risk of cardiovascular disease than other (apo)lipoproteins (18-21). In the present study apolipoprotein B level and the ratio apolipoprotein A1/apolipoprotein B were

not found to be significantly different between groups. Furthermore, in contrast to earlier studies, we found that HDL_3 cholesterol and apolipoprotein A2 levels in offspring of CAD+ fathers and particularly the sons of CAD+ fathers show important differences. Differences in results between earlier studies and the present study are that (apo)lipoproteins in offspring were studied in relation to myocardial infarction in the parents (19-21) and not specifically to atherosclerosis, which may reflect other mechanisms in addition to those leading to atherosclerosis. Another explanation for these differences may be that in the present study the offspring was older than in other studies (18-19).

An inverse relation between HDL cholesterol and risk of ischemic heart disease is well established (22-25). HDL cholesterol has been shown to aggregate within families of patients with myocardial infarction (26,27) and, recently, HDL cholesterol subfractions and apolipoprotein A1 as well as apolipoprotein A2 have been reported to be strongly and independently associated with risk of myocardial infarction (28,29). The results of the present study are in line with these studies and suggest that HDL cholesterol, its subfractions and related apolipoproteins play a role in atherogenesis early in life. The specific role that the HDL subfractions HDL_2 and HDL_3 play in the inverse relation with ischemic heart disease still has to be elucidated, although HDL_2 is thought to be the most important factor in this respect. In the present study total HDL cholesterol levels are somewhat lower in sons of CAD+ fathers and not materially different between the two groups of daughters, and HDL_2 cholesterol tends to be higher in both the sons and daughters of CAD+ fathers, which is suggestive of a shift from HDL_3 to HDL_2 cholesterol in sons and daughters of CAD+ fathers.

In summary, we have compared lipoprotein and apolipoprotein levels in the offspring of patients suffering from severe, angiographically proven, coronary atherosclerosis with levels in offspring of patients with angiographically minimal coronary atherosclerosis. Lower levels of HDL_3 cholesterol and apolipoprotein A2 were found in the offspring and particularly in the sons of patients suffering from severe coronary atherosclerosis. Our findings add to the growing evidence that changes in lipoprotein and apolipoprotein levels can be detected relatively early in life. It is concluded that sons of patients with severe coronary sclerosis have reduced levels of HDL_3 cholesterol and apolipoprotein A2 and that these reduced levels may play a role in the development of coronary artery disease.

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CHAPTER 4

FAMILIES AND NATURAL HISTORY OF LIPIDS IN CHILDHOOD: AN 18 YEAR FOLLOW-UP STUDY

4. Families and Natural History of Lipids in Childhood: an 18 year Follow-up Study

Abstract

Parental levels of total cholesterol were related to repeated measurements of total cholesterol and lipoprotein-cholesterol in their offspring in the EPOZ study. In the EPOZ study (1975 - 1978) risk indicators for chronic diseases were studied in families of which the members were 5 years and over who were living in two districts in the Dutch town of Zoetermeer. A sample of youngsters was subsequently followed up for 18 years (average follow-up: 13.8 years) with yearly measurements of cardiovascular risk factors. Positive associations were found between age-adjusted tertiles of parental cholesterol levels and offspring cholesterol and low density lipoprotein-cholesterol levels, which persisted throughout childhood and young adulthood. Offspring with both parents in the upper cholesterol tertile had almost 1 mmol/l higher cholesterol levels compared to offspring with both parents in the lowest tertile. Differences between offspring of parents with high total cholesterol levels and parents with low levels were stable with increasing age of the offspring. There was considerable change with age of offspring cholesterol levels, with a dip around puberty. For high density lipoprotein-cholesterol and its subfractions no relations with parental cholesterol level were found.

In this study the aim was to assess parental cholesterol level as a determinant of offspring lipid level and change in lipid level over time. A strong positive association was shown between parental cholesterol levels and offspring cholesterol and low density lipoprotein-cholesterol levels. This association was already detectable at a young age and persisted with increasing age.

Introduction

The natural history of lipids and lipoproteins in children and adolescents (1-5) suggests that serum lipids and lipoproteins show tracking from childhood into young adulthood, with lipid and lipoprotein levels remaining at relatively stable ranks within distributions over time. Thus, lipid and lipoprotein levels measured at a young age may be predictive of adult levels. Furthermore, in a recent prospective study (6), total cholesterol levels in young men were shown to be related to coronary heart disease much later in life. Preventive measures might therefore already be taken in childhood and adolescence. Lipids and lipoproteins tend to

aggregate within families (7) and selective cholesterol screening may, among other indications, be recommended for children having a parent with a high blood cholesterol level (8). Therefore, it is important to know if offspring of parents with high risk factor levels have high levels themselves and to what extent these high levels persist throughout childhood and young adulthood.

In this study the aim is to assess the relation between parental cholesterol level and offspring lipid levels and change in offspring lipid levels with increasing age.

Methods

Subjects

All residents of ages 5 years and over who were living in two districts in the Dutch town of Zoetermeer were invited to participate in a study of risk indicators for chronic diseases (Dutch acronym: EPOZ study) (9-11). Zoetermeer is a suburban residential community near The Hague in The Netherlands, of at that time about 55,000 inhabitants. Between 1975 and 1979, 4,649 persons aged 5 - 19 years took part in the study (82% of those invited). In 1980 and 1981 all migrants moving to Zoetermeer were also asked to participate in the study. From this total group a random sample of 596 children was selected for annual follow up in a study of cardiovascular risk factors and its determinants. This paper deals with 483 subjects (81%), 252 males and 231 females, who took part in the follow up study. The average follow-up period was 13.8 years (range 3 to 18 years). Data on parents of these 483 subjects were obtained at the baseline study (1975 - 1981). Complete data were available for 425 fathers and 454 mothers. In 1991, 352 subjects still took part in the follow up study.

Measurements

Both parents and offspring were seen at baseline (1975 - 1981). Subsequently, the offspring were seen yearly and measurements were performed by one research assistant throughout the whole follow-up period. At each examination systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a random zero sphygmomanometer (Hawksley) as described in detail elsewhere (12). Serum blood samples were drawn by antecubital venipuncture for measurement of total cholesterol, low density lipoprotein-cholesterol, high

density lipoprotein-cholesterol, and its subfractions high density lipoprotein2-cholesterol and high density lipoprotein3-cholesterol. Height and weight were measured without shoes and heavy clothing. At each examination, the offspring answered a questionnaire about use of medication, alcohol intake, coffee consumption and smoking habits. Additionally, daughters were asked about use of oral contraceptives, menstrual cycle and pregnancies.

Laboratory analysis

Serum total cholesterol at baseline was measured with an automated enzymatic method (13). During follow-up a modified reagent was used (CHOD/PAP High Performance, Boehringer Mannheim, Germany). In the transition period both reagents have been used simultaneously, obtaining an excellent correlation ($r>0.99$). The overall coefficient of variation was 2.5% at baseline and 2.3% during follow-up. Cholesterol determinations at follow-up were performed on serum samples stored at -20°C for up to 4 years. Repeated measurements performed by our laboratory in frozen serum showed no significant changes up to 4 years after sampling compared to frozen samples measured within one week after venipuncture. The standard deviation of these duplicate measurements did not exceed 3.0% in all instances and did not show a significant drift.

High density lipoprotein-cholesterol measurements were started in 1979 and low density lipoprotein-cholesterol measurements in 1984. High density and low density lipoprotein-cholesterol were measured by the same method after precipitation. For high density lipoprotein-cholesterol the phosphotungstate method according to Burstein (14), with a minor modification as described by Grove (15) was used. For low density lipoprotein-cholesterol precipitation was carried out with polyvinylsulphate (Boehringer Mannheim, FRG). All measurements were carried out in the laboratory of the Department of Epidemiology & Biostatistics (Erasmus University Medical School), which participates since 1978 in the lipid standardization program of the WHO Regional Lipid Reference Centre in Prague (Dr. D. Grafnetter) and from 1979 in the Dutch National Cholesterol standardization program (KCA foundation), initiated in analogy to the program of the CDC Lipid Standardization Laboratory in Atlanta. In addition, during the baseline period quality control was indirectly checked on the CDC protocol by monthly comparison with cholesterol determination using the Abell-Kendall method (Gaubius Institute TNO, Leiden). Both accuracy and precision of total and high density lipoprotein-cholesterol measurements were within acceptable limits (CDC/WHO) over the whole period. All automated analyses were initially carried out on a Technicon Auto

Analyzer-II system (Technicon Instruments, Tarrytown, USA) and from 1989 on a Kone Specific Analyzer (Kone Instruments, Espoo, Finland) using frozen (-20°C) serum samples. From 1987 high density lipoprotein2 and high density lipoprotein3-cholesterol subfractions in serum were assayed as described by Gidez et al. (16) with slight modifications. High density lipoprotein2 and high density lipoprotein3-cholesterol subfractions were separated using stepwize precipitation of apolipoprotein B containing lipoproteins with heparin/Mn^{2+} in two steps and high density lipoprotein2 with dextran-sulphate.

Data analysis

The data analysis aimed to assess parental total cholesterol level as a determinant of offspring lipid level, and change in offspring lipid level over time. Mean values and standard deviations of baseline characteristics of parents and offspring were calculated. Tertile cut off values of the age-adjusted serum total cholesterol distributions were obtained for fathers and mothers separately. Baseline characteristics of sons and daughters were calculated according to both paternal and maternal tertiles of total cholesterol. In the offspring mean individual cholesterol and lipoprotein levels in 5 year age intervals were obtained by averaging for each subject all yearly measurements within that category. Thus, values from individual subjects could contribute to different age categories. Subsequently, mean total cholesterol and lipoprotein values were calculated according to tertiles of parental total cholesterol level. Offspring cholesterol levels were analyzed according to total cholesterol levels in fathers and mothers separately as well the combination of their total cholesterol levels, where both the father and the mother were in the same gender-specific tertile of their respective total serum cholesterol distributions. For testing group differences the lowest parental tertile values are taken as a reference category.

Individual lipid level change with age was determined by calculating slopes of regression lines through total cholesterol and lipoprotein values in each of the above mentioned 5 year intervals. Again, subjects could contribute to values calculated for different age-categories. Differences in change of offspring lipid levels between parental tertiles were tested using Student's t-test with values in the lowest parental tertile as reference category. Group comparisons of changes in offspring lipid levels were weighed for the individual number of lipid measurements within age-categories.

Results

Table 4.1 shows antropometric characteristics, systolic and diastolic blood pressure and serum total cholesterol levels of fathers (n=425) and mothers (n=454). Mean levels are also given by tertiles of parental total cholesterol levels. The cutoff-points of parental total cholesterol corresponding to the tertiles were 5.4 mmol/l and 6.2 mmol/l in fathers and 5.2 mmol/l and 6.0 mmol/l in mothers. In both fathers and mothers, Quetelet-index, and systolic and diastolic blood pressure were higher in those with higher total cholesterol levels. During follow-up, familial hypercholesterolemia was diagnosed in one mother and both her sons taking part in the present investigation. One of the sons started taking symvastatine in 1990 and his later follow-up data were excluded from the analysis.

Baseline characteristics of sons and daughters are shown in table 4.2, according to paternal tertiles of total cholesterol. Table 4.3 shows the same characteristics by maternal tertiles of total cholesterol. None of the differences between parental tertiles in table 4.2 and table 4.3 was statistically significant.

In table 4.4 total cholesterol and low density lipoprotein-cholesterol levels in offspring are shown by parental tertiles of serum total cholesterol levels. Thus, total cholesterol levels in offspring are given according to level in the father, irrespective of the total cholesterol level in the mother and vice versa. Total cholesterol and low density lipoprotein-cholesterol levels in children were strongly related to total cholesterol levels in their parents. For total cholesterol levels statistically significant differences were present between the highest and the lowest tertile. The differences persisted in the same direction in all age-categories but the last, while values in the medium tertile were consistently intermediate between values according to the lowest and the highest tertile. Differences between offspring total cholesterol levels by parental tertiles were fairly constant with increasing offspring age. In all tertiles the lowest offspring cholesterol levels were found in the age groups of 10 - 14 years and 15 - 19 years, suggesting a dip in cholesterol levels around puberty. Data according to paternal and maternal tertiles of total cholesterol are essentially similar, although the differences between mean offspring cholesterol levels by maternal tertiles seemed slightly larger than differences by paternal tertiles, amounting to more than 0.5 mmol/l between the highest and lowest tertiles. The differences between tertiles were already present for the youngest age category and persisted into adult age-categories. Statistically significant differences in low density lipoprotein-cholesterol levels were found between the highest and lowest paternal tertile in the age categories from 15 to 29 years. Numbers on low density lipoprotein cholesterol measurements in the younger age categories (5 - 9 and 10 - 14 yrs) were too

Table 4.1. Characteristics of fathers and mothers, who took part in the study (1975 - 1981): overall and by age-adjusted tertiles of total cholesterol.

	Fathers				Mothers			
	Overall	Tertiles total cholesterol			Overall	Tertiles total cholesterol		
		Low	Medium	High		Low	Medium	High
Number of participants	425	142	141	142	454	150	152	152
Age (yrs)	44.3 ± 7.9	44.6 ± 8.2	44.0 ± 7.7	44.4 ± 7.8	41.5 ± 7.5	40.6 ± 6.9	42.1 ± 7.9	41.8 ± 7.6
Height (cm)	176.1 ± 7.0	176.8 ± 7.0	176.1 ± 7.1	175.2 ± 6.8	164.5 ± 6.1	164.5 ± 6.0	164.4 ± 6.2	164.6 ± 6.1
Weight (kg)	76.4 ± 10.2	74.9 ± 10.4	76.3 ± 10.8	77.9 ± 9.3	65.6 ± 10.2	62.8 ± 8.2	65.7 ± 10.5	68.2 ± 10.9
Quetelet-index (kg/m^2)	24.6 ± 2.8	24.0 ± 3.1	24.5 ± 2.7	25.4 ± 2.5	24.2 ± 3.5	23.2 ± 2.9	24.3 ± 3.6	25.2 ± 3.7
Systolic blood pressure (mmHg)	128.2 ± 15.3	125.2 ± 14.4	128.2 ± 15.5	131.4 ± 15.3	125.2 ± 16.7	120.5 ± 15.6	126.4 ± 16.6	128.8 ± 16.7
Diastolic blood pressure (mmHg)	79.2 ± 11.1	77.6 ± 10.4	78.8 ± 11.1	81.3 ± 11.4	79.2 ± 12.0	75.5 ± 9.8	80.3 ± 12.2	81.7 ± 12.8
Total cholesterol (mmol/l)	5.9 ± 1.14	4.8 ± 0.5 range 3.4 - 5.4	5.8 ± 0.2 range 5.4 - 6.2	7.1 ± 0.9 range 6.2 - 10.0	5.7 ± 1.37	4.6 ± 0.5 range 2.5 - 5.2	5.6 ± 0.2 range 5.2 - 6.0	6.9 ± 1.6 range 6.0 - 24.1

Values are mean ± SD.

Table 4.2. Baseline characteristics of sons and daughters by tertiles of age-adjusted serum total cholesterol of fathers.

	Sons			Daughters		
	Tertiles total cholesterol fathers			Tertiles total cholesterol fathers		
	Low	Medium	High	Low	Medium	High
Number of participants	68	75	80	74	66	62
Age (yrs)	13.6 ± 0.46	13.1 ± 0.49	12.9 ± 0.50	12.5 ± 0.44	13.1 ± 0.50	13.0 ± 0.56
Height (cm)	159.7 ± 2.33	156.5 ± 2.73	156.5 ± 2.63	151.2 ± 1.93	152.0 ± 2.26	150.2 ± 2.18
Weight (kg)	47.3 ± 1.95	45.7 ± 2.08	45.9 ± 2.17	43.4 ± 1.50	43.6 ± 1.88	43.1 ± 1.87
Quetelet-index (kg/m^2)	17.8 ± 0.29	17.5 ± 0.28	17.8 ± 0.34	17.9 ± 0.30	18.0 ± 0.38	18.2 ± 0.38
Systolic blood pressure (mmHg)	103.9 ± 1.45	103.3 ± 1.50	103.4 ± 1.57	98.5 ± 1.06	97.8 ± 1.28	97.8 ± 1.38
Diastolic blood pressure (mmHg)	58.0 ± 0.96	55.4 ± 0.98	57.0 ± 0.92	56.3 ± 0.92	55.6 ± 0.81	56.0 ± 1.07
Ever smoked (%)	22.4	26.4	23.8	24.8	22.3	20.3
Current smoking (%)	16.7	20.0	20.5	21.2	20.1	21.2
Use of oral contraceptives (%)				27.3	23.7	20.1

Values are mean ± SEM.

Table 4.3. Baseline characteristics of sons and daughters by tertiles of age-adjusted serum total cholesterol of mothers.

	Sons			Daughters		
	Tertiles total cholesterol mothers			Tertiles total cholesterol mothers		
	Low	Medium	High	Low	Medium	High
Number of participants	81	68	84	69	84	68
Age (yrs)	13.2 ± 0.48	13.2 ± 0.52	13.2 ± 0.46	12.2 ± 0.48	13.2 ± 0.45	12.7 ± 0.50
Height (cm)	158.2 ± 2.37	157.2 ± 2.59	156.5 ± 2.61	149.5 ± 1.99	153.5 ± 2.01	150.3 ± 2.13
Weight (kg)	46.7 ± 1.91	46.3 ± 2.19	46.9 ± 2.05	40.7 ± 1.65	44.6 ± 1.64	42.9 ± 1.74
Quetelet-index (kg/m^2)	17.7 ± 0.29	17.8 ± 0.34	18.0 ± 0.28	17.4 ± 0.32	18.1 ± 0.34	18.2 ± 0.36
Systolic blood pressure (mmHg)	103.9 ± 1.43	104.6 ± 1.61	104.1 ± 1.40	98.5 ± 1.16	97.5 ± 1.13	98.6 ± 1.21
Diastolic blood pressure (mmHg)	56.6 ± 1.01	57.4 ± 1.02	56.9 ± 0.82	54.7 ± 0.92	56.3 ± 0.83	55.9 ± 0.94
Ever smoked (%)	20.6	21.0	24.0	24.0	23.1	21.7
Current smoking (%)	19.6	19.2	18.7	21.2	21.7	21.2
Use of oral contraceptives (%)				24.5	25.5	20.8

Values are mean ± SEM

Table 4.4. Total cholesterol and LDL cholesterol in offspring by tertiles of total cholesterol in fathers and in mothers.

		Tertiles of total cholesterol in fathers			Tertiles of total cholesterol in mothers		
	Age	Low	Medium	High	Low	Medium	High
Total cholesterol (mmol/l)	5 - 9	4.6 ± 0.06	4.6 ± 0.1	5.0 ± 0.09 ‡	4.6 ± 0.06	4.8 ± 0.10	4.9 ± 0.09 *
	10 - 14	4.5 ± 0.07	4.7 ± 0.07 *	4.8 ± 0.07 †	4.6 ± 0.08	4.7 ± 0.06 *	4.9 ± 0.07 ‡
	15 - 19	4.5 ± 0.07	4.7 ± 0.06 *	4.8 ± 0.06 †	4.4 ± 0.06	4.6 ± 0.05 *	4.9 ± 0.07 ‡
	20 - 24	4.9 ± 0.08	5.0 ± 0.07	5.2 ± 0.07 †	4.8 ± 0.06	5.0 ± 0.06 †	5.3 ± 0.08 ‡
	25 - 29	5.1 ± 0.09	5.4 ± 0.09 *	5.5 ± 0.09 †	5.0 ± 0.07	5.4 ± 0.09 †	5.6 ± 0.10 ‡
	30 - 37	5.2 ± 0.17	5.3 ± 0.11	5.6 ± 0.14	5.1 ± 0.14	5.5 ± 0.14 *	5.6 ± 0.16 *
LDL cholesterol (mmol/l)	15 - 19	2.7 ± 0.12	3.0 ± 0.15	3.0 ± 0.10 *	2.8 ± 0.12	2.9 ± 0.09	3.1 ± 0.14 *
	20 - 24	3.0 ± 0.09	3.3 ± 0.11 *	3.4 ± 0.09 †	2.9 ± 0.08	3.2 ± 0.08 *	3.6 ± 0.11 ‡
	25 - 29	3.2 ± 0.11	3.5 ± 0.10	3.7 ± 0.10 †	3.1 ± 0.09	3.5 ± 0.10 *	3.7 ± 0.11 ‡
	30 - 37	3.4 ± 0.18	3.4 ± 0.11	3.4 ± 0.16	3.3 ± 0.14	3.6 ± 0.15	3.7 ± 0.16 ‡

Values are mean ± SEM. LDL = low density lipoprotein; * = $p<0.05$, † = $p<0.01$, ‡ = $p<0.001$, reference category: lowest parental tertile.

small to allow a meaningful analysis. The direction and magnitude of the differences found for low density lipoprotein-cholesterol levels were quite similar to those found in total cholesterol levels and again differences according to maternal tertiles were higher than according to paternal tertiles.

High density lipoprotein-cholesterol, high density lipoprotein2-cholesterol and high density lipoprotein3-cholesterol levels did not show differences by paternal and maternal tertiles nor were they detectable with increasing age. Pooled levels (over age) for males were: high density lipoprotein-cholesterol 1.2 ± 0.01 mmol/l (mean ± SEM); high density lipoprotein2-cholesterol 0.26 ± 0.010 mmol/l and high density lipoprotein3-cholesterol 0.90 ± 0.008 mmol/l. Pooled values (over age) for females were 1.4 ± 0.02 mmol/l; 0.44 ± 0.014 mmol/l and 0.98 ± 0.011 mmol/l respectively.

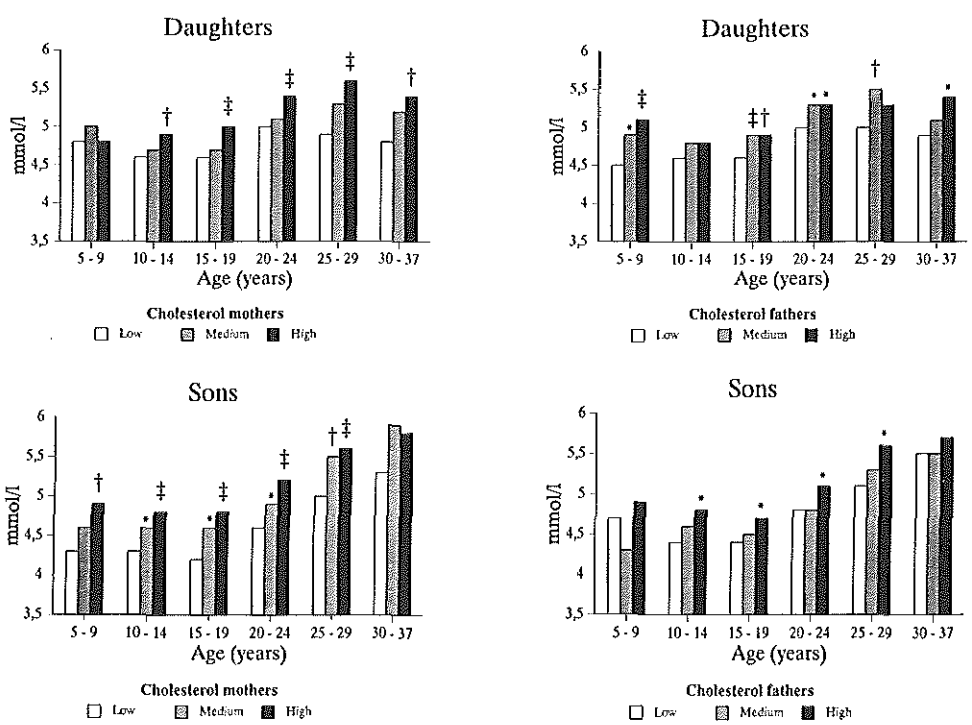

Figure 4.1. Total cholesterol in daughters and sons by tertiles of total cholesterol in mothers and fathers. * = p<0.05, † = p<0.01, ‡ = p<0.001, with lowest tertile as reference category.

Figure 4.1 shows total cholesterol levels separately in daughters and sons by paternal and maternal tertiles of total cholesterol. There were no major differences between the gender specific data and the overall analyses. However, in sons the data were suggestive of a steeper rise in total cholesterol levels after age 15 in both paternal and maternal tertiles.

In figure 4.2 serum total cholesterol levels in offspring are shown according to tertiles of total cholesterol in the fathers (upper left), the mothers (upper right), and both

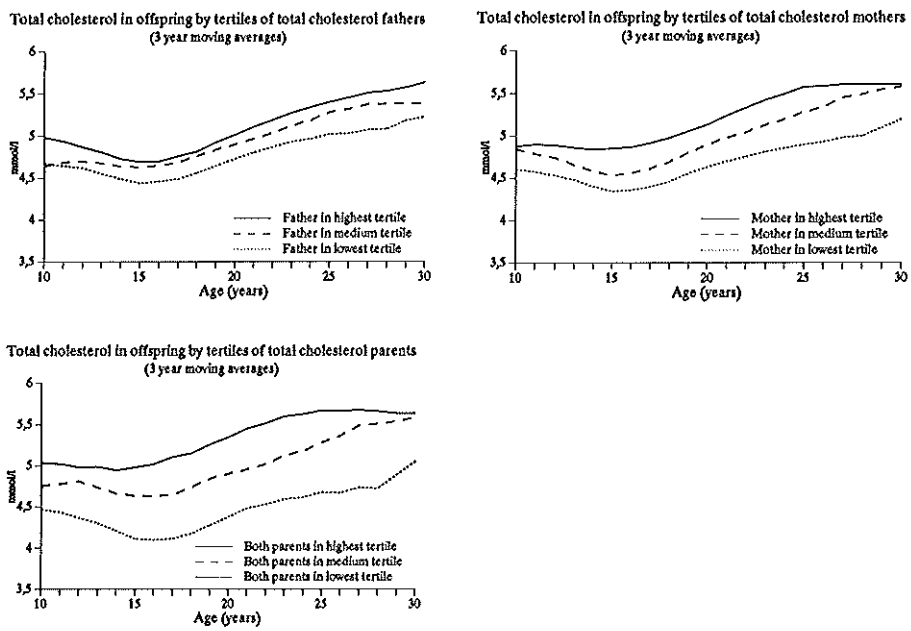

Figure 4.2. Serum total cholesterol levels in offspring according to tertiles of total cholesterol levels in fathers (upper left), mothers (upper right), and both parents (lower).

fathers and mothers (lower panel). When offspring was analyzed that had both fathers and mothers in the same gender specific tertile the offspring differences between parental tertiles increased to almost 1 mmol/l between the lowest and highest tertile. Again, total cholesterol levels dipped between 15 and 20 years of age, most clearly for the lowest two tertiles and showed a gradual increase thereafter.

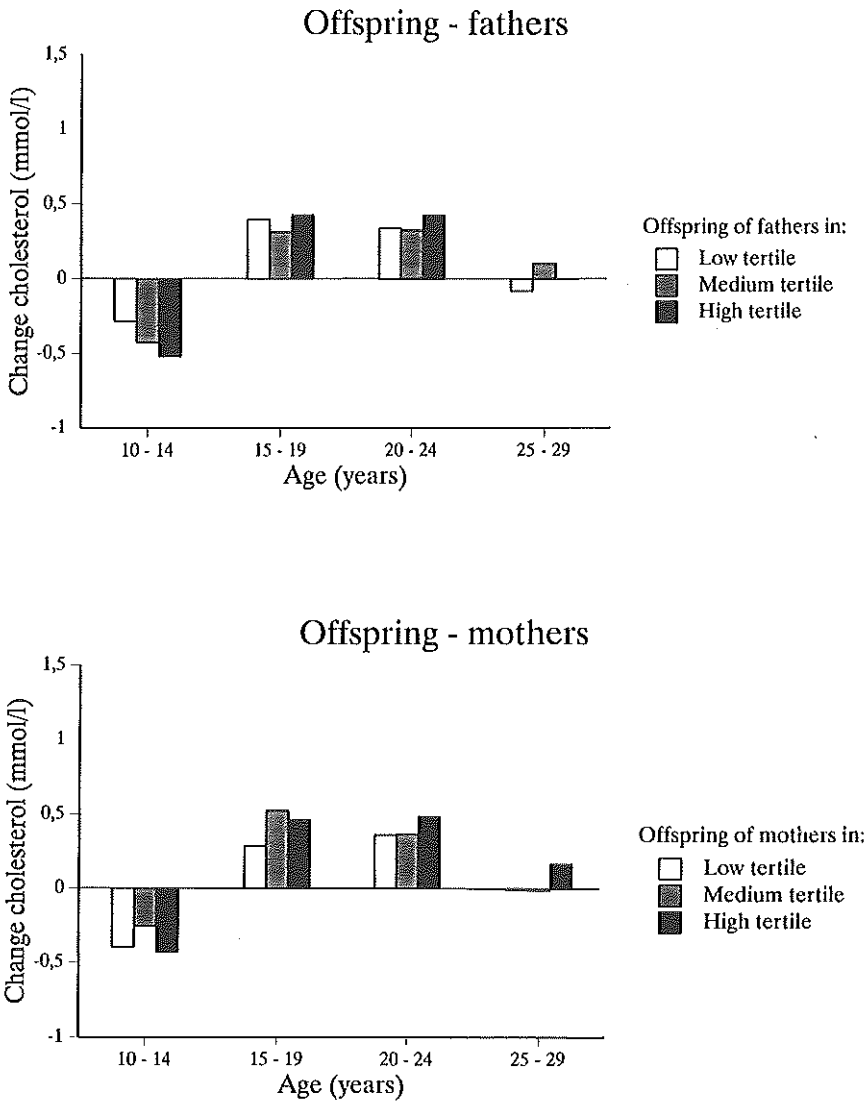

Figure 4.3. Change of total cholesterol level in offspring by tertiles of paternal (upper panel) and maternal (lower panel) total cholesterol. Bars represent mean 5-year change.

Figure 4.3 shows the age-specific change in total cholesterol in offspring by paternal tertiles (upper panel) and maternal (lower panel) tertiles of total cholesterol. It shows that in each

tertile there is a decrease of total cholesterol levels up to age 15 and a rise in total cholesterol levels from age 15 to 25. None of the differences in change between parental tertiles was statistically significant.

Before 1991, there were 131 offspring who terminated their participation in the study. Separate analyses were performed to evaluate if this group of offspring was differentially distributed over parental groups. In the fathers there were 44 offspring from the low group who terminated participation, 35 offspring from the medium group and 33 from the high group. These offspring groups showed differences in total cholesterol levels by parental tertiles for the 5 year age groups which were similar to those found for still participating subjects. In the age category 15 - 19 years the low paternal group (n=41) had mean total cholesterol levels of 4.4 ± 0.11 mmol/l, the medium group (n=26): 4.6 ± 0.12 mmol/l, and the high group (n=26): 4.9 ± 0.15 mmol/l. Similar differences were found when analyzed according to maternal level.

Discussion

In the present study, we have found a strong positive relation between parental levels of serum total cholesterol and levels of serum total cholesterol and low density lipoprotein-cholesterol levels in their offspring. Offspring group differences in total cholesterol and low density lipoprotein-cholesterol were present from childhood and persisted into young adulthood, while throughout this period these group differences remained rather constant. No associations between parental total cholesterol levels and offspring high density lipoprotein-cholesterol and its subfractions were found. The association between maternal total cholesterol levels and offspring total and low density lipoprotein-cholesterol levels appeared slightly more pronounced than for paternal total cholesterol levels. The largest effects were observed when cholesterol levels of both parents were taken into account. The association for daughters and sons did not differ materially.

Before we can interpret these findings some methodological aspects of the study have to be discussed. The present analyses are based on data obtained in a random sample of youngsters initially examined in a population survey and subsequently invited for longitudinal follow-up. It is felt that response rates were high enough to consider this sample representative of the source population. Loss-to-follow-up would have biased our results if offspring loss-to-follow-up would be differentially (with regard to offspring groups) associated with risk factor levels. However, there was no evidence from the data for such differential loss-to-follow-up.

During the whole follow-up period laboratory measurements quality was controlled by the CDC/WHO (total cholesterol and high density lipoprotein-cholesterol) and by KCA (for total cholesterol measurements) and no drift of measurements over time was observed.

Coronary heart disease tends to aggregate in families (17-19). In two recent prospective studies it was shown that a parental history of myocardial infarction is an independent risk factor for coronary artery disease (20,21). A number of cross-sectional studies has shown that the offspring of patients with cardiovascular disease have less favorable (apo)lipoprotein profiles as compared to offspring of non-patients (22-25). This familial aggregation of coronary heart disease may reflect shared genetic factors, shared environmental factors, or both. Coronary heart disease risk factors themselves have also been shown to aggregate within families (7,26,27). The cross-sectional relation between total cholesterol levels in parents and offspring total cholesterol and lipoprotein-cholesterol levels throughout childhood and young adulthood has been reported earlier (28), including in the present cohort (29). Tracking of lipids at a young age has been demonstrated in a number of studies in general populations of children and young adults (1-5). In the present study the parent-offspring relation with regard to cholesterol and lipoprotein-cholesterol levels is further shown to persist longitudinally. This indicates that the influence of parental cholester levels on cholesterol levels in their offspring is apparent particularly at a young age and much less thereafter. In general, the origins of parent-offspring similarity may be genetic, acquired or both. Genetic explanations for familial similarity among relatives of adverse lipid levels are the genetic dyslipoproteinemias, of which the autosomal dominant disorders familial hypercholesterolemia, leading to impaired low density lipoprotein-receptor function and familial combined hyperlipidemia are the most frequent. Familial hypercholesterolemia was confirmed in one family taking part in the present study. The finding that there were no significant differences in change with increasing age of total cholesterol and low density lipoprotein-cholesterol levels between the groups of offspring suggests that parental cholesterol level, although strongly related to offspring level, is not a major determinant of change in offspring levels. This means that offspring group differences in total cholesterol and low density lipoprotein-cholesterol by parental levels of total cholesterol are not only found in cross-sectional analyses but also longitudinally. Thus, on an individual level, children of high risk parents have on average higher levels of the studied risk factors than children of low risk parents irrespectivem of their age. The phenomenon of familial aggregation of cardiovascular risk factors may underlie aggregation of coronary heart disease and it may further provide a tool for its prevention at an early age.

In conclusion, our longitudinal data show a strong positive association between parental cholesterol levels and the levels of total and low density lipoprotein-cholesterol levels in their

offspring.

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CHAPTER 5

GENERAL DISCUSSION I

5. General discussion I

Introduction

In the studies described in this thesis the focus is on etiology; lipids and lipoproteins as putative causal risk factors of atherosclerosis. Common to the definitions of causality in medical science is the notion that exposures antecede health outcomes. As atherosclerosis is a life-long process, a point of departure in the study of causality is to ensure that factors of interest do indeed antecede atherosclerosis. What are the suitable options in study design that meet this requirement? Let us examine the example of postprandial lipid metabolism and coronary artery disease. One option would be to measure postprandial lipids in a group of children and young adults and follow these subjects until manifest coronary artery disease occurs in some of them. This cohort design, although valid in itself, would obviously require a very long study period and large numbers of subjects and thus is rather impractical. Still, such a study has been performed for the classical cardiovascular risk factor serum total cholesterol in young men who were followed for 27 to 42 years (1).

Alternatively, a study may be considered in which postprandial lipid metabolism is examined in groups of diseased and non-diseased subjects. In general, such a study cannot exclude the possibility that the process of atherosclerosis itself influenced postprandial lipid metabolism or that atherosclerotic events and symptoms change behavior, which in turn may influence postprandial lipid metabolism. There is one exception for which this design could be used and the time-sequence criterion could unequivocally be met. If, hypothetically, an unfavorable postprandial lipid metabolism was known to be completely genetically determined and would express at a young age, studying postprandial lipids in patients with coronary artery disease would lead to valid results, in the sense that the exposure, unfavorable postprandial lipid metabolism, precedes the outcome, coronary atherosclerosis, in time. However, as the genetic contribution and mechanistic pathways to postprandial lipids is not fully understood, such a study design will not inherently meet the time-sequence criterion.

A valid and efficient alternative is provided by the patient-offspring study as described in chapters 1 and 2 of this thesis. In the patient-offspring study, risk factors are measured in offspring of patients with the disease and compared with risk factors measured in offspring of parents who do not have the disease. This design allows to study the relation between a risk factor and disease, such as coronary atherosclerosis, where the risk factor is measured in the offspring who have not yet developed coronary atherosclerosis. In the next sections the essential characteristics of this design are discussed.

The patient-offspring study

Design

In general, a study in parents and their offspring may be used to assess the relation between:

1. a risk factor (R) in offspring and the same factor in parents (R-R), or
2. an outcome (D) in offspring and the same outcome in parents (D-D), or
3. a risk factor in offspring and disease in parents: the patient-offspring study

The underlying assumptions in these three types of study are familial aggregation of risk factors (R-R), of disease (D-D), or both. In the first type of study the assumption is similarity of risk factor levels in parents and their offspring, in other words familial aggregation of risk factor levels (R-R). In the second type of study it is expected to find more disease among offspring if in their parents the disease has occurred, in other words familiar aggregation of disease (D-D).

The third type of study will be referred to as the patient-offspring study. In the patient-offspring study the goal is to study risk factors for a disease before the occurrence of the disease. The study design as described in chapters 1 and 2 of this thesis is depicted in figure 5.1. In these studies, a group of fathers with coronary artery disease and a reference group of fathers without coronary artery disease was selected, irrespective of the disease status in the mothers. Levels of putative risk factors were compared between the sons of both groups of fathers.

Premises

1. Familial aggregation of risk factors

One point of departure in a patient-offspring study is the assumption of familial aggregation of disease (D-D aggregation). In this paragraph we will examine how this relates to causes for disease in patients and offspring.

In a multifactorial disease such as coronary artery disease we may distinguish familial risk factors and non-familial risk factors. The familial risk factors may be genetic factors or acquired factors that aggregate in families (R-R aggregation). Non-familial risk factors for

coronary artery disease are not transmitted from one generation to another. The disease is

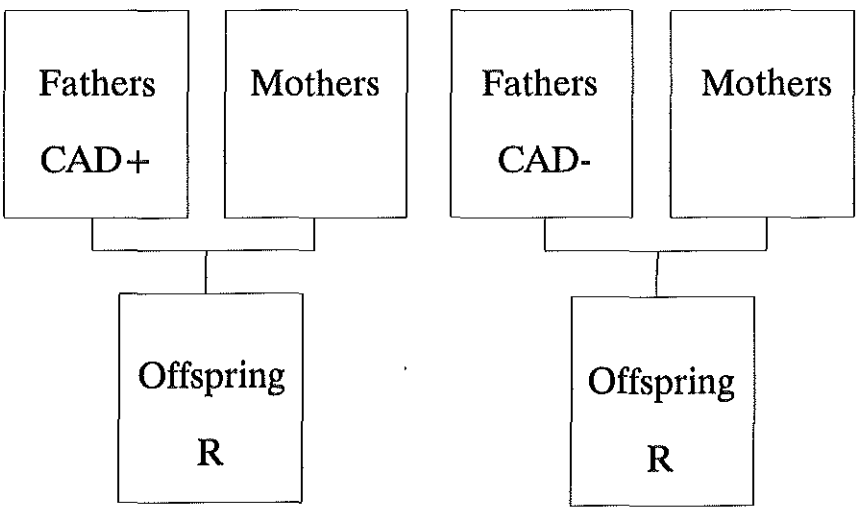

Figure 5.1. The patient-offspring study: a risk factor is studied in offspring of parents with and without coronary artery disease (CAD).

caused by: a set of familial risk factors; or a set of non-familial risk factors; or combinations of familial and non-familial risk factors. Every patient with coronary artery disease has at least one of these three combinations of risk factors.

In an unselected group of patients with coronary artery disease, some patients will have familial and non-familial risk factors, other patients will have familial risk factors only and yet other patients will only have non-familial risk factors. Suppose that we now follow a group of healthy offspring of patients with coronary artery disease from the present into the future and select those who developed coronary artery disease. These offspring will not necessarily have the same distribution of causes for coronary artery disease as the total group of diseased parents. In these offspring we will particularly find familial risk factors, perhaps in combination with non-familial risk factors. In some of the offspring with coronary artery disease we will find sets of non-familial risk factors only. A smaller proportion of the offspring of controls, as compared to offspring of cases, will also develop coronary artery disease in the future. However, because they have healthy parents, familial risk factors,

exclusively or in combination with non-familial risk factors, will be found less frequent in these diseased offspring of controls than in the diseased offspring of patients. In the diseased offspring of controls a much larger proportion of disease will be due to sets of non-familial risk factors. If the familial and non-familial risk factors are not correlated, the percentage of the unselected offspring of controls with non-familial risk factors only will be similar to the percentage of the unselected offspring of cases with non-familial risk factors only. Therefore, in offspring of patients we will find the familial risk factors to be different from those in offspring of controls. The non-familial risk factors may be found in both groups of offspring, but none of these will be differently distributed in offspring of cases compared with controls.

This example shows that the patient-offspring study can only detect differences between offspring groups in risk factors that are familial and not in risk factors that are non-familial. Familial aggregation of the disease thus is not sufficient as a basis for the patient-offspring study, the premise is familial aggregation of risk factors.

2. Tracking by parental risk

Given that a risk factor is familial, the patient-offspring study aims to detect differences in risk factor levels between offspring of patients and offspring of controls. It would facilitate the patient-offspring study if these risk factor differences between offspring groups can be detected in a large age range of the offspring. This requires that the risk factor levels are higher in offspring of patients than in offspring of controls over long periods of time. The group differences may change in magnitude but should remain over time. The examination of such 'tracking by parental risk' requires a study as described in chapter 4 of this thesis, which evaluated whether offspring of high risk parents (based on high cholesterol levels) have high cholesterol levels over time. Risk factors that do not track by parental risk are much more difficult to detect in a patient-offspring study.

Quantification of the effect

In each of the three types of study relating characteristics in parents to characteristics in their offspring, the parent to offspring transmission has to be considered. In the patient-offspring study, given that the disease is multifactorial, the risk factor under study will probably explain only part, if any, of the occurrence of the disease. The subgroup of parents with the risk factor will transmit it to a proportion but not to all of their offspring. In the

hypothetical situation of a purely Mendelian inheritance of a risk factor the expected proportion of offspring with the risk factor can be calculated and is it possible to infer the strength of the true risk factor-disease association (2). Also, if the risk factor were completely acquired it seems unlikely that an environment that has led to high levels of the risk factor in the parents will in all instances lead to similarly high levels in their offspring. Consequently, if in the patient-offspring study a relation is found between a risk factor in offspring and parental disease it will probably always underestimate the strenght of the occurrence relation that one would find in the patients themselves. In other words, the relation between offspring risk factor levels and parental disease is likely to reflect a dilution or smaller derivative of the real occurrence relation.
The extent to which a risk factor aggregates in families (R-R) is an indicator of the underestimation of the occurrence relation. Familial aggregation of disease (D-D) may be predominantly explained by other familial risk factors than the one under study. Thus, familial aggregation of the risk factor is more indicative of the magnitude of underestimation than familial aggregation of disease.

Inference

Except for ecological studies, risk factor and disease status in epidemiologic research are assessed in the same individuals. In the patient-offspring study, however, risk factors and disease are measured in different individuals; offspring and parents. In this type of study it is not immediately apparent what the conceptual unit of observation is. In most studies the increased risk for disease in offspring is taken as the starting point in lipid research (3-7) and, for instance, hypertension research (see for review 8). Thus, many investigators set out to relate offspring risk factor levels to increased offspring disease risk. Here, the offspring are taken as the conceptual units of observation and parental disease is taken as a proxy for disease in the offspring. This will be referred to as the offspring-perspective. This perspective is graphically depicted for atherosclerosis in figure 5.2. It shows the hypothetical situation where there is familial aggregation of atherosclerosis. In the patient-offspring study parents are selected according to their presence of coronary atherosclerosis and risk factors are measured in their offspring at some point in time (T_0). In the cases (upper panel), parents have high degrees of atherosclerosis. In the controls (lower panel), parents have low degrees of atherosclerosis. Based on D-D type aggregation, a proportion of the offspring of cases (upper panel) will at some point in their future develop atherosclerosis. Most of the control offspring

(lower panel) will maintain low degrees of atherosclerosis. Suppose that in the offspring-perspective familial as well as non-familial risk factors are examined in relation to the proxy of disease. As shown earlier non-familial risk factors will not be different between offspring

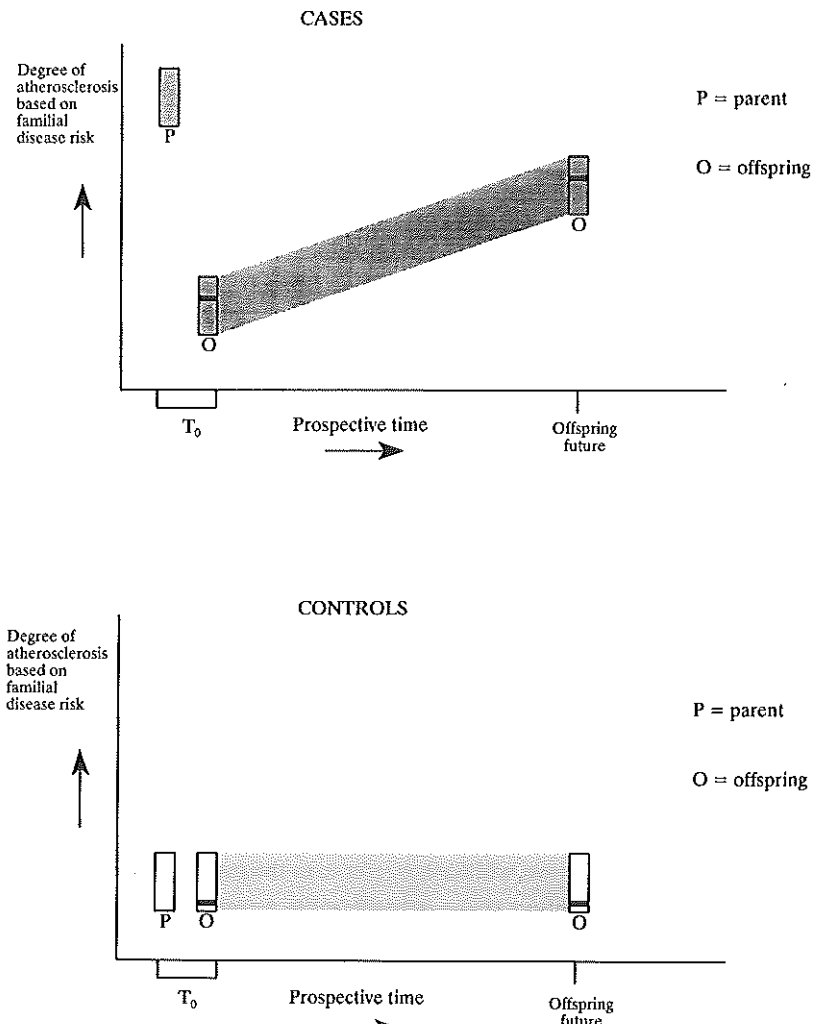

Figure 5.2. Schematical representation of the offspring-perspective to a patient-offspring study. Risk factor levels are indicated by lines within bars.

groups even though they may be causally related to the disease.

From the offspring-perspective, however, it is not uncommon that it will be concluded that non-familial risk factors are not related to the disease, without considering the alternative explanation that such risk factors are not familial. An example of such a pitfall is presented in a landmark report on lipoproteins and apolipoproteins that was published in the New England Journal of Medicine by Freedman and colleagues (7). In this study on data from the Bogalusa Heart Study, the authors report on the assessment of lipoprotein-cholesterol levels and apolipoprotein A-1 and B levels in children of fathers who reported to have had a myocardial infarction as compared with children whose fathers did not report such a medical history. Logistic regression was used to predict on the basis of levels of offspring serum variables the probability of myocardial infarction in the parents. Because the authors stated that this study design may provide "relevant information concerning the future risk of disease", they presumably had the intent to use parental myocardial infarction as a proxy of offspring disease. No offspring differences were found for lipoprotein-cholesterol fractions. Adverse levels of apolipoproteins A-1 and B were found in offspring of fathers with myocardial infarctions. It was concluded that apolipoproteins may be more strongly related to the risk of cardiovascular disease than lipoprotein-cholesterol fractions. However, apolipoproteins A-I and B could show different levels of familial aggregation than lipoprotein-cholesterol fractions. Apolipoprotein levels are probably more genetically determined than lipoprotein-cholesterol levels (9). Differences in familial aggregation between apolipoproteins and lipoprotein-cholesterol fractions could provide an alternative explanation. The question is if there is a better approach to the patient-offspring study that will prevent this type of erroneous inference.

We introduce the parent-perspective as a better approach to the patient-offspring study. In this approach, offspring levels of a risk factor are taken as a proxy for parental levels of this risk factor when they were of the age of their offspring. Parents are chosen as units of observations, an orientation that we will further refer to as the parent-perspective. The parent-perspective to the patient-offspring study is graphically depicted in figure 5.3. The same patient-offspring study as depicted in figure 5.2 is performed at T_0. Now, however, estimates are made of parental risk factor levels in their past through offspring risk factor levels measured at T_0. These estimates are related to parental current disease, with the classical case control study as the paradigm. At T_0, the cases are parents with high degrees of atherosclerosis (upper panel). At T_0, the controls are parents with low levels of atherosclerosis. Atherosclerosis in both offspring of cases and of controls is still minimal.

From the parent-perspective in the patient-offspring study only familial risk factors will be

studied. Non-familial risk factors will not be studied, because they are not a proxy for parental levels of these risk factors. Information about these non-familial risk factors must be obtained

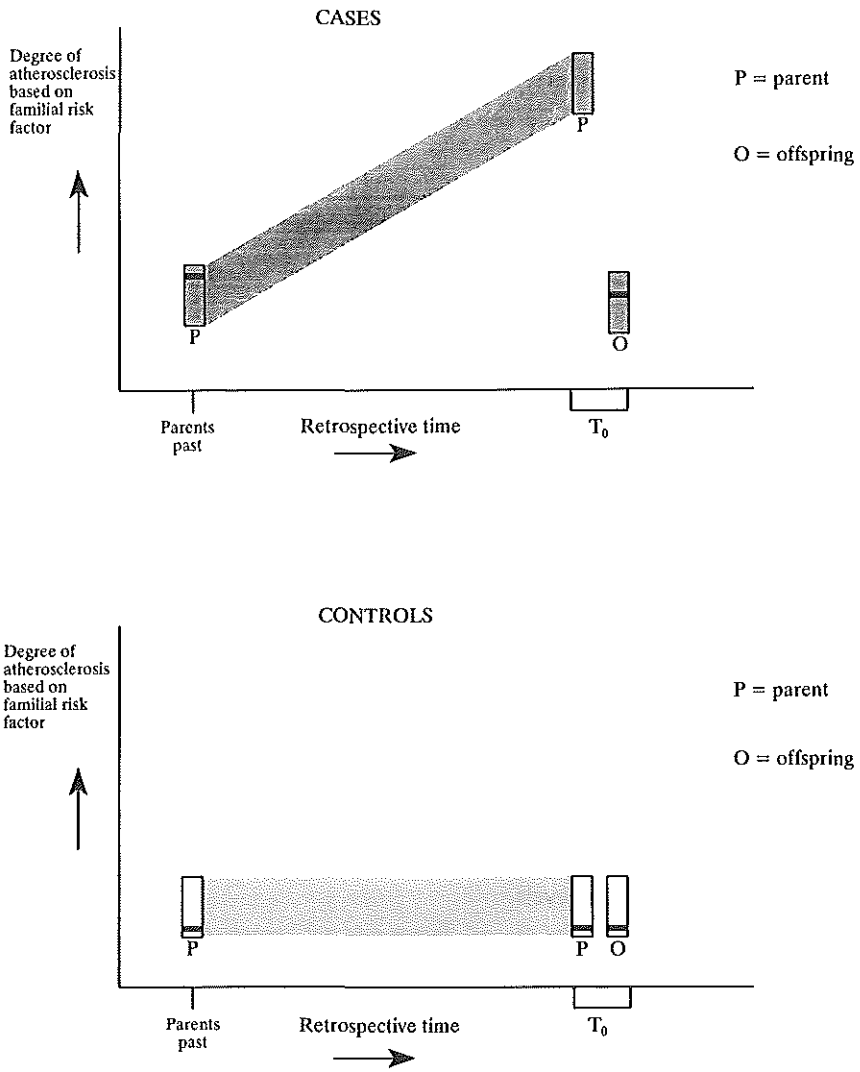

Figure 5.3. Schematical representation of the parent-perspective to a patient-offspring study. Risk factor levels are indicated by lines within bars.

from other research.

Thus, contrary to the offspring-perspective, the parent-perspective to the patient-offspring study inherently meets its prior premise, the familial aggregation of the risk factor under study.

Consideration of familial aggregation of the risk factor is a logical step in the parent-perspective which protects the investigator from the pitfalls of interpretation. Therefore, the parent-perspective provides a better orientation to the patient-offspring study than the offspring perspective.

Summary

The time-sequence of risk factors and subsequent disease occurrence is an important criterion for causality. In the patient-offspring study risk factor levels in offspring are related to disease in their parents and aim to meet the time-sequence criterion. It gives a valid and feasible tool for studying etiological relations between risk factors and a multifactorial disease. While in the patient-offspring study measurements of risk factors and disease take place at one point in time it can detect relationships between risk factors and disease that would otherwise require studies with long follow-up and large numbers of subjects for their assessment.

In this chapter we discussed the premises for the patient-offspring study. It was shown that disease aggregation is not sufficient as a point of departure for the patient-offspring study. A first premise for the patient-offspring study is that there is familial aggregation of the risk factor (R-R). If this premise of familial aggregation risk factor is not met, the absence of familial aggregation of a risk factor should be considered as an alternative explanation for a zero finding. A second premise is that there is 'tracking by parental risk' from young into late adulthood, which facilitates the possibility for a patient-offspring study to detect offspring group differences in risk factor levels.

If there is familial aggregation of the risk factor it is not known to what extent risk factors are transmitted from one generation to the next generation. Therefore, the patient-offspring study will not provide exact information about the strengths of risk factor - disease associations. If risk factors differ in familial aggregation, they will differ with respect to the strenght of association with the disease. This sets limitations to conclusions from the patient-offspring study about which of these risk factors is best suitable for intervention.

The offspring-perspective to the patient-offspring study takes parental disease as a proxy for offspring future disease. Because the disease-proxy involves a selection of causes, in particular

causes involving familial risk factors alone or in combination with non-familial risk factors, this offspring-perspective may lead to wrong inference with respect to non-familial risk factors. The parent-perspective uses offspring risk factor levels as a proxy for parental risk factor levels in the past. Because in the parent-perspective it is a logical step to evaluate the premise of familial aggregation of the risk factor it is considered less vulnerable to erroneous interpretation of results.

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CHAPTER 6

GENERAL DISCUSSION II

6. General discussion II

In general discussion I essential aspects of the patient-offspring study were discussed in relation to familial aggregation of disease and familial aggregation of risk factors. In this chapter familial aggregation of cardiovascular disease and its risk factors will be discussed.

Familial aggregation of cardiovascular disease and cardiovascular risk factors

Heart attacks occur more often in certain families, an observation that dates back decades before the concept of coronary risk factors. Already in the 50's and 60's family history studies demonstrated that coronary heart disease aggregates in families (1-3), particularly when the proband had coronary heart disease at a relatively young age (3-5). Where cardiovascular disease runs in families, the same is true for classical risk factors for cardiovascular disease such as hypertension (6,7), smoking (8), and cholesterol levels as was also shown longitudinally in chapter 4 of this thesis. Other factors related to coronary artery disease including diabetes mellitus (9,10), obesity (11) and physical fitness (12) have been reported to aggregate in families as well. In cardiovascular research a question has been to what extent, if at all, familial aggregation of coronary heart disease may be attributed to familial aggregation of the known cardiovascular risk factors. In a number of prospective studies (13-19), cardiovascular risk profiles were related to the occurrence of cardiovascular disease in subjects who had family relatives with cardiovascular disease and thus had a positive family history. Overall, these studies have indicated that familial aggregation of cardiovascular disease can only partially be attributed to classical cardiovascular risk factors. The question remains how this may be explained. It may be that members of families in which cardiovascular disease aggregates have an increased susceptibility to the effects of classical risk factors. Thus, this increased susceptibility may be an unknown risk factor in itself. Alternatively, the familial aggregation of cardiovascular disease may be related to other, as yet unknown, risk factors. Such new risk factors and their relation with familial cardiovascular disease may be studied with the patient-offspring study. In chapters 2 and 3 the angiographic patient-offspring study was used to assess relatively unexplored risk factors such as changed postprandial lipoprotein metabolism and apolipoproteins. Familial aggregation of postprandial responses to an oral lipid load has, to our knowledge, not been studied. There are, however, some indications for familial aggregation of an increased triglyceride response to an oral lipid load in families of probands with coronary heart disease. Familial aggregation of lipids, cholesterol and its subfractions has been reported in several studies, including determinants

of postprandial lipid metabolism such as triglyceride level and high density lipoprotein-cholesterol level (20-25).

Tracking of risk factors

The phenomenon of tracking of a risk factor for a disease concerns the stability of individual's ranks of the risk factor level within the distribution of this risk factor over time. In general discussion I it was pointed out that 'tracking by parental risk' is one of the premises for the patient-offspring study.

A number of longitudinal studies in children and young adults has shown tracking for total cholesterol and low density lipoprotein cholesterol in childhood and young adulthood, with weaker levels of tracking for high density lipoprotein cholesterol and triglycerides (26-35). The apolipoproteins A-I and B were shown to track to a similar extent as high density lipoprotein cholesterol and low density lipoprotein cholesterol respectively (36). Recently, 3-year tracking was shown for high density lipoprotein$_2$ and to a lesser extent high density lipoprotein$_3$-cholesterol (37), high density lipoprotein$_2$ being considered a determinant of postprandial triglyceride metabolism (38). Thus, there is a large body of evidence from population studies that lipoproteins and apolipoproteins track over time. To our knowledge there have been no reports actually showing tracking of postprandial lipids and lipoproteins over time. Repeated measurements of postprandial triglyceride responses suggested some degree of stability of ranking, although these were performed in small numbers of subjects and over short time intervals (39,40).

In the study of tracking by parental risk, categories are made of parental risk factor levels and the ranks of offspring risk factor levels are followed over time according to these parental categories. Parent-offspring similarity of total cholesterol values throughout childhood and early adulthood has been shown previously (23,41). The study in chapter 4 on the EPOZ population describes the yearly follow-up of total cholesterol and lipoprotein-cholesterol levels in offspring according to tertiles of total cholesterol levels in their parents. It illustrates that total cholesterol and low density lipoprotein cholesterol values in offspring are related to parental levels of total cholesterol. There were clear differences between offspring of parents with high cholesterol levels as compared to parents of low cholesterol values, for both total cholesterol and low density lipoprotein cholesterol. The differences appear to remain fairly constant throughout childhood. This is further substantiated by the analysis of change of cholesterol levels during childhood within subjects. Low density lipoprotein-cholesterol and

high density lipoprotein-cholesterol were shown to track to a similar extent as their main constituent apolipoproteins B and A-I, respectively (45). One might speculate that tracking by parental risk of these apolipoproteins resembles tracking by parental risk of their corresponding lipoproteins. For the angiographic patient-offspring study on apolipoproteins and coronary atherosclerosis as described in chapters 2 and 3 of this thesis more direct information on tracking by parental risk was available. Studies on the relation between apolipoprotein levels in offspring and myocardial infarction in their parents indicated tracking by parental risk of apolipoprotein levels (42-44). There have been no studies on tracking by parental risk of postprandial lipoproteins. Tracking of postprandial lipid response to an oral lipid load may be related to the tracking of determinants of this response such as triglyceride level and high density lipoprotein$_2$ (37). However, inference about tracking of this response by parental risk is speculative.

Summary

In this chapter the familial aggregation of cardiovascular disease and of cardiovascular risk factors were discussed. Familial aggregation of cardiovascular risk factors within families and tracking of such factors by parental risk are the main premises for a patient-offspring study. Current evidence shows that lipids and lipoproteins are familial, and that they as well as apolipoproteins show tracking. In previous studies, tracking by parental risk was demonstrated for apolipoproteins in offspring of parents with myocardial infarction. For the studies in chapter 2 and 3 of this thesis the premise of tracking by parental risk was thus met for apolipoproteins but speculative for postprandial lipoproteins.

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CHAPTER 7

SUMMARY

7. Summary

Cardiovascular disease is the leading cause of morbidity and mortality in western industrialized countries. Numerous studies have shown that classical risk factors such as hypertension, high levels of total and low density lipoprotein-cholesterol, low high density lipoprotein-cholesterol and smoking are related to the occurrence of cardiovascular disease. The atherosclerotic process starts early in life and from an etiologic and a preventive point of view it is important to know if and to what extent this process and the diseases resulting from it are related to risk factors measured in the young. Cardiovascular disease aggregates in families, which implies that healthy relatives of patients with cardiovascular disease have an increased risk for developing the disease themselves. Current evidence suggests that if in certain families patient(s) have cardiovascular disease, otherwize healthy relatives of those patients have an increased risk of the disease, a relation which is seemingly not predominantly caused by aggregation of classical cardiovascular risk factors. Risk factors for cardiovascular disease are also known to aggregate in families, meaning that there is a resemblance of the levels of cardiovascular risk factors among family members. A method for assessing the relation between putative risk factors and cardiovascular disease is to study such factors in offspring of patients with the disease. The general research question is if this new cardiovascular risk factor is causally related to the occurrence of the disease in the parents.

Chapter 2 describes a study on postprandial triglyceride metabolism in 80 sons of fathers with angiographically established coronary sclerosis and in 55 sons of fathers who had at most only minor coronary atherosclerotic lesions at coronary angiography. Both groups of sons received an oral lipid load and the subsequent postprandial handling of this lipid load was studied in their serum for 12 hours after lipid loading. The sons of coronary artery disease patients had a prolonged postprandial hypertriglyceridemia.

Chapter 3 describes a study on lipoprotein-cholesterol levels and apolipoprotein levels in 188 offspring of fathers with angiographically established severe coronary sclerosis and in 115 offspring of fathers who had at most only minor coronary lesions at coronary angiography. In this study sons of patients with coronary artery disease had significantly lower mean levels of high density lipoprotein3-cholesterol and of apolipoprotein A2. Although similar trends for high density lipoprotein3-cholesterol and apolipoprotein A2 levels were observed in daughters of patients none of the differences was statistically significant.

Chapter 4 describes an 18-year follow-up study on cardiovascular risk factors in 252 male and 231 female children and young adults. These subjects were a random sample of youngsters initially examined in the cross-sectional EPOZ study, a population based study on risk factors for chronic diseases, conducted in 1975 - 1978 in the Dutch town of Zoetermeer,

and who were subsequently invited to take part in the follow-up study. In the initial EPOZ study, the parents of these subjects were also examined. Total cholesterol levels measured in the parents were then related to levels and change of levels of total cholesterol and its lipoprotein-subfractions, which were repeatedly measured on a yearly basis. The results show a strong cross-sectional as well as longitudinal relationship between parental total cholesterol levels and levels of total cholesterol and low density lipoprotein-cholesterol throughout childhood and adolescence. Overall, levels showed an initial tendency to decrease untill about 15 years of age with a rise in levels thereafter. Despite these changes in lipid levels differences between groups of offspring contrasted by total cholesterol levels in their parents appeared to be stable, indicating that parental total cholesterol level is not a major determinant of change of cholesterol and lipoprotein-cholesterol levels during childhood and adolescence. No offspring group differences were found for high density lipoprotein-cholesterol and its subfractions.

In chapter 5 some considerations are given for the use of the patient-offspring study (chapter 2 and chapter 3) as a means to study the putative causal relationship between some factor as measured in offspring, and disease as measured in parents. It is postulated that familial aggregation of the risk factor as well as tracking by parental risk of the factor in offspring are premises for the patient-offspring study. An often used approach to the patient-offspring study is the offspring-perspective which means that offspring of parents with cardiovascular disease are at increased risk for the disease and risk factors measured in offspring are related to their increased disease risk. It is shown in chapter 5 that familial aggregation of disease is not sufficient as a premise for the patient-offspring study. The parent-perspective to the patient-offspring study considers risk factor levels in offspring as an approximation of the level of the factor in parents when they were of the age of their offspring. Thus, consideration of the premise of familial aggregation of the risk factor is a logical step in this perspective and, therefore, the parent-perspective is postulated to be a preferable approach to the patient-offspring study.

In chapter 6, the patient-offspring studies as described in chapters 2 and 3 are discussed in the context of the current evidence for the relation between cardiovascular risk factors and familial aggregation of cardiovascular disease. The study as described in chapter 4 is discussed as being further evidence for familial aggregation of cardiovascular risk factors such as total cholesterol and low density lipoprotein-cholesterol and as a model to investigate the premises for the patient-offspring study.

7. Samenvatting

Hart- en vaatziekten vormen de belangrijkste oorzaak van morbiditeit en mortaliteit in westerse geïndustrialiseerde landen. Vele studies hebben aangetoond dat klassieke risicofactoren zoals hypertensie, verhoogde plasma gehalten van totaal en low density lipoproteine-cholesterol en roken zijn gerelateerd aan het ontstaan van hart- en vaatziekten. Het proces van atherosclerose begint vroeg in het leven. Het is daarom van belang vanuit etiologisch en preventief oogpunt om na te gaan in hoeverre dit proces en de daaruit resulterende ziekten samenhangen met risicofactoren bij jongeren. Hart- en vaatziekten aggregeren in families, hetgeen suggereert dat gezonde verwanten van patiënten met hart- en vaatziekten zelf een verhoogd risico hebben op deze ziekten. Ook risicofactoren voor hart- en vaatziekten aggregeren in families, hetgeen betekent dat niveau's van deze risicofactoren in met elkaar verwante familieleden overeenkomen. Onderzoek laat zien dat gezonde verwanten van patiënten een verhoogd risico op hart- en vaatziekten hebben, waarbij kan worden aangetekend dat deze relatie niet uitsluitend kan worden verklaard op basis van familiaire aggregatie van cardiovasculaire risicofactoren.

Een methode om de relatie tussen veronderstelde risicofactoren en hart- en vaatziekten te onderzoeken is het bepalen van zulke risicofactoren in nakomelingen van patiënten die lijden aan dergelijke ziekten. De algemene onderzoeksvraag is daarbij of er een causaal verband bestaat tussen deze risicofactoren en het ontstaan van de ziekten.

Hoofdstuk 2 beschrijft een onderzoek naar het postprandiale triglyceriden metabolisme in 80 zonen van vaders met angiografisch aangetoonde coronair atherosclerose en in 55 zonen van vaders die bij angiografie hoogstens minimale coronairafwijkingen hadden. Beide groepen zonen kregen een orale vetbelasting toegediend en de postprandiale verwerking daarvan werd bestudeerd in het bloed gedurende 12 uur na toediening van de vetbelasting. Zonen van vaders met coronair atherosclerose hadden ten opzichte van zonen uit de controle groep een verlengde postprandiale hypertriglyceridaemie.

Hoofdstuk 3 beschrijft een onderzoek naar niveau's van lipoproteine-cholesterol en apoproteinen in 188 nakomelingen van vaders met angiografisch aangetoonde coronair atherosclerose en in 115 nakomelingen van vaders met angiografisch hoogstens minimale coronaire afwijkingen. Zonen van patiënten hadden gemiddeld statistisch significant lagere waarden van high density lipoproteine3-cholesterol en van apoproteine A2. Hoewel vergelijkbare trends voor waarden van high density lipoproteine3-cholesterol en apoproteine A2 te zien waren bij dochters van patiënten waren de groepsverschillen niet statistisch significant.

Hoofdstuk 4 beschrijft een vervolgstudie, uitgevoerd over een periode van 18 jaar, naar

cardiovasculaire risicofactoren in 252 mannelijke en 231 vrouwelijke kinderen en jonge volwassenen. Deze groep kinderen vormde een random sample uit jongeren die zijn onderzocht in het Epidemiologisch Preventief Onderzoek Zoetermeer (EPOZ). Het EPOZ onderzoek is een populatie onderzoek naar risicofactoren voor chronische ziekten, dat in de periode 1975 - 1978 is uitgevoerd in Zoetermeer. Deze groep heeft deelgenomen aan een vervolgstudie waarbij serum totaal cholesterol gehalten en lipoproteinen-fracties jaarlijks zijn gemeten. In het EPOZ populatie onderzoek zijn serum totaal cholesterol gehalten tevens eenmalig gemeten bij de ouders van deze groep kinderen. De gehalten in de ouders zijn bestudeerd in relatie tot de herhaald gemeten gehalten bij hun kinderen. De resultaten laten zien dat er een sterk cross-sectioneel en ook longitudinaal verband bestaat tussen ouderlijke totaal cholesterol gehalten en de totaal cholesterol en low density lipoproteine-cholesterol gehalten bij kinderen en jonge volwassenen. In alle groepen kinderen vertoonden de totaal cholesterol gehalten een daling tot ongeveer het 15e jaar en daarna een stijging. Ondanks deze veranderingen bleven de verschillen tussen de groepen kinderen, ingedeeld naar cholesterol niveau's van hun ouders, gelijk. Dit wijst erop dat ouderlijke cholesterol gehalten wel een belangrijke determinant zijn van de niveau's van totaal cholesterol en low density lipoproteine-cholesterol tijdens de jeugd, maar niet van de veranderingen hierin in de tijd. Voor high density lipoproteine-cholesterol en de subfracties daarvan werden geen verschillen bij de kinderen waargenomen.

In hoofdstuk 5 worden enkele overwegingen gegeven voor het onderzoeken van patienten en nakomelingen (patient-offspring onderzoek, hoofdstukken 2 en 3) als methode om het veronderstelde oorzakelijk verband te bestuderen tussen een risicofactor zoals gemeten in kinderen en de ziekte zoals gemeten in hun ouders. Besproken wordt dat familie aggregatie van een risicofactor alsmede "tracking naar ouderlijk risico" van de risicofactor in de kinderen aannamen zijn voor de patient-offspring studie. Een vaak toegepaste benadering van de patient-offspring studie is de benadering vanuit de kinderen (offspring-perspective). Dit houdt in dat kinderen van patienten met cardiovasculaire ziekten zelf een verhoogd risico op deze ziekten hebben en risicofactoren, gemeten in de kinderen, worden gerelateerd aan dit verhoogde ziekte risico. In hoofdstuk 5 wordt gedemonstreerd dat familie aggregatie van ziekte als aanname niet voldoende is voor de patient-offspring studie. Bij de benadering vanuit de ouders (parent-perspective) worden niveau's van risicofactoren in kinderen beschouwd als een schatting van de niveau's van deze factoren in de ouders toen zij de leeftijd van hun kinderen hadden. In deze benadering is het een logische stap om rekening te houden met de aanname van familie aggregatie van de risicofactor. Om deze reden zou in de patient-offspring studie de benadering vanuit de ouders moeten worden verkozen boven die vanuit de kinderen.

In hoofdstuk 6 worden de patient-offspring studies zoals beschreven in de hoofdstukken 2 en 3 geplaatst in het licht van de huidige kennis over de relatie tussen risicofactoren en familie aggregatie van hart- en vaatziekten. Het in hoofdstuk 4 beschreven onderzoek wordt besproken als nieuwe aanwijzing voor familie aggregatie van risicofactoren voor hart- en vaatziekten zoals totaal cholesterol en low density lipoproteine-cholesterol gehalten. Tevens wordt dit onderzoek beschreven als model om de aannamen voor de patient-offspring studie te onderzoeken.

Dankwoord

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