Progression and Regression of Coronary Atherosclerosis

Role of Diet, Lipoproteins and Lipases

Proefschrift-begeleidingscomissie

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Progressie en regressie van coronaire atherosclerose
De rol van het dieet, de lipoproteïnen en de lipases

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
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List of abbreviations

HDL	high-density-lipoprotein
IDL	intermediate-density-lipoprotein
LDL	low-density-lipoprotein
TC	total-cholesterol
TG	triglycerides
HL	hepatic lipase = LLA = liver lipase activity
LPL	lipoprotein lipase
T_4	thyroxine
T_3	triiodothyronine
CAD	coronary artery disease
CHD	coronary heart disease
AMI	acute myocardial infarction
ECG	electrocardiogram

Contents

Success has many fathers, failure is an orphan.

CHAPTER I

Short summary of the literature and discussion of the results of the investigations

The evolution and natural course of human coronary artery disease and risk factor mitigation programs can be studied by comparing sequential coronary angiograms of the same patient at different moments in time.[1] Although tens of thousands of angiograms are performed each year to elucidate the possible anatomical substrate of clinical manifestations, a very limited number is carried out to assess the effects of an intervention with the aim to decelerate the natural progression of coronary artery disease. As having a distorted lipid metabolism is one of the main risk factors which tend, if uncorrected, to accelerate the natural course of coronary atherosclerosis, several studies have been published that focus on the effect of lipid lowering on coronary atherosclerosis. A clear relationship between lipoprotein concentrations and severity of atherosclerosis has been shown to exist,[2,3] and the results of nine intervention studies with regard to coronary atherosclerosis, carried out between 1975 and 1985 are summarizes in Table I. A brief description of these follows.

Cohn et al.[4] used clofibrate in 40 severely atherosclerotic diseased patients. No diet was prescribed and the angiograms were assessed by visual interpretation. Sequential angiography with an interval of one year, showed regression to occur (data not shown). Blankenhorn et al.[5] studied femoral arteries. For intervention they used a lipid lowering diet and (mainly) clofibrate. With a computerized measuring technique the authors noted that after one year, regression of atherosclerosis had occurred in 14 of 25 patients. Rafflenbeul et al.[6] used a Vernier caliper to measure coronary diameters on the two sequential angiograms of patients with unstable angina carried out with an interval of one year. Patients were given an 'optimal' medical treatment. Five of the 25 patients studied showed signs of coronary atherosclerotic regression. No specific lipid lowering therapy or diet was administered. Kuo et al.[7] used diet and colestipol in the treatment of hyperlipidemic patients. The average interval for sequential coronary angiography was three years. In twenty-one of the 25 patients studied atherosclerotic lesions stabilized. Nash et al.[8] had similar results when he prescribed colestipol to 42 patients for 2 years. Duffield et al.,[9] studying femoral arteries, used diet, cholestyramine, nicotinic acid and clofibrate for intervention. With repeat angiograms performed after 18 months, 71 out of the 300 segments studied showed signs of regression of atherosclerosis. Nikkilä et al,[10] used diet, clofibrate and nicotinic acid as a lipid lowering therapy. When the repeat angiogram was performed after three years the therapy resulted in a slower rate of progression of coronary artery disease. Brensike et al,[11] who used diet and cholestyramine found 5 years later definite signs of regression of

coronary atherosclerosis in the repeat angiograms in 18 of the 116 patients studied. Arntzenius et al.,[12] with a computerized measuring technique to 'read' coronary angiograms found definite regression to occur in 7 of the 39 patients who participated in the study, while stabilization of the lesions was seen in 11 patients. Sequential angiography was performed after two years of study and (lipid lowering) intervention consisted solely of diet.

All of the above mentioned studies with the exception of those of Cohn and Rafflenbeul showed a definite correlation between the anatomical changes observed and serum cholesterol levels. Since the natural history of coronary artery disease when no intervention is undertaken, seems to be one of an unequivocal progression,[3,14] any possibility to decelerate lesion growth should be viewed with great interest. Even a slight deceleration if extrapolated on a lifetime period, could mean an amelioration of the quality of life.[15] Further research should be directed towards a thorough analysis of the relation between angiographic findings and pathological substrate of coronary stenosis.[16-18] However, we should keep in mind that the physiological state of the patient (during coronary angiography) may substantially influence the results. Dynamic changes in luminal diameter due to varying vasomotor tone, platelet aggregation, thrombolysis or the state of the local atherosclerotic disease itself may alter the angiographic apperance of the disease.[19,20] With the development of computerized quantitive measuring methods of arterial luminal width on coronary angiograms a more sophisticated and reliable tool has come to hand to limit bias and variations in angiograms interpretations.[21,22]

There is growing evidence that the process of atherosclerosis e.g. lesion growth of the plaques can be halted or perhaps even be reversed.[23,24] Nine studies that made use of interventions, and that were probably by no means optimal, tended to show that regression of atherosclerosis is feasible. Larger controlled studies are required to show by which type of intervention (diet and/or drugs) regression of atherosclerosis can be achieved most efficaciously. Before we are able to study the process of progression and regression of atherosclerosis, we should review the origins of the disease before we are capable to make decisions that may influence the natural course.

Large epidemiological studies have shown that lipid disorders are strongly correlated to morbidity and mortality of cardiovascular diseases.[16,17] Cholesterol, the most important lipid, is mainly transported in the low-density-lipoprotein (LDL) fraction of the lipoproteins. Other lipid fractions are triglycerides, which are mainly carried by chylomicrons and very-low-density-lipoprotein (VLDL) and phospholipids which are principally found in the high-density-lipoproteins (HDL). The distinction between these before mentioned lipoprotein fractions, is made on the basis of variations in size, density, lipid composition and apolipoprotein content.

LDL(-cholesterol) is positively correlated to coronary artery disease (CAD), while HDL-cholesterol (HDL-C) has an inverse correlation to this process. There-

TABLE I
Arteriographic assessment of interventions studies on atherosclerosic lesion growth

Author	Year published	Number of patients involved	Artery studied	Duration	Drug used	Diet	Controlled study	Measurements techniques*	Correlation of lesion change to serum cholesterol value	Definite regression
Cohn et al	1975	40	coronary	1 yr	clofibrate	−	+	visual (4)	−	−
Blankenhorn et al	1978	25	femoral	1 yr	clofibrate (+ neomycin)	+	−	visual + computer	+	14/25 pts.
Rafflenbeul et al	1979	25	coronary	1 yr	optimal med. treatm.	−	−	vernier caliper	−	5/25 pts.
Kuo et al	1979	25	coronary	3 yrs	colestipol	+	−	visual (3)	+	−
Nash et al	1982	42	coronary	2 yrs	colestipol	−	−	visual (2)	+	−
Duffield et al	1983	24	femoral	1.5 yrs	cholestyr., nicot. acid clofibrate	+	+	visual + computer	+	71/300 segm.
Nikkilä et al	1984	30	coronary	3 yrs	clofibrate nicot. acid	+	−	visual (2)	+	−
Brensike et al	1984	116	coronary	5 yrs	cholestyr.	+	+	visual (3)	+	18/113 pts.
Arntzenius et al	1985	39	coronary	2 yrs	−	+	−	visual (2) + computer	+	7/39 pts.

* number of independent observers
segm. = segments
pts. = patients

fore, they are respectively considered as 'atherogenic' cholesterol and 'anti-atherogenic' cholesterol.[18,19] Other fractions like chylomicrons and VLDL are not conclusively correlated to atherosclerosis development.[20,21] As a high LDL-level is accelerating CAD development and a high HDL-level is retarding this growth, an investigation was undertaken to assess the risk factor profile in CAD in male survivors one year after they had suffered a first acute myocardial infarction (AMI). The results of this investigation indicate that, contrary to the patients with age in the general population, total-cholesterol (TC) and triglycerides (TG) were significantly higher in the younger patients than in the older ones while HDL was significantly lower as compared to the older age groups (Chapter II). Although these findings are in accordance with the risk factor principle they are nevertheless disappointing. Apparently during the one year period after the AMI, lipid disorders were not sufficiently assessed and adequately treated. Although the most firmly established risk factor for CAD development remains a disordered lipid metabolism, cigarette smoking and systemic hypertension appear also to be of great importance. The exact mechanism by which smoking is harmful to the heart is

unknown.[22] Nevertheless, accelerated coronary atherosclerosis has been reported in smokers.[23,24] A specific pattern of coronary atherosclerosis as assessed by coronary arteriography has been established under these circumstances.[25] An interesting aspect concerning smoking and CAD is the fact that a survivor of an AMI who gives up smoking has been shown to improve the life expectancy. Two distinct mechanisms of the harmful effects of smoking can be described. One, that induces cardiac arrhythmias and one, that acts slowly and is compatible with an accelerated rate of the atherosclerotic process. In the before mentioned risk factor analysis study in which male survivors of an AMI were evaluated, a significant difference was found between the three age groups assessed (Chapter II). The youngest group of patients, forty years or younger, all smoked before they suffered an AMI. Other risk factors considered were hyperlipoproteinemia and hypertension. Much has been said about hypertension and its treatment in cardiovascular diseases. The fact that hypertension is strongly and positively correlated to cerebrovascular diseases but not whith CAD, is of particular interest.[26,27] Other points worth noting are the metabolic side effects of antihypertensive drugs. Therefore, the gain in risk factor lowering may be lost due (to facilitation) of the induction of atherogenic metabolic disorders.[28] It is noteworty that the etiology of hypertension is unknown, that treatment is almost always possible and that other factors like lipid disorders must play a role, before hypertension can express itself in CAD.[29,56] Nevertheless, in view of the assessment of the high frequency of lipid disorders in a group male survivors of an AMI, an evaluation of the incidence of risk factors is of great importance given the life expectancy of these young patients. In this evaluation study, hypertension was more prevalent in the younger patients than in the older age groups (Chapter II). This finding should be viewed while keeping in mind the fact that due to an AMI, left ventricular muscular mass is lost and as a result, the individual is less capable to develop systemic hypertension. How hypertension in CAD may accelerate atherosclerosis when hyperlipidemia is present, remains to be investigated, although several proposals have been made.[30]

Recently several studies have been published, stressing the importance of treatment of (even) mild hypertension.[31-33] From these studies one may derive that antihypertensive treatment does not affect mortality or morbidity of CAD. Antihypertensive treatment affects the incidence of cerebrovascular diseases, although even here the cost-benefit ratio is high.

Coronary prone behavior or type 'A' behavior has been described as an important and independent risk factor for CAD.[34] This behavioral pattern has been significantly more prevalent in young male survivors of an AMI than in older age groups (Chapter II). Encouraging results have recently been published of attemps to mitigate this kind of behavior.[35]

Although many more coronary risk factors may contribute to CAD, with regard to their relatively lower grade of importance, these factors have not been investigated by us.[36]

As serum lipoproteins remain the most important determinants of coronary atherosclerosis growth, modulators of lipoprotein metabolism are thought to play a prominent role. The postheparin lipoprotein lipase activities are lipoprotein modulators and can be divided into lipoprotein lipase (LPL) and liver lipase activity or hepatic lipase (HL). Both are located in the endothelial cells lining blood vessels. LPL may regulate the catabolism of triglyceride rich lipoproteins, chylomicrons and VLDL,and may therefore modulate to a certain extent part of LDL metabolism.[37] Recently published reviews indicate the importance of LPL as HDL modulator.[38] The exact function of the other postheparin lipoprotein lipase, HL is less clear.[39] Data from several studies have indicated that HL may be associated with the conversion of intermediate-density-lipoprotein (IDL) to LDL.[40] Another function may be clearing of cholesterol into bile through an intervention in the HDL metabolism.[39]

In a controlled study that compared male patients with diffuse severe atherosclerosis on the coronary angiogram with angiographically assessed coronary atherosclerosis-free individuals, LPL and HL were measured. In the atherosclerotic group HL was significantly lower than in the other group and LPL had identical (normal) values in both groups (Chapter III).

In the Leiden Intervention Trial (Chapter IV), in which sequential coronary angiography was performed to assess progression of atherosclerosis during vegetarian dietary intervention, HL and LPL were measured. By multivariate analysis HL was shown to be the variable correlated most strongly with regression of atherosclerosis (Chapter V and VI). Although aspects of Chapter V contain some data used in Chapter VI, only the former paper contains the crucial description of the methodology used to assess quantitative coronary atherosclerosis measurements. Furthermore, only Chapter V contains the data and the discussion concerning different response patterns to dietary interventions on the lipoprotein lipases values, and all atherosclerosis linked hormones (Chapter V, Table VI).

Those patients who showed initially a high TC/HDL-C ratio and who showed benefit from dietary intervention (with significant lowering of TC/HDL-C ratio) by showing a regression or no progression of coronary atherosclerosis had normal HL values, while the patients who showed further progression of the atherosclerotic process at least ultimately, had low HL values. The LPL values were not different in the groups with progression and regression (or no progression) of coronary atherosclerosis, respectively.

Another aspect which may further elucidate the mechanism of regression of atherosclerosis is the fact that in the patients with regression or no progression of atherosclerosis triiodothyronine (T_3) was significantly higher than in the group with progression of atherosclerosis, independent from the lipid fractions (HDL, LDL, TG, TC, VLDL) and the lipoprotein lipases, HL and LPL. The T_4 fraction is considered the thyroid hormone 'pool' from which in the peripheral tissues, mainly the liver, the metabolically active T_3 is formed by conversion.[41] It is well known that

bètablocking agents inhibit this conversion. No significant difference could be found in the number of patients treated with these agents in the groups with progression and regression or no progression of atherosclerosis, respectively (Chapter VI). It is well known that hypothyroidism induces an elevation of LDL and that thyroid hormone substitution normalizes this lipoprotein fraction.[42] This effect was clinically tested in the Coronary Drug Project by the administration of thyroxine to euthyroid patients suffering from coronary atherosclerosis, but this study had to be stopped prematurely due to the development of cardiac arrhythmias.[43] No assessment of the development of coronary atherosclerosis and its complications was performed. In the Leiden Intervention Trial no significant correlation between thyroid hormone levels and postheparin lipoprotein lipases could be demonstrated (Chapter VI).

Another aspect of the epidemiology of CAD is the striking higher in incidence in men when compared to pre-menopausal women. Post-menopausal women have the same incidence of CAD as men.[44] This observation together with the fact that there is evidence that a high estradiol level in men may promote AMI,[45] has led to investigate sex hormones in coronary atherosclerosis. In an earlier investigation we could not find a relationship between plasma testosterone and the HDL-cholesterol fraction.[46] In another study (Chapter III) we found a significantly higher estradiol/testosterone ratio in normolipemic men with severe diffuse coronary atherosclerosis than in men angiographically free from coronary atherosclerosis. It should be stressed that the patients in this study represented two extremes of the atherosclerosis spectrum, while in the Leiden Intervention Trial (Chapter IV), where we did not observe such a difference between the progression and the regression group, there was a relatively small difference in the severity of the atherosclerotic process. Nevertheless, we feel that in men sex hormones, by modulating the lipid fractions, may directly or indirectly play a role in the progression of atherosclerosis.[47]

Glucagon, a biologic antagonist of insulin, is a hormone that to a large extent regulates blood sugar levels in response to sudden changes in the provision of energy.[48] Glucagon, therefore, may be a risk factor in the process of coronary atherosclerosis. Insulin is supposed to be atherogenic in adipose people.[49] Therefore, we assessed both glucagon and insulin levels in the above mentioned two groups of men. The group with coronary atherosclerosis did show a significantly higher glucagon level than the coronary angiographically atheroslerosis-free individuals. Insulin was not different between the groups (Chapter III). Other studies have indicated the importance of insulin for the HDL-cholesterol metabolism.[50] In this respect we did not find a difference between the groups that showed progression and regression of CAD, respectively.

Cortisol has been found to be related to type 'A' behavior,[51] that constitutes an independent risk factor for CAD and early atherosclerosis in general.[52] In our study comparing men with advanced atherosclerosis with men who were free from

coronary atherosclerosis, no significant difference in plasma cortisol levels were found between the two groups. Likewise, no difference in plasma cortisol was observed between the progression and regression group of patients in the Leiden Intervention Trial (Chapter IV). This intervention study was undertaken with the aim of assessing the influence of a vegetarian lipid lowering diet enriched in polyunsaturated fatty acids.

As a large groups of patients for a long period of time are required to evaluate the effects of the intervention on complications of coronary atherosclerosis, sequential coronary arteriographies were chosen as means of assessing the effect on atherosclerosis lesion growth. With the development of quantitative computerized analysis methods, a reliable method became available.[1,53-55] The natural history of the disease has been described as almost unequivocally progressive,[56] but the methods used in most intervention programs are too unreliable to make statements with regard to the influence of an intervention.[57] Review articles have established that the evaluation of coronary atherosclerotic regression is feasible in all species including humans.[58-60] In the analysis of a coronary angiogram change in the vasomotor tone has been included.[61] After the development of a reliable quantitative computerized method, the Cardiovascular Angiographic Analysis System (CAAS), a scoring system had to be developed. This coronary scoring system was based on the relative importance of blood flow in the different coronary segments.[62] (Chapter V).

Although complications of the natural progressive nature of coronary atherosclerosis may be an acute expression of the disease,[63] the underlying slowly progressive process should be halted or at least the annual increment diminished.[64] As strong relationships exist between diets and lipids on one hand and between lipids and progression of CAD on the other hand,[65] a vegetarian diet enriched in polyunsaturated fatty acids and low in cholesterol with a twice a week allowance of lean fish was chosen.[66-71]

The principal outcome of The Leiden Intervention Trial, that regression of coronary atheroslcerosis can be induced by (lipid lowering) dietary means only, is of great importance. The clinical significance of these results show that regression of coronary atherosclerosis in men is significantly correlated with survival and disappearance of the orginal anginal complaints (Chapter VII).

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CHAPTER II

Cardiovascular risk factors in men (49 years or younger), survival one year after a first myocardial infarction.

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orginal publication in dutch entitled: Cardiovasculaire risicofactoren bij mannen (49 jaar of jonger) één jaar na het eerste myocardinfarct (Ned T v Geneesk 1985; 129: 1378-1381). Appendix paper I.

Summary

Risk factors (hypertension, total serum cholesterol, HDL-cholesterol, triglycerides, a positive family history for cardiovascular diseases, smoking and Type 'A' coronary prone behavior were investigated in men who had suffered a first myocardial infarction.

Three age groups were compared (40 years of age or under, between 41 and 49 years and 50 years and above). Results show a higher incidence of risk factors in the younger age groups both before and after myocardial infarction. Lipid disorders were especially implicated. Our findings are discussed with special focus on the possibility to mitigate these risk factors.

Introduction

Myocardial infarction is a complication of a heart disease in which risk factors are considered to contribute to the progression of the underlying coronary atherosclerotic disease.

Major risk factors are the male sex, hypertension, hyperlipoproteinemia, smoking, glucose intolerance, a positive family history of cardiovascular diseases and a certain type of psychological behavior.[1-3] Results of the modification of these risk factors in large scale studies are inconclusive.[4,5] A subgroup, however, with a high incidence of risk factors may be prone to benefit from intervention. Male patients under the age of 50, who had sustained a myocardial infarction can be considered to be such a group, apparently at high risk. We studied the risk factor profile in a series of these patients one year after they had suffered a first myocardial infarction.

Patients and methods

All male patients over a period of one year, who were 49 years of age or younger at

the time of their myocardial infarction and were treated in the Coronary Care Unit of the Thoraxcenter Rotterdam, The Netherlands, were asked to participate in the study. In this period (from June 1981 – June 1982), 252 male patients and 70 female patients with a respective average age of 58.2 years and 61.9 years were admitted for a first myocardial infarction out of 1121 admissions. Thirty-six men and 16 women had died in the hospital phase leaving 216 men and 54 women eligible for the follow-up study. Of the total male population (n=216), 52 patients were 49 years or younger at the time of the myocardial infarction. Five patients (9.6%) had died during the one year interval period. Furthermore during this period two patients has suffered a recurrent myocardial infarction, two patients had undergone a coronary bypass grafting and one patient had emigrated to a foreign country. A total of 42 patients under the age of 50 years were available for our investigations. Thirty patients participated in the study at the out-patient clinic of the Thoraxcenter in Rotterdam. The remaining twelve patients, who did not want to participate were alive and well at the time of the survey period. Of the thirty participants under the age of 50 years, twelve were 40 years or younger and eighteen were between 40 and 50 years of age. As a control group, a random sample of 20 patients out of the remaining 200 patients of 50 years or older was taken. The same procedure was performed to check the risk factors in the respective three age groups.

The patients were asked to report back to the out-patient clinic one year after the myocardial infarction. At this follow-up visit a physical examination was performed, a questionnaire was filled out by the patient, and a fasting bloodsample was collected for measurements of several lipid fractions (total-cholesterol,[6] HDL-cholesterol,[7,8] triglycerides,[9]) and glucose. None of these patients were known to have diabetes mellitus or a familiar hyperlipoproteinemia before they suffered a myocardial infarction.

The questionnaire contained the following questions:

1. Are you now on any form of diet?
2. Are you using any tobacco now and were you using any before your myocardial infarction? If so, in what form and how much a day?
3. Are you known to have been suffering from hypertension?
4. Does coronary heart disease run in your family? If so, please give details.
5. Do you feel you were experiencing excessive emotional stress before your myocardial infarction, like living under time pressure limits, restlessness or unexplained feeling of depression?

One risk factor was considered to be present if the patient showed one of the following:

1. a serum total-cholesterol value of 290 mg/dl or above.
2. a serum high-density-lipoprotein (HDL)-cholesterol value of 30 mg/dl or below.
3. smoking more than 5 cigarettes a day.
4. a blood pressure in excess of 150 mmHg systolic and/or 90 mmHg diastolic.

5. a positive family history for cardiovascular diseases, if a first degree relative had a myocardial infarction, a cerebrovascular accident, or died suddenly under the age of 60 years.
6. signs and symptoms of coronary prone behavior (type 'A'-behavior).

If present, one point per risk factor was added to the risk factor sum of the patient.

All patients studied were of Dutch descent.

Results

Risk Factor Profile (Table I):

No significant difference was found between the three groups for the risk factors hypertension and the number of cigarettes smoked per day. The percentage of smokers was significantly different in the sense that in the younger age groups more patients had smoked. In the oldest age group (above 50 years or above) the percentage of patients who showed coronary prone behavior (20%) or had a positive family history for cardiovascular disease (10%) was remarkably low in comparison with the younger age groups.

No patient was on diet at the follow-up visit examination. No glucose intolerance was demonstrated by the presence of an elevated fasting blood glucose level.

TABLE I
Risk factor profile in different age groups in men one year after suffering a first acute myocardial infarction

Age (years)	≤ 40 (n = 12)	41 - 49 (n = 18)	≥ 50 (n = 20)
Bloodpressure of ≥ 150/90 mmHg	4	4	4
Positive hypertension history	6	5	5
Cigarettes smoking			
– Before myocardial infarction	12	15	12
– After myocardial infarction	3	9	4
Numbers of cigarettes smoked per day (S.D.)			
– Before myocardial infarction	24 (10)	17 (12)	18 (15)
– After myocardial infarction	4 (6)	5 (6)	3 (4)
Positive family history for cardiovascular diseases	10	9	2
Coronary related emotional stress perception (type 'A' behavior)	8	13	4

n = number of patients

Blood lipids (Table II):

Total serum cholesterol was significantly higher in the youngest age group (under 41 years) in comparison to the other groups of patients. HDL-cholesterol nor triglycerides were significantly different between the three age groups.

TABLE II
Lipid values in the different age groups one year after suffering a first acute myocardial infarction

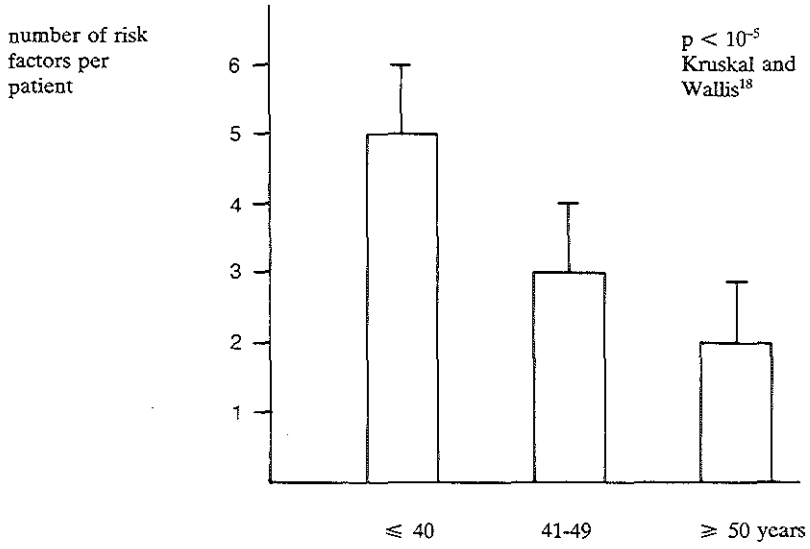
Age (years)	⩽ 40 (n = 12)	41 - 49 (n = 18)	⩾ 50 (n = 20)
Total-Cholesterol (mg/dl)	358** (250-625)	277** (227-328)	274 (174-302)
HDL-Cholesterol (mg/dl)	35 (30- 46)	39 (27- 50)	42 (34- 53)
Triglycerides (mg/dl)	300 (62-698)	229 (119-548)	215 (129-374)

P-value Student's t test, $^{**}p < 0.01$
all values mean and range
n = number of patients.

Patients at risk (Figure 1):

When all risk factors were considered as a whole, the younger patients showed a significantly higher sum than the older age group of patients.

FIGURE 1
Total accumulation of risk factors considered



All values means ± standard deviation of the total number of risk factors present in each group.

Discussion

Although the three groups of patients are small and conclusions should be made only with great caution, especially considering the fact that in weighting the risk

factors we assigned equal contributing weight to the several risk factors in the profile. These findings may nevertheless stimulate further discussion regarding the treatment of risk factors of special groups with coronary artery disease. Certainly one should consider the fact that surviving a myocardial infarction is already a selection for the better.

Serum total-cholesterol tends to be higher in an older population (carrying the 'atherogenic' LDL-cholesterol). In our study the youngest age group showed a serum cholesterol that was higher than the older age groups, implicating a 'special' atherogenic aspect in the youngest age group. The HDL-cholesterol, considered to be 'anti-artherogenic' as it is inversely correlated to the coronary heart disease,[11] was not significantly different in the three groups of patients, adding no further risk to the different groups. A tendency of a higher value in the older group seems worth mentioning. Whether triglycerides are a separate risk factor for coronary artery disease, remains to be determined, a trend towards a higher level in the younger group appears to be the case in our study.

As to the other risk factors, of special interest seems to be the fact that all patients in the youngest age group smoked cigarettes before suffering an infarction and a relatively high percentage (33%) of the patients were supposed to be hypertensive before the infarction as measured by a positive history of the patient for hypertension. After the myocardial infarction, the younger patients usually quit smoking and were less frequent found to be hypertensive as measured at physical examination. The high frequency of a positive family history for cardiovascular disease and coronary related emotional stress for the younger group is worth noting (Table I).

Several studies in different countries measured risk factors in young patients who had symptomatic coronary artery disease. Dolder and Oliver.[12] in their Nine Countries Study, found a high frequency of elevated total-cholesterol of 280 mg/dl or higher in young male patients after their first myocardial infarction in countries with a western style of living. The frequency varied extensively between Israel 45% and India 1%; the risk factor hypertension was present in 23% in Australia and 7% in Singapore. Smoking varied from 86% in Singapore to 96% in the U.S.A. Rosskamm et al.[13] in a similar group of patients as in our study found an elevated serum cholesterol (300 mg/dl or higher) in 73% of the patients; 93% smoked cigarettes before the myocardial infarction and 30% were hypertensive, Vanhaelke et al.[14] found similair results in a Belgium population. Glover et al.[15] established in a younger population in the U.S.A., that 98% were smoking cigarettes and 21% were hypertensive, while a positive history for hyperlipidemia was found in 20%. In another U.S.-study by Goldstein et al.[16] the percentage of lipid abnormalities in survivors of myocardial infarction was highest in young male patients under 40 years of age (60%).

To summarize: The young patient who survived a myocardial infarction smoked cigarettes (100%), had a elevated serum total-cholesterol (78%), had a lower

HDL-cholesterol (34%). A positive family history for cardiovascular diseases, coronary prone behavior (type 'A') and hypertension were also significantly higher. Especially noteworthy, seems to be the finding of a significant inverse relation between age and total numbers of risk factors present (Figure 1). In view of the sometimes lively debates that followed the publication of the results of studies like the MRFIT[4,17] one may ask, are not we obliged to treat these young male patients at high risk with extra care and vigor? The answer for us is clearly affirmative. On the other hand it seems that coronary artery disease in the older patient is possible part of the aging process itself and may be in need of an different approach than the younger patient who may have a different etiology of the pathogenesis of atherosclerosis itself.

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CHAPTER III

Post-heparin Lipases, Lipids and Related Hormones in Men Undergoing Coronary Arteriography to Assess Atherosclerosis

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Summary

Post-heparin lipase activities were measured in normolipemic men with complaints suggestive of symptomatic coronary artery disease. A study group, who showed severe diffuse atherosclerotic narrowing of the coronary vessels, as assessed by a quantitative computer-assisted analysis method, had a lowered hepatic lipase in comparison with a group with normal angiograms. Lipoprotein lipase valuess were lower in the study group but well within the normal range and not statistically different from the control group. Some atherosclerotic related hormones (cortisol, estradiol, testosterone and glucagon) were different in the two groups while others (insulin, human growth hormone, prolactin, thyroid hormones) were not. The results are discussed in view of the proposed role of hepatic lipase in the uptake of HDL-cholesterol by the liver.

Introduction

Serum cholesterol, predominantly transported in the low-density-lipoprotein (LDL) fraction, is thought to play a major in the development of atherosclerosis,[1-7] which is reflected in a marked positive correlation between serum (LDL) cholesterol levels and the risk of atherosclerosis. High-density-lipoprotein (HDL)-cholesterol concentrations are inversely correlated to this risk, and a protective role of this fraction against the progression of atherosclerosis has been suggested.[8-11]

The metabolism of the different lipoprotein fractions is closely interrelated.[8-11] Alterations in the metabolism of one of these lipoproteins may reflect changes in the overall lipid transport through the plasma and contribute to cholesterol deposition leading to atherosclerosis. Therefore, it is of interest to study the mechanisms involved in the lipoprotein metabolism in connection with atherosclerosis.

Two lipases that can be released into the circulation by heparin infusion, lipoprotein lipase (LPL) and hepatic lipase (HL), may play important roles in the

lipid transport system. Lipoprotein lipase does so by catalysing the hydrolysis of triglycerides of chylomicrons and very-low-density-lipoproteins (VLDL). This can lead to the formation of lipoproteins relatively enriched in cholesterol.[13-14]

Although the role of 'liver lipase' or 'hepatic triglyceridase' is not yet established, it seems that this enzyme plays a key function in lipoprotein metabolism, as inhibition of this enzyme activity in situ leads to major changes in plasma lipoprotein concentrations.[15-18]

Previous studies have shown a decreased activity of post-heparin plasma lipids in atherosclerotic patients.[19,20] In these studies, however, lipoprotein lipase and hepatic lipase have not been measured separately. We studied both enzyme activities in patients with or without signs of atherosclerosis as assessed by coronary arteriography. We also measured serum levels of a number of hormones which are known to influence lipid transport and lipoprotein metabolism, and which have been suggested to be factors in the development of atherosclerosis.

Patients

Male patients under the age of 50 years and of normal weight, in whom coronary arteriography has been performed because of complains of severe chest discomfort, were selected after reviewing the coronary angiogram. It was required that they be normolipemic (total-cholesterol < 8.0 mmol/l, triglycerides < 2.5 mmol/l and phospholipids < 3.7 mmol/l). Excluded were those patients who showed a complete occlusion of a main vessel, in order to avoid acute effects like thrombosis or hemorrhage in the vessel wall. All patients with such known chronic diseases as diabetes mellitus and hypertension, or any other disease for which medication had to be taken, were excluded. Kidney function as measured by BUN, and creatinine and liver functions as measured by bilirubin, SGOT, SGPT, LDH and γGT had to be normal.

Patients in whom the angiogram showed diffuse sclerotic thickening of all the vessel walls and in at least two of the three main coronary arteries (right coronary artery, left anterior descending artery and the left circumflex artery) a stenosis of >70% in diameter, were considered to have coronary artery disease (CAD). Patients were considered to belong to the control group if no visual detectable coronary stenosis could be found on the angiogram. Patients with one-vessel disease or left main disease, or a history of myocardial infarction, were discarded.

The two groups were comparable in age, weight, complaints and lipid profiles. Absolute diameter measurements of the coronary angiogram were performed by a computer-assisted analysis method.[32]

Methods

Blood was collected after an overnight fast of 14 h and at least 24 h after coronary

arteriography. A blood sample was drawn 30 min after the introduction of an intravenous catheter. From these blood samples glucagon, insulin, thyroxine, triiodothyronine, T_3-resin uptake, human growth hormone, prolactin, testosterone, 17β-estradiol and lipids were measured. Immediately thereafter, heparin 50 IU/kg body weight (Tromboliquine, Organon, Oss) was administered intravenously. Blood was collected in disodium EDTA (2.7 mmol/l) on ice 5 and 40 min after the administration of heparin. The blood was centrifuged at 4°C for 30 min at 3000 r.p.m. and the plasma was stored –20°C. The lipid contents were determined in the samples according to the following methods: total-cholesterol,[33] triglycerides.[34] and phospholipids.[22] HDL-cholesterol and HDL-phospholipids were measured in plasma, after precipitation of VLDL and LDL with $MnCl_2$ and heparin.[23,24] LPL and HL were measured in plasma 5 and 40 min after heparin infusion.[25] Blind samples were measured in duplicate for all patients.

In each series of measurements 2 standard sera with known lipase activity were included to control the enzyme essay. Hormone concentrations with known or suspected relation to the lipolytic enzymes[30,31] were measured in plasma samples according to the following methods: glucagon (Novo Industries, Denmark), cortisol (I.R.E., Holland), insulin (I.M.C., Holland, MRC 66/304), thyroxine,[28] triiodothyronine,[29] T_3-resin uptake,[28] human growth hormone (I.R.E., Holland), prolactin (I.R.E., Holland), testosterone,[26] and 17β-estradiol.[27]

The assessments of diameters in the coronary angiograms were performed with the assistance of a computerized coronary analysis method[32] as to minimise problems in interpretation.

Statistical methods

Student's *t*-test was used to compare measurements between the two groups. For each variable a *P* value is given. All results are expressed as means ± standard deviation ($\bar{x} \pm SD$). As far as applicability of the *t*-test is concerned, no important differences arose when the *P*-values were compared with those obtained by applying Mann-Whitney's test.

Results

Lipid analyses

Serum lipid values for the two groups are presented in Table I. All levels are within the normal range and the only significantly different value was that of total-cholesterol.

Post-heparin plasma lipases

LPL reaches a maximal value 40 min. after heparin administration and HL, which is more readily released, reaches a maximal value after 5 min. Consequently values at 40 and 5 min. are given in Table II.

TABLE I
Serum lipids for the atherosclerotic group (study) and atherosclerotic-free group (control)

Serum lipids	Study (n = 11)	Control (n = 9)	P
Total-cholesterol	6.90 ± 0.77	5.96 ± 0.94	0.025
Triglycerides	1.48 ± 0.69	1.74 ± 0.70	0.386
Phospholipids	3.27 ± 0.57	2.85 ± 0.53	0.084
HDL-cholesterol	1.23 ± 0.21	1.21 ± 0.19	0.988
HDL-phospholipids	1.65 ± 0.45	1.55 ± 0.33	0.282

All values are means ± standard deviation in mmol/l

TABLE II
Lipolytic lipases (mU/ml) for the two groups studied

	Study (n = 11)	Control (n = 9)	P
LPL (40 min.)	39 ± 10	48 ± 11	0.4810
HL (5 min.)	125 ± 28	176 ± 40	0.0070

All values are means ± standard deviation
1 mU = nmoles free fatty acids released/min.

TABLE III
Hormone concentrations for the two groups studied

	Study (n = 11)	Control (n = 9)	P
Testosterone (nmol/l)	17.0 ± 3.2	22.7 ± 5.1	0.002
17β-Estradiol (pmol/l)	128 ± 39	156 ± 43	0.001
Thyroxine (T_4) (nmol/l)	105 ± 10.6	87 ± 8.7	0.048
Triiodothyronine (T_3) (nmol/l)	1.66 ± 0.30	1.50 ± 0.22	0.369
T_3-resin uptake (%)	25.0 ± 2.1	27.3 ± 3.7	0.089
Cortisol (nmol/l)	516 ± 74	397 ± 55	0.046
Glucagon (ng/l)	60.1 ± 29.6	36.6 ± 15.1	0.003
Insulin (mU/l)	11.8 ± 5.0	11.3 ± 4.8	0.673
Human growth hormone (ng/l)	1.15 ± 0.40	0.79 ± 0.35	0.604
Prolactin (ng/l)	2.6 ± 0.7	3.6 ± 0.9	0.398

All values are means ± standard deviation

Hormone concentrations

Hormone values for the two groups are presented in Table III. All values are within the normal range. Significantly higher levels of cortisol, glucagon and thyroxine were found in the study group. Testosterone and 17β-estradiol concentrations were lower in the study group but the estradiol/testosterone ratio was increased in these patients ($P = 0.036$).

Weight and age

The mean age of the study group was 45 (35-59) years vs. the control groups 44

(32-50) years. The body weight in the study group was 71 (63-82) kg vs. 72 (62-81) kg in the control group.

Discussion

Although the two groups were comparable with respect to complaints, age, weight, biochemical status and lipid profiles, it cannot be excluded that the two groups differed in parameters other than the presence of atherosclerosis. Therefore, the interpretation of the *P* values in this non-randomized study is uncertain. They are used here as a basis set for the formulation of hypotheses. It is also questionable to what extent the control group had an indication for catheterization. Finally, from this type of design it is impossible to determine whether the lipase abnormalities observed are induced temporal precursors of atherosclerosis or if they simply emerge when atherosclerosis develops. Furthermore, extra care was taken to avoid bias, by blinding all measurements and results until the study was terminated.

The purpose of the study was to assess post-heparin plasma lipase activities and possible related hormones in normolipemic men with severe and diffuse coronary atherosclerosis.

Post-heparin plasma lipase activity has been shown to be lower in atherosclerosis,[19,20] but, no differentiation between lipoprotein lipase and hepatic lipase was made. The present paper suggests that hepatic lipase is lowered in male patients with diffuse coronary atherosclerosis, as compared to a group of control patients with no signs of coronary atherosclerosis. The implications of the present findings are not unequivocally clear. The lowered HL may be the result of the atherosclerotic process itself, However, this enzyme has been suggested to play a role in the removal of HDL-cholesterol from plasma,[15-18,31]

Cholesterol may be mobilised from peripheral cells by HDL and transported to the liver, where it may be converted to bile acids.[36] A lowered HL may impair this system and thus lead to a less efficient removal of cholesterol from the body and possibly to an enlarged body pool of cholesterol. Therefore, as has been suggested before,[30] a lowered HL may contribute to the progression of atherosclerosis.

It has also been proposed that increased lipoprotein lipase may be atherogenic.[37] Our results do not support this hypothesis. However, LPL originates from several tissues and local activities are not known. Recent studies have shown that HL is influenced by steroids. In men estrogens have been found to lower HL,[35] while anabolic steroids increase HL.[41] Hypercortisolism lowers HL,[31] and as hypercortisolism can lead to hyperglucagonism,[38] an increased glucagon/insulin concentration ratio has been suggested to be responsible for the lower HL in atherosclerotic heart disease.[30] For this reason we studied these hormones which are thought to be related to a lowered HL. The higher estradiol/testosterone ratio, the higher cortisol levels, and the higher glucagon levels in the study group may thus all have been contributing to the lowered HL.

In conclusion, hepatic lipase was found to be lowered in male patients with

In conclusion, hepatic lipase was found to be lowered in male patients with diffuse coronary atherosclerosis. The underlying cause may be of endocrine origin as a result of a 'stress'-induced disregulation of HL by relatively higher levels of cortisol, glucagon or estradiol. It remains a subject for further study whether a lowered HL contributes to the origin of progession of coronary atherosclerosis or whether it is the result of the atherosclerotic process itself.

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CHAPTER IV

Diet, lipoproteins, and the progression of coronary atherosclerosis

The Leiden Intervention Trial

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Summary

We studied the relations between diet, serum lipoproteins, and the progression of coronary lesions in 39 patients with stable angina pectoris in whom coronary arteriography had shown at least one vessel with 50 per cent obstruction before intervention. Intervention consisted of a two-year vegetarian diet that had a ratio of polyunsaturated to saturated fatty acids of at least 2 and that contained less than 100 mg of cholesterol per day. Dietary changes were associated with a significant increase in linoleic acid content of cholesteryl esters and a significant lowering of body weight, systolic blood pressure, serum total-cholesterol, and the ratio of total to high-density-lipoprotein (total/HDL) cholesterol. Angiographic examination was performed after 24 months; angiograms were assessed visually (with blinding) and by computer-assisted image analysis. Both types of assessment indicated progression of disease in 21 of 39 patients but no lesion growth in 18. Coronary lesion growth correlated with total/HDL cholesterol ($r = 0.50$, $P = 0.001$) but not with blood pressure, smoking status, alcohol intake, weight, or drug treatment. Disease progression was significant in patients who had values for total/HDL cholesterol that were higher than the median (>6.9) throughout the trial period. No coronary-lesion growth was observed in patients who had lower values for total/HDL cholesterol (≤6.9) throughout the trial or who initially had higher values (>6.9) that were significantly lowered by dietary intervention.

Introduction

The Leiden Intervention Trial is a study of the effect of lowering cholesterol on coronary artery atherosclerosis. Most previous studies have shown that serum total-cholesterol can be lowered by changes in diet and by drugs, but have been inconclusive with respect to a reduction of the incidence of coronary heart disease.[1] Recently, the Lipid Research Clinics Coronary Primary Prevention Trial has shown

that lowering of serum total-cholesterol and low-density-lipoprotein cholesterol by diet and cholestyramine was followed by a significant reduction in the incidence of coronary heart disease and death.[2,3] An alternative strategy to evaluate the efficacy of lowering lipid levels is to measure the rate of change in arterial lesions as an end point.[4] Eight studies of the latter type have been published.[5-12] In six of these, coronary arteries were evaluated by repeat arteriography, and in two, femoral arteries were examined. Atherosclerotic changes were shown to be associated with serum lipid levels in the two studies of femoral arteries and in four of the six studies of coronary arteries. In all eight trials intervention consisted of drug treatment, with or without diet. The Leiden Intervention Trial differs from these trials in that lipid levels were lowered solely by dietary measures. The objective of the study was to investigate the relations of diet and the ratio of serum total-cholesterol to high-density-lipoprotein cholesterol (total/HDL cholesterol), to the growth of coronary lesions in patients with stable angina pectoris.

Methods

Patients with stable angina pectoris in whom coronary angiography had demonstrated severe narrowing of the diameter (≥50 per cent) of one of more major coronary arteries were selected for the trial. They had not undergone coronary bypass surgery, either because the angina was stable and only one of two vessels were diseased, or because the arteries were unsuitable for grafting and three vessels were diseased. The protocol excluded patients with disease of the left main coronary artery, coexisting valvular or nonischemic myocardial disease, or insulin-requiring diabetes. Of the 61 persons, all under 60 years of age, invited to attend two introductory meetings with physicians and dietitians, 53 gave informed consent to enter the study. Full clinical examinations, including biochemical evaluation, 12-lead electrocardiography, and measurement of weight and blood pressure, were carried out twice on two different days just before dietary intervention. The means of the values from these examinations were used as base-line readings.

The trial was conducted from 1978 to 1982. Each patient followed a diet for two years, during which the usual, appropriate individual therapy was also given for angina pectoris, elevated blood pressure, cardiac arrhythmias, and symptoms of heart failure – e.g., betablockers, diuretics, digitalis, nitroglycerin, and anticoagulants. The participants were seen 15 times during the two-year period by cardiologists and dietitians. Of the 53 patients who entered the study, 4 died during the first year – 3 of acute myocardial infarction and 1 suddenly. In seven patients with two-vessel disease, the angina became labile and necessitated coronary surgery; this occurred in six of these patients in the first year of the study. The data for the seven patients were not used in analysis, since the angiographic techniques applied for their operations were not identical and since the rate of change of obstruction in grafted arteries is known to be different from that in ungrafted vessels.[13,14] Repeat

angiography was not carried out in three patients; malignant disease had developed in two, and the second investigation was refused by one. Thus, only data for 39 patients could be used for analysis and are presented here. The 35 men and 4 women were between 33 and 59 years of age (mean, 48.9).

The study was approved by the Research Ethical Committee of Leiden University Hospital.

Diet

Intervention consisted of a vegetarian diet in which the ratio of polyunsaturated to saturated fatty acids (P/S ratio) was at least 2 and the dietary cholesterol was less than 100 mg per day. Much emphasis was placed on continuous supervision and dietary instruction for the patients and their spouses. Instruction was individualized and based on 24-hour recall of food intake, which was recorded before intervention. To evaluate adherence one year after the trial had begun, we collected information on diet but were able to obtain it for only 28 of the 39 patients. It is unlikely that these 28 patients represented biased selection from among the 39, because at base line the food intake of the 28 did not differ significantly from that of 10 patients who participated in the base-line analysis only. The 28 patients weighted their food and drinks for one week under the supervision of a dietitian. All foods and drinks were coded and analyzed according to the Uniform Food Encoding System, recently developed in the Netherlands.[15]

Lipid Measurements

Serum total-cholesterol and high-density-lipoprotein cholesterol were measured when the patients were not fasting, according to the method of Abell et al. (Gaubius TNO Research Laboratory, Leiden).[16] High-density-lipoproteins were isolated by precipitating other lipoproteins with magnesium phosphotungstate.[17] The fatty acid content of cholesteryl esters was analyzed by thin-layer and gas chromatography (Unilever Research Laboratory, Vlaardingen). For base-line readings, we recorded the mean of the two measurements made before intervention, and for results at two years, the mean of the six measurements made during the trial.

Coronary Angiography

Coronary arteriography had been performed in all patients immediately before entry. The initial visual assessment of the angiograms was conducted by a clinical angiographer (B.B.). Patients with at least one obstruction of 50 per cent or more were invited to enroll in the study. The examination was repeated, as scheduled, after two years of dietary intervention. The same technique (Judkins percutaneous femoral-artery approach) and the same radiographic equipment and cine-angiographic techniques were used in obtaining the first and the second agiograms. With few exceptions, the procedure was performed by the same arteriographer (B.B.).

The angiograms were assessed both visually and by computerized image analysis.

Films were assessed visually by two experienced observers who had no knowledge of the lipid values and who could not distinguish between the first and second angiograms since identical techniques had been used and since the dates of the films were concealed. They estimated the percentage of obstruction by each lesion in 30 segments of coronary arteries, in increments of 5 percentage points, as described by Bruschke et al.[18] The severity of coronary lesions was scored by calculating the mean of the percentages of obstruction in each patient.

Computer-assisted analysis was carried out with the computer-based Coronary Angiography Analysis System (CAAS, Thorax Center, Rotterdam). This system permits the accurate delineation of the contours of user-selected coronary arterial segments by means of automated edge-detection algorithms.[19] Coronary lesions were measured in 9 segments of the major epicardial branches: the proximal, middle, and distal portions of the right coronary artery; the left main coronary artery; the proximal, middle, and distal portions of the left anterior descending artery (Figure 1.), and the proximal and distal portions of the left circumflex coronary artery. Since the accuracy of acquisition and measurement of an arterial vessel decreases when its diameter is less than 0.8 mm, side branches of the major epicardial arteries were not analyzed.

The severity of coronary obstruction was expressed relatively, as the percentage of reduction of a vessel's diameter, and absolutely, as the minimal diameter (in millimeters) at the site of obstruction. We preferred the absolute value and used it throughout, since the relative value expresses the severity of the stenosis with respect to a user-defined reference position, whose location is arbitrary. Also, the reference diameter may change over time, thus possibly obscuring or overemphasizing the dimensional changes of the lesion.

In order to evaluate the variability of the measurements, enddiastolic cine frames of 13 routine coronary angiograms were analyzed twice by one technician, with a median interval of 28 days between analyses. The average difference between duplicate measurements was found to be 0.00 mm; the variability, defined as the standard deviation of the differences between repeated measurements, was found to be 0.10 mm.[20]

Statistical Analysis

Means and their standard deviations were used as descriptive statistics for the total group or subgroups of patients. Relations between continuous variables were analyzed by linear-regression analysis. Means were compared by the two-sample t-test (two-sided) or, when appropriate, by paired t-test. Dichotomous variables were analyzed by the chi-square test.

FIGURE 1.
Example of Computer Assessment of Obstruction of the Proximal Part of the Left Anterior Descending Artery (Right Anterior Oblique Projection).
Detected contours are superimposed on the image. The 'diameter function' is presented in the lower left-hand section of the image; the minimal diameter at the site of obstruction equals 2.69 mm, and the percentage of diameter narrowing with respect to the user-defined proximal reference diameter equals 37 per cent. The extent of the obstruction is indicated in the diameter function by the two dotted lines and in the arterial segments by the two lines connecting opposite contours of the vessel.

Results

Twenty-seven of the 39 patients with stable angina pectoris had previously had a myocardial infarction. At base line, 10 of the 39 patients had systemic hypertension (systolic blood pressure $\geq$ 160 mm Hg, diastolic $\geq$ 95 mm Hg, or both), 16 had hypercholesterolemia (cholesterol $\geq$ 7.2 mmol per liter [$\geq$ 280 mg per deciliter]), 1 was obese (Quetelet index > 27 kg per square meter of body-surface area), and 18 were smokers.

At base line the diet contained an average of 2002 kcal per day, had a P/S ratio of 0.91, and had a cholesterol content of 88.6 mg per 1000 kcal. In the Dutch population in general, the P/S ratio is 0.37 and dietary cholesterol is 140 mg per 1000 kcal.[21] These data suggest that the participating patients were already following some type of cholesterol-lowering diet before they entered the study. They were then placed on the vegetarian diet with a P/S ratio of at least 2 and a dietary cholesterol intake of less than 100 mg per day. Compliance was measured by dietary surveys and by analyses of the serum linoleic acid content of the cholesteryl esters. The surveys showed that after one year of intervention the P/S ratio was 2.54 and the dietary cholesterol intake 29.5 mg per 1000 kcal (Table I). These results indicate that the patients reduced their intake of saturated fat by about 50 per cent and doubled their intake of polyunsaturated fat. The latter finding was confirmed by a significant increase – from 52.4 to 60.8 per cent – in the linoleic acid content of the serum cholesteryl esters. On the basis of changes in the fatty acid and cholesterol content of the diet, the expected decrease in average serum total cholesterol level could be calculated according to Keys' equation.[22] The expected decrease did not differ from the observed (–0.79 vs. –0.82 mmol per liter [–30.5 vs. –31.7 mg per deciliter]). Body weight, Quetelet index, serum total-cholesterol, total/HDL cholesterol, and systolic blood pressure dropped significantly during the intervention period (Table II).

TABLE I
Energy, fatty acids, and dietary cholesterol at base line and after one year of intervention in 28 of the 39 patients.*

Variable	At base line	At 1 year
Energy (kcal)	2002 ± 518	2003 ± 581
Saturated fat (% energy)	11.6 ± 4.8	6.6 ± 1.5 †
Polyunsaturated fat (% energy)	8.5 ± 3.6	16.8 ± 4.2 †
P/S ratio	0.91 ± 0.62	2.54 ± 0.47 †
Dietary cholesterol (mg/1000 kcal)	88.6 ± 23.5	29.5 ± 11.5 †
Linoleic acid content of cholesteryl esters (%)	52.4 ± 6.2	60.8 ± 4.0 †

* Values means ± S.D. P/S ratio denotes ratio of polyunsaturated fat to saturated fat.
† Significantly different from base-line value ($P<0.001$).

Coronary Artery Lesions

According to visual assessment, a total of 307 lesions (lowest degree of measured stenosis, 5 per cent) were detected in the 30 segments of coronary arteries. On average, the patient had 7.9±2.7 lesions. The mean percentage for diameter narrowing by the lesions was 44.1±31.6 per cent on the first arteriogram and 48.6±30.9 per cent on the second, demonstrating a mean progression of disease in the 39 participants ($P<0.001$).

According to computer assessment, a total of 166 lesions (of which the least

stenosis measured 0.14 mm) were detected in the 9 major epicardial coronary segments. On average, the patients had 4.26±1.52 lesions. Seven patients had one-vessel disease, 11 had two-vessel disease, and 21 had three-vessel disease. The mean diameter of vessels at a total of 166 sites of obstruction was 2.12±0.99 mm on the first arteriogram and 1.99±1.01 mm on the second, illustrating, as also shown by visual assessment, that on average, the coronary lesions of the 39 patients progressed in the two years of observation ($P<0.01$). The four complete obstructions (100 per cent) seen at the first examination, which were slightly reduced at the second, were not included in the analysis, since recanalization was considered to be a different phenomenon.

TABLE II
Change in values for risk factors in the 39 patients over two years.*

Risk factor	At base line	At 2 years	Change
Body weight (kg)	74.5 ± 11.4	73.5 ± 11.3	−1.2 ± 3.6 †
Quetelet index (kg/m^2)	24.0 ± 2.4	23.7 ± 2.6	−0.4 ± 1.1 †
Serum total-cholesterol (mmol/liter) ‡	6.9 ± 1.4	6.2 ± 1.3	−0.7 ± 0.7 §
HDL-cholesterol (mmol/liter) ‡	1.01 ± 0.21	0.98 ± 0.16	−0.03 ± 0.14
Total/HDL-cholesterol	7.1 ± 1.8	6.4 ± 1.6	−0.6 ± 1.2 ¶
Blood pressure (mm Hg)			
Systolic	130.4 ± 17.4	126.3 ± 14.2	−4.2 ± 11.2 †
Diastolic	84.4 ± 11.1	82.1 ± 7.6	−2.4 ± 8.5
Smoking (no. of patients)	18	18 ‖	0

* Values are means ± S.D.
† Significant difference ($P<0.05$).
‡ To convert to milligrams per deciliter, multiply by 38.7.
§ Significant difference ($P<0.001$)
¶ Significant difference ($P<0.01$)
‖ Includes four patiens who stopped smoking during the second year of intervention.

Progression of Coronary Lesions

Progression of lesions was defined as a decrease of 0.1 mm or more in the mean coronary diameter (computer assessment) or as an increase in the mean percentage of narrowing (visual assessment). Both visual and computer-assisted assessments showed progression in 21 of the 39 patients but no lesion growth in 18 patients. The degree of reduction of total-cholesterol (1.1 mmol per liter) and total/HDL-cholesterol (1.0) was greater in patients with progression of a lesion than in those without progression (0.6 and 0.1 mmol per liter, respectively). However, the 18 patients with no progression of coronary lesions had significantly lower values for total-cholesterol and total/HDL-cholesterol and higher values for high-density-lipoprotein cholesterol both at base line and during the two years of intervention (Table III). No difference were found between these two groups with respect to

age, smoking status, alcohol intake, systolic or diastolic blood pressure, Quetelet index, or fatty acid composition of the diet at base line or during intervention. Also, the use of anticoagulants, beta-blocking agents, digitalis, diuretics, and nitroglycerin was unrelated to progression of coronary lesions. Since patients entered the trial with at least one 50 per cent obstruction, progression of major obstructions (≥50 per cent) was analyzed separately from that of minor lesions (<50 per cent). A mean progression of –4.29 per cent occurred in the 124 minor lesions, and a mean regression (+3.47 per cent) took place among the 42 major obstructions.

TABLE III
Relation between coronary lesion growth (computer assessment) and total-cholesterol, HDL-cholesterol, and total/HDL-cholesterol at base line and after two years of intervention.*

	No growth (18 patients)	Lesion growth (21 patients)
Total-cholesterol (mmol/liter) †		
At base line	6.4 ± 1.1	7.4 ± 1.5 ‡
At 2 years	5.8 ± 0.9	6.5 ± 1.5 ‡
HDL-cholesterol (mmol/liter) †		
At base line	1.1 ± 0.2	0.9 ± 0.2 ‡
At 2 years	1.0 ± 0.1	0.9 ± 0.2 ‡
Total/HDL-cholesterol		
At base line	5.8 ± 1.2	8.1 ± 1.7 §
At 2 years	5.7 ± 1.2	7.1 ± 1.7 §

* Values are means ± S.D.
† To convert to milligrams per deciliter, multiply by 38.7.
‡ Significantly different from value for patients with no lesion growth ($P<0.01$).
§ Significantly different from value for patients with no lesion growth ($P<0.001$).

Correlation between Values for Total/HDL-Cholesterol and Coronary Lession Growth

Computer-measured lesion growth correlated positively with the mean of values for total/HDL-cholesterol at two years ($r = 0.39$, $P = 0.01$); visual assessment showed a positive association between these features that was of the same magnitude ($r = 0.38$, $P = 0.02$). When the coronary obstruction that was the largest but was less than 99 per cent (the obstruction that qualified a patient to enter the trial) was measured, the correlation between lesion growth and total/HDL-cholesterol was again of the same magnitude (computer assessment: $r = 0.38$, $P = 0.02$). Coronary lesion growth, no matter by what technique it was measured, proved to be even more strongly correlated with base-line total/HDL-cholesterol (computer assessment: $r=0.55$, $P<0.001$) and the average of base-line and two-year means ($r=0.50$, $P=0.001$) (Figure 2).

This finding prompted us to look at the difference in base-line characteristics between the 19 'low' patients – i.e., those whose total/HDL-cholesterol was lower

than the median (<6.9) – and the 20 'high' patients – those with higher values (>6.9). At base line the 20 high patients consumed on average 350 kcal per day more than the 19 low patients ($P<0.05$), but the groups did not differ in fatty acid content of the diet, blood pressure, weight, smoking status, or number of severity of coronary lesions (Table IV). Over the ensuing two years the mean coronary diameters of the 19 low patients remained the same (mean change, +0.002 mm), but those of the 20 high patients showed significant disease progression (mean change, –0.237 mm).

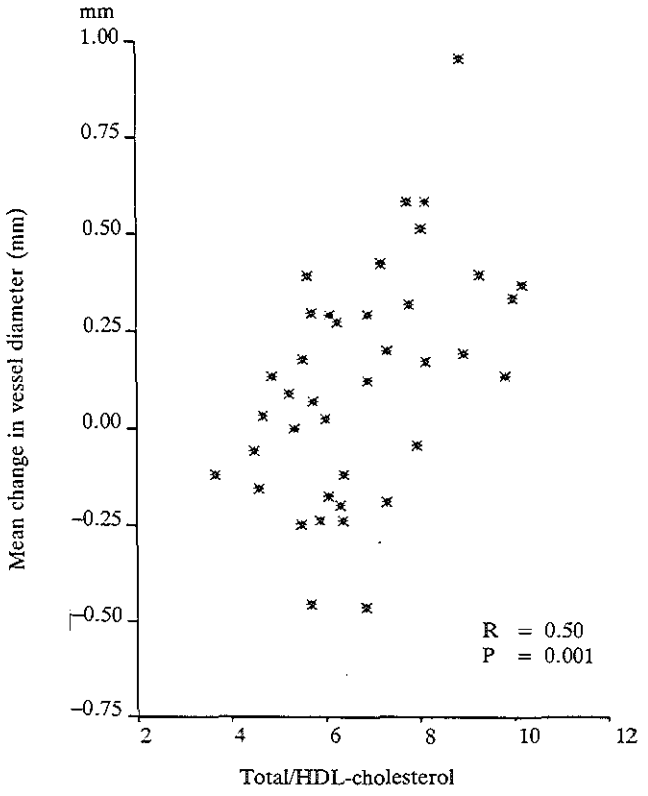

FIGURE 2
Association between change in coronary lesion and ratio of total to high-density-lipoprotein (total/HDL) cholesterol, according to individual patient (computer assessment).
Values for total/HDL-cholesterol are averages of base-line and mean two-year values for 39 patients. Progression of lesion growth (values above 0.1 mm) is associated with relatively high values for total/HDL cholesterol (2 patients). Mainly, patients with relatively low values for total/HDL-cholesterol had no disease progression – some even had regression.

Diet, Total/HDL-Cholesterol, and Coronary Lesion Growth

Because there was no control group, the effect of dietary intervention on

lipoprotein levels and coronary lesion growth could not be assessed directly. To obtain indirect information on the effect of the diet, the data were stratified according to the 'low' and 'high' patient groups. In addition, the 20 high patients were divided in two subgroups: 'the 'high-high' subgroup – 11 patients whose total/HDL cholesterol remained high during the two years of intervention – and the 'high-low' subgroup – 9 patients whose ratio became low over the two years.

TABLE IV
Relation between serum total/HDL-cholesterol at base line and characteristics of coronary lesions at base line and after two years of intervention.*

	Total/HDL-cholesterol at base line	
	≤6.9 (19 patients)	>6.9 (20 patients)
Lesions/patient		
Visual assessment	7.74 ± 2.80	8.15 ± 2.76
Computer assessment	4.37 ± 1.74	4.15 ± 1.31
Vessel diameter (mm) †		
At base line	2.15 ± 0.58	2.30 ± 0.47
At 2 years	2.15 ± 0.61	2.07 ± 0.45
Change in vessel diameter		
Absolute (mm)	0.002 ± 0.23	− 0.237 ± 0.32 ‡
Relative (%)	2.2 ± 5.8	− 5.0 ± 6.7 §

* Values are means ± S.D.
† Computer assessment.
‡ Significantly different from value for patients with ratio ≤6.9 ($P<0.01$).
§ Significantly different from value for patients with ratio ≤6.9 ($P<0.001$).

The fatty acid and cholesterol content of the diet was expressed as the so-called Φ (phi) value, bases on Keys' equation[22]:

$$\Phi = (2S - P) + 1.5\sqrt{Z},$$

where S denotes saturated fatty acids (per cent energy), P polyunsurated fatty acids (per cent energy), and Z dietary cholesterol (milligrams per 1000 kcal) (Figure 3). The values for saturated fatty acids and cholesterol content elevate the Φ value, whereas the value for polyunsaturated fatty acids does the opposite. At base line in the present study the highest Φ value (45.3) was found among the nine high-low patients; it was closest to that of the Dutch population (60.0).[21] The base-line Φ values of the low group and the high-high subgroup were lower (35.8 and 31.4, respectively). After one year of intervention, no difference between the Φ values of the three groups were observed. The change in the Φ value (resulting from a lower saturated-fat and cholesterol intake) was largest in the nine persons in the high-low group ($P<0.05$). No progression of coronary lesions (mean change, –0.043 mm) was observed in these nine patients (Figure 3). However, the coronary arteries of the 11 high-high patients showed considerable and significant progression of disease (mean change in diameter, –0.396 mm; $P = 0.01$). These findings suggest

that in patients with high values for toal/HDL-cholesterol and high Φ values, the possibilities for benefit from intervention are greater than in patients with low values.

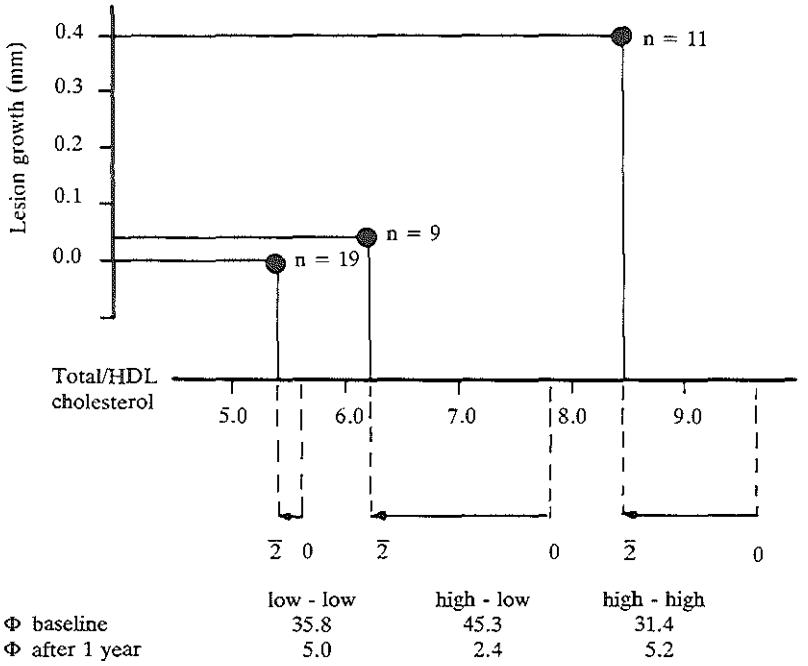

FIGURE 3
Association between change in coronary lesion and total/HDL-cholesterol, according to patient group or subgroup.
Values for Φ (content of fatty acids and cholesterol in diet, based on Keys' equation[22]) are also presented according to group. Values at base line are denoted by 0, and mean values at two years by 2̄: in the low group they are 5.52 and 5.40, respectively; in the high-low subgroup, 7.79 and 6.17; and in the high-high subgroup, 9.16 and 8.46. Disease progression occurred in the high-high subgroup, but not in the low group or the high-low subgroup ($t = 2.87$, $P = 0.01$).

Discussion

The purpose of the Leiden Intervention Trial was to analyze the relations of diet and total/HDL-cholesterol to coronary lesion growth as assessed angiographically. Fifty-three patients with stable angina were enrolled in the study, but data on 14 (7 underwent grafting, 4 died, 2 had neoplasms, and 1 refused repeat angiography) could not be used for analysis. This loss of a quarter of the patient population weakened the study results, but the 14 patients' base-line status for risk factors did not differ from that of the 39 who could be evaluated.

It should be pointed out that coronary lesion growth itself cannot as yet be measured. We used the term only for brevity's sake. The change in coronary diameter was the variable that we estimated. This variable is not specific for

atherosclerosis and may be influenced by other biologic variables, such as vascular tone. We used two different and independent techniques, computer assessment and visual assessment. With visual assessment 307 lesions were detected in all segments of coronary arteries, or roughly 10 lesions per segment. The computer technique measured 166 lesions in the 9 major epicardial segments, or roughly 18 lesions per segment. This is in keeping with the fact that major branches generally have more lesions than smaller branches. The visual and computer techniques produced a similar – i.e., the finding that 18 of the 39 patients had no coronary lesion growth. Also, the correlation between values for total/HDL-cholesterol and coronary lesion growth was similar on computer assessment ($r=0.39$, $P=0.01$) and on visual assessment ($r=0.38$, $P=0.02$). These results give the study an internal consistency.

Total/HDL-Cholesterol and Coronary Lesions

The results of the study suggest that the values for total/HDL-cholesterol in the 39 patients with stable angina were associated with lesion growth of coronary atherosclerosis, as assessed either by a computer-assisted technique or visually. This relation was noted for lipid levels at two years as well as for base-line total/HDL-cholesterol. The association was stronger for base-line values, possibly because the range of values for total/HDL-cholesterol (95 cent of values between 3.46 and 10.68) was larger at base line than during the two years of intervention (3.32 and 9.56), since all patients were put on the same diet (Table I). A strong correlation was observed between total/HDL-cholesterol at base line and at two years ($r = 0.78$, $P<0.001$). We therefore conclude that in the present study coronary lesion growth was strongly related to the total/HDL-cholesterol. Previous intervention studies aiming at lowering lipids, notably clinical trials by Kuo (1979), Nash (1982), Nikkilä (1983), and Levy (1984) and their colleagues, also demonstrated associations between serum cholesterol levels and the likelihood of progression of coronary atherosclerosis lesion growth as assessed by repeat coronary arteriography.[8-12].

In the present study no lesion growth was observed in the patients with low total/HDL-cholesterol at base line (≤ 6.9) that was reduced by 2 per cent during two years of intervention. Similar results were obtained in patients with high total/HDL-cholesterol at base line (>6.9) that was reduced by 13 per cent – to below 6.9 – during intervention. Patients with initially high total/HDL-cholesterol that was eventually reduced by 8 per cent only and that stayed above 6.9 had significant disease progression (Figure 3). The question whether coronary lesion growth among these patiens was less than among untreated patients with high total/HDL-cholesterol cannot be answered, because a control group was lacking. Lack of a control group complicated the analysis and interpretation of the results of the present trial. It is possible that the relation between total/HDL-cholesterol and lesion growth can be explained by other risk factors. Information was collected on

blood pressure, smoking status, alcohol intake, weight (Quetelet index), and drug treatment – e.g., anticoagulants, beta-blockers, diuretics, digitalis, and nitroglycerin. Coronary lesion growth was unrelated to these risk factors. This strengthens the conclusion that total/HDL-cholesterol was related to coronary lesion growth, although the possibility that some unidentified risk factor may a part cannot be definitely ruled out.

Diet, Total/HDL-Cholesterol, and Coronary Lesions

In intervention studies, information on compliance with diet is crucial. In the Leiden trial, compliance with the vegetarian, linoleic acid-enriched diet was measured by dietary surveys and by analysis of the linoleic acid content of cholesteryl esters. Dietary data could be obtained at base line in 38 of 39 patients and at one year of intervention in 28 patients. The base-line energy and nutrient intake of the 10 patients who did not participate in the second survey did not differ from that of the 28 participating patients. Comparison of the base-line and one-year data showed a significant increase in the P/S ratio, from 0.91 to 2.54. The increase in dietary linoleic acid in the 39 patients was confirmed by a significant 16 per cent increase in linoleic acid content of cholesteryl esters. These results indicate that patients adhered well to the diet. The finding that the observed mean decrease in total serum cholesterol (–0.82 mmol per liter) did not differ from the expected value as calculated by Keys' equation (–0.79 mmol per liter) provides evidence for the validity of the dietary information collected among these patients.

To obtain information about the efficacy of the dietary intervention, the 39 patients were divided into three groups bases on their values for total/HDL-cholesterol. In the 19 patients with low values at base line and the 9 patients with high values at base line but low values during intervention, coronary artery lesions were stable. The 11 patients with high values throughout the trial had significant disease progression. The Φ value of the diet at base line was high among the patients whose values for total/HDL-cholesterol were high at base line and low during intervention. The Φ value was low among the patients in the two other groups. These results suggest that when total/HDL-cholesterol is low in patients with angina, their prospects with respect to coronary lesion growth are favorable. When total/HDL-cholesterol is high and the Φ value before intervention is rather low, coronary lesions are likely to progress. When, however, total/HDL-cholesterol is high and the Φ value of the fatty acid and cholesterol content of the diet is also high (e.g., similar to values in the general population of industrialized Western countries), dietary intervention may reduce the rate of coronary lesion growth.

Further research should be directed toward early indentification of patients with high values for total/HDL-cholesterol and Φ, in whom dietary intervention may be successful, as well as patients with equally high total/HDL-cholesterol but low Φ values – a group to which most of the heterozygous patients with familial hyper-

cholesterolemia undoubtedly belong and in which diet alone is insufficient to prevent progression of coronary atherosIcerosis.

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CHAPTER V

Quantitative Coronary Angiography in a Lipid Intervention Study

The Leiden Intervention Trial

Summary

Postheparin lipoprotein lipase activities, lipid fractions and atherosclerosis linked hormones were studied in 35 male patients with advanced coronary artery disease, who participated in a two year diet only, lipid lowering intervention program, The Leiden Intervention Trial. Sequential angiographies, one at the beginning of the trial and one two years later, were performed to assess the degree of progression or regression of the disease; the coronary angiograms were analyzed with the Cardiovascular Angiographic Analysis System 'CAAS'. In each patient film a total of nine coronary segments in the major coronary arteries were analyzed in at least two angiographic views. From the obstruction diameters and mean diameter values in nonobstructed segments, an absolute coronary score was derived. A patient was considered to have progressive disease, if the absolute coronary score showed a decrease over the study period. No lesion growth or regression of the disease was assumed if the coronary score showed an increase. Specific response patterns to dietary therapy were measured for total cholesterol, triglycerides, HDL-cholesterol and the postheparin lipases, sex-, and thyroid hormones, insulin and cortisol.

At termination of the study fourteen men showed no lesion growth or regression of atherosclerosis, whereas twenty-one men showed further progression of their underlying disease. Lipoprotein lipase (LPL) was not significantly different in the two groups studied, while liver lipase activity (LLA) was significantly lower ($p < 0.001$) in the progression group (298 ± 190 mU/ml) as compared to the no lesion growth group; (499 ± 146 mU/ml) LLA was inversely correlated with the degree of progression. ($r = -0.601$, $p < 0.01$); Cholesterol and triglycerides were higher and HDL-cholesterol lower in the progression group as compared to the no lesion growth group. The measured hormones, testosterone, estradiol- 17β, cortisol, insulin, thyroid stimulating hormone, thyroxine and T_3-resin uptake were not different between the two groups of patients. Triiodothyronine (T_3) appeared to be significantly higher in the no lesion growth group. (2.24 ± 0.36 vs. 1.86 ± 0.28) Linear regression analysis showed a poor correlation between LLA and T_3. ($r = 0.283$, $p =$ n.s.) When the individual responses to dietary intervention were assessed, LLA appeared to be an important indicator of an inducibility of regression of atherosclerosis by dietary means.

We infer from this study, in which for the first time a lipid lowering diet therapy in coronary atherosclerosis was prescribed, in which sequential coronary angiograms were analyzed quantitatively and in which atherogenesis linked hormones,lipid fractions and lipoprotein lipases were measured, the following:

1. Regression of coronary atherosclerosis due to dietary therapy appears to be feasible.
2. Liver lipase activity is a possible marker for the success of dietary intervention to induce a deceleration of the natural course of coronary atherosclerosis.
3. Testosterone, estradiol, thyroxine, thyroid stimulating hormone, cortisol and insulin seem to be of no great additional importance, when lipid fractions are manipulated by dietary means to induce regression of coronary atherosclerosis. The triiodothyronine level seems to reflect the body's metabolic status and adds an additional aspect, if raised as to the presence of regression of coronary atherosclerosis.

Introduction

Coronary atherosclerosis resulting in angina pectoris, myocardial infarction and sudden death remains a major contributor to the epidemic of cardiovascular disease in the industrialized world.[1] One of the major factors that determine the rate of development of progression of coronary atherosclerosis is a disordered lipid metabolism.[2-5]

The total cholesterol (TC) is considered to be causal to coronary artery disease (CAD) for the reasons as stated by the WHO Expert Committe on the Prevention of Coronary Heart Disease:[6] 'The manifestation of its strength, graded character, consistency and independence, the demonstration that the trait precedes the disease; the coherence with clinical and experimental data; and the fact that logical mechanisms have been deliniated for the effect'.

The major lipid fraction within TC is the low-density-lipoprotein (LDL) fraction. This fraction has been implicated as the 'atherogenic' fraction, while another lipoprotein fraction, the high-density-lipoprotein (HDL), has been considered as 'anti-atherogetic' for many reasons, among these the inverse relationship to coronary atherosclerosis and its clinical manifestations.[7] Modulaters of lipoprotein metabolism, that codetermine the ratio between 'good' (HDL) and 'bad' (LDL) cholesterol are therefore of great importance. Postheparin lipoprotien lipase activiteis are important modulators in the process of atherosclerotic development.[8-12] Another group of factors that influence directly or indirectly this process are centain hormones, e.g. the sex-, thyroid- and stress-hormones.[13-17]

Until recently only more or less specific clinical manifestations of the atherosclerotic lesion growth, such as angina pectoris, acute myoccardial infarction or sudden death, could be studied. It is also well-known that a direct positive relationship exists between the number of vessels diseased and the level of the ratio of total-

cholesterol to HDL-cholesterol.[18] However, an accurate method to study growth or possibly regression of atherosclerotic plaques itself, which is strongly related in living human subjects to the clinical manifestations mentioned above, was unavailable.

Visual interpretation of sequential coronary angiographies was one method by means of which one has tried to measure directly the natural growth of coronary atherosclerosis in humans. By this means one did not want to wait for manifestations of atheroclerosis, but attempted to observe the process of growth itself. However, the method of visual interpretation is very much limited by the relatively large inter- and intra-observer variations in the judgement of the serverity of the disease; these variations in itself are usually larger than the absolute anatomical changes present in the serial angiograms.[19-21]

With the CAAS, differences in the atherosclerotic plaques between two sequential angiograms can be measured with high accuracy. To demonstrate the applicability and the value of the CAAS in such studies, the coronary angiograms in the Leiden Intervention Trial were measured with this system.[22]

Methods

Patients

Thirty-five male patients with chronic stable angina pectoris who had undergone coronary arteriography to assess their suitability for coronary bypass grafting surgery were selected for the study after having been rejected for surgery for technical reasons.[22] They were offered to participate in a diet intervention study. At the end of the two year study period a repeat coronary arteriography was performed.

Although both sexes could be admitted according to the original design of the study, in practice only four women entered the study. As postheparin lipoprotein lipase activities and hormone levels differ between the sexes, only the 35 men who completed the study were ultimately evaluated. The study lasted from 1978 until 1982 and each patient was on dietary intervention for a period of 2 years, with -if needed - individual therapy for angina pectoris or hypertension.

All patients were seen monthly during the first half year at the outpatient clinic and bimonthly thereafter. They were seen by a cardiologist and a dietician for a total of fifteen times during the two year period. At each visit lipid fractions were measured, and a routine physical examination and dietary re-evaluation were performed.

Diet

A diet advice was given to all patients participating in the study and active encouragement to comply with this advice was also give to the partners of the patients. The diet was vegetarian with a total fat content of 34 energy(cal) percent

(e%) with al low saturated fat content of 7.0e%. It contained 100 mg cholesterol or less and was low caloric, if appropriate. Adherence to the diet was assessed by the determination of the fatty acid content of cholesterol esters.

Dietary instruction was individualized and based on a 24 hour recall which was carried out in the first weeks following study admittance. Adherence to the diet was further checked by evaluation of the food consumed during a period of one week. Further measures that were undertaken to try to mitigate coronary risk factors were the following. Smoking was strongly discouraged. An advice to exercise at least half an hour daily was given. Hypertension (RR≥160/95 mmHg) if present, was treated with hygienic methods; if this failed, a step-up medication was prescribed consisting of beta-blocking agents, diuretics, and vasodilating drugs.

Biochemical measurements

Serum total cholesterol (TC) and HDL-cholesterol (HDL-C) were determined at the Gaubius TNO Research Laboratory in Leiden in accordance with Abell's method.[23] HDL-C was isolated by precipitating other lipoproteins with magnesiumphosphotungstate; cholesterol was determined in the supernatant.[24]

Baseline readings were defined by the mean values of the measurements on two different days prior to entry into the study; for the interpretation of the results due to the intervention, the mean values of the total of fifteen measurements performed over the following two years were determined.

Triglycerides were measured at the end of the study following an overnight fast in a basal resting state.[25] Also at the termination of the study, postheparin lipoprotein lipase activities - liver lipase activity (LLA) and lipoprotein lipase (LPL) - were measured as follows: Thirty minutes after introduction of an intravenous catheter in the right brachial vein, heparin (50 IU/kg bodyweight) was administered intravenously. Blood was collected from the left brachial vein in disodium EDTA (2.7 mmol/l) on ice. Twenty minutes after the administration of heparin, blood was centrifuged at 4° C for 30 minutes at 3000 r.p.m. and plasma was stored at −20°. LPL and LLA were measured at the Department of Internal Medicine III of the Erasmus University Rotterdam.[26]

At the end of the study hormone measurements were performed in blood drawn 30 minutes after introduction of an intravenous catheter prior to heparin administration according to the following methods: cortisol (C), estradiol 17β (E2),[27] testosterone (T),[28] triiodothyronine (T_3),[29] thyroxine (T_4),[30] T_3-resin-uptake,[29] thyroid stimulating hormone (TSH),[30] and insulin (In).

Quantitative coronary arteriography

The coronary angiographic investigation was performed at the Department of Cardiology of Leiden University via the Judkins technique. The initial and sequential coronary arteriographies were performed by the same angiographer. At the second arteriography special care was taken to obtain projections identical to those

of the first angiography. At the time of the first angiography the angulations of the X-ray systems in the different projections had been registered. Angiograms were recorded on 35 mm cinefilm using the 6 inch mode of a Philips image intensifier.

All patients had constant dose maintenance therapy, thereby reducing possible effects on diameter measurements due to a change in vasomoter tone.

Quantitative analysis of coronary arterial segments was carried out with the CAAS at the Thoraxcenter in Rotterdam. An example of the quantitative analyses of the pre- and post-intervention coronary angiograms is shown in Figure.1. The severity of a coronary obstruction was expressed as a percentage diameter reduction with respect to a user-defined reference position proximal or distal to the stenosis and by means of the absolute value of the minimal obstruction diameter. For arterial segments showing no focal obstruction the mean diameter value over a user-defined length was computed.

Cineframes to be analyzed were selected as closely as possible to the end-diastolic phase. In cases of overlap of a particular segment with other vessels, a frame was selected at another instant in time.

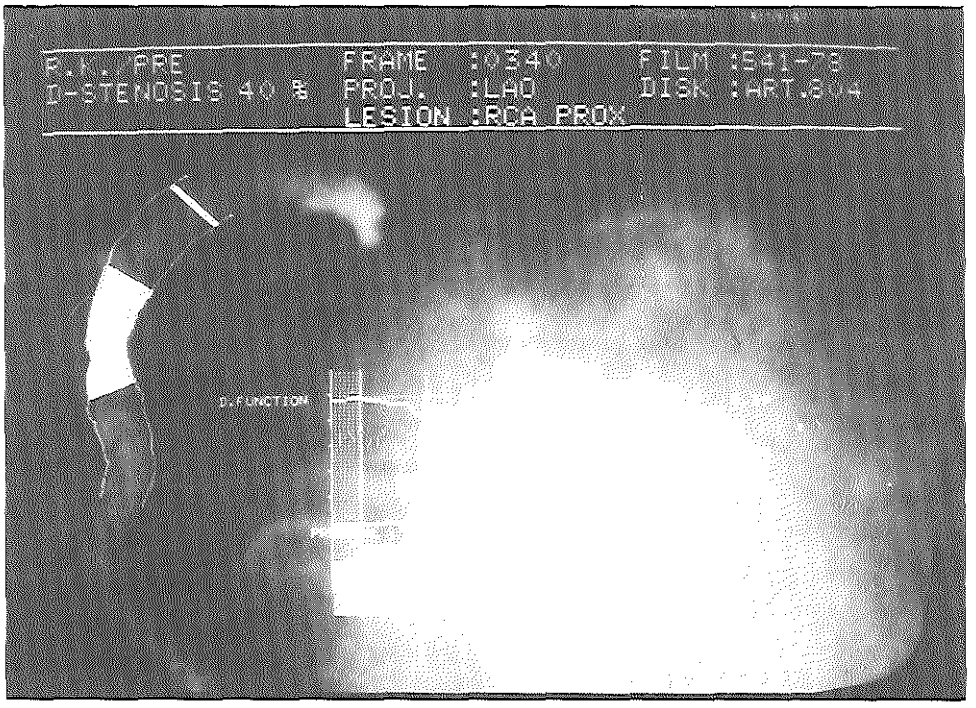
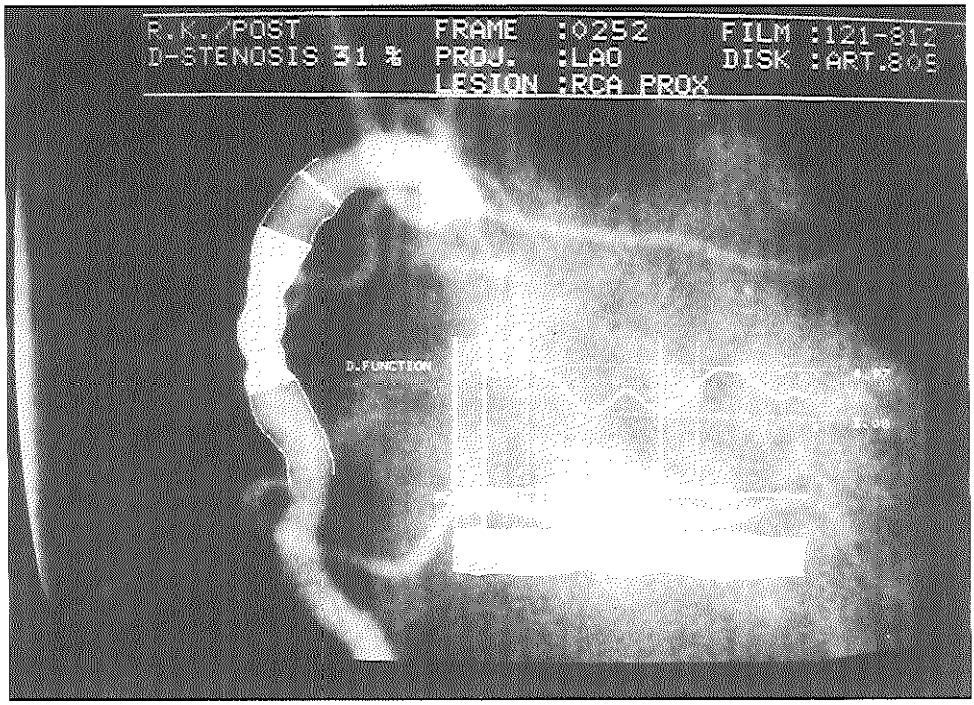


FIGURE 1
Example of quantitative results of one particular coronary segment (proximal part of right coronary artery) in the pre- and post-study situation. Note that a decrease of approximately 0.1 mm in the minimal obstruction diameter was found at the end of the study (obstruction diameter 3.08 mm) as

Coronary angiographic scoring method

The large coronary arteries were divided into a total of nine coronary segments according to the recommendations by the American Heart Association (right coronary artery: proximal, mid and distal portions; left anterior decending artery: proximal, mid and distal portions; left circumflex artery: proximal and distal portions; and the main-stem.[31] These nine coronary segments were analyzed in at least two angiographic views. For each analyzed coronary segment the severity of an obstruction, if present, was computed in terms of relative and absolute measures in addition, the mean diameters over the remaining normal proximal and distal parts were computed. From all data two coronary scores were derived, a percentage coronary score and an absolute coronary score; an example is given Table I. The percentage score was determined as follows: for each obstruction the percentage area stenosis was computed from the percentages diameter stenoses assessed from two projections, assuming elliptical cross sections, and multiplied by a flow dependent weighting factor specific for that particular coronary segment.[32] The total percentage coronary score was by adding these weighted area stenosis values for all obstructions in the nine coronary segments.



compared to the initial situation (obstruction diameter 3.18 mm). Due to the fact that the reference diameter showed a larger decrease in size than the obstruction diameter, the percentage diameter stenosis decreased as well from 40% to 31%.

TABLE I
Example computation of percentage and absolute coronary scores.

Percentage coronary score Analyzed lesion		Pre-study			Post-study		
Branch	Section	%-A sten.	WF	Cor. score	%-A sten.	WF	Cor. score
RCA	mid 1	0.31	1.0	0.31	0.23	1.0	0.23
RCA	mid 2	0.15	1.0	0.15	0.39	1.0	0.39
RCA	mid 3	0.89	1.0	0.89	0.88	1.0	0.88
RCA	mid 4	0.87	1.0	0.87	0.78	1.0	0.78
LAD	prox	0.64	3.5	2.24	0.53	3.5	1.86
LAD	mid 1	0.93	2.5	2.33	0.91	2.5	2.28
LAD	mid 2	0.92	2.5	2.30	0.90	2.5	2.25
LCX	dist 1	0.45	1.0	0.45	0.42	1.0	0.42
LCX	dist 2	0.73	1.0	0.73	0.45	1.0	0.45
			Total	10.27		Total	9.54

Mean obstr. diameter	Pre	Post	Mean diameter art. segm.	Pre	Post
RCA	2.50	2.11	RCA	3.59	3.54
MAIN	–	–	MAIN	4.66	4.27
LAD	1.65	1.75	LAD	2.77	2.80
LCX	2.21	2.22	LCX	3.42	3.01

Absolute coronary score (mm)	Pre	Post
RCA	2.50	2.11
MAIN	4.66	4.27
LAD	1.65	1.75
LCX	2.21	2.22
Absol. score	11.02	10.35

Abbreviations: RCA: right coronary artery; LAD: left anterior descending artery; LCX: circumflex artery; %-A sten: percentage area stenosis computed from the measured percentages diameter stenosis in two views; WF: flow dependent weighting factor.

The absolute coronary score was determined as follows: for each of the four large coronary arteries mentioned above the mean obstruction diameter in mm was computed from the available views. If no obstruction was present in a coronary artery, the mean value of the computed average diameter measurements for the different segments of this artery was substituted. Adding these 4 mean diameter values resulted in the absolute coronary score. The changes in the percentage and absolute coronary scores over the two year diet period were simply determined as the differences in the pre- and post-diet coronary scores.

A patient was considered to have progressive disease, if the absolute coronary

score showed a decrease. No lesion growth or regression of the disease was assumed if the coronary score showed an increase. Since we were particularly interested in changes of absolute dimensions of coronary obstructions and of nonobstructed segments, only absolute measurements were used for this study.

Statistical Analysis

Mean values and their standard deviations were used as descriptive statistics for the total group of subgroups of patients. Mean values were compared by the two sample t-test (two-sided) or when appropriate by paired t-tests. Relations between continuous variables were analyzed by linear regression analysis. All results are expressed as mean ± standard deviation ($\bar{x}\pm$s.d.). Multivariate analysis was performed by stepwise discriminant analysis.[33]

Results

No significant difference was found in the initial coronary scores between the no lesion growth group of patients (n=14) and the progression group (n=21) (Table II;) neither were weight, age, blood pressure or smoking habits significantly different. Persistance of anginal complaints in the progression group was much higher than in the no lesion growth group.

TABLE II
Clinical data and coronary scores in patients showing no lesion growth and those with progression of coronary atherosclerosis.

	No lesion growth group (n = 14)		Progression group (n = 21)	
Age (years; $\bar{x}$ ± s.d.)	49.8 ± 8.0		52.4 ± 7.8	
Number of patients	Pre-study	Post-study	Pre-study	Post-study
Smoking	9 (64%)	6 (42%)	10 (48%)	5 (23%)
Angina pectoris	14 (100%)	6 (42%)	21 (100%)	17 (80%)
Syst. BP (mmHg; mean)	130	124	131	128
Diast. BP (mmHg; mean)	83	80	85	85
Quetelet index (kg/m^2; mean)	23.9	23.9	24.1	23.4
CS (mm; mean ± s.d.)	10.17 ± 1.72	11.14 ± 1.79	10.38 ± 2.29	9.17 ± 2.12
ΔCS (mm; mean ± s.d.)	+ 1.02 ± 0.69		− 1.20 ± 0.96	

Abbreviations: n, number of patients; BP, blood pressure; CS, coronary score; ΔCS, the difference (mm) in absolute coronary scores between the pre- and post-study angiograms.

In Table III the pre- and post-study values of several lipid fractions and of the postheparin lipoprotein lipase activities are given for both groups of patients. At the start of the intervention trial total-cholesterol (TC) was significantly higher in the progression group, while high-density-lipoprotein cholesterol (HDL-C) was almost the same in both groups. At the end of the study a significant difference was

observed in the triglycerides (TG) values. TC levels showed a relatively larger drop in the progression group than in the no lesion growth group (although not significant). Liver lipase activity (LLA) in the progression group was significantly lower than in the no lesion growth group of patients, while lipoprotein lipase (LPL) was not different in the two groups of patients. LLA was inversely correlated with the degree of progression in the total population (Figure 2). LPL did not show any significant linear regression relation to the changes in coronary scores of the total population. Multivariate stepwise discriminant analysis showed LLA to be the most powerful parameter to progression or regression of atherosclerosis, independently from other pre- or post-study parameters (TC, HDL-C, TG). The change in the degree of coronary atherosclerosis expressed in terms of differences in absolute coronary scores, was not related to the change in TC or to the change in the TC to HDL-C ratio (r= −0.196) (Figure 3).

TABLE III
Serum lipids and lipases in patients showing no lesion growth and those with progression of coronary atherosclerosis.

	No lesion growth group (n = 14)		Progression group (n = 21)	
	Pre-study	Post-study	Pre-study	Post-study
		$p < 0.05$		
TC	6.37 ± 1.05	-*- 5.80 ± 1.03	7.22 ± 1.57 -*-	6.43 ± 1.55
			$p < 0.05$	
HDL-C	1.00 ± 0.15	1.01 ± 0.19	0.99 ± 0.11	0.96 ± 0.11
TG		1.59 ± 1.14	---*---	2.02 ± 1.04
LLA		499 ± 146	---***---	298 ± 190
LPL		86 ± 36		87 ± 34

Abbreviations: TC: total-cholesterol (normal values: 4.5-8.0 mmol/l); HDL-C: high-density-lipoprotein-cholesterol (normal values: 0.80-1.20 mmol/l); TG: triglycerides (normal values: 0.50-2.50 mmol/l9; LLA: liver lipase activity (mU/ml) (normal values: 472 ± 127); LPL: lipoprotein lipase (mU/ml) (normal values: 87 ± 32); 1 mU = mmoles free acids released/min. All values are mean ± s.d.; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table IV shows the absence of any significant difference in basal cortisol, estradiol-17β, testosterone and insulin values between the no lesion growth and progression groups of patients. Similarly, there were no significant differences in for, T_3-resin-uptake and the thyroid stimulating hormone between these two groups of patients (Table V). However, triiodothyronine (T_3), appeared to be significantly ($p<0.01$) higher in the no lesion growth group, thyroxine (T_4) was lower in the no lesion growth group ($p < 0.01$) although T_3 and T_4 remained within the normal ranges for both groups. Linear regression analysis showed a poor correlation between LLA and T_3 ($r = 0.283$, $p =$ n.s.).

When the total male population was categorized according to the lipid lowering

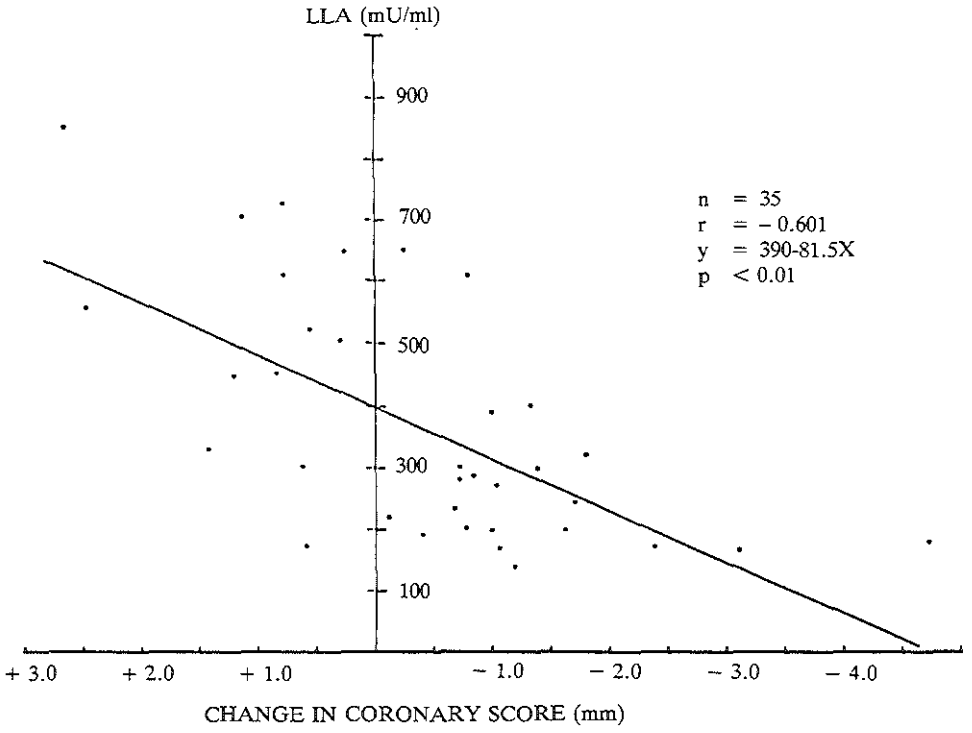


FIGURE 2
This figure shows the relationship between the change in absolute coronary score (mm) and liver lipase activity at the end of the study period. LLA = liver lipase activity (mU/ml), 1 mU = mmoles free fatty acids released/min.

effects of the diet, three response patterns became apparent (Table VI). The group (n = 15 patients) with an initial low TC to HDL-C ratio ($\leq$ 6.9) remained so during dietary treatment; this behavior was accompanied by a normal LLA value. The second group (n = 11 patients) showed an initial high value of the TC to HDL-C ratio (> 6.9), which was nonresponsive to the diet, and a low LLA value. Of particular interest is the third group (n = 9 patients) in which an initial high TC to HDL-C ratio (> 6.9) fell to below 6.9 in reaction to the dietary intervention, while the LLA was as higher than in the first mentioned group of patients. LPL was not different in these three groups of patients showing different responses to dietary intervention.

The values of the endocrine parameters in relation to the three different response patterns are shown in Table VII. No specific major effect on dietary lowering of TC could be found for insulin, estradiol-17β, testosterone, cortisol, thyroxine, T_3-resin uptake and the thyroid stimulating hormone. In the group of patients that were responsive to the dietary intervention, triiodothyronine was higher than in both other groups.

Table VIII shows the average initial coronary score values and the mean changes over the study period for the three different lipid lowering response patterns.

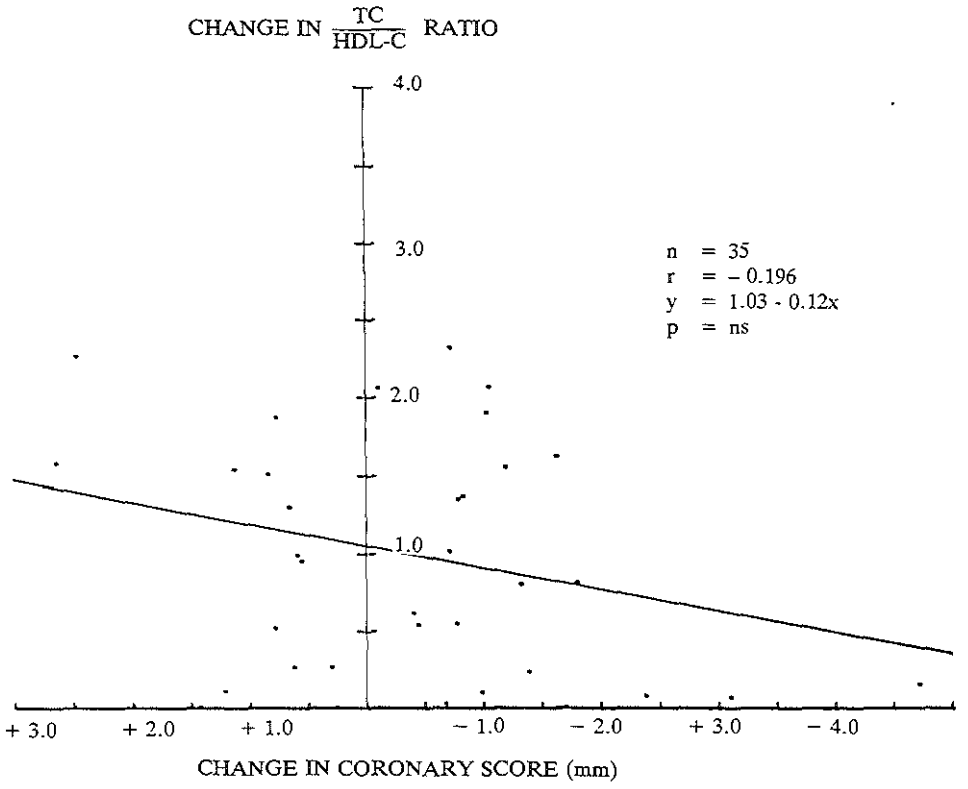


FIGURE 3
In this figure the relationship between the change in absolute coronary scores (mm) and the change in the ration of total cholesterol (TC) to HDL-C from baseline values is shown. HDL-C = high density lipoprotein cholesterol (mmol/l).

Discussion

Although this study is hampered by the fact that no pre-study endocrine and postheparin lipoprotein lipase activities were available, the following conclusions may be drawn taking into account comparable data from other studies:[34]

Firstly, in the presence of advanced coronary atheroclerosis, progression of the disease i.e. its natural progressive course, apparently may be halted or even be reversed by dietary intervention.

Secondly, LLA and not LPL is negatively correlated to the degree of progression of coronary atherosclerosis, suggesting a key role for the former enzyme.[35] The levels of all analyzed hormones, with the exception of T_3 and T_4 were not different

when the no lesion growth group was compared to the progression group.

With respect to the first conclusion, it should be noticed that although the two groups studied were comparable with respect to complaints, age, weight, biochemical status and lipid profile (except for a initial slightly higher TC-value in the

TABLE IV
Insulin, sex hormones and cortisol in the no lesion growth and progression groups of patients.

	No lesion growth group (n = 14)	Progression group (n = 21)
Testosterone (nmol/l)	23.6 ± 12.0	22.3 ± 10.6
Estradiol - 17β (pmol/l)	118 ± 37	129 ± 31
Cortisol (nmol/l)	455 ± 97	479 ± 138
Insulin (mU/l)	12.4 ± 1.3	13.9 ± 1.9

All values: means ± s.d.

TABLE V
Thyroid hormone values in the no lesion growth and progression groups of patients at termination of the study.

	No lesion growth group (n = 14)	Progression group (n = 21)
T_3 (nmol/l)	2.24 ± 0.36 - $p < 0.01$ -	1.86 ± 0.28
T_4 (nmol/l)	99 ± 14 - $p < 0.01$ -	115 ± 18
T_3-resin uptake (%)	27.3 ± 2.5	28.0 ± 3.1
TSH (mU/ml)	1.9 ± 2.5	2.4 ± 2.8

Abbreviations: T_3, Triiodothyronine; T_4, Thyroxine; TSH, Thyroid Stimulating Hormone. All values: means ± s.d.

TABLE VI
Dietary lipid lowering effects on the postheparin lipoprotein lipase activities and on lipid fractions.

TC/HDL ratio			
Initial value	> 6.9	> 6.9	≤ 6.9
Value during study	≤ 6.9	> 6.9	≤ 6.9
Number of patients	(n = 9)	(n = 11)	(n = 15)
TC/HDL-C	6.20 ± 0.70 -***-	8.38 ± 1.09 -**-	5.10 ± 0.72
		$p < 0.05$	
TG	1.21 ± 0.54 -**-	2.46 ± 1.04 -**-	1.13 ± 0.42
		n.s.	
LLA	480 ± 149 -***-	244 ± 104 -**-	426 ± 144
		n.s.	
LPL	85 ± 38 -n.s.-	78 ± 20 -n.s.-	89 ± 23
		n.s.	

Abbreviations: TC, total-cholesterol (mmol/l); HDL-C, high-density-lipoprotein-cholesterol (mmol/l); TG, triglycerides (mmol/l); LLA, liver lipase activity (mU/ml); LPL, lipoprotein lipase (mU/ml); 1 mU = mmoles free fatty acids released/min, All values: means ± s.d.; n.s., not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

progression group), it cannot be excluded that the two groups of patients differed in other parameters. The interpretation of the results from this study is also limited to some extent by the lack of a control group.

However, as has been shown before, coronary atherosclerosis is an almost unequivocally progressive disease and therefore any evidence of nonprogression may be considered as an indication of a deviation from the natural course of the disease.[36,37] Difficulties in the quantitative evaluation of atherosclerosis have been described elsewhere.[38,39]

The purpose of this study was threefold:

1. To assess the relationship between the diet induced changes in lipid values and the progression of coronary atherosclerosis.

TABLE VII
Dietary lipid lowering effects and endocrine parameters.

TC/HDL ratio			
Initial value	> 6.9	> 6.9	⩽ 6.9
Value during study	⩽ 6.9	> 6.9	⩽ 6.9
Number of patients	(n = 9)	(n = 11)	(n = 15)
In (mU/ml)	11.2 ± 1.4	13.7 ± 2.9	14.3 ± 40
		$p < 0.05$	
E2 (pmol/l)	114 ± 37	120 ± 38	124 ± 4.3
T (nmol/l)	19.6 ± 4.2	18.2 ± 75	19.9 ± 4.3
C (nmol/l)	398 ± 89	480 ± 134	472 ± 121
T_3 (nmol)	2.4 ± 0.4 -***-	1.7 ± 0.2	1.9 ± 0.9
		-**-	
T_4 (nmol/l)	90 ± 19	109 ± 24	106 ± 16
T_3-resin uptake (%)	26.1 ± 2.6	28.8 ± 2.9	28.1 ± 2.7
TSH (mU/ml)	2.2 ± 2.4	1.0 ± 2.6	1.8 ± 2.0

Abbreviations: In, Insulin; E2, Estradiol-17β; T, Testosterone; C, Cortisol; T_3, Triiodothyronine; T_4, Thyroxine; TSH, Thyroid Stimulating Hormone; All values: means ± s.d.; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Other relations were not significantly different.

TABLE VIII
Dietary lipid lowering effect and the initial average coronary score and the response pattern changes over the study period.

TC/HDL ratio			
Initial value	> 6.9	> 6.9	⩽ 6.9
Value during study	⩽ 6.9	> 6.9	⩽ 6.9
Number of patients	(n = 9)	(n = 11)	(n = 15)
Average initial coronary score	10.88 ± 1.52 -n.s.-	10.71 ± 1.40 -$p < 0.05$-	0.64 ± 1.92
		$p < 0.05$	
Mean change in mm	+ 0.38	− 1.09	+ 0.07
Range of changes	(− 1.09 to + 2.47)	(− 3.10 to + 0.26)	(− 1.25 to + 2.05)

All values: mean ± s.d.

2. To assess the relationship between the postheparin lipoprotein lipase activities and the progression of coronary atherosclerosis on one hand and the diet induced lipid value changes on the other hand.
3. To assess the role of several atherosclerosis related hormones and the progression of coronary atherosclerosis.

Concerning the first question, a clear and powerful positive relationship has been established between diet (the amount of cholesterol and poly unsaturated fatty acids) lipid level changes and the rate of progression of coronary atherosclerosis.[22] As had been stated before, the postheparin lipoprotein lipase activities are important modulators in lipid metabolism determining to a certain degree the ratio between HDL and LDL cholesterol.

To study the possible relationship between LLA and LPL, and the degree of change of coronary atherosclerosis in men with advanced atherosclerosis, a lowered LLA and a normal LPL level may be assumed for reasons mentioned before.[34] This seems valid as the initial coronary atherosclerotic values, expressed in terms of coronary scores, were essentially not different (Table II). As may be derived from the results, LLA was significantly higher in the no lesion growth group of patients but still within the normal range, independently from all other lipoprotein parameters including LPL (Table III).

The results may suggest a possible threshold value for cholesterol for the induction of nonprogression of advanced coronary atherosclerosis; this is based on the fact that the decrease in TC level in the progression group was even larger than in the no lesion growth group (Table III). A lowered LLA may impair the mobilization of TC from peripheral cells by HDL by hampering with the excretion of cholesterol in the liver to be converted to bile acids. Therefore, a lowered LLA may contribute to a higher cholesterol pool and as a result contribute to the progression of atherosclerosis.

Although LLA was only measured at the end of the study, results clearly demonstrate that those patients that responded to diet with a lowering of the TC values, showed no further lesion growth or possibly even regression. This may indicate those patients that may benefit from the prescribed diet and those who probably do not. In the future a period of only three to six months may be sufficient to discriminate those who may profit from diet and those who do not by measuring the LLA level, although this obviously requires further study.

It is of interest to note that those patients with an initial and continuing low TC/HDL-C ratio, had the lowest coronary scores, hence the most extensive atherosclerotic disease (Table VIII). Since they did not appear to be the patients with the greatest tendency to regression of the atherosclerotic process, as measured by the average change (mm) in coronary scores, other factors besides the lipoproteins and lipases, have to be assumed (Table VIII).

The Leiden Intervention Trial showed that LLA measured following dietary intervention is strongly correlated to changes in coronary atherosclerotic lesion size

(Figure 2). However, whether a causal relationship may be assumed between LLA and the changes observed cannot be concluded from these data. A strong relationship between the changes in TC/HDL-C ratio and those in the coronary scores was not observed (Figure 3).

As far as the third question concerning endocrine parameters, a differentiation must be made between the various possibly atherosclerotic growth related hormones and the progression of coronary atherosclerosis. Diabetes mellites or abnormal levels of insulin (relative or absolute) are correlated te acceleration of progression of coronary atherosclerosis.[40] Cortisol, the main adrenal cortical stress hormone, is also positively related to the rate of progression of coronary atherosclerosis.[41] No significant difference between the groups mentioned could be established at the end of our study, suggesting that no important role for both of these atherogenic hormones exists (Table IV).

Estradiol-17β (E2) has been considered to be anti-atherogenic and was even administered in pharmacologic doses in the Coronary Drug Project,[42] but was later withdrawn for reasons that in those patients receiving estradiol a significantly higher percentage suffered a sudden death. Nevertheless, E2 which in men is mainly derived from testosterone (T), is known to show a positive correlation to the degree of coronary atherosclerosis.[43] Our study showed that after two years of dietary intervention no significant difference could be found in E2 and T values between the no lesion growth and the progression groups (Table IV).

Of particular interest is thyroid hormone action in relation to atherosclerosis. The TC measurement used to be a marker for the metabolic state. When TC was high, a low thyroid function was assumed.[44] Although it is wellknown that in hypothyroidism coronary atherosclerosis is severe, a clear advantage from thyroxine administration has not been shown to benefit a general population. The Coronary Drug Project even withdrew thyroxine administration as a therapeutic drug after the establishment of severe side effects in coronary atherosclerosic patients.[45]

Nevertheless, it is of interest to derive from the results that patients showing no lesion growth apparently have a normal although higher metabolic state than the progression group patients, as measured by T_3 levels (Table V). A possible explanation may be that at the end of the study, those patients showing progression were using bèta blocking agents to a greater degree than the patients from the no lesion growth group. As bèta blocking agents inhibit the conversion of T_4 to T_3, and as no significant different number of patients on bèta blocking agents existed, the different metabolic state of these patients in the no lesion growth group as compared to the progression group cannot be explained by the usage of bèta blockers.

Finally, it may be concluded from this study, that the levels of insulin, cortisol, estradiol, and testosterone with the exception of T_3 and T_4, were not essentially different in the no lesion growth and progression groups, suggesting no major atherogenic modulating effects of these before-mentioned hormones.

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CHAPTER VI

Progression and Regression of Coronary Atherosclerosis. The role of lipoproteins, lipases and thyroid hormones in coronary lesion growth.

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Summary

Relations between lipoprotein fractions, postheparin lipoprotein lipase activities, thyroid hormones and coronary lesion growth were studied among 35 male patients with severe cononary atherosclerosis and stable angina pectoris, who had participated in the lipid lowering Leiden, Intervention Trial. Coronary arteriographies were performed at the beginning of the study and 2 years later at termination. The lesions were quantitated with a computer-based analysis system to assess the progression rate of coronary lesions on the basis of the absolute arterial dimensions in a patient's coronary tree: for these purposes an absolute coronary score was computed. On the basis of the absolute coronary scores, the entire group of patients could be divided into a no lesion growth group (14 patients) and a progression group (21 patients). Lipoprotein fractions, lipoprotein lipases and thyroid hormones were determined after two years of intervention. No significant difference was found between the no lesion growth or regression and progression groups of patients for total-cholesterol (TC) and LDL-cholesterol (LDL). The VLDL-cholesterol (VLDL) and the triglycerides (TG) were significantly higher ($p<0.05$) and HDL-cholesterol (HDL-C) was almost significantly lower ($p<0.10$), in the progression group. Lipoprotein lipase (LPL) values were not significantly different in the groups with and without lesion growth Hepatic lipase (HL) values, however, were significantly higher in the no lesion growth group, as compared to the progression group. The biologically active thyroid hormone, triiodothyronine (T_3) was significantly lower ($p<0.01$) in the progression group as compared with the no lesion growth group. Multivariate regression analysis showed HL to be the most important determinant of changes in coronary atherosclerosis. T_3 and HDL were also independently inversely related to

coronary lesion growth. The probably enhanced conversion of thyroxine (T_4) to the more metabolic active T_3 could imply that the reversed cholesterol transport mechanism may take place at a higher rate in the no lesion growth group than in the progression group.

Introduction

Coronary atherosclerosis resulting in angina pectoris, myocardial infarction or sudden death remains a major contributor the epidemic of cardiovascular diseases. One of the most important factors that determine the rate of progression of coronary atherosclerosis is a disordered lipid metabolism.[1-3] A positive correlation has been demonstrated between the morbidity and mortality of coronary heart disease and the serum lipid fractions.[4-5] Serum levels of certain lipoprotein fractions such as the low density lipoprotein (LDL), the major carrier of cholesterol, have a strong positive correlation to the incidence of coronary atherosclerotic heart disease (CHD). The fraction of the high-density-lipoproteins (HDL) is considered 'anti-atherogenic' as this fraction is inversely correlated tot development of CHD.[6]

The postheparin lipoprotein lipase activities belong to the class of modulators of lipoprotein metabolism affecting the ratio between HDL and LDL-cholesterol.[7-9] These enzymes, located in endothelial vascular cells are released into the blood-stream by intravenous heparin administration.[7] While lipoprotein lipase (LPL) plays a major role in the removal of plasma triglycerides (TG), the role of hepatic lipase (HL) is less clear. Both enzymes play a role in HDL-metabolism. It has been established that LPL is positively correlated with HDL-cholesterol,[10] while the other lipase, HL, is negatively correlated with this fraction.[11] As HL may play a role in HDL-cholesterol transport to the liver and/or in the metabolism of the atherogenic intermediate-density-lipoproteins (IDL), it has been proposed that conditions with a low(ered) HL are unfavorable with regard tot atherosclerotic conditions.[12,13,33] This knowledge together with the inverse relationship of HL with HDL(-cholesterol) implicates that a high HDL-cholesterol level may not always be advantageous. In line with these considerations we found that normolipedemic men with angiographically defined coronary atherosclerosis have a lower HL than controls without coronary atherosclerosis.[14]

Thyroid hormone, more specifically triiodothyronine (T_3), is a factor known to alter lipid metabolism[46]. In hypothyroidism, LDL rises and HDL falls. With thyroid substitution therapy, a normalization of the lipid disorder ensues.[36] The serum level to thyroid hormone is also known to be correlated to the postheparin lipoprotein lipases[38] and part of the serum lipid level changes may be due to changes in the activities of these enzymes.

Recently the effect of dietary intervention on the development of the atherogenic process was studied in a group of patients with severe coronary atherosclerosis: The Leiden Intervention Trial.[21] End points for this study were the measurements

of the rates of change of arterial dimensions as assessed by coronary arteriography.[15] Conventional visual interpretation of the severity of arterial lesions by coronary angiography is hampered by large inter- and intra-observer variability.[16-18] To circumvent these problems, in the Leiden trial a quantitative computer assisted image processing technique was employed to assess the severity of coronary obstructions from 35 mm cine-angiograms in a highly objective and reproducible way.[19-20] At the final examination of the trial, lipoproteins, LPL, HL and thyroid hormones were determined in all 35 male patients. These variables were studied in relation to coronary lesion growth during an observation period of two years. The results of these analyses are reported here.

Methods

Thirty-five male patients with chronic stable angina pectroris were selected to participate in a lipid lowering dietary intervention program (The Leiden Intervention Trial). At the end of the two-year intervention period – the study lasted from 1978 tot 1982 – a repeat coronary arteriography was performed.

The intervention consisted of a vegetarian diet with a polyunsaturated to saturated fatty acids (P/S) ratio of 2 and with a total fat content of 34 energy percent. It contained 100 mg cholesterol per day or less and was low caloric, if appropriate.

Serum total-cholesterol (TC) and HDL-cholesterol (HDL-C) were determined according to Abell's method;[22] HDL-C was isolated by precipitating other lipoproteins with magnesiumphosphotungstate and cholesterol was determined in the supernatant.[23] Triglycerides (TG) were measured at the end of the study, after an overnight fast in a basal resting state.[24] The cholesterol concentration in the VLDL lipoproteins was calculated according to Friedewald et al[37] by dividing serum triglycerides by 5. LDL-cholesterol was calculated by subtracting HDL- and VLDL-cholesterol from total cholesterol. Postheparin lipoprotein lipase activities were measured as follows: thirty minutes after introduction of an intravenous catheter in the right brachial vein heparin (50 IU/kg bodyweight, Thromboliquine, Organon, Oss) was administered intravenously. Blood was collected from the left brachial vein in disodium EDTA (2.7 mmol/l) on ice twenty minutes after the administration of heparin. Blood was centrifuged at 4°C for 30 minutes at 3000 r.p.m. and plasma was stored at -20°C. LPL, HL were measured as described by Huttunen et al.[25]

All biochemical parameters were measured at the final examination.

Coronary arteriography

The coronary angiography procedure was performed with the Judkins technique.[26] The initial and sequential coronary arteriographies were performed by the same angiographer. At the second arteriography special care was taken to obtain projections identical to those of the first angiography.

Computer processing of coronary angiograms

Quantitative analysis of coronary arterial segments was carried out with the computer-based Cardiovascular Angiography Analysis System (CAAS); details of the analysis procedure and the results of a validation study have been described elsewhere.[20,27] The system allows the accurate delineation of the contours of user-selected coronary arterial segments by means of automated edge-detection algorithms. An example of the quantitative analyses of the pre- and post-intervention coronary angiograms of one particular coronary segment is shown in Figure 1. Diameter values are expressed in absolute terms (mm) by using the intracardiac catheter as a scaling device. The severity of a coronary obstruction was expressed as a percentage diameter reduction with respect to a user-defined reference position proximal or distal to the stenosis and by means of the absolute value of the diameter at the site of maximal obstructions. For arterial segments showing no focal obstruction, the mean diameter value over a user-defined length was computed.

Cineframes to be analysed were selected as closely as possible to the enddiastolic phase. When a praticular segment of a coronary artery overlapped with another vessel, a cineframe was selected at another instant in time.

The variablility of repeated analysis of cine-angiograms in terms of absloute arterial dimensions of the CAAS system has been found to be less than 0.11 mm and in terms of percentage coronary arterial narrowing for coronary obstructions less than 2.74%.[20] A total of nine major coronary segments were assessed according to the recommendations by the American Heart Association (right coronary artery, RCA: proximal, mid and distal portions; left anterior descending artery, LAD: proximal, mid and distal portions; left circumflex artery, LCX: proximal and distal protions; and the main stem, main). These nine coronary artery segments were analyzed in at least two, preferably orthogonal projections. For each analyzed coronary segment the severity of an obstruction, if present, was computed in terms of relative and absolute measurements, as well as the mean diameter over one or more nonobstructed portions of this segment. The absolute size of an obstruction was computed as the mean diametervalue of the obstruction diameters assessed in the different views. The absolute coronary score was determined as follows: for each of the four large coronary arteries (RCA, main, LAD, LCX) the mean vascular diameter at the different sites of the obstructions as assessed from the available views was computed in mm. If no obstruction in a coronary artery was present, the mean value of the computed average diameter measurements for the different segments of this artery was subsitituted. Adding these 4 mean diameter values resulted in the absolute coronary score. The changes in absolute coronary scores over the two year diet period were simply determined as the differences in the pre- an postdiet scores.

A patient was considered to have a progressive disease, if the absolute coronary score showed a decrease. No lesion growth or regression of the disease was assumed if the coronary score showed an increase.

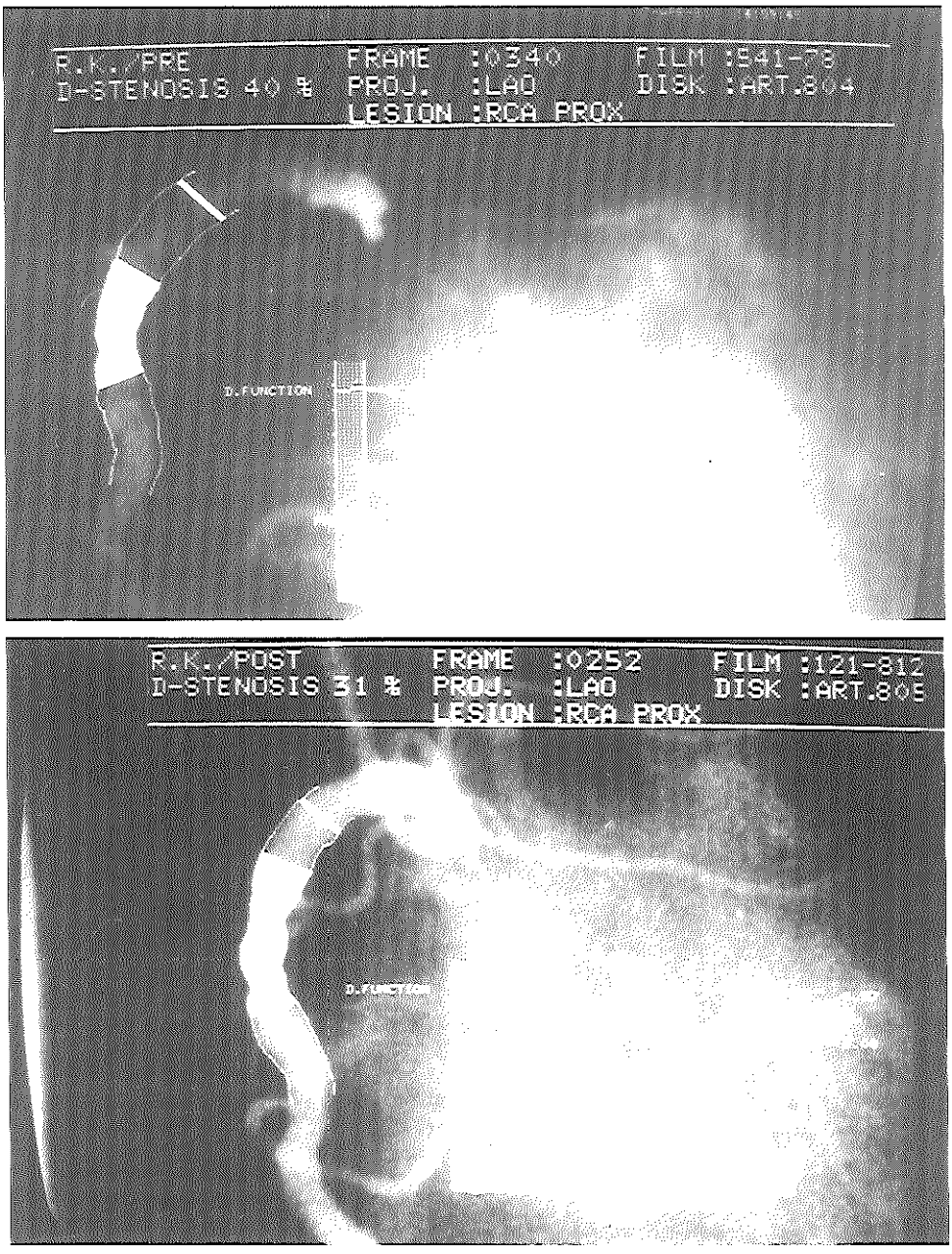

FIGURE 1
Example of quantitavive results of one particular coronary segment (proximal part of right coronary artery) in the pre- (above) and post- (below) study situation. In this particular case of a proximal segment of a right coronary artery the luminal obstruction diameter decreased from 3.18 mm to 3.08 mm. Due to the fact that the reference diameter showed a larger decrease in size than the obstruction diameter, the percentage diameter stenosis decreased as well from 40% to 31%.

Statistical methods

Means were compared by the two sample Student's t-test (two-sided). Relations between continuous variables were analyzed by linear regression analysis. The number of possible determinants of coronary lesion growth was reduced by using a backward elimination program.[29] Independent variables remained in the regression equation when their p-value was lower than 0.10.

Results

Twenty-one of the 35 patients showed progression of coronary atherosclerosis and 14 patients showed no lesion growth or even regression (Table I). No significant difference was found between the progression and no lesion growth group with respect to their initial coronary lesion scores. Furthermore, significant differences were not found for weight, age, blood pressure and smoking habits. Persistence of anginal complaints at the end of the trial was, however, significantly ($p < 0.01$) higher in the progression group (Table I).

Table II shows the lipoprotein fractions, the lipoprotein lipases and the thyroid hormones at the termination of the study. LDL cholesterol levels were similar in both groups, but VLDL cholesterol was significantly higher among the men who showed progression. HDL cholesterol was lower among the men who showed progression compared to the men without progression. This difference was almost statistically significant ($p < 0.10$). HL was significantly higher ($p<0.001$) in the no lesion growth group while LPL was not different in the two groups. The mean HL levels were also significantly lower than normal. No significant correlations could be found between HL and HDL-C, VLDL-C and LDL-C values. Of the thyroid hormones, T_3 was significantly higher and T_4 was significantly lower in the no lesion growth group. Both parameters remained within the normal range. T_3-resin uptake dit not differ between the two groups.

After univariate analysis, multiple lineair regression analysis was performed using coronary lesion growth as dependent and lipoprotein fractions, hepatic lipase and thyroid hormones as independent variables. Hepatic lipase, T_3 and HDL-cholesterol were independently significantly inversely related to coronary lesion growth (Tabel III).

Discussion

At termination of the study, lipoprotein fractions (TC, TG and HDL-C), lipoprotein lipases (LPL and HL) and thyroid hormones (T_3 and T_4) were measured, as these parameters may be related to progression of atherosclerosis.[5,14,21,39,46].

In our study, calculated LDL-cholesterol, the main component of TC, was not significantly different in the progression and the no lesion growth groups. This

TABLE I
Clinical data and coronary scores in patients showing no lesion growth or progression of coronary atherosclerosis at termination of the study.

	no lesion growth group (n = 14)	progression group (n = 21)
Age (years)*	49.8 ± 8.0	52.4 ± 7.8
Number of patients:	poststudy	poststudy
Smoking	6 (42%)	5 (23%
Angina pectoris	6 (42%)	17 (80%)
Syst. BP (mmHg)	124	128
Diast. BP (mmHg)	80	85
Quetelet index (kg/m^2)	23.9	23.4
CS* (mm)	11.14 ± 1.79	9.17 ± 2.12
ΔCS* (mm)	+1.02 ± 0.69	−1.20 ± 0.96

n = number of patients
CS = coronary score (mm)
ΔCS = the difference (mm) in absolute coronary scores form the pre- and post-study angiograms
* mean ± S.D., otherwise mean values

seems to be in contrast to previous findings where TC is an important risk factor for coronary artery disease.[39] However, in the prospective analysis of the Leiden Intervention Trial, the TC level was significantly related to occurrence of progression of atherosclerosis.

The fact that no significant difference was found in our study for LDL-cholesterol between the two groups is in agreement with Vlietstra et al[40] and Jenkins et al.[41] However, one has to take into account that these patients had been on an intervention diet for two years.[11] The variation between patients may therefore be reduced. This may be an explanation for observing no relation between the calculated LDL at termination of the trial and coronary lesion growth. However LDL values were higher at termination of the trial in the no lesion growth group when compared with the progression group. These results suggest that reversed cholesterol transport is enhanced in the no lesion growth group. Hypothyroidism is known to be associated with elevated TC values and TC normalization[36] follows thyroid hormone substitution therapy. Patients who died with clinical symptoms of hypothyroidism had significantly more extensive coronary atherosclerosis than controls.[43] Thyroxine (T_4), quantitatively the main secreted thyroid hormone, is converted to the metabolically active T_3 in the peripheral tissues.[44] In our study T_3 was significantly higher and T_4 significantly lower in the no lesion growth group, implying a higher metabolic state. Bèta blocking agents intervene with the conversion of T_4 tot T_3.[45] No significant difference was found in the use of these agents in the two groups studied: eleven out of fourteen in the no lesion growth group and twelve out of twenty-one in the progression group.

The post-heparin lipoprotein lipases, LPL and HL, are important modulators of the lipid metabolism.[7-10] The extent of coronary atherosclerosis has been shown to

TABLE II

Lipids, lipoproteins, lipoprotein lipases and thyroid hormones values at the final examination of the Leiden Intervention Trial in patients showing no lesion growth or progression of coronary atherosclerosis.

	no lesion growth group n = 14	progression group n = 21
TC	233 ± 52	254 ± 76
TG	97 ± 74	162 ± 86*
HDL-C	39 ± 7	37 ± 4
LDL-C	162 ± 44	188 ± 76
VLDL-C	21 ± 16	36 ± 19*
LPL	86 ± 36	87 ± 34
HL	499 ± 146	298 ± 190***
T_3	2.24 ± 0.36	1.68 ± 0.28**
T_4	99 ± 14	115 ± 18**
T_3-resin uptake	27.3 ± 2.5	28.0 ± 3.1

TC = total-cholesterol (mg/dl)
TG = triglycerides (mg/dl)
LDL-C = low-density-lipoproteins (mg/dl)
HDL-C = high-density-lipoprotein cholesterol (mg/dl)
VLDL-C = very low density lipoprotein-cholesterol (mg/dl)
HL = hepatic lipase (mU/ml) (normal values 472 ± 127)
LPL = lipoprotein lipase (mU/ml) (normal values 87 ± 32)
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$
T_3 = triiodothyronine (ng/l)
T_4 = thyroxine (ng/l)
T_3-resin uptake (%)
n = number of patients, all values are mean ± s.d.

1 mU = mmoles fatty acids released/min.

TABLE III

Univariate and multivariate standardized regression coefficients for HDL cholesterol, hepatic lipase and T_3 in coronary lesion growth.

	standardized regression coefficients	
	UV	MV
HDL-cholesterol (mg/dl)	−0.30	−0.30*
Hepatic lipase (mU/ml)	−0.55***	−0.47**
Triiodothyronine (T_3) (ng/l)	−0.48**	−0.33*
R^2		0.51

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
UV = univariate analysis
MV = multivariate analysis
R^2 = proportion of explained variance in the dependent variable.

be inversely correlated to the HL level.[14] On the other hand HL and LPL have also been shown to be correlated to thyroid hormone levels.[46] In our study HL was significantly higher in the no lesion growth (or regression) group as compared to the progression group. Multivariate analysis indicated that HL was the most powerful predicting parameter with regards to regression of angiographically defined coronary atherosclerosis (Table III). Next to HL, T_3 and HDL-C were also important in predicting regression of atherosclerosis. All these parameters were independent contributors to the process, defined as regression of coronary atherosclerosis and explained more than 50% (Table III), in the variance of lesion growth. This means that T_3 not only influences the atherosclerotic regression proces by virtue of its possible stimulating effect on HL but the fact that HL is the most powerful predictor points to an important role of HL in the cholesterol transport mechanism.[33]

What the exact role of HL in lipoprotein metabolism is, remains yet to be clarified. This enzyme has been suggested to play a role in the uptake of cholesterol from HDL by the liver.[33] There are indications that this lipase may play a role in the conversion of IDL to LDL.[32] A lowered HL would thus either lead to an impaired cholesterol removal by the liver and reverse cholesterol transport mechanism, and/or to an accumulation of the highly atherogenic IDL, leading to atherogenic conditions.[13]

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CHAPTER VII

Clinical significance of progression and regression of coronary atherosclerotic lesions

The Leiden Intervention Trial

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Summary

The clinical significance of progression and regression of coronary atherosclerosis was studied in the Leiden Intervention Trial. Quantitative measurements of coronary arterial dimensions assessed at the beginning of the study with a computer-based analysis system and after a dietary intervention period of 2 years were performed. Progression of lesions occurred in 21 out of the 39 patients, 11 patients remained stable, while 7 patients showed clear signs of regression. The effect of the intervention diet was stronger on the severe coronary lesions (> 50%) than on lesser ones ($p < 0.02$). ECG as a parameter of coronary atherosclerosis was coded according to the Cardiac Infarction Injury Score (CIIS). The CIIS score was significantly ($p < 0.05$) correlated with coronary atherosclerosis both before and after two years of intervention.

Persistent exertional angina pectoris at the end of the study was significantly ($p < 0.01$) correlated with progression of coronary atherosclerosis. Vital status, 3.5 years after final examination was also significantly ($p = 0.05$) correlated with progression of coronary atherosclerosis. We infer from these findings that progression and regression of coronary atherosclerosis as measured over a period of two years, have important clinical implications for the individual patient.

Introduction

There is evidence that under certain circumstances the luminal diameters of stenotic, presumably atherosclerotic arteries may widen over the years. The underlining process – distinct from release of spasm – has been named 'regression of atherosclerosis'. When we try to relate regression to our understanding of the pathogenesis of atherosclerosis fundamental questions are encountered, as Malinow has pointed out:[1,2] What is the anatomical substrate for the phenomena? Are

certain lesions more likely to regress than others? What variables define regression in different circumstances? Does regression (arrest or progression) influence the clinical course in patients with cardiovascular arterial disease?

When one wants to try and find answers to these questions a number of problems are encountered. The most serious disadvantage of present day investigation techniques is that for the documentation of the evolution of coronary atherosclerotic narrowing, serial angiography is required, which almost rules out the possibility to observe pro- and regression of atherosclerotic lesions in symptom-free persons. A second important problem is caused by the fact that with angiographic films we cannot as yet study the tissue surrounding the arterial lumen. Diagnosis of regression of atherosclerosis can thus be inferential only. A third obstacle or rather group of obstacles concerns the methodology of quantitation of lesions in repeat angiography, which is foremost, as stipulated by Blankenhorn et al.[3,4] For example, great care should be taken in obtaining the same angiographic projections at the time of the sequential angiographies. Completeness of filling by contrast material in the vessel and timing in relation to the cardiac cycle as well as vascular tone are yet another group of important factors which should be taken seriously into account. Quantitation of changes in diameters has been essentially improved by the development of computerized edge-detection techniques.[5-7] So far, three serial prospective angiographic studies with computer-supported analysis on the possible occurence of regression have been published.[6,8,9] In two of these femoral arteries were studied and in the third one coronary arteries: The Leiden Intervention Trial.

Reversibility of atherosclerosis is important for the prevention of clinical sequelae of coronary atherosclerosis. The purpose of the present study was to relate pro- and regression of coronary atherosclerotic lesions to symptoms and signs of the disease e.g. ECG-findings, angina pectoris and mortality.

Patients and methods

The Leiden Intervention Trial protocol and findings have already been published in detail.[9] In brief, the relations between diet, serum lipoproteins and the progression of coronary lesions were studied in 39 patients with stable angine pectoris in whom coronary arteriography at the time before the intervention had demonstrated single or multiple vessel disease with at least one obstruction with a greater than 50% diameter narrowing. Intervention consisted of the use of a vegetarian diet for two years. The diet had a ratio of polyunsaturated to saturated fatty acids of 2 and furnished a dietary cholesterol intake of less than 100 mg per day.

Computer-assisted analysis of the coronary cine-angiograms was carried out with the Cardiovascular Angiography Analysis System (CAAS, Thoraxcenter, Rotterdam, The Netherlands,[7]). This system permits the accurate delineation of the contours of user-selected coronary arterial segments by means of automated edge-detection algorithms. The reproducibility of the analyses has been shown to be

excellent.[7] This reproducibility was assessed on the basis of end-diastolic cineframes of 13 routine coronary angiograms which were analyzed twice by one technician with a median interval of 28 days between analyses. The average difference between duplicate measurements was found to be negligible (< 0.001 mm); the variability, defined as the standard deviation of the differences between repeated measurements, was found to be 0.10 mm.[5]

Electrocardiograms (ECG) of the patients at rest were interpreted according to the Cardiac Infarction Injury Score (CIIS).[10] The CIIS is a multivariate decision-theoretic electrocardiogram classification scheme. It uses 11 discrete and 4 continuously measured ECG variables and is based on the use of the standard 12-leads ECG. Each feature contributes to the total score according to its weight for specified ranges of feature values. The weight coefficient of a continuous feature is multiplied by its measured value and the product is added to (or subtracted from) the score. The higher the final score, the higher the chance of cardiac injury; the scale ranges fron –20 to +80. In the Leiden Intervention Trial electrocardiograms were recorded and coded prior to intervention and twelve times during the two years of intervention.

Persistend exertional angina was analyzed by a routine questioning method.

The vital status of all patients was verified on April 1, 1985. For all patients the follow-up period after the final angiogram amounted to at least 3.5 years.

Statistical methods

Differences between groups were evaluated by Student's t-test when the dependent variable was continous and the independent variable was categorical. When both the dependent and independent variables were categorical, the χ^2 test was used. The relation between progression of coronary sclerosis and vital status was analyzed by Wilcoxon test. All statistical analyses were carried out with the SPSS-X statistical package.[21]

Results

According to the computer assessment of the pre-intervention cinefilms, a total of 166 lesions were detected in the 9 major epicardial coronary segments of the 39 patients. On average, each patient had 4.26 ± 1.52 lesions. Seven patients had one-vessel disease, 11 two-vessel disease and 21 patients had three-vessel disease. The mean ± s.d. of the minimal diameter of the vessels at the 166 sites of obstruction was 2.12 ± 0.99 mm in the first arteriogram and 1.99 ± 1.01 mm in the second, illustrating that on average, the coronary lesions of the 39 patients, progressed during the two years of obstruction ($p < 0.01$) (Table I).

Progression of coronary atherosclerosis was defined as a decrease of 0.1 mm or more in the mean coronary diameter (computer assessment). Regression of lesions

was defined as a mean increase of 0.2 mm or more, which is two times the standard deviation of the difference between repeat measurements. This definition was chosen to be certain that in regression an important deviation from the natural progressive course had happened. Therefore, in progression only one standard deviation was considered sufficient.

TABLE I
Number of segments that showed progression, regression or remained stable, when the two sequential mean diameter values were compared after two years of intervention.

Segments studied	progression	stable	regression	total
(Number of segments)				
RCA proximal	21	1	9	31
mid	14	0	11	25
distal	6	4	9	19
Total RCA lesions	41	5	29	75
main stem	1	1	1	3
LAD proximal	15	0	9	24
mid	8	0	11	19
distal	5	0	2	7
Total LAD lesions	28	0	22	50
LCX proximal	9	2	6	17
distal	11	5	2	15
Total LCX lesions	20	7	8	35
Grand total	90	13	60	163
Lesions with suspected recanalisation				3
		Total lesions		166

RCA = Right Coronary Artery
LAD = Left Arterial Descending coronary artery
LCX = Left Circumflex Artery

Using the above criteria, progression of coronary lesion growth occured in 21 out of the 39 patients and regression in 7 patients. In 11 out of the 39 patients neither progression nor regression was seen to occur, a situation which we called 'stable' (Figure 1). Only in 9 patients all lesions progressed, in all other patients both progression and regression of lesions were seen to occur in the same individual coronary tree.

Association between computer-assessed dimensions of coronary obstructions for each patient and the CIIS score of the ECG, both at onset and at final examination, proved to be significant ($r = 0.33$, $p = 0.04$ and $r = 0.38$, $p = 0.02$ respectively).

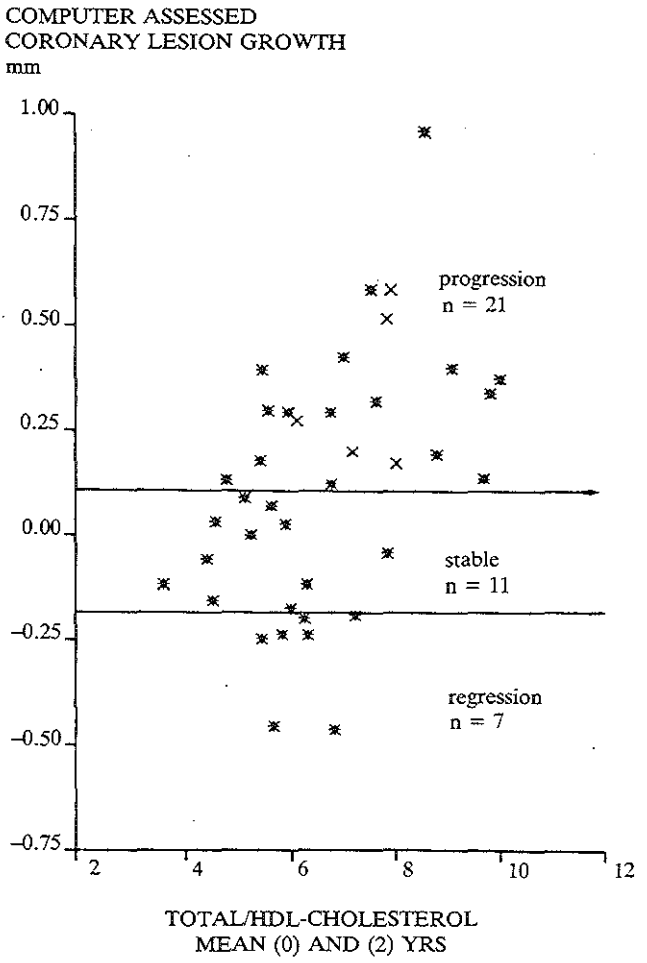

FIGURE 1
Total-cholesterol to HDL-cholesterol ratio mean value between (0) and (2) years as related to the computer assessed coronary lesion growth.

After 24 months of dietary intervention 23 out of the 39 patients still had typical angina pectoris on exertion. When these findings were related to the computer-assessed coronary changes over the two years period, it was concluded that persistent angina pectoris was present in 17 of the 21 patients with progression of disease, in 5 out of 11 patients with stable lesions and in 1 out of 7 patients with regression. Statistical analysis showed that persistent angina pectoris was significantly correlated with progression of coronary lesions (Table II).

The inquiry showed that 5 patients had died and 34 were alive 3.5 years after the

final examination (Figure 1). The five deaths all had occured in the group of 21 patients with progression of disease. The mean change of the coronary obstruction diameters in the 5 patients over the 2 years period was + 0.35 mm, thus showing considerable progression. The mean change in coronary obstructions of the remaining 34 patients still alive, was found to be + 0.09 mm (p = 0.05).

TABEL II
Relation between computer assessed changes in coronary diameters and exertional angina pectoris.

	Progression n = 21	Stable n = 11	Regression n = 7
Mean change (mm) in obstruction size	+ 0.35	− 0.08	− 0.29
Angina after study	17	5	1 (p = 0.005)

n = number of patients

Discussion

Seven prospective angiographic studies have shown serum lipid levels to be associated with atherosclerotic lesion growth.[3,8,9,11-14] In the Leiden Intervention Trial for instance, coronary lesion growth correlated significantly with the total-cholesterol/HDL-cholesterol ratio (r=0.50, p=0.001).[9] Disease progression was significant in patients who had values for total/HDL-cholesterol that were higher than the median (>6.9) throughout the trial period. No coronary lesion growth was observed in patients who had lower values for total/HDL-cholesterol than the median (≤6.9) throughout the trial or who initially had higher values that were significantly lowered by dietary intervention.

It is also important, however, to examine whether the slight changes, that did take place in the coronary diameters at the sites of the stenotic lesions have had clinical significance for the individual patient. For this purpose we have analyzed the electrocardiograms of the 39 patients, the persisting exertional angina pectoris and the vital status of the participants 3.5 years after the intervention period.

The diagnosis of coronary heart disease from electrocardiographic findings has been a clinical problem of long standing. To derive reliable information on the severity of coronary atherosclerotic lesions from ECG's at rest is even more difficult. Yet, each clinical cardiologist values the 12-leads ECG when assessing the patient with angina. Little has been published, however, on the relationship between ECG and coronary angiographic findings. Flaming et al,[15] in a multivariate analysis of angiographic, histologic and ECG data, found that ECG abnormalities were more related to the state of the myocardium (fibrosis, etc.) than to the stenotic lesions. However, Rautaharju et al,[10] the orginator of the CIIS coding, found his scoring system to be considerably more accurate for the diagnosis of coronary heart disease than the conventional ECG classifications. Bart et al[16]

compared the CIIS code findings to the Minnesota Code with the use of a data set of 542 ECG's of which 244 were derived from patients with proven myocardial infarction. Results showed that indeed the diagnostic accuracy of CIIS was greater than that of the Minnesota Code. In the Leiden Intervention Trial, as reported here, the CIIS scoring of the 39 patients both at the onset of the study and at the final examination correlated significantly, although not strongly, to the computer-assessed coronary lesions, satisfies the clinical notion that electrocardiograms of anginal patients have some bearing on the severity of the disease.

The presumed association between angina pectoris and coronary stenotic lesions has been considered of great importance, but angina pectoris is not a disease but a symptom. Therefore, it is of importance to understand and derive from the symptom the underlying disease.

In the Leiden Intervention Trial all 39 patients had angina pectoris when they enrolled in the study; and angiographic assessment had shown in each of them an atherosclerotic diameter obstruction of 50% or more. None of the patients had other heart abnormalities as valvular disease, hypertrophic cardiomyopathies or pericarditis. Therefore, it seems perfectly valid to presume that the angina pectoris of which the 39 patients suffered could be attributed to their coronary atherosclerotic lesions. It seems also fair, but indeed not proven, that any changes in the intensity of the anginal symptoms will be derived from changes in the coronary atherosclerotic lesions.[17] Most cardiologists have the above presumed association in their mind when they take the anatomic changes that they see on the angiograms as the principle yardstick in their clinical judgement of patients with angina pectoris.[18] It is true that other factors than the degree of narrowing of the coronary lumen can be of importance, e.g. the presence of collaterals.[19] But the severity of the lesions remains the most important factor which is linked to the formation of collaterals.[20] As angina is considered a symptom of myocardial ischemia and, since, without intervention, coronary atherosclerosis is almost unequivocally progressive in nature, a change in the process will have great clinical implications. It would thus appear that after 2 years of dietary intervention no longer exertional angina pectoris was present in 16 of the 39 patients and may point to an efficacious diet for angina pectoris. It is unlikely that other influences have played a major role in the outcome of the clinical course of the anginal symptoms, as anti-anginal drug-medication was not altered drastically in any of the patients. Of far greater importance, as far as changes of symptoms is concerned, however, is the finding that there is a strong and significant correlation between persisting exertional angina and progression of coronary lesions. The fact that angina persisted in 17/21 patients with progression, in 5/11 in those with stable lesions and only in 1/7 patients whose coronary lesions showed regression, is of significance for the individual patient. Clearly, the patient with regression of coronary atherosclerosis has a better chance on an improved quality of life.

Three and a half years after the intervention period 5/39 patients had died. In

order to put this figure into perspective, we have applied the findings of Storstein et al[22] to our data. Storstein analyzed the national history of coronary artery disease, studied by coronary angiography in 795 consecutive patients with anginal complaints and of a similar age group as that of the Leiden patients. Like most investigators, she found that prognosis was greatly determined by the extent of coronary artery involvement. The mean annual mortality rates of patients with one-, two- and three-vessel disease were 2.4, 4.9 and 6.2%, respectively. If we apply these data to the patients of the Leiden study with one- (n=7), two- (n=11) and three-vessel disease (n=21) and calculate mortality for the 3.5 years of follow-up, then 7 patients should have died in this period. In the Leiden study the mortality was of similar magnitude with five deaths. The fact that all five deaths occurred in the 21 patients with progression of disease underscores the clinical importance of the angiographic finding of even small changes in coronary diameters. We infer from this that regression and arrest of progression did influence the clinical course of the patient. Slight changes in the growth rate of atheromatous plaques thus may have far reaching implications for survival.

Blankenhorn et al.4,[23] pointed out that an American and European men who died of myocardial infarction at the age of 40, the lesions have spread over coronary endothelial surface with a rate of 3 per cent per year. By the same token, men who died of myocardial infarction at 80 years of age had lesions that grew to cover 1 percent more of the endothelial coronary surface each year. The implication is that a small reduction in the growth rate of coronary lesions may produce a major delay in death from coronary disease. The arrest of progression of disease in 18/39 patients (including 7/39 with regression) of the Leiden Trial may thus have important implications. It shows that diet may modify the course of the process that at the present time is the most important cause of death in the adult population of the industrialized world. This can only be proven, however, if further investigations are facilitated by non-invasive technology to study the course of atherosclerosis also in its early stages. We then may be able not to understand better the pathogenesis of progression but also the process of regression.

Regression does not necessarily follow the exact pathway of progression in the opposite direction. Of interest is the finding of Vartiainen at al.[24] that during the war 1940-1945 the elevated atheromatous aortic plaques had diminished by 20-40% post-mortum, when compared to the 1933-1938 findings, but the incidence of fatty streaks had not decreased. Vartiainen's observation is in keeping with Strøm and Jensen's data[25] which show that in 1941, soon after the beginning of World War II, the death rate from coronary heart disease decreased abruptly and considerably in Norway, a finding which seems to point towards regression, particularly of advanced plaques. Similar results were obtained in the Leiden Intervention Trial. The 2 years application of the cholesterol lowering diet had significantly more effect on the severe (% D-stenosis >50%) lesions than on the lesser ones (<50%).[9] In the 41 severe obstructions the mean change of diameters of the obstructions was -3.47%,

signifying that on average regression had occurred in all the severe lesions of the 39 participants. Of the 124 less severe lesions the mean change of diameter was +4.29%, thus showing progression of disease (p=0.02). It should be pointed out that each patient had at least one severe and one or more less severe lesions. This rules out the possibility that the mean regression of the more severe lesions and the mean progression of the lesser ones could be ascribed to individual characteristics, such as better adherence to diet.

On the basis of the post-mortum observations of Vartianen et al on aortic lesions, the epidemiologic data of Strøm and Jensen and the angiographic findings of the Leiden Trial, we conclude that their is evidence that with dietary intervention atherosclerotic lesions in an advanced stage are more likely to regress than early ones. This statement undoubtedly needs confirmation, but it raises hope for the prevention of clinical sequelae of coronary artery disease not only in patients with early lesions, but even more so in those that have advanced atherosclerotic plaques.

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APPENDIX PAPER I

Cardiovasculaire risicofactoren bij mannen (49 jaar of jonger), één jaar na het eerste myocardinfarct

J.D. Barth, G.T. Meester en J. Lubsen

Samenvatting

Cardiovasculaire risicofactoren (hypertensie, totaal-cholesterol, HDL-cholesterol, triglyceridengehalte in het serum, een positieve familie-anamnese voor hart- en vaatziekten, roken en (type 'A'-gedrag) werden onderzocht bij mannen (49 jaar of jonger), een jaar nadat deze een eerste myocardinfarct hadden doorgemaakt. In drie leeftijdsgroepen, respectievelijk 40 jaar of jonger, tussen 40 en 50 jaar, en 50 jaar en ouder werd dit onderzoek verricht na beoordeling van de totale groep patiënten die op de afdeling Hartbewaking gedurende een jaar (juni 1981-juni 1982) met een myocardinfarct waren opgenomen.

De resultaten lieten een verhoogd aantal risicofactoren in vooral de jongere groepen zien. Op grond daarvan moet overwogen worden of het niet aan te bevelen is, mede omdat met name vetstofwisselingsstoornissen bij de jongere groepen vaak voorkomen, een actief beleid te gaan voeren ter beïnvloeding van deze risicofactor. Daarnaast lijkt een mitigeren van andere aanwezige risicofactoren, gezien het mogelijke gunstige effect op langere termijn, aanbevelenswaardig.

Inleiding

Volgens de huidige inzichten is het hartinfarct een multifactorieel bepaald ziektebeeld, waarvan gedurende een langere periode de cardiovasculaire risicofactoren mede de voortgang of de progressie bepalen. Gesuperponeerd op het basale atherosclerotische proces kan peracuut een afsluiting van een reeds vernauwde kransslagader ontstaan, bijvoorbeeld door een thrombus of een spasme. Het resultaat is dan het hartinfarct.

Er zijn vele cardiovasculaire risicofactoren die de progressie van het atherosclerotische proces bepalen, zoals geslacht, vetstofwisselingsstoornissen, hypertensie, roken, diabetes mellitus, positieve familieanamnese en een bepaald psychologische gedragspatroon (het zogenaamde type 'A'-gedrag).[1-3]

Modificatie van risicofactoren heeft in grotere onderzoekingen tot op heden niet altijd tot een duidelijke verlaging van het risico geleid.[4] Patiënten met ziekte van de kransslagaderen hebben over het algemeen veel risicofactoren. Uit het Lipid Research Clinics-onderzoek is naar voren gekomen dat verlaging van het choleste-

rolgehalte de cardiovasculaire morbiditeit en sterfte kan verminderen. De implicaties daarvan voor de patiënt met een kransvataandoening verdienen heroverweging.[5,6]

Patiënten die hun eerste myocardinfarct overleven, vormen een groep met een verhoogde kans op een recidief infarct. Wij hebben dergelijke patiënten ruim 1 jaar na hun eerste myocardinfarct opnieuw poliklinisch onderzocht en daarbij hun cardiovasculaire risicofactorprofiel samengesteld.

Patiënten en methoden

Van de in totaal 1121 patiënten die in de periode van 1 juni 1981 tot en met 1 juni 1982 op de Coronary Care Unit van het Thoraxcentrum te Rotterdam waren opgenomen, bleken 252 mannen en 70 vrouwen een myocardinfarct te hebben doorgemaakt. De gemiddelde leeftijd van de mannen was 58,2 jaar, van de vrouwen 61,9 jaar. In het ziekenhuis overleden 36 mannelijke patiënten en 16 vrouwelijke patiënten, zodat voor nader onderzoek in principe 216 mannen en 54 vrouwen beschikbaar waren. Van deze 216 mannen waren er 52 ten tijde van hun eerste myocardinfarct 49 jaar of jonger. Ruim één jaar na het myocardinfarct bleken inmiddels 5 patiënten te zijn overleden; daarnaast hadden 2 een recidief myocardinfarct doorgemaakt, 2 een aortacoronaire bypass-operatie ondergaan en 1 patiënt was geëmigreerd. Uiteindelijk kwamen 42 patiënten onder de 50 jaar voor ons onderzoek in aanmerking. Van hen verschenen 30 op de polikliniek van het Thoraxcentrum. De overige 12 waren ten tijde van het na-onderzoek allen nog in leven, maar niet gemotiveerd eraan deel te nemen. Van deze 30 patiënten hadden 12 de leeftijd van 40 jaar of jonger, 18 de leeftijd tussen 41 en 49 jaar. Als controle werd een aselecte steekproef van 20 patiënten genomen uit de patiënten boven de 50 jaar.

Tijdens het polikliniekbezoek werd een algeheel lichamelijk onderzoek verricht en een cardiovasculaire vragenlijst afgenomen, gericht op risicofactoren. Tevens werd totaal-cholesterolgehalte,[7] HDL-cholesterolgehalte,[8,9] en het triglyceridengehalte[10] in het serum bepaald. Bij geen van deze patiënten was een familiaire hyperlipoproteïnemie en (of) diabetes mellitus vóór het hartinfarct bekend.

De vragenlijst die aan de patiënten werd voorgelegd, bevatte verder de volgende vragen:

1. Rookt u op het ogenblik meer dan 5 sigaretten per dag en hoeveel rookte u voor het infarct?
2. Heeft u hoge bloeddruk en (of) wordt u hiervoor behandeld (bloeddrukgrenswaarde 150/90 mmHg)?
3. Komen hart- en vaatziekten frequent in uw familie voor? (Komen er in uw directe familie leden voor die op 60-jarige leeftijd of nog jonger aan een hart- en vaatziekte zijn overleden of er aan lijden?).
4. Heeft u het idee dat u op het ogenblik onderhevig bent aan hevige emotionele

spanningen, die duidelijk gerelateerd zijn aan het door u doorgemaakte myocardinfarct (onbegrepen woede, rusteloosheid, een gevoel van een chronisch tekort aan tijd, snelle irritatie)?

Resultaten

Zoals uit tabel I valt op te maken, is er een duidelijk verschil in frequentie en ernst van vetmetabolismestoornissen tussen de jongere groepen en de groep die 50 jaar of ouder is. In de jongere groep is het cholesterolgehalte duidelijk hoger terwijl het HDL-cholesterolgehalte lager is dan bij de ouderen.

Uit tabel II blijkt dat alle patiënten uit de jongste groep vóór het hartinfarct sigaretten rookten en dat de frequentie na het infarct weliswaar lager was, maar niet geheel tot nul was gedaald. In de oudere groepen nam het aantal rokers sterk af. Hypertensie kwam in de 3 groepen even vaak voor. Een positieve familieanamnese voor hart- en vaatziekten kwam vooral in de jongste groep voor, ook het zogenaamde type 'A'-gedrag kwam in de jongere groepen vaker voor dan in de oudere groep.

In de figuur is het totale aantal cardiovasculaire risicofactoren per groep weergegeven. Het hoogste aantal risicofactoren is in de jongste groep te zien.

TABEL I
Serumlipidegehalte in de verschillende leeftijdsgroepen van mannelijke patiënten, één jaar na het eerste hartinfarct

Gehalten in mmol/l (gem. en uiterste waarden)	Leeftijdsgroep in jaren		
	≤ 40 jr. (n = 12)	41 - 49 jr. (n = 18)	≥ 50 jr. (n = 20)
Totaal-cholesterol	9,30** (6,50 - 16,20)	7,20** (5,90 - 8,51)	7,11 (4,52 - 7,83)
HDL-cholesterol	0,91 (0,79 - 1,19)	1,02 (0,71 - 1,29)	1,09 (0,89 - 1,38)
Triglyceriden	3,81 (0,78 - 8,73)	2,87 (1,49 - 6,86)	2,69 (1,62 - 4,68)

** $p < 0.01$ (t-toets)

Beschouwing

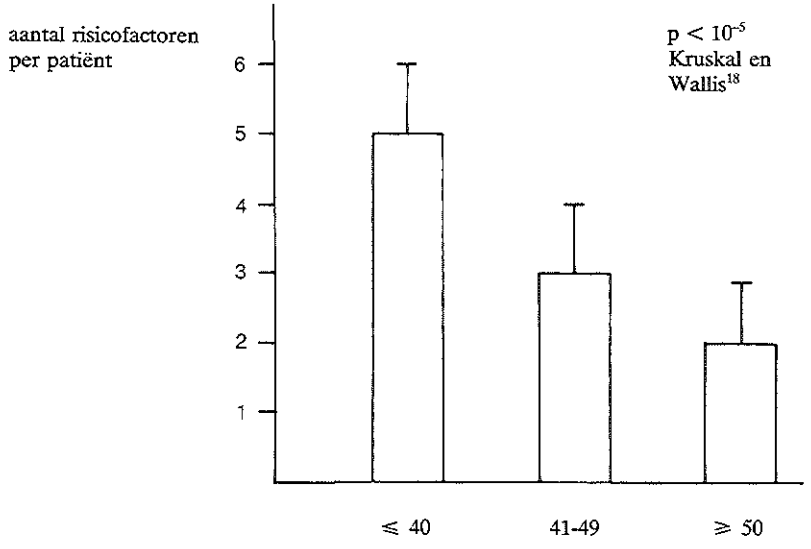
Hoewel de 3 groepen van patiënten relatief klein zijn zodat conclusies slechts met terughoudendheid geformuleerd kunnen worden, lijkt het toch frappant dat er ruim één jaar na het doormaken van het eerste myocardinfarct nog steeds een groot aantal risicofactoren bij jonge mensen aanwezig blijft. Het hoge tot verhoogde cholesterolgehalte in de jongste groep is des te ernstiger gezien het feit dat dit vrij zeker met de leeftijd verder zal gaan stijgen.[11] In onze patiëntengroep had

de jongste groep de hoogste cholesterolwaarden. Het HDL-cholesterolgehalte, door velen als 'anti-atherogeen' beschouwd aangezien het negatief geassocieerd is met het vóórkomen van hart- en vaatziekten,[12] was niet significant verschillend in de 3 groepen, wel was er een tendens tot lagere waarden in de jongere groep. Of de triglyceridenspiegels een aparte risicofactor voor hart- en vaatziekten zijn, is nog niet duidelijk. Wel is er in dit onderzoek sprake van een trend tot een hogere waarde in de jongere leeftijdsgroep.

TABEL II
Risicofactoren in verschillende leeftijdsgroepen van mannelijke patiënten, één jaar na het eerste hartinfarct

Risicofactoren	Leeftijdsgroep in jaren		
	⩽ 40 (n = 12)	41 - 49 (n = 18)	⩾ 50 (n = 20)
Bloeddruk > 150/90 (mmHg)	4	4	4
Hypertensie in voorgeschiedenis	6	5	5
Sigaretten roken vóór	12	15	12
Sigaretten roken na	3	9	4
Aantal sigaretten vóór (SD)	24 (10)	17 (12)	18 (15)
per dag gem. na (SD)	4 (6)	5 (6)	3 (4)
Positieve familie-anamnese	10	9	2
Type 'A'-gedrag	8	13	4

FIGURE 1
Aantal risicofactoren per leeftijdsgroep (gemiddelde en standaard deviatie)



Belangrijk lijkt verder de constatering dat alle patiënten uit de jongste leeftijdsgroep sigaretten hadden gerookt voor het hartinfarct en dat een groot aantal ook nog na het infarct hypertensie had. Dit terwijl het hartinfarct waarschijnlijk door een daling van de linker-ventrikelfunctie zelf reeds een bloeddrukverlagend effect heeft. Ook een positieve familie-anamnese voor een cardiovasculaire aandoening en een type 'A'-gedrag kwamen in de jonge groep vaker voor.

In verscheidene onderzoekingen in verschillende landen is het voorkomen van risicofactoren bij jonge hartpatiënten nagegaan. Dolder et al. in the Nine Countries Study vonden een verhoogd totaal-cholesterolgehalte (7,25 mmol/l of hoger) bij jonge mannen na een eerste infarct, vooral in geïndustrialiseerde landen. Het percentage varieerde echter aanmerkelijk; zo bedroeg het percentage patiënten met verhoogd totaal-cholesterolgehalte in Israël 45%, in India slechts 1%. Hypertensie als risicofactor bestond bij 23% van de patiënten in Australië en bij 7% in Singapore. Roken varieerde van 86% in Singapore tot 96% in de Verenigde Staten.[13] Roskamm et al. bestudeerden bij mannen onder de 40 jaar ook de frequentie van cardiovasculaire risicofactoren. Zij vonden een verhoogd cholesterolgehalte (8,0 mmol/l of hoger) bij 73% van hun patiënten, terwijl 93% voor het myocardinfarct sigaretten rookte en 30% hypertensie had.[14] Vanhaelke et al. hadden gelijksoortige bevindingen bij een Belgische patiëntengroep.[15] Glover et al. vonden in de leeftijdsgroep van 36 jaar en jonger in de Verenigde Staten dat 98% van de patiënten die een hartinfarct hadden doorgemaakt, sigaretten had gerookt voor het infarct en dat 21% hypertensie had gehad. Bij 20% kwam hyperlipidemie in de anamnese voor.[16] Goldstein et al. tenslotte vonden voor hyperlipoproteïnemie bij gezonde mannen onder de 40 jaar een percentage van 60.[17]

Gezien het risicoprofiel van onze postmyocardinfarctpatiënten, mede gezien de recente resultaten van de Lipid Research Clinics,[5,6] lijkt een actiever beleid, gericht op secundaire preventie, op zijn plaats. Hierbij moet worden aangetekend dat van beïnvloeding van de hypercholesterolemie op dit moment geen eenduidig effect bekend is. Mede hierom lijkt helaas de twijfel zoals die o.a. door Oliver is uitgesproken over het nut van risicofactormodificatie, gerechtvaardigd.[18] Hiertegenover staat dat er sinds het Coronary Drug Project, waarbij slechts met clofibraat en nicotinezuur gewerkt is,[19] meer inzicht in het vetmetabolisme is gekomen en dat men nu meer keuze heeft om het juiste medicament bij een specifieke aandoening te gebruiken.

Ten slotte moet gesteld worden dat, gezien het verschillend voorkomen van cardiovasculaire risicofactoren in de verschillende leeftijdspgroepen, er ook sprake kan zijn van een andere wijze van ontstaan bij jongere en oudere hartinfarctpatiënten.

Conclusie

In de jongste groep (onder de 41 jaar) mannelijke patiënten die een hartinfarct hadden overleefd en ruim één jaar na dit doorgemaakte hartinfarct opnieuw werden onderzocht, bleek dat alle patiënten voor het hartinfarct hadden gerookt. Zij hadden ook een significant hoger totaal-cholesterolgehalte in het serum dan de oudere leeftijdsgroepen. Een positieve familie-anamnese voor een cardiovasculaire aandoening hadden 10 van de 12, terwijl van 6 patiënten bekend was dat ze hypertensie hadden. Tevens kwam het zogenaamde type 'A'-gedrag meer bij hen voor (8 van de 12).

Gesteld kan worden dat bij patiënten die 49 jaar of jonger zijn ten tijde van hun eerste myocardinfarct meer risicofactoren voorkomen dan bij oudere patiënten. Hoewel bij deze patiënten na het doormaken van het hartinfarct actief getracht is de risicofactoren te mitigeren, bleken deze één jaar later echter nog weinig veranderd te zijn. Vooral misschien bij relatief jonge patiënten, zeker in geval van hoge tot verhoogde totaal-cholesterolgehalten, moet overwogen worden deze medicamenteus te beïnvloeden, als dieetmaatregelen onvoldoende resultaat hebben.

Summary

Cardiovascular risk factors in men (49 years or younger), one year after their first myocardial infarction. - Cardiovascular risk factors (hypertension, total serum cholesterol, HDL-cholesterol, serum triglycerides, a positive family history of cardiovascular disease, smoking and type 'A' = coronary prone behavior) were studied in men (49 years or younger), one year after they had had their first myocardial infarction. In three age groups, 40 years or younger, 41-49 years and 50 years and older, respectively, this investigation was carried out after assessment of the total group of patients who had been admitted with a myocardial infarction to the coronary care unit in a period of one year (June 1981- June 1982).

The findings revealed an increased number of risk factors especially in the younger groups. For this reason, the question should be considered, partly in view of the fact that abnormalities of the lipid metabolism are frequent, especially in the younger age groups, whether it would not be advisable to start an active compaign to influence this risk factor. Mitigation of the other risk factors present is also to be recommended, because of the possible favourable longterm effect.

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Progression and regression of coronary atherosclerosis.

Thesis Jacques D. Barth, Rotterdam, 1986.

Summary

Manifestations of coronary heart disease like angina, pectoris acute myocardial infarction and sudden death are rapid sudden processes of a slower, generally progressive underlying coronary atherosclerotic disease. Important factors that may influence atherosclerosis are the plasma lipoproteins. According to their density, which is determined by the lipid-protein ratio, the lipoproteins can be divided into 4 subclasses: chylomicrons, very-low-density-lipoproteins (VLDL), low-density-lipoproteins (LDL) and high-density-lipoproteins (HDL). The level of the LDL fraction is strongly positively correlated to a progressive course of atherosclerosis and HDL on the other hand is inversely correlated to the natural (progressive) course of atherosclerosis. The higher the blood LDL fraction level, the more rapid the progression of coronary artery disease; the higher the blood HDL fraction level, the slower the progression of the disease. This thesis describes several investigations in patients with severe coronary atherosclerosis. The incidence of a disordered lipid metabolism is evaluated. The biochemical data in the process of atherosclerosis are assessed and the results of a lipid lowering diet intervention study on progression of coronary atherosclerosis and its complications are discussed. Although the complications of coronary artery disease, as mentioned above, are clinicaly important phenomena, the underlying disease remains the most important factor in the etiology of these manifestations. Our investigation into risk factors one year after a first acute myocardial infarction in all patients who had been admitted to the coronary case unit during the period of one year undertaken proving the point that the younger the patient, the more lipid risk factors were present (a significantly higher 'atherogenic' total-cholesterol and a lower 'anti-atherogenic' HDL-cholesterol). The total sum of risk factors was also the highest in the youngest age group (Chapter II).

As postheparin lipoprotein lipase activities, hepatic lipase (HL)* and lipoprotein lipase (LPL), are important modulators of the lipoprotein metabolism, notably of the ratio LDL- to HDL-cholesterol, two matched groups of patients with and without angiographic evidence of coronary atherosclerosis have been studied with respect to the possible lipid risk factors, the postheparin lipoprotein lipases and hormones suspected of being atherosclerosis related. When a group of normolipemic patients who had undergone coronary angiography without apparent atherosclerosis was compared biochemically with a matched group of patients who showed

* Synonym of liver lipase activity (LLA).

angiographically severe and diffuse coronary atherosclerosis, the most striking difference between the groups was the significant different activity of one of the postheparin lipoprotein lipases. HL was significantly lowered in the diffuse and severe atherosclerotic group of patients, while normal values were observed in atherosclerosis-'free' subjects. No significant difference could be observed for the LPL-values (Chapter III).

As several hormones may influence lipid transport and atherosclerosis, the following hormones were measured and compared in both groups of patients: glucagon and the estradiol – 17β – testosterone ratio were significantly higher in patients with diffuse severe atherosclerosis while thyroid hormone, insulin, prolactin, human growth hormone and cortisol values were not essentially different between the two groups of patients (Chapter III).

As specific lipoprotein fractions are important endogeneous factors that determine to a certain extent the rate of progression of coronary atherosclerosis, diet seems to be one of the most important exogeneous regulating factors, especially a diet low in cholesterol content and enriched in polyunsaturated fatty acids. Other studies have shown that in all species investigated including the human species, an elevation of the intake of atherogenic food constituents enriched in saturated cholesterol resulted in a more rapid development of atherosclerosis in comparison with groups of animals and men in which this dietary measure was not taken. As lipid metabolism of men differs from that in animal study models, a trial with the aim to decelerate the natural progressive course of coronary atherosclerosis and its acute superimposed manifestations in men is a great challenge to meet. In an effort to try to answer the underlying basic question 'Can a lipid lowering diet decelerate progression or even induce regression of coronary atherosclerosis?', a secundary diet intervention program was started, called the Leiden Intervention Trial. To study the natural course of coronary atherosclerosis sequentional angiographies were performed.

However, investigations of the natural course of coronary atherosclerosis by sequential angiograms by visual assessment are not reliable enough to suit the aim of the study, due to the large inter- and intra-observator variations in interpretation. Therefore, a computerized quantitative method that allows the measurement of absolute and relative arterial dimensions from coronary cineangiograms was used for the above mentioned trial. By this method two identical coronary segments of the same patient on two arteriograms with a two-year interval can be compared with sufficient accuracy and precision. A scoring coronary system has been developed for comparison of the arterial dimensions pre- and post-intervention; a flow dependent weighting factor corrects for the different sizes of the arterial segments. (Chapter V).

In the 39 participating patients, who had undergone 2 sequential coronary angiograms, an assessment was made as to the effect of the prescribed diet on lipid fractions and atherosclerotic lesion growth (Chapter IV). In 21 patients progres-

sion of the disease occurred while in 18 a stabilization or even regression of coronary atherosclerosis was observed. A positive and significant correlation was found between the total-cholesterol/HDL-cholesterol ratio and coronary atherosclerotic lesion growth (Chapter III). The patients, who eventually benefitted the most from the dietary intervention, were those patients who showed an initial high total-cholesterol/HDL-cholesterol ratio and orginally had a high dietary intake of saturated fatty acids (Chapter IV).

As has been mentioned before, several enzymes and hormones are directly or indirectly involved in the natural progressive course of coronary atherosclerosis. Several atherogenesis linked hormones were studied and no difference could be established between the progression and the no lesion growth or regression group for insulin, testosterone, estradiol and cortisol. A significantly higher triiodothyronine (T_3) value was found in those patients that showed no lesion growth or even regression of coronary atherosclerosis (Chapter IV). Lipoprotein lipase activities as important lipid modulators were assessed and correlated to coronary atherosclerotic lesion growth. In a linear regression analysis a significant positive correlation was established between hepatic lipase and the coronary score. This implies that the lower the hepatic lipase value, the more progressive the nature of the disease. By multivariate analysis, hepatic lipase was shown to be the variable correlated most strongly with regression of coronary atherosclerosis.

However, T_3 and HDL are also important and independent factors that add to the probability to induce regression of coronary atherosclerosis (Chapter VI).

To conclude, the clinical significance of the changes in vascular lumen diameter can be made by assessing three clinical aspects:

1. The presence of angina pectoris at termination of the trial.
2. Survival of patients after termination of the study.
3. The electrocardiogram at the beginning and at termination of the trial.

The analysis of angina pectoris showed that the severity of complaints had diminished in the total patient population. At termination of the trial, a significantly positive correlation was found between regression of atherosclerosis and the disappearance of anginal complaints.

Concerning the survival three and a half year after termination of the study, a total of 5 (out of 39) patients had died, all belonging to those that showed lesion growth.

The electrocardiogram is considered an indirect method for assessing myocardial perfusion. The following results were observed: both the initial ECG and the ECG that was performed at termination showed a positive correlation to the severity of coronary atherosclerosis (Chapter VII). We infer from these findings that even minor changes in coronary atherosclerotic stenoses may have important clinical implications for individual patients. Of special interest seems to be the observation that especially the most severe stenoses are the ones most liable to benefit from the intervention.

Derived from the above mentioned data we propose as hypothesis that a different mechanism exists for regression and progression of coronary atherosclerosis. An acceleration of the reversed cholesterol transport by HDL-C and an increased excretion of cholesterol into bile by HL may explain the phenomena of regression of atherosclerosis. T_3 as an indicator of the metabolic activity could place this process on a higher turnover level.

Progressie en regressie van coronaire atherosclerose. De rol van het dieet, de lipoproteïnen en de lipases.

Dissertatie Jacques D. Barth, Rotterdam, 1986.

Samenvatting

Complicaties van coronaire atherosclerose zoals het acute myocardinfarct, de plotse dood of angina pectoris zijn mogelijk snelle veranderingen die optreden in of op de vaatwand bij een chronisch langzaam progressief atherosclerotisch proces. Vele factoren beïnvloeden dit langzame onderliggende lijden. Een belangrijke factor hierbij is een gestoord vetmetabolisme. Verscheidene fracties zijn al naar gelang hun samenstelling, (vet en eiwitverhouding) en gewicht te onderscheiden. In totaal worden vier hoofdklassen van deze fracties of lipoproteïnen onderscheiden. Zo zijn er met toenemend gewicht en afname van grootte de volgende fracties: de chylomicronen, de very-low-density-lipoproteins (VLDL), de low-density-lipoproteins (LDL) en de high-density-lipoproteins (HDL). Aangezien de LDL-fractie sterk positief gecorreleerd is met de frequentie van cardiovasculaire aandoeningen, wordt deze fractie als atherogeen beschouwd, terwijl de HDL-fractie, die juist negatief met deze aandoeningen is gecorreleerd, als anti-atherogeen wordt gezien. Vastgesteld is dat hoe hoger het LDL-gehalte is en/of hoe lager het HDL-gehalte, hoe sneller de progressie van coronaire atherosclerose is. Deze dissertatie betreft onderzoekingen bij patienten met ernstige coronaire atherosclerose; naar de ernst van de vetstofwisselingsstoornissen, de biochemische parameters bij het proces van atherosclerose en vooral of met een cholesterol verlagend dieet een verdere voortgang van de coronaire atherosclerose en de complicaties hiervan tot staan konden worden gebracht. Uiteraard zijn de complicaties van het coronair vaatlijden uiterst belangrijk echter, het onderliggende proces blijft datgene dat beïnvloed moet worden (Hoofdstuk I).

Om allereerst tot inventarisatie te komen van het voorkomen van vetstofwisselingsstoornissen en andere risicofactoren voor cardiovasculaire aandoeningen, werden alle mannelijke patienten, die op de hartbewaking gedurende één jaar opgenomen waren geweest, beschouwd één jaar nadat ze een eerste hartinfarct hadden doorgemaakt. Er werd met name naar leeftijd gekeken, want een lange termijn interventie, indien noodzakelijk, lijkt vooral bij jongere patienten op zijn plaats. De conclusies van deze studie zijn ernstig te noemen in die zin dat juist de jongste leeftijdsgroep de meeste risicofactoren heeft en dat met name het atherogene totaal-cholesterol verhoogd en het anti-atherogene HDL-cholesterol verlaagd

waren, ook in vergelijking met voor de bijpassende leeftijdsgroep behorende normaalwaarden (Hoofdstuk II).

Een volgende stap om te komen tot inventarisatie van modulatoren bij coronaire atherosclerose was ons onderzoek waarbij twee groepen mannelijk patienten werden vergeleken aan de hand van het wel of niet aanwezig zijn van diffuse ernstige coronaire atherosclerose, zoals aangetoond bij coronair-angiografie. Bij deze studie werd met name gekeken naar het endotheel gelocaliseerde lipoproteine lipase en het leverlipase die bij het LDL en het HDL metabolisme een belangrijke rol spelen. Tevens werden in beide groepen verscheidene hormonen bepaald die aan atherosclerose gerelateerd zijn (Hoofdstuk III). Geconcludeerd werd dat het lever lipase of hepatische lipase duidelijk significant verlaagd is bij mannen met uitgebreide en diffuse coronaire atherosclerose. De activiteit van lipoproteïne lipase was niet verschillend in beide groepen. De glucagon-spiegel en de estradiol – 17β – testosteron-verhouding waren significant hoger in de atherosclerotische groep. Schildklierhormoon, insuline, prolactine, groeihormoon en cortisol waren niet wezenlijk verschillend in beide groepen.

Zoals reeds eerder gesteld werd, zijn sommige lipiden-fracties met atherosclerose gecorreleerd. Daar cholesterol zowel endogeen wordt geproduceerd als exogeen via het voedsel wordt opgenomen, lijkt het zinvol om tot vermindering van vetrijke maaltijden, met name van verzadigde vetzuren, te komen, aangezien deze als atherogeen beschouwd worden. Een vegetarisch dieet met een hoge verhouding van meervoudig-onverzadigde tot verzadigde vetzuren bij een geringe hoeveelheid cholesterol, zou mogelijk tot teruggang van atherosclerose kunnen leiden. Een probleem dat bij een dergelijk onderzoek naar voren komt, heeft te maken met het natuurlijke beloop van coronaire atherosclerose. Dit is een langzaam progressief proces waarbij het vele jaren duurt voordat hierop gesuperponeerde complicaties optreden. Om de cruciale vraag 'Kan door middel van een cholesterol-verlagend dieet een vermindering van de voortgang of zelfs teruggang van coronaire atherosclerose bereikt worden?' te kunnen beantwoorden, werd een secundaire interventie studie met dieet alleen geëntameerd: de Leidse Interventie Trial. Voordat deze vraag beantwoord kon worden, moesten de volgende problemen betreffende o.a. de betrouwbaarheid van de methode die in de studie gebruikt werd, opgelost worden.

Een in de laatste jaren ontwikkelde methode om het groeiproces van atherosclerose in de patient te volgen is het bij herhaling verrichten van coronair-angiografie. Hoewel een coronair-angiografie met behulp van een contrast middel slechts dat aanduidt wat zich in het lumen bevindt, kan men met deze methode toch het natuurlijke beloop (de progressie) van het proces van atherosclerose observeren. Een volgend probleem dat naar voren kwam is het feit dat indien men met relatief korte tussenliggende perioden coronairangiografieën herhaalt, de mate van nauwkeurigheid van het volgen van veranderingen afneemt. Visuele interpretatie alleen is, gezien de grote verschillen bij herhaalde interpretatie van dezelfde angiografie

door dezelfde waarnemer en bij herhaalde interpretatie door verscheidene waarnemers, voor een onderzoek met herhaalde angiografieën ongeschikt. Een kwantitatieve methode ter analyse van de diameters van de coronaire vaten is daarom vereist. Voor deze studie was een gecomputeriseerde kwantitatieve analyse beschikbaar gekomen waarvan de nauwkeurigheid van het systeem groter is dan de normale biologische variatie, zodat dit systeem geschikt geacht werd om gebruikt te worden bij deze interventiestudie, waarbij d.m.v. sequentiele coronair-angiografieën de veranderingen van de diameter van het lumen onder behandeling met een vegetarisch dieet waargenomen werden. Hiervoor werd rekening houdend met de mate van bloedstroming door een betreffend vasculair lumen een waarderingssysteem ontwikkeld (Hoofdstuk V).

Van 39 patienten, waarbij uiteindelijk twee angiografieën waren gemaakt, werd de invloed van het dieet op met name het vetmetabolisme en de coronaire atherosclerose onderzocht (Hoofdstuk IV). Bij 21 patienten vond verdere progressie van hun lijden plaats, terwijl bij 18 patienten stabilisatie of zelfs teruggang van de ernst van coronaire atherosclerose bereikt werd. Alle patienten hadden op het eerste coronair-angiogram een ernstige lumenvernauwing. Een manier om tot een score te komen moest ontwikkeld worden waarbij rekening gehouden kon worden met 9 belangrijke coronaire segmenten. Bij deze waarderingsmaat werd er vanuitgegaan dat bepaalde segmenten, waar meer bloed doorheen moet kunnen stromen (aangezien een grote spiermassa voorzien moet worden) belangrijker waren dan andere (Hoofdstuk V). Op grond van deze twee jaren durende studie werd vastgesteld dat behandeling met dieet alleen regressie of stabilisatie van de coronaire atherosclerose kan bewerkstelligen. Er was een duidelijke positieve correlatie tussen de totaal-cholesterol – HDL-cholesterol – verhouding enerzijds en de gemiddelde verandering van de absolute coronairscore anderzijds (Hoofdstuk V). Bij nadere analyse bleek dat die patienten die het meeste baat bij dit dieet hadden, de groep patienten was, die aanvankelijk een hoge serum totaal-cholesterol/HDL-cholesterol-verhouding had en relatief veel verzadigd vet hadden genuttigd (Hoofdstuk IV).

Zoals eerder werd gemeld, zijn verscheidene enzymen en hormonen direct of indirect van belang bij de progressie van coronaire atherosclerose. T.a.v. de hormonen die onderzocht werden op hun eventuele relatie tot progressie van coronaire atherosclerose werd er geen verschil gevonden tussen de groepen met respectievelijk zonder progressie wat betreft insuline, testosteron, oestradiol en cortisol. Wel werd een significant hoger triiodothyronine (T_3) gevonden in de groep zonder progressie (Hoofdstuk V en VI). Voorts zijn in het interventieonderzoek als modulatoren van het lipiden metabolisme, de postheparine lipoproteine lipasen en hun relatie met het al of niet optreden van coronaire diameterveranderingen betrokken. Hierbij werd vastgesteld dat er een significant lineair positief verband aanwezig was tussen de leverlipase activiteit en de toename van de coronaire 'score', d.w.z. de regressie van het atherosclerotische proces. De groep

zonder progressie had significant hogere leverlipase waarden en bij 'multivariate' analyse kwam het leverlipase naar voren als de variabele die het sterkst gecorreleerd was met het optreden van regressie van coronaire atherosclerose. Daarnaast spelen T_3 en HDL-C ook een belangrijke rol (Hoofdstuk VI).

Tot slot werd de klinische betekenis van deze veranderingen in de diameter van de coronair arteriën onderzocht. Er werden drie klinische parameters beschouwd.

1. De aanwezigheid van angina pectoris aan het einde van de studie.
2. Het al dan niet in leven zijn van de participanten geruime tijd na beëindiging van de studie.
3. Het ECG bij het begin en aan het einde van de studie.

Uit de analyse van de gegevens bleek dat de ernst van de angina pectoris in de totale groep patienten aanmerkelijk verminderd was. Bij differentiatie naar de diameterverandering werd vastgesteld dat er aan het einde van de studie significant minder angina pectoris in de groep met regressie voorkwam dan bij de overige patienten. Drie en half jaar na beëindiging van het onderzoek waren 5 patienten overleden, allen behorend tot de groep met duidelijke progressie. Het ECG als indirecte maat voor de myocardiale perfusie vertoonde het volgende: zowel het initiële ECG als het ECG dat bij beëindiging van de studie werd gemaakt, kwamen redelijk goed overeen met de ernst van de coronaire atherosclerose, dit zowel bij het begin als aan het einde van het onderzoek (Hoofdstuk VII). Geconcludeerd werd dat voor de individuele patient zelfs een geringe verandering van de diameter van de coronaire arteriën grote klinische implicaties heeft. Daarbij hebben veranderingen ter plaatse van de ernstigste stenosen de grootste klinische consequenties.

Onze hypothese is dat eventuele regressie van coronaire atherosclerose veroorzaakt wordt door versnelling van het 'reversed' cholesterol transportmechanisme onder invloed van het leverlipase, waarbij meer cholesterol in de gal wordt uitgescheiden. Dit gehele proces kan wellicht verder gestimuleerd worden door een verhoging van het metabolisme weergegeven door een hoge T_3-waarde.

Chapter III post-heparin lipases, lipids and related hormones in men undergoing coronary arteriography to assess atherosclerosis. Atherosclerois 1983; **48** : 235-241 is used with permission from Elsevier Science Publishers B.V. Biomedical Division, Amsterdam, the Netherlands.

Chapter IV Diet, lipoproteins and the progression of coronary atherosclerosis. The Leiden Intervention Trial N. Engl. J Med. 1985; **312** : 805-811 is used with permission from the Massachusetts Medical Society 1985 Waltham, MA 02254, USA.

Chapter V Quantitative coronary angiography in a lipid intervention study: The Leiden Intervention Trial pp 408-425 from Quantitative Coronary and Left Ventricular Cine-angiography eds. Reiber J.H.C. Serruys P.W. and Slager C.J. is used with permission from Martinus Nijhoff Publishers B.V. Dordrecht, the Netherlands.

Appendix paper I Cardiovasculaire risicofactoren bij mannen (49 jaar of jonger), één jaar na het eerste myocardinfarct Ned. T. v. Geneeskd. 1985; **129** : 1378-1381 is used with permission from the Nederlands Tijdschrift voor Geneeskunde Amsterdam, the Netherlands.

Curriculum vitae

Jacques D. Barth werd op 28 januari 1948 te Amsterdam geboren. In 1968 behaalde hij het HBS-B-diploma aan het Maimonides Lyceum te Amsterdam. Na een jaar 'premedical studies' aan Yeshiva University te New York City, studeerde hij verder medicijnen aan de Medische Faculteit van de Erasmus Universiteit Rotterdam, alwaar in 1975 het artsexamen werd afgelegd. Hierna volgde de opleiding tot internist op de Afdeling Inwendige Geneeskunde III (hoofd: Prof. Dr. J.C. Birkenhäger) van het Academisch Ziekenhuis Rotterdam Dijkzigt. Vervolgens werd de opleiding tot cardioloog aan het Thoraxcentrum van het Academisch Ziekenhuis Rotterdam Dijkzigt ondernomen (hoofd: Prof. P.G. Hugenholtz). Na registratie op 1 januari 1984 als cardioloog, was hij verbonden aan de Afdeling Klinische Epidemiologie (hoofd: Prof. Dr. J. Lubsen) van het Thoraxcentrum van de Erasmus Universiteit Rotterdam.
Na ruim een jaar als cardioloog in het Zuiderzeeziekenhuis te Lelystad gefunctioneerd te hebben, volgde zijn benoeming tot Universitair Docent aan de Katholieke Universiteit Nijmegen op de Afdeling Cardiologie (hoofd: Prof. Dr. T. van der Werf). Sinds augustus 1985 is hij hoofd polikliniek Cardiologie van het Sint Radboudziekenhuis te Nijmegen.