A COMPARISON OF VARIOUS CHOLESTEROL LOWERING DIETS IN YOUNG HEALTHY VOLUNTEERS; EFFECTS ON SERUM LIPOPROTEINS AND ON OTHER RISK INDICATORS FOR CARDIOVASCULAR DISEASES

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TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
DR. C.C. OOSTERLEE,
HOOGLERAAR IN DE VEETEELTWETENSCHAP,
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Voorwoord

In dit proefschrift worden twee onderzoeken beschreven naar de invloed van de soort en de hoeveelheid vet in de voeding op enkele risiko-indicatoren voor hart- en vaatziekten bij gezonde vrijwilligers. De resultaten zijn weergegeven in de vorm van vijf artikelen (hoofdstuk 2 t/m 6). In een bijlage is geprobeerd om niet-vakgenoten enig inzicht te geven in het verband tussen voeding en hart- en vaatziekten.

Het onderzoek werd uitgevoerd op de vakgroep Humane Voeding van de Landbouwhogeschool, met financiële steun van de Nederlandse Hartstichting.

Bij het onderzoek zijn veel mensen betrokken geweest, die hier niet onvermeld mogen blijven.

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STELLINGEN

1. In de Nederlandse situatie is zowel een drastische beperking van de hoeveelheid vet in de voeding als een matige vetbeperking gepaard gaande met verhoging van de linolzuurconsumptie effectief in het verlagen van het serum cholesterolgehalte. Drastische vetbeperking leidt echter tot lagere HDL-cholesterol- en hogere nuchtere serum triglyceridengehalten.

   Dit proefschrift.

   Antonis, A. and Bersohn, I. The influence of diet on serum triglycerides in South African White and Bantu prisoners. Lancet i: 3, 1961.

2. Het HDL-cholesterolgehalte wordt meer bepaald door de totale hoeveelheid vet dan door het aandeel van meervoudig onverzadigde vetzuren in de voeding.

   Dit proefschrift.

3. Bij het uitbrengen van voedingsadviezen dient tegelijkertijd inzicht gegeven te worden in de mate van bescherming tegen ziekte die het opvolgen van die adviezen oplevert.

4. Linolzuurrijke produkten zijn bij uitstek geschikt voor een serum cholesterol verlagend dieet; het aanprijzen van dergelijke produkten ter verlaging van de bloeddruk is echter voorbarig.

   Dit proefschrift.

5. Bij de interpretatie van de resultaten van experimenten, waarin gebruik gemaakt is van vloeibare voedingen, wordt vaak onvoldoende rekening gehouden met het effect van dergelijke voedingen op de darmfysiologie.

6. De toename in lichaamsgewicht die vaak optreedt wanneer men stopt met het roken van sigaretten vormt een beduidend kleiner risico voor de gezondheid dan het roken zelf.

   Sorlie, P., Gordon, T. and Kannel, W.B. Body build and mortality. The Framingham Study. J. Am. Med. Ass. 243: 1828, 1980.

7. Het instellen van wijkraden van overheidswege is in de huidige praktijk strijdig met het beoogde doel: burgers meer directe invloed op de plaatselijke bestuurlijke beslissingen te geven.

8. Verhoging van de militaire investeringen leidt tot verlies van arbeidsplaatsen.

9. Bij de inrichting van werkruimten wordt er onvoldoende van uitgegaan dat het werk in deeltijd verricht moet kunnen worden.

10. Voeding is geen wetenschap.

Proefschrift J.H. Brussaard.
A comparison of various cholesterol lowering diets in young healthy volunteers; effects on serum lipoproteins and on other risk indicators for cardiovascular diseases.
Wageningen, 4 december 1981.

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De illustraties in de bijlage werden op prettige wijze verzorgd door Loet van Moll.

Alle anderen die op een of andere wijze aan het onderzoek hebben bijgedragen en hier niet met name genoemd zijn wil ik bij deze ook bedanken.

Summary

This thesis deals with the effect of type and amount of dietary fat on the concentration and composition of serum lipoproteins, colonic function, plasma glucose and serum insulin levels and blood pressure in healthy human volunteers.

Two experiments were carried out. In the first experiment with 60 volunteers a moderate fat diet with a high proportion of polyunsaturated fatty acids (as recommended by several advisory bodies), was compared with three other diets during a test period of 5 weeks. One diet was low in total fat with a low proportion of polyunsaturated fatty acids, one diet was high in total fat with a high proportion of polyunsaturated fatty acids and one was high in total fat with a low proportion of polyunsaturated fatty acids. In the second experiment with 35 volunteers the moderate fat diet rich in polyunsaturated fatty acids and the low fat, low polyunsaturated fatty acid diet were compared again, but this time the test period lasted 13 weeks. The diets were composed of regular foodstuffs and differed in carbohydrate and fat content or fatty acid composition only. There were only minor differences in intake of dietary fiber and other nutrients known to affect cholesterol metabolism.
Subjects in both studies were under strict dietary control. All foodstuffs, except for 100 kcal (0,4 MJ) per day were supplied individually according to each person's energy need. Actual food intake was measured by food records and analysis of double portions. The fatty acid composition of cholesterol esters in serum was analysed in the second experiment in order to check adherence to the diets.

The serum lipoprotein composition and concentration observed during the experiments are given in Chapters 2 and 3. In serum, total cholesterol, triglycerides, apolipoprotein-A_I and -B were measured; in high-density-lipoprotein (HDL), cholesterol was measured; in low-density-lipoprotein (LDL), cholesterol and triglycerides were measured and in very-low-density-lipoprotein (VLDL), triglycerides, apolipoprotein-B and the apolipoprotein-C_{II}/C_{III} ratio were measured. From these experiments two conclusions can be drawn. Firstly, that both a low fat diet, low in polyunsaturated fatty acids and a moderate fat diet, high in polyunsaturated fatty acids lower total serum cholesterol levels when compared with the habitual diet of affluent communities. Secondly, that a low

fat diet causes lower HDL-cholesterol and higher fasting VLDL-triglyceride levels than a moderate fat diet, high in polyunsaturated fat.

It is by no means certain that changes in the concentration of HDL and VLDL really result in changes in the risk of death from cardiovascular diseases. In long-term intervention trials, such a hypothesis has not been tested; only the effect of changes in serum total cholesterol has been studied in intervention trials.

Chapter 4 deals with effects on colonic function. No changes in mean transit time through the gut, fecal wet and dry weight, frequency of stools and concentration of fecal steroids were found. This shows that the intake of dietary fiber had been roughly equal in all diet groups within each experiment. Because there were no consistent short- or long-term changes in fecal bile acid or neutral steroid excretion, it is concluded that changes at the intestinal level do not explain the changes in total serum cholesterol concentration.

Chapter 5 gives the results of measurements of fasting and postprandial serum insulin and glucose concentrations. The results show, that neither the amount nor the type of dietary fat had a strong influence on these variables in healthy subjects.

Chapter 6 describes the effect of type and amount of dietary fat on blood pressure as well as the effect of dietary fiber from various sources and type of protein. None of these dietary components had a demonstrable effect on blood pressure in young normotensive subjects.

The results of these experiments do not call for changes in the dietary recommendations of the Netherlands Nutrition Council, as far as the risk of death from cardiovascular diseases is concerned.

1. Introduction

This thesis deals with the effect of the type and amount of dietary fat on a number of risk indicators for cardiovascular diseases. Cardiovascular diseases are the cause of death for 45% of all people who die each year in the Netherlands. Thirteen percent of deaths from cardiovascular diseases occur in people before the age of 60 years and 8 percent between the age of 60 and 65 years. Within the broad category of cardiovascular diseases, ischemic heart diseases and cerebrovascular diseases account for 75% of all deaths. Mortality from these diseases is four to six times greater in men below the age of 60 than in women of similar age. Of the people who cease work for health reasons each year, 19% do so because of cardiovascular diseases (1, 2).

The principal cause of ischemic heart diseases and cerebrovascular diseases is atherosclerosis. A number of hypotheses concerning the pathogenesis of atherosclerosis have been proposed (3) but the most important within the scope of this introduction is the 'response-to-injury' hypothesis (4).

The 'response-to-injury' hypothesis

To understand this hypothesis one must recall that normal arteries are composed of three layers or tunicas as outlined in Figure 1.

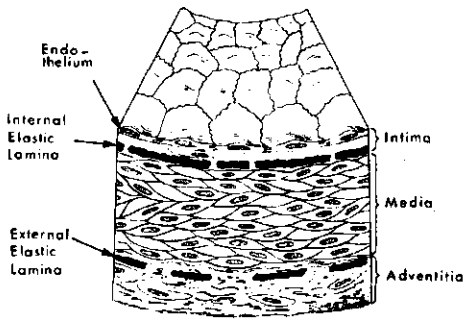

Figure 1. The structure of a small artery, free of atherosclerosis

The inner layer is the tunica intima; the endothelial lining which provides a smooth, low friction, inner surface in contact with the blood stream. The tunica media is a thick middle layer composed of smooth muscle and elastic fibers intermingled and arranged both lengthwise and circumferentially. The outer layer, the tunica adventitia, is formed by fibrous collagenous tissue. According to the 'response-to-injury' hypothesis factors such as hyperlipidemia, the increased stress in hypertension, carboxyhaemoglobin (resulting from cigarette smoking) and a number of other factors may 'injure' the endothelium. This alters the nature of the endothelial barrier to the passage of blood constituents into the arterial wall and possibly causes focal desquamation of the endothelium, resulting in exposure of the underlying intimal connective tissue. This is followed by adherence of platelets to subendothelial collagen, after which microthrombi are formed by platelet aggregation. The platelets then release the contents of their granules. The massive infiltration of platelet factors, plasma lipoproteins and possibly other plasma constituents at the site of injury leads to the focal proliferation of arterial smooth muscle cells, the formation of large amounts of connective tissue matrix by these cells and to the deposition of lipids both within the cells and in their surrounding connective tissue matrix. If the injury occurs only once, the lesion may heal and regress leaving a slightly thickened intima. Repeated or chronic injury to the endothelium may lead to a complicated lesion protruding into the arterial lumen. In such a lesion, deposition of lipid would occur, smooth muscle cells would proliferate, and connective tissue would be laid down and eventually the whole lesion would become rigid as a result of calcification. This sequence of events could lead ultimately to such clinical symptoms as angina pectoris or infarction, resulting from the lesion obstructing the artery or from a thrombus lodging in the partially obstructed artery.

Risk factors

Many epidemiological studies have been carried out to explore the characteristics of people or the environment in which they live in relation to the risk of developing cardiovascular diseases. A number of factors have been found to be associated with such risk and these can be classified, as suggested by Stamler and coworkers (5), as follows:

- those involving social environment and life style, e.g. a habitual diet high in saturated fats, cholesterol, and energy; cigarette smoking; sedentary living.

- those involving endogenous biochemical and physiological regulatory mechanisms, but amenable to exogenous influences such as diet or pharmaceuticals. Examples of such endogenous mechanisms are: hyperlipoproteinemia, hypertension, diabetes, hyperglycemia, hyperuricemia, and rapid resting heart rate.
- those involving organ pathology, e.g. ECG abnormalities, hypothyroidism, and renal disease.
- those involving fundamental biological properties not generally amenable to exogenous influences, e.g. age, sex, heredity.

Some of these factors occur simultaneously and are often interrelated. From multivariate analysis (6) of data from several studies it became apparent that most of the risk for developing cardiovascular diseases can be predicted from three factors; hypercholesterolemia, cigarette smoking and hypertension. From the foregoing explanation of the 'response-to-injury' hypothesis it can be seen that these factors might also be causally involved in the proces of atherogenesis. In long-term intervention trials reduction of both hypercholesterolemia and hypertension have been shown to lower mortality from cardiovascular diseases (7-10).

In recent years is has become clear that not only is the total serum cholesterol concentration important, but that the cholesterol distribution between the various lipoprotein fractions is also important. Over the last two decades prospective epidemiological studies have shown that the risk of cardiovascular diseases increases with increasing plasma concentrations of total cholesterol and also low density lipoprotein (LDL) cholesterol (11) and decreases with increasing concentrations of high density lipoprotein (HDL) cholesterol (12-14). There are indications that LDL promotes deposition of cholesterol in the arterial wall, while HDL does the reverse. It should be noted however that in affluent societies, the level of serum total cholesterol mainly reflects the concentration of LDL-cholesterol.

A controversy still remains about the possible benefits of lowering serum cholesterol concentrations. This may arise partially from misunderstandings about what can be expected in terms of lowering the risk of cardiovascular diseases. Thus, an example will be given of an estimate of the possible benefit of lowering serum cholesterol concentrations on the incidence of myocardial infarction. The estimate, which can only be calculated for groups of persons on average, was made by Whyte (15) based on data from the Framingham Study, which is a large population study in the United States. Whyte calculated that if 100 men who are non-smokers, with normal blood pressure and electrocardiogram,

lower their plasma cholesterol from 8 to 6.7 mmol/l starting at 35 years of age, 6 could potentially benefit by avoiding a coronary incident before the age of 55, 94 would be likely to follow the regimen without apparent benefit, and 8 of these would have an attack within 20 years despite adherence to the regimen. The potential benefit is less for women and for those who start the regimen at an older age. It is greater if cholesterol is lowered further and if other risk factors are present: for instance, 29 of 100 men starting at age 35 who are cigarette smokers with moderate hypertension and left-ventricular hypertrophy and who reduce their plasma-cholesterol concentration from 8 to 5.4 mmol/l would benefit.

These examples show that lowering the concentration of serum cholesterol does not give 100% protection against cardiovascular diseases but also that the protection bestowed is not negligable. Thus recommendations, including recommendations concerning diet, aimed at reducing the incidence of ischemic heart diseases in particular and cardiovascular diseases in general have been drawn up by authorities in a number of countries.

Dietary recommendations

Dietary factors, especially saturated fatty acids and cholesterol influence the risk of cardiovascular diseases probably via their effect on the level of cholesterol in serum but perhaps also through other factors such as blood pressure and blood clotting. A controversy still exists about which type of diet should be recommended to the general population for the optimalization of serum lipid levels. The Netherlands Nutrition Council (16) recommends a diet moderate in total fat (33% of daily energy) in which the ratio of saturated, mono-unsaturated and polyunsaturated fatty acids is 1 : 1 : 1. However, objections have been raised against recommending diets rich in polyunsaturated fatty acids since there has been no long-term experience with such diets on a large scale. As an alternative, lowering the consumption of total fat has also been proposed (17). Since the effects of such diets on serum lipids have been studied much less thoroughly than those of diets rich in polyunsaturated fatty acids, two studies were carried out.

Experiments described in this thesis

In this thesis the results of these studies are reported. In the first experiment a diet containing a moderate amount of fat rich in polyunsaturated fatty acids, as recommended by the Netherlands Nutrition Council was compared

with 3 other diets during a test period of 5 weeks; a high carbohydrate diet, low in fat which was low in polyunsaturated fatty acids and two high fat diets, one high and one low in polyunsaturated fatty acids.
In the second experiment, the high carbohydrate low fat diet and the moderate fat 'recommended' diet were compared again, but this thime the test period lasted 13 weeks.
In both studies, the diets differed only in the proportion of carbohydrate and fat present and in the fatty acid composition of the fat. The dietary intake of the participants was strictly controlled. Relatively large numbers of participants were involved in the studies; in the first experiment there were 60 people, and in the second, 35. The subjects were healthy young male and female students who volunteered to participate in the study.

Five publications resulting from this work are reproduced in this thesis. The first two papers (Chapters 2 and 3) describe the composition and concentration of the lipoproteins in the two experiments. Chapter 4 deals with the effects of the type and amount of dietary fat on colonic function. In Chapter 5, the fasting and postprandial serum insulin and glucose concentrations in both experiments are presented. Finally, Chapter 6 deals with the influence of various dietary factors on blood pressure.

The overall conclusions were that a low fat diet, low in polyunsaturated fatty acids and a moderate fat diet high in polyunsaturated fatty acids both lower total serum cholesterol levels when compared with the habitual diet of affluent communities. However, the low fat diet produced lower HDL-cholesterol levels and higher fasting VLDL-triglyceride levels than the recommended moderate fat diet high in polyunsaturated fatty acids. It may well be that many low fat diets consumed by people in the community differ in their composition from the one used here and thus may not produce the adverse effects on the concentration of HDL-cholesterol and VLDL-cholesterol as observed in the experiments described.
Changes in total serum cholesterol concentrations could not be explained by changes in the fecal excretion of degradation products of cholesterol.
Neither the type nor the amount of dietary fat had a demonstrable effect on blood pressure or serum glucose or insulin levels in the young healthy men and women studied in these experiments.
The results do not call for changes in the dietary recommendations of the Netherlands Nutrition Council, at least as far as risk of death from cardiovascular diseases is concerned.

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Atherosclerosis, 36 (1980) 515—527

2. Effects of amount and type of dietary fat on serum lipids, lipoproteins and apolipoproteins in man. A controlled 8-week trial.

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Summary

We have studied whether a low-fat diet is as effective in lowering some risk factors for atherosclerosis as a diet rich in polyunsaturated fat (PUFA). During a 2.5 week control period, 60 volunteers were given a moderate-fat diet (MOD) providing 30% of the daily energy intake (energy %) in the form of fat, one-third of which was PUFA. For the next 5 weeks subjects were divided into 4 groups and received diets providing varying amounts of total fat and of PUFA: for group LO, 20 energy % fat and 3 energy % PUFA; group MOD, 30 energy % fat and 11 energy % PUFA; group HIPUF, 40 energy % fat and 19 energy % PUFA; and group HISAT, 40 energy % fat and 3 energy % PUFA. The diets contained the same amounts of cholesterol, phytosterols, oligosaccharides and other nutrients, known to affect serum lipid levels. All food was prepared daily and weighed out for each individual appropriate to his energy needs. Nutrient intakes were checked by 7-day records and by chemical analysis of double portions.

On diet LO, total serum cholesterol concentration increased by 0.25 mmol/l while HDL cholesterol concentration did not change significantly. The

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Abbreviations: CVD, cardiovascular disease; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PUFA, polyunsaturated fatty acids; energy %, percent of daily energy intake.

HDL cholesterol/apoprotein-A_1 ratio fell, and VLDL and LDL triglyceride concentrations were elevated.

On the HIPUF diet, total serum cholesterol concentration was not significantly lower, but HDL cholesterol concentration increased by 0.10 mmol/l. On the HISAT diet, total serum cholesterol concentration went up by 0.38 mmol/l; 0.12 mmol/l of this was due to HDL.

LDL cholesterol/serum apoprotein-B ratios were unaffected by any of the diets.

It was concluded that after 5 weeks, the influence of a low-fat, high-carbohydrate diet on the concentrations of serum lipoproteins was less favourable than that of moderate- or high-fat diets rich in polyunsaturated fatty acids.

Key words: *Apolipoproteins — Carbohydrates — Dietary fat — HDL cholesterol — Linoleic acid — Serum cholesterol*

Introduction

During the last two decades, prospective epidemiological studies have shown that the risk of cardiovascular disease (CVD) increases with increasing plasma concentrations of total or LDL cholesterol [1] and decreases with increasing HDL cholesterol [2—4]. Plasma HDL cholesterol concentration is reduced in several conditions associated with an increased risk of future CVD namely hypercholesterolemia, hypertriglyceridemia, obesity and diabetes mellitus [5,6]. Subjects with existing clinical atherosclerotic disease also have lower levels of HDL than healthy subjects within the same community [5,7,8].

In order to elucidate the metabolic origin of differences in lipoprotein lipid levels interest has focussed recently on the measurement of the apolipoproteins of LDL and HDL. Apo-B, the main apoprotein of plasma LDL, is substantially increased in the plasma of subjects with hypercholesterolemia, while endogenous hypertriglyceridemia is associated with a mild increase in plasma levels of apo-B [9,10]. In the latter instance the increase in serum apo-B is mainly located in the VLDL fraction.

Apo-A_1, the main apoprotein of plasma HDL, is found to be reduced in survivors of myocardial infarction as compared with controls [11] and the HDL cholesterol/apo-A_1 ratio was also lower in this condition [8]. At the other end of the risk scale, HDL cholesterol/apo-A_1 ratios were higher in a low-risk group of vegetarians than in a control group [12].

Many short-term experiments have shown that on diets in which saturated fat is replaced by polyunsaturated fat the serum cholesterol and triglyceride levels are lowered; in long-term intervention trials a reduction in the incidence of cardiovascular disease was also noted [13,14]. As a result of such studies, diets high in polyunsaturated fat have been advocated for the prevention of CVD, and consumption of vegetable oils in the U.S. has indeed risen considerably during the last decade [15]. There are fewer controlled studies in man on the effect of replacing saturated by polyunsaturated fat on the concentration and composition of separate lipoproteins, while studies on the effect of natural low-fat diets in healthy subjects are quite rare.

We have investigated the effects of a low-fat diet on serum lipids, lipoprotein composition, blood pressure and faecal steroids as compared with a moderate-fat, high-polyunsaturated diet, containing the same amounts of cholesterol and other nutrients that may affect serum lipid levels. In the present paper results of lipoprotein, apo-A_1 and apo-B analyses will be presented.

Subjects and Methods

Selection of subjects

Thirty-seven male and 23 female university students, aged 18—28 years participated in this study. The subjects were all volunteers who satisfied the following criteria:

apparently healthy, as judged by a detailed questionnaire;
serum cholesterol concentration below 5.7 mmol/l;
serum triglyceride concentration below 1.7 mmol/l;
diastolic blood pressure below 90 mm Hg;
percentage of body fat below 23% in males and 30% in females as determined by skin fold measurements.

Only two of the females used hormonal anticonception.

Experimental design, diets and control of food intake

During the first 2.5 weeks of the study all subjects consumed a control diet (MOD) which was moderate in fat and relatively high in PUFA (30 energy % total fat, 10 energy % PUFA) in agreement with the recommendations of The Netherlands Nutrition Council. After this period, 4 subgroups were formed which received diets as presented in Fig. 1. The groups were matched for initial serum cholesterol, energy intake and sex. Mean body weights per group were 67.2, 66.6, 64.9 and 66.0 kg for groups LO, MOD, HIPUF and HISAT respectively. Throughout the 8-week period all foodstuffs were weighed out for each person in quantities appropriate to his energy needs, except for 100 kcal/day, which the subjects were free to spend at will. Average energy intake during the control period was 11.1 ± 3.6 MJ (±1 SD) (2643 ± 854 kcal). Actual nutrient intakes were checked by 7-day individual food records using Dutch food composition tables [16], and by double-portion analysis [17] for one imaginary person of average energy intake on each diet. Mean daily intakes on nutrients are given in Table 1. Analysis of double portions revealed a slightly

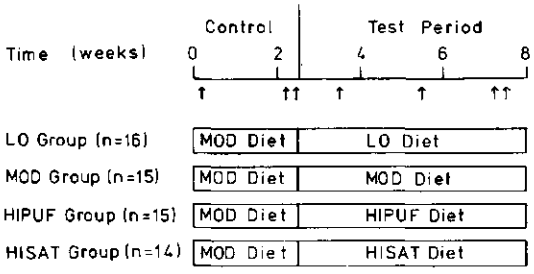

Fig. 1. Experimental design. Blood sampling is indicated by arrows.

TABLE 1

MEAN DAILY INTAKE OF NUTRIENTS ACCORDING TO INDIVIDUAL FOOD RECORDS [a]

	Habitual intake before experiment [b]	Control [c] period	Experimental period [d]			
		MOD	LO	MOD	HIPUF	HISAT
Energy (MJ)	11.4	11.1	10.6	10.5	10.7	10.3
(kcal)	2734	2646	2517	2517	2552	2455
Protein (energy %)	13	14	13	14	14	13
vegetable	6	7	6	7	7	6
animal	7	7	7	7	7	6
Total fat (energy %)	32	30	22	30	40	39
saturated	15	10	8	10	11	18
monounsaturated	11	8	10	8	8	16
polysaturated	5	11	3	11	19	3
Carbohydrates (energy %)	50	54	64	54	44	46
sugars	24	22	21	21	21	23
polysaccharides	27	32	43	33	23	23
Alcohol (energy %)	4	1	0	1	1	2
Vitamin C (mg)	109	110	100	103	100	107
Cholesterol (mg/1000 kcal)	106	114	102	113	104	86
Phytosterols (mg/1000 kcal) [e]		160	98	158	107	214
Dietary fiber (g/1000 kcal)	15.0	10.0	9.7	9.4	9.7	10.5

[a] The food records were elaborated using the Netherlands food composition tables.
[b] 3-day records.
[c] 2-day records.
[d] 5-day records.
[e] Measured by analysis of duplicate portions providing 2700 kcal/day.

higher proportion of fat in the total daily energy intake in all groups, but the differences between groups in fat intake, fatty acid composition and other nutrients agreed well with the food records. Body weight was recorded weekly. Energy intake was adjusted when necessary in order to avoid changes in body weight of more than 2 kg.

The mean change per group in body weight over the 5 weeks of the test period ranged from -0.4 ± 1.1 to -0.8 ± 1.4 kg.

Stools were collected for 7 days at the end of the control and the test periods. There were no differences between the groups in intestinal transit time [18], faecal output and moisture content or frequency of stools in either period.

Blood sampling and analysis

Blood samples were taken after an overnight fast before and during the experimental period as indicated in Fig. 1. No alcohol was allowed during 24 h prior to blood sampling. Serum was obtained by low-speed centrifugation. Serum cholesterol was measured according to Huang et al. [19] using serum calibrators calibrated as described by Abell et al. [20]. Reproducibility for blind control sera provided by the Center for Disease Control, Atlanta, GA, U.S.A., was ±0.9% and accuracy was within 1.4% of the "true" (target) values. HDL cholesterol was determined after manganese-heparin precipitation of apo-B containing lipoproteins [21] as previously described [22]. At the end of the control and the test periods, sera were collected for apolipoprotein-A_1 and

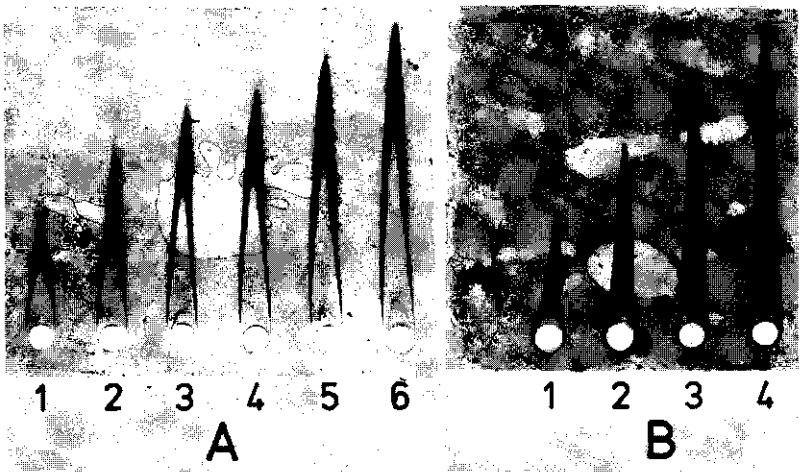

Fig. 2. Coomassie-blue stained immunoprecipitates (rockets) of a pooled human serum with anti-A_1 (A) and anti-B (B) goat serum. Conditions of the electroimmunoassay were : (A) 2.0% goat anti-human apo-A_1 serum; antigen wells 1—6 contained 15 μl human serum diluted with 150 mM NaCl (1 plus 119, 1 plus 74, 1 plus 54, 1 plus 42, 1 plus 34 and 1 plus 29 respectively). Surface area/dilution, $r = 0.999$. (B) 0.5% goat anti-human apo-B serum; antigen wells 1—4 contained 15 μl diluted human serum (1 plus 16, 1 plus 8, 1 plus 6, and 1 plus 4 respectively). Surface area/dilution, $r = 0.998$.

B determinations and for lipoprotein fractionation by the procedure of Redgrave et al. [23] with several modifications. To visualize the lipoprotein bands after ultracentrifugation, 3 ml serum was mixed with 0.3 ml 0.02% (w/v) Sudan Black (Difco Laboratories) dissolved in dimethylsulphoxide. The background density of the mixture was raised to 1.210 with solid KBr (0.9552 g). On top of the serum a discontinuous density gradient was built up in a centrifuge tube (14 × 89 mm, 13 ml) with salt solutions of d = 1.063 (4.5 ml) followed by d = 1.006 (4.5 ml). These salt solutions contained EDTA (0.1 mg/ml) and were prepared from NaCl and KBr [24]. Centrifugation was carried out at 20° C and 40 000 rpm ($RCF_{max} = 272{,}700 \times g$) for 16 h and the clearly visible bands of VLDL, LDL and HDL were harvested by tube slicing [25]. Lipoprotein cholesterol concentrations were determined according to Abell et al. [20]. Serum VLDL and LDL triglyceride concentrations were measured as described by Soloni [26].

Apoproteins were measured in whole serum by rocket immunoelectrophoresis [27] (see Fig. 2). Monospecific antisera against human apoprotein-B and apoprotein-A_1 were raised in goats using pure apo-B in LDL ($1.040 < d < 1.053$ mg/ml) and pure apo-A_1 [28,29] as antigens. Electrophoresis was performed at pH 8.6 using 0.8% agarose (apo-B assay) or 0.8% agarose, 5% dextran T 10 (apo-A_1 assay) in 50 mM diethylbarbiturate buffer as supporting medium for the antisera.

The electrophoresis was performed at 2.5 V/cm for 16 h (apo-B) or at 3.5 V/cm for 10 h (apo-A_1). Serum samples were analysed in three dilutions. For one subject the samples obtained at the start and at the end of the test period were always analysed on one plate in order to minimise the effect of inter-assay variability. Because isolated LDL solutions become turbid during prolonged

preservation, day to day calibration standards of a pooled serum were prepared in four dilutions. The apo-B and apo-A_1 contents in this secondary calibration standard were determined with freshly isolated pure LDL and pure apo-A_1 and redetermined periodically. The protein content in the primary standards was determined by quantitative amino acid analysis. Analytical error was monitored by analysing a sample from a control pool on each plate. The combined within-day and between-day coefficients of variation for this control serum were 7% and 9% for the apo-B and the apo-A_1 assays respectively.

Statistical evaluation

The individual values at the beginning and the end of the test period were compared per group, using a paired two-tailed *t*-test. Each subject thus served as his own control.

Results

The time courses of serum total cholesterol, serum total triglycerides and HDL cholesterol are presented in Figs. 3, 4 and 5 respectively.

All groups received the moderate fat, high PUFA MOD diet for the initial 2.5 week control period. By this time the mean serum total cholesterol concentration had decreased significantly by 0.46 mmol/l (Fig. 3), of which 0.06 mmol/l was located in HDL (Fig. 5). Serum total triglycerides had decreased by 0.12 mmol/l (Fig. 4). Although the groups were matched for initial total serum cholesterol there were slight differences in initial values of HDL cholesterol and total serum triglycerides (Figs. 3 and 4). However, neither these initial values nor the changes in these parameters during the control period differed significantly between the groups. When the subjects changed from the MOD diet to the test diets, the concentrations of serum cholesterol remained unchanged in groups MOD and HIPUF and increased significantly in groups LO and HISAT, by 0.25 and 0.38 mmol/l respectively. HDL cholesterol concentration was unchanged in groups LO and MOD, but increased significantly in groups HIPUF and HISAT, by 0.10 and 0.12 mmol/l respectively. Total triglyceride concen-

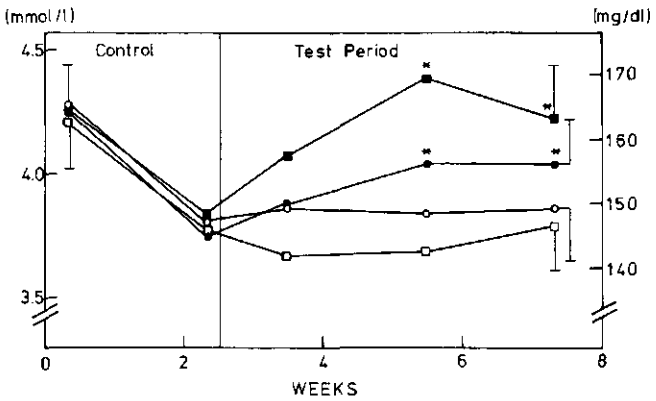

Fig. 3. Mean total serum cholesterol concentrations during the experiment. ●———●, LO; ○———○, MOD; □———□, HIPUF; ■———■, HISAT. Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P < 0.05$ or 0.01) from the value at the end of control period.

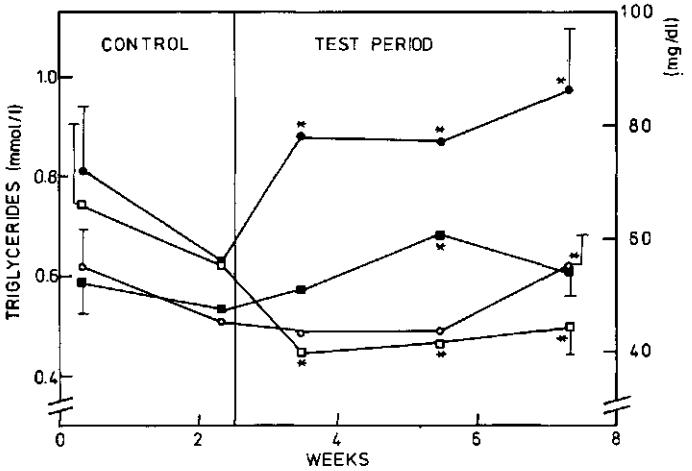

Fig. 4. Mean total serum triglycerides during the experiment. ●———●, LO; ○———○, MOD; □———□, HIPUF; ■———■, HISAT. Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P < 0.05$ or 0.01) from the value at the end of control period.

tration increased significantly in group LO by 0.35 mmol/l and in group MOD by 0.11 mmol/l.

Data for lipoprotein lipids and serum apolipoproteins are presented in Tables 2, 3 and 4 respectively. In all groups HDL cholesterol and serum apo-A_1 concentrations were slightly but not significantly higher for females than for males.

As HDL cholesterol was unchanged in group LO the rise in total serum cholesterol in this group was entirely located in the LDL and VLDL fractions (0.38 and 0.09 mmol/l respectively). This was accompanied by an increase of 118 ± 60 mg/l in serum apo-B concentration, but the LDL/apo-B ratio remained the same (Table 4). Although HDL cholesterol remained constant, HDL composition changed as judged by the HDL cholesterol/apo-A_1 ratio, which decreased significantly (Table 4). VLDL triglycerides increased significantly by 0.14 mmol/l and LDL triglycerides by 0.08 mmol/l (Table 2).

The MOD group, which continued on the control diet during the test period,

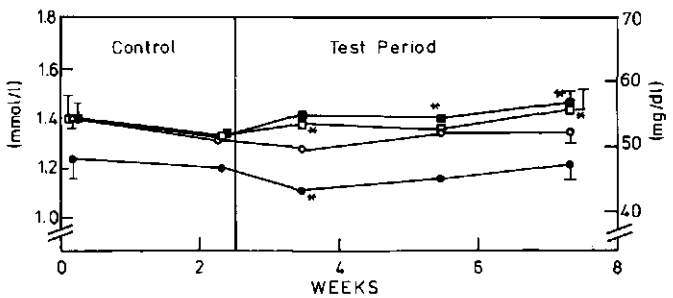

Fig. 5. Mean serum HDL cholesterol during the experiment. ●———●, LO; ○———○, MOD; □———□, HIPUF; ■———■, HISAT. Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P < 0.01$ or 0.05) from the value at the end of control period.

TABLE 2

CONCENTRATIONS IN SERUM OF CHOLESTEROL AND TRIGLYCERIDES IN LOW-DENSITY AND VERY-LOW-DENSITY LIPOPROTEINS AT THE END OF THE CONTROL AND THE TEST PERIODS

Values expressed as mean ± SD. Group MOD was not measured

Group	LDL				VLDL			
	Cholesterol (mmol/l)		Triglycerides (mmol/l)		Cholesterol (mmol/l)		Triglycerides (mmol/l)	
	Control	Test	Control	Test	Control	Test	Control	Test
LO	1.93 ± 0.53	2.31 ± 0.57 **	0.20 ± 0.04	0.28 ± 0.09 **	0.24 ± 0.13	0.33 ± 0.09 **	0.41 ± 0.26	0.55 ± 0.24 *
HIPUF	1.91 ± 0.40	1.98 ± 0.60	0.21 ± 0.06	0.18 ± 0.03	0.27 ± 0.13	0.23 ± 0.12	0.47 ± 0.34	0.37 ± 0.18
HISAT	2.07 ± 0.80	2.33 ± 0.59 **	0.19 ± 0.04	0.21 ± 0.07	0.22 ± 0.05	0.27 ± 0.12	0.31 ± 0.10	0.35 ± 0.20

* $P < 0.05$, ** $P < 0.01$; significantly different from control period according to paired two-tailed t-test.

TABLE 3

CONCENTRATIONS IN SERUM OF APOLIPOPROTEIN-A_1 AND APOLIPOPROTEIN-B AT THE END OF THE CONTROL AND THE TEST PERIODS

Values expressed as mean ± SD

Group	apo-A_1 (mg/l)		apo-B (mg/l)	
	Control	Test	Control	Test
LO	1068 ± 151	1138 ± 149 **	627 ± 163	732 ± 165 **
MOD	1089 ± 150	1201 ± 149 **	544 ± 143	541 ± 122
HIPUF	1271 ± 127	1295 ± 109	580 ± 103	574 ± 120
HISAT	1178 ± 205	1278 ± 162 **	664 ± 202	718 ± 175 **

** $P < 0.01$; significantly different from control period according to paired two-tailed t-test.

The correlation coefficient between LDL cholesterol and serum apo-B was $r = 0.87$ ($n = 60$); the correlation coefficient between HDL cholesterol and serum apo-A_1 was $r = 0.58$. Rather low correlation coefficients between serum apo-A_1 and HDL cholesterol in normolipemics have also been published by other authors [28,45—47].

showed very little change. Although serum total and HDL cholesterol concentration remained unchanged, the HDL cholesterol/apo-A_1 ratio decreased significantly. Total triglycerides increased by 0.11 mmol/l, after an initial decrease of 0.11 mmol/l in the control period (Fig. 4).

Surprisingly, the effects of the HIPUF diet, in which an additional 10 energy % of carbohydrates had been replaced by PUFA, did not differ greatly from the MOD diet. Total serum cholesterol remained reasonably constant compared with during the control period, but HDL cholesterol increased significantly by 0.10 mmol/l (Fig. 5). There was a minor increase in the HDL cholesterol/apo-A_1 ratio (Table 4). Total triglycerides decreased significantly by 0.13 mmol/l (Fig. 4); this decrease mainly took place in VLDL (0.09 mmol/l).

The high-fat low-PUFA diet caused a significant increase in serum cholesterol concentration. It is noteworthy, however, that of the total increase of 0.38 mmol/l, about 30% was accounted for by HDL. Total serum triglycerides

TABLE 4

CHOLESTEROL/APOLIPOPROTEIN RATIOS AT THE END OF THE CONTROL AND THE DIETARY TEST PERIODS

Apoprotein values are for whole serum. HDL cholesterol was determined after manganese-heparin precipitation and LDL cholesterol after density gradient centrifugation. Values expressed as mean ± SD.

Group	HDL cholesterol/apo-A_1 (mmol/g)		LDL cholesterol/apo-B (mmol/g)	
	Control	Test	Control	Test
LO	1.13 ± 0.19	1.07 ± 0.18 *	3.2 ± 0.5	3.2 ± 0.5
MOD	1.22 ± 0.18	1.13 ± 0.15 *	—[a]	—[a]
HIPUF	1.05 ± 0.17	1.11 ± 0.14 *	3.3 ± 0.3	3.4 ± 0.4
HISAT	1.14 ± 0.12	1.15 ± 0.11	3.1 ± 0.6	3.2 ± 0.3

[a] Not measured.

* $P < 0.05$; significantly different from control period according to paired two-tailed t-test.

increased by 0.07 mmol/l, and serum apo-B concentration by 54 mg/l (Table 3).

In all groups, the LDL cholesterol/apo-B ratio remained constant as compared with the control period (Table 4).

Discussion

Participants in this study were well-motivated, normolipidemic students of the Agricultural University. We have repeatedly found [17,30] that the fat and cholesterol consumption in this population is lower than the average intake in this country (Table 1). Thus the change from 'habitual' diet to the rigidly controlled MOD diet in the control period of our study mainly involved an increase in the P/S ratio (Table 1). The observed decrease in serum cholesterol and triglycerides is in agreement with earlier studies [31], although the fall in total cholesterol is somewhat larger than expected [31]. This could be due to adaptation to laboratory conditions and to an inaccurate estimation of their habitual fat intake.

At this level of fat intake (30% of energy), our data showed a decrease in HDL cholesterol on the high P/S diet, a result previously described by Shepherd et al. [32] in dietary studies in normo- and hyperlipidemic men. However, as discussed below, we were unable to demonstrate such a change under controlled conditions at a higher fat intake level (40% of energy).

The effects of a change from MOD to LO, HIPUF and HISAT diets on serum lipoproteins were studied under rigidly controlled conditions as was the effect of prolonged consumption of the MOD diet. In the latter (MOD) group no changes in serum total cholesterol, HDL cholesterol and serum apo-B concentrations were observed between 3 and 8 weeks, indicating that these parameters were stabilised already by the end of the control period. However, this was not the case for HDL composition as serum apo-A_1 concentrations rose significantly during the test period.

Changing from the MOD to the LO diet involved a replacement of 8 energy % PUFA by carbohydrates. VLDL increased significantly by this treatment, probably by carbohydrate-induced synthesis [33,34] which may have been reinforced by the decrease in PUFA.

A pertinent finding was the increase in LDL, reflected by the simultaneous rise in LDL cholesterol and serum apo-B concentrations (+17%). Whether the rise in LDL is mediated by an increased conversion of VLDL into LDL [36–38] or by a delayed LDL degradation [39] (the phospholipid moiety of LDL may be more saturated on this carbohydrate-rich diet [35]) is at present not known.

One might speculate that the decreased HDL cholesterol/apo-A_1 ratio in the LO group reflected a shift from the cholesterol-rich HDL_2 fraction to the HDL_3 fraction [42].

Changing from the MOD to the HIPUF diet implied that 8 energy % of carbohydrates were replaced by PUFA. The further increase in dietary linoleic acid did not cause significant changes in LDL and VLDL cholesterol values or serum apo-B. Although this indicates that the HIPUF diet was no more effective in depressing serum LDL levels than the MOD diet, total serum

triglycerides decreased on the HIPUF diet and a significant rise in HDL cholesterol was observed, which was not accompanied by an increase in serum apo-A_1 concentration.

On changing from the MOD to the HISAT diet, 8 energy % PUFA plus 8 energy % carbohydrates were both replaced by saturated fat resulting in a significant increase in serum LDL concentrations judged from the proportional change in LDL cholesterol and serum Apo-B concentrations. HDL cholesterol as well as serum apo-A_1 concentrations were also significantly increased. At this level of fat intake (40 energy %), no differences in HDL cholesterol and apo-A_1 concentrations between the HISAT and the HIPUF diet were present. Lower VLDL and LDL levels on PUFA-rich diets such as MOD and HIPUF, compared with saturated fat-rich diets, such as HISAT, have been reported repeatedly. VLDL synthesis and conversion into LDL may be decreased on high P/S diets [36—38] although a recent report points to increased LDL catabolism [39].

Although different amounts and types of fat in the diet will also affect postprandial chylomicron production [40], it is unlikely that this will lead *directly* to changes in serum LDL concentrations. Small quantities of LDL can indeed be formed by chylomicron degradation but it can be calculated that the contribution of this pathway to the overall LDL synthesis is very limited.

Potential effects of diet on serum HDL are important since serum HDL cholesterol concentrations have been inversely correlated with risk of cardiovascular disease [2—4]. Recently the effect of a diet rather similar to our MOD diet on serum HDL cholesterol concentrations in hyperlipidemic subjects has been studied by Hjermann et al. [41] and compared with a HISAT type diet. In this intervention study a 20% increase in HDL cholesterol on the MOD-type diet was observed after 4 years. However, it cannot be excluded that this rise was due to the decreases in serum triglyceride concentrations, obesity and cigarette smoking rather than to the diet.

In addition to changes in HDL cholesterol, the average composition of HDL in our experiment was obviously sensitive to diet, although the changes were small.

In order to illuminate the effects of the different diets, we calculated the ratio of the concentration of HDL cholesterol to total cholesterol and of apo-A_1 to apo-B (Table 5). If LDL is "atherogenic" and HDL is "anti-athero-

TABLE 5

HDL/TOTAL CHOLESTEROL AND APO-A_1/APO-B RATIOS AT THE END OF THE CONTROL AND TEST PERIODS

Values expressed as mean ± SD

Group	HDL/total cholesterol		Apo-A_1/Apo-B	
	Control	Test	Control	Test
LO	0.33 ± 0.07	0.31 ± 0.08 **	1.82 ± 0.55	1.67 ± 0.61 **
MOD	0.35 ± 0.07	0.36 ± 0.05	2.14 ± 0.65	2.29 ± 0.41
HIPUF	0.35 ± 0.06	0.38 ± 0.06 **	2.24 ± 0.38	2.32 ± 0.37
HISAT	0.36 ± 0.06	0.35 ± 0.05	1.93 ± 0.64	1.86 ± 0.42

** $P < 0.01$; significantly different from control period according to paired two-tailed t-test.

genic" then these ratios may have predictive value for atherogenic risks. On the HISAT diet the ratios showed surprisingly little change compared with the MOD diet. The low level of dietary cholesterol may have contributed to this result; there are indications [43] that satured fat and dietary cholesterol reinforce each other's effect on serum (LDL) cholesterol levels; the effects of a high saturated fat intake might have been more pronounced if the experiment had been performed at a higher level of cholesterol consumption. The HIPUF diet showed a trend towards more favourable ratios in contrast to the LO diet where unfavourable changes were observed. However, the latter could in part be due to the relatively short test period of 5 weeks. It is known that after 2—3 months on high carbohydrate diets serum triglyceride concentration will go down again [44]. It is not known if similar long-term changes in HDL cholesterol and apolipoproteins could appear; this should be investigated in long-term dietary studies.

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3. Serum lipoproteins of healthy persons fed a low fat diet or a polyunsaturated fat diet for three months: a comparison of two cholesterol-lowering diets.

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Atherosclerosis, submitted

SUMMARY

In a controlled 16-week trial we have compared the effects on some risk factors for atherosclerosis of two cholesterol-lowering diets, one low in total fat and low in polyunsaturated fat (LO) and one moderate in total fat but high in polyunsaturated fat (MOD) as recommended by various advisory councils.

Thirty-five volunteers were given diet MOD, during a 2.5 week control period. This provided 31% of the daily energy intake (energy%) as total fat, one-third of which was polyunsaturated fat (PUFA). The subjects were then randomized into 2 groups, one of which continued on diet MOD, while the other group received diet LO, providing 21 energy% as total fat and 4 energy% as PUFA. The diets contained the same amounts of cholesterol, phytosterols, oligosaccharides and other nutrients known to affect serum lipid levels. All food was prepared daily and weighed out for each individual in amounts appropriate to his/her energy needs. Nutrient intakes were checked by 7-day records and by chemical analysis of double portions. VLDL plus LDL cholesterol decreased by 0.66 mmol/l during the control period while HDL-cholesterol level was unchanged. Total serum triglycerides decreased by 0.33 mmol/l. Total serum cholesterol increased by 0.21 ± 0.41 mmol/l on the control diet MOD during the test period of 13 weeks. 0.09 ± 0.11 mmol/l of this was due to HDL cholesterol. Total serum triglycerides remained constant during the test period in group MOD.
On diet LO total serum cholesterol remained stable during the test period, but HDL cholesterol was lowered by -0.06 ± 0.20 mmol/l. The difference in HDL cholesterol between the diet groups was mainly located in HDL_2. Total serum triglyce-

rides increased by +0.32 ± 0.31 mmol/l in group LO; the increase in VLDL triglycerides was 0.22 ± 0.18 mmol/l.

The depression of HDL on diet LO was also reflected in the ratios of HDL to total cholesterol which decreased by -0.02 ± 0.05 and was unchanged on diets LO and MOD respectively and in the ratio of apo-A_I to apo-B (-0.17 ± 0.50 and constant on diets LO and MOD respectively). LDL-cholesterol/LDL-apo-B ratios were not affected by the diets. Combination of these data with those from a preceding study (Brussaard J.H. *et al.*, Atherosclerosis 36 (1980) 515) leads to the following conclusions:

1. Both diets lower total serum cholesterol levels when compared with the habitual diets of affluent communities.
2. The high-carbohydrate, low-fat diet causes lower HDL and higher fasting VLDL triglyceride levels than the recommended moderate fat-high PUFA diet.

INTRODUCTION

A controversy exists about which type of diet should be recommended to the general population for the optimalization of serum lipid levels. Diets high in linoleic acid have been shown to lower serum cholesterol and triglyceride levels; in long term intervention trials a reduction in the incidence of cardiovascular diseases was also noted (1, 2). However, objections have been raised against recommending diets rich in polyunsaturated fatty acids because there is no long term experience with such diets on a large scale. As an alternative, lowering of total fat consumption has been proposed (3). However, the effects of such diets on serum lipids have been studied much less thoroughly than those of PUFA-rich diets.

We have recently reported (4) the effect of diets differing in type and amount of dietary fat on serum lipids and lipoproteins in young healthy volunteers during a test period of 5 weeks. It was found that replacing polyunsaturated fat by carbohydrates caused an increase in LDL cholesterol and serum apolipoprotein B concentration and a lowering of HDL-cholesterol. Total serum triglycerides and VLDL concentration increased, but it was uncertain whether this was a transient phenomenon. Antonis and Bersohn (5) also reported an increase of serum triglycerides on carbohydrate-rich diets, but the hypertriglyceridemia subsided after 3-4 months on this diet.

In order to verify our earlier findings with respect to the effects on serum lipoproteins of replacing polyunsaturated fatty acids by starch we have

now carried out a similar experiment lasting 16 weeks. Dietary intake was strictly controlled during the whole period.

This paper presents the results of lipoprotein lipid, apolipoprotein A_I, B and C_{II}/C_{III} ratio analyses.

SUBJECTS AND METHODS

Subjects

Twelve female and twenty-three male university students aged 19 to 30 years participated in this study. The subjects were all unpaid volunteers. They were thoroughly informed about the nature of the experiment before they gave their informed consent. They were apparently healthy, as judged by a detailed questionnaire and had diastolic blood pressures below 90 mm Hg.

Mean body weights per group were 68.4 ± 6.2 kg and 71.6 ± 11.2 kg for MOD and LO respectively; body fat percentages were 14 ± 4% and 13 ± 4% for males and 26 ± 4% and 26 ± 3% for females in groups MOD and LO respectively.

None of the volunteers had glucosuria or proteinuria. Two of the females used hormonal anticonception in each dietary group.

Experimental design, diets and control of food intake

The experiment lasted from 27th August until 14th December 1979. During the first 2.5 weeks of the study all subjects consumed a control diet (MOD) which was moderate in fat and high in PUFA (31% of daily energy as total fat, 11% of daily energy as PUFA) in agreement with the recommendations of The Netherlands Nutrition Council, the Dietary Goals for the US and other official recommendations. After this period the subjects were randomized into two subgroups stratifying for initial serum total and HDL cholesterol level, male/female ratio and energy intake. One group (MOD) continued on the moderate fat high-PUFA control diet, the other group (LO) received a low-fat test diet (21% of daily energy as total fat, 4% of daily energy as PUFA) for a period of 13 weeks (Fig. 1).

Throughout the whole sixteen-week period all foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs, except for 100 kcal/day, which the subjects were free to spend at will. Average energy intake during the control period was 10.1 MJ (2416 kcal) per day.

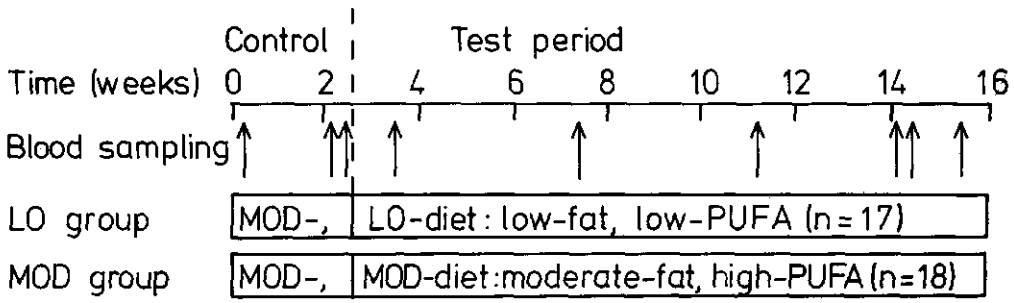

Fig. 1. Experimental design.

Actual nutrient intakes were checked in all subjects by 7-day individual weighed records using Dutch food composition tables (6) to which analyses had been added of 5 special products (breads and margarines) used in this experiment and by analysis of double portions (7) for one imaginary person of average energy intake on each diet.

Mean daily intakes of nutrients are given in Table 1. Analysis of double portions revealed a slightly higher absolute proportion of fat in the total daily energy intake in both groups, compared with computations from food tables but the differences between the two groups in fat intake, fatty acid composition and other nutrients agreed well with the food records.

As an independent check on dietary fat consumption the fatty acid composition of cholesterol esters in serum was analysed (8, 9). The results are given in Table 2. During the test period the fatty acid composition in the control group MOD was unchanged while in the LO group the percentage of linoleic acid decreased markedly, with compensatory increases in monounsaturated and saturated fatty acids.

Body weight was recorded weekly. Energy intake was adjusted when necessary in order to maintain body weight. The mean change per group in body weight over the 13 weeks of the test period was -1.4 ± 2.6 kg in group MOD and -1.7 ± 1.7 kg in group LO. Three subjects in each group had lost between 3 and 7 kg. However, eliminating the data of these subjects from the analysis caused only negligeable changes in all parameters and did not affect the conclusions from the experiment.

Urine was checked monthly for glucose and protein. Hemoglobin was measured monthly and was normal and stable in both groups during the experiment.

Stools were collected during the first and the last seven days of the test

TABLE 1. MEAN DAILY INTAKE OF NUTRIENTS ACCORDING TO INDIVIDUAL FOOD RECORDS[a)]

	Habitual intake before experiment[b)]	Control[c)] period (MOD)	Test period[d)] MOD	Test period[d)] LO
Energy (MJ)	11.1	10.1	10.1	10.3
(kcal)	2653	2416	2470	2460
Protein (energy%)	13	15	15	16
vegetable	5	7	7	8
animal	7	8	8	8
Total fat (energy%)	33	31	31	21
total saturated	15	8	9	7
C_{12}-C_{16} saturated	10	6	6	5
monounsaturated	12	10	10	8
ci-cis linoleic	4	11	10	3
total polyunsaturated	5	11	11	4
Carbohydrates (energy%)	48	50	50	60
sugars	23	20	20	20
starch	24	29	29	40
Alcohol (energy%)	6	1	1	1
Vitamin C (mg/day)	128	69	86	88
Cholesterol (mg/1000 kcal)	112	108	108	106
Phytosterols (mg/1000 kcal)[e)]	-	143	153	109
Dietary fiber (g/1000 kcal)	12.8	15.9	15.7	18.6
Polygalacturonic acid (g/1000 kcal)	1.2	1.0	0.9	1.2

a) The food records were elaborated using the Netherlands food composition tables supplemented with analysis of special breads and margarines.

b) 3-day records

c) 2-day records

d) 10-day records

e) Measured by analysis of duplicate portions providing 2700 kcal/day.

TABLE 2. MEAN FATTY ACID COMPOSITION OF CHOLESTEROL ESTERS IN SERUM AT THE END OF THE CONTROL PERIOD AND DURING THE TEST PERIOD (g/100 g fatty acid methylesters; mean ± SD)

Fatty acid	After 2 weeks of control period		After 5 weeks of test period		After 12 weeks of test period	
	MOD (n=16)	LO (n=17)	MOD (n=11)	LO (n=13)	MOD (n=16)	LO (n=17)
C_{14}	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.6 ± 0.2
C_{16}	11.5 ± 1.0	11.3 ± 1.5	10.8 ± 1.6	13.2 ± 1.2[x]	11.0 ± 1.3	13.1 ± 0.9[x]
$C_{16:1}$	2.4 ± 0.6	2.0 ± 0.5	1.8 ± 0.4	3.0 ± 0.7[x]	2.0 ± 0.1	3.3 ± 0.9[x]
C_{18}	2.4 ± 0.5	2.4 ± 0.6	2.9 ± 0.6	2.4 ± 0.5	2.7 ± 0.9	2.4 ± 0.4
$C_{18:1}$	18.5 ± 1.5	17.2 ± 0.9	18.5 ± 1.5	24.1 ± 1.8[x]	18.5 ± 2.0	25.0 ± 2.6[x]
$C_{18:2}$	58.2 ± 2.5	59.9 ± 2.1	59.0 ± 3.8	50.0 ± 3.3[x]	58.9 ± 4.2	49.4 ± 3.6[x]
$C_{20:4}$	6.6 ± 1.3	6.5 ± 1.1	6.7 ± 1.6	6.8 ± 1.3	6.9 ± 1.4	6.4 ± 1.3

[x] Change over test period significantly different from change in control group MOD ($p < 0.05$; t-test).

period. There were no significant differences between groups in intestinal transit time (10), faecal output and moisture content or frequency of stools in either period. This confirms the conclusion that the difference in starch intake had not resulted in differences in dietary fibre consumption (see also Table 1).

Blood sampling and analysis

Blood samples were taken after an overnight fast before and during the experimental period at the intervals indicated in Fig. 1.
No alcohol consumption was allowed for 24 h prior to blood sampling. Serum was obtained by low-speed centrifugation.

Serum cholesterol was measured with Liebermann-Burchardt reagent (11) using serum calibrators calibrated according to Abell *et al.* (12). The lipid laboratory was certified by the Center for Disease Control, Atlanta, G.A., USA, as meeting the WHO criteria. Reproducibility for blind control sera provided by the Center for Disease Control was 1.4% (coefficient of variation) and the absolute level (accuracy) was within 1.2% of the 'true' (target) values.

HDL cholesterol was determined after manganese-heparin precipitation of apo-B containing lipoproteins (13) as previously described (14). For blind control sera obtained in the Center for Disease Control Survey of HDL-cholesterol Measurement (15) a reproducibility of 2.2% (coefficient of variation) was found. The bias with regard to the overall survey mean was on average 0.1%.
VLDL was isolated for analysis of VLDL-apolipoprotein-C_{II}/C_{III} ratios by selective precipitation with Mg-heparine according to (16) and apo-C_{II}/C_{III} ratios were analysed by polyacrylamide slab gel electrophoresis in urea-containing buffers (17). Protein bands were stained with Fast Green FCF and quantified by densitometer with 640 and 750 nm as sample and reference wavelength respectively.

Lipoproteins were fractionated by density gradient ultracentrifugation according to the procedure of Redgrave *et al.* (18) with several modifications as described previously (19) but without lipoprotein prestaining.
Lipoprotein bands were harvested by tube slicing ($d < 1.006$ g/ml for VLDL, $d = 1.006$-1.070 for LDL, $d = 1.070$-1.125 for HDL_2 and $d > 1.125$ for HDL_3.
The upper density limit of LDL was increased slightly over the convential value of 1.063 so as to reduce contamination of HDL by sinking pre-beta lipoproteins.

Cholesterol concentrations in ultracentrifuged fractions were determined enzymatically (CHOD-PAP kit, Boehringer) using serum calibrators as described

above. The mean recovery was 99% and the day-to-day variation coefficient for control sera 1%. Serum total, VLDL and LDL triglyceride concentrations were measured as described by Soloni (20).
The changes in HDL cholesterol as measured after Mn-heparin precipitation were equal to the changes in cholesterol in total HDL as obtained by density gradient centrifugation. The percentual differences between HDL concentrations according to the two methods were 3.5%, 1.5% and 3.5% at the end of the control period, after 5 weeks of test period and after 12 weeks of test period respectively. The average correlation coefficient was 0.93.

Apolipoprotein-B was measured in whole serum and in fresh ultracentrifugally isolated VLDL fractions by rocket immunoelectrophoresis (21) as described (4). For apo-A_I analysis, serum was first delipidated with redistilled tetramethylurea and diluted with 8 M urea, 10 mM Tris-HCL, pH 8.2 (22). Apolipoprotein-A_I was then measured by rocket immunoelectrophoresis as described (4) using rabbit anti-serum against apo-A_I. The combined within-day and between-day coefficients of variation for control serum were 8.7% and 4.1% for the apo-B and the apo-A_I assays, respectively.

Statistical evaluation

The multiple values obtained at the end of the control period and of the test period (Fig. 1) were averaged per person. The difference between these two averages, yielded the change over the test period. The mean of the changes in the LO group was then compared with the mean of the changes in the MOD control group, using a 2-tailed Student's t-test.

RESULTS

Changes during the control period

The time courses of serum total cholesterol, total triglyceride and HDL cholesterol concentration are given in Figs. 2, 3 and 4 respectively. Both groups received the moderate fat, high PUFA diet (MOD) during the first 2.5 weeks of the study. During this control period average serum total cholesterol for all subjects combined decreased significantly by -0.68 ± 0.44 mmol/l (Fig. 2) of which on average only 0.02 ± 0.13 mmol/l was located in HDL (Fig. 4). This was accompanied by a decrease of total serum apolipoprotein B of -131 ± 94 mg/l and a decrease of total serum apolipoprotein A_I of -131 ± 143 mg/l on average.

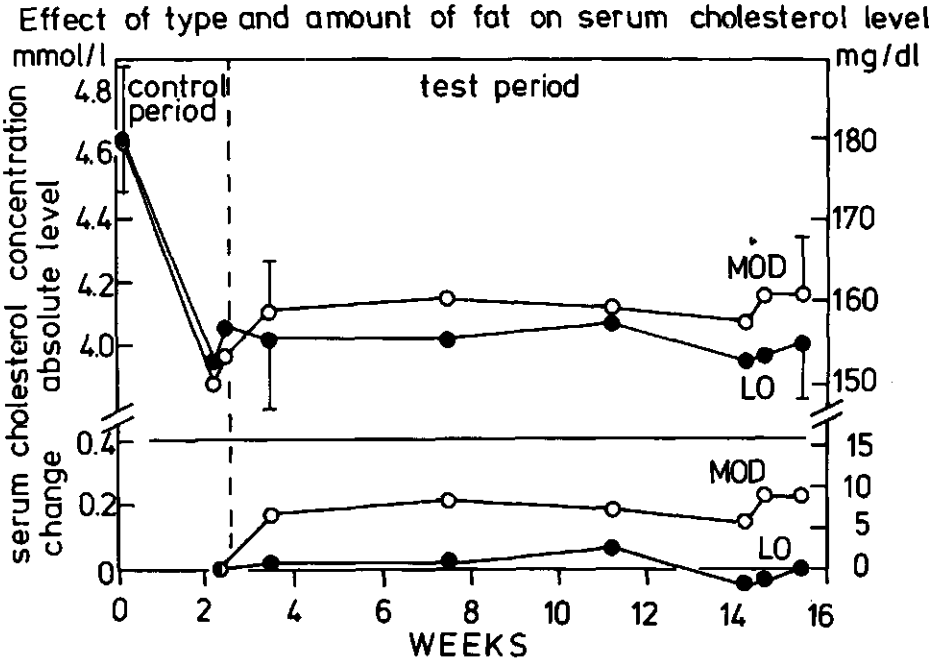

Fig. 2. Mean total serum cholesterol concentrations during the experiment and mean change in total serum cholesterol concentration during the test period. ●——● LO; ○——○ MOD (control).
Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($p < 0.05$ or 0.01) between the changes in the control group MOD and the LO group.

Total serum triglycerides decreased by 0.33 ± 0.41 mmol/l (Fig. 3). We did not stratify for total serum triglycerides which unfortunately resulted in a difference in absolute level between the groups.
During the control period, the changes in those subjects who were later to consume the low fat diet were not significantly different from those in the subjects randomized into the control diet group.

Changes during the test period

During the test period total serum triglycerides (Fig. 3) did not change significantly in the control group MOD but increased by 0.31 ± 0.33 mmol/l in group LO (+61%) which was significantly different from the change in group MOD (p 0.05). Total serum cholesterol (Fig. 2) increased by +0.21 ± 0.41 mmol/l (+5%) in the control group MOD during the test period but remained stable in

the group which switched over to the low fat diet.

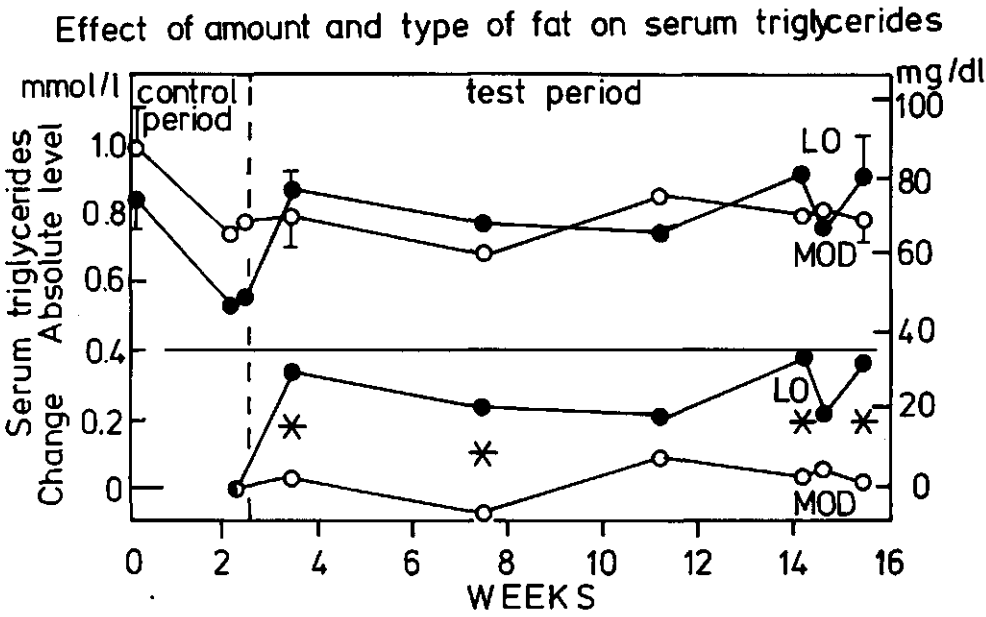

Fig. 3. Mean total serum triglyceride concentrations during the experiment and mean change in total serum triglyceride concentrations during the test period. ●——● LO; ○——○ MOD (control).
Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($p < 0.05$ or 0.01) between the changes in the control group MOD and the LO group.

Data for changes in lipoprotein concentration and composition during the test period are presented in Table 3.

Changes in High Density Lipoproteins

HDL cholesterol increased in the control group by +7% and decreased on the low fat diet by -4%. The changes in HDL cholesterol in group MOD were mainly located in HDL_2. In group LO HDL_3 cholesterol had also changed, the change being smaller after 12 weeks than after 5 weeks.
Apolipoprotein-A_I concentrations were stable in both groups. The ratio of total HDL cholesterol to apo-A_I had increased in the control group by +5% after 12 weeks and was unchanged in group LO, after an initial decrease of -3%.

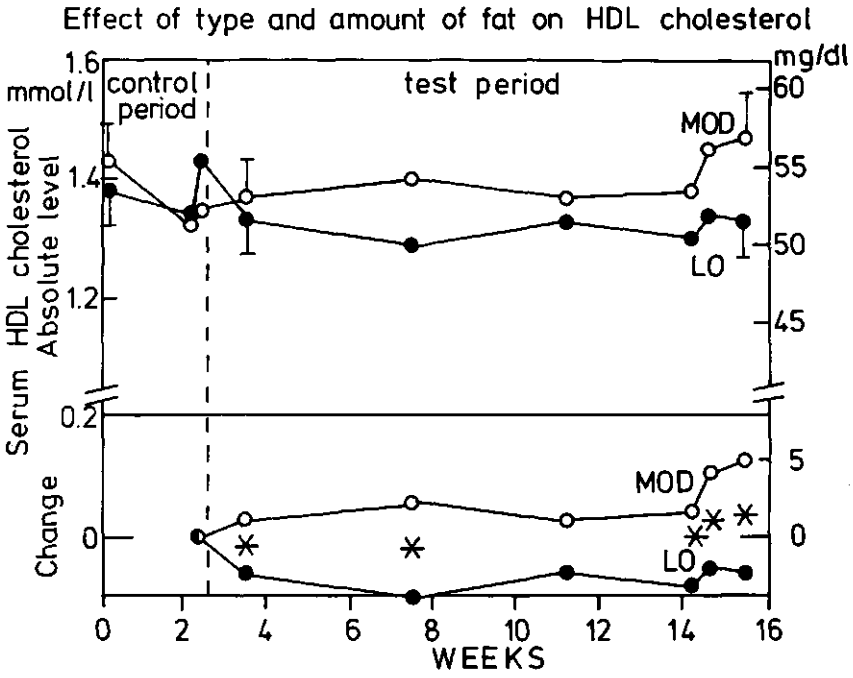

Fig. 4. Mean serum HDL-cholesterol concentrations (after Mn-heparin precipitation) during the experiment and mean change in serum HDL-cholesterol concentrations during the test period. ●——● LO; ○——○ MOD (control).
Vertical bars indicate ± SEM; an asterisk denotes a significant difference ($p < 0.05$ or 0.01) between the changes in the control group MOD and the LO group.

Changes in Low Density Lipoproteins

The concentration of LDL cholesterol remained rather stable in the group changing from the control to the low fat diet but increased slightly in the MOD control group. LDL triglyceride levels had not clearly changed. Changes in LDL-apo-B were minimal as were the changes in the ratio of LDL-cholesterol to apo-B.

Changes in Very Low Density Lipoproteins

After the transition from the moderate fat high PUFA control diet MOD to the low fat diet LO the mean concentration of cholesterol, triglycerides and

TABLE 3. EFFECT OF A LOW FAT DIET (LO) ON LIPOPROTEIN COMPOSITION AND CONCENTRATION AS COMPARED WITH A MODERATE FAT DIET (MOD) RICH IN LINOLEIC ACID. Values expressed as mean ± SD.

	End of control period Absolute level		Absolute change over test period (% of control level) After 5 weeks		After 12 weeks	
	MOD	LO	MOD	LO	MOD	LO
HDL						
Total HDL-chol.[a] (mmol/l)	1.34 ± 0.24	1.35 ± 0.21	+0.06 ± 0.14 (+ 4)	-0.10 ± 0.20[e] (- 7)	+0.09 ± 0.11 (+ 7)	-0.06 ± 0.20[e] (- 4)
HDL_2-chol.[b] (mmol/l)	0.73 ± 0.25	0.79 ± 0.25	+0.02 ± 0.15 (+ 3)	-0.07 ± 0.20 (- 9)	+0.06 ± 0.16 (+ 8)	-0.11 ± 0.17[e] (-14)
HDL_3-chol.[b] (mmol/l)	0.62 ± 0.11	0.61 ± 0.08	0.00 ± 0.11 (0)	-0.06 ± 0.12 (-10)	0.00 ± 0.14 (0)	-0.03 ± 0.09 (- 5)
Apolipoprotein-A_I[c] (mg/l)	1390 ± 140	1401 ± 247	+20 ± 121 (+ 1)	-18 ± 162 (- 1)	+51 ± 179 (+ 4)	-14 ± 193 (- 1)
Total HDL-chol/apo-A_I (mmol/g)	0.95 ± 0.11	0.96 ± 0.11	+0.04 ± 0.06 (+ 4)	-0.03 ± 0.12 (- 3)	+0.05 ± 0.07 (+ 5)	0.00 ± 0.09 (0)
LDL						
Cholesterol (mmol/l)	2.25 ± 0.53	2.44 ± 0.80	+0.31 ± 0.53 (+14)	+0.06 ± 0.47 (+ 2)	+0.15 ± 0.54 (+ 7)	-0.04 ± 0.53 (- 1)
Triglycerides (mmol/l)	0.20 ± 0.05	0.18 ± 0.05	0.00 ± 0.03 (0)	+0.03 ± 0.05 (+17)	+0.04 ± 0.08 (+20)	+0.01 ± 0.03 (+ 6)
Apolipoprotein-B[d] (mg/l)	494 ± 130	515 ± 197	+32 ± 93 (+ 6)	-1 ± 64 (0)	+42 ± 102 (+ 9)	+13 ± 87 (+ 3)
Cholesterol/apo-B (mmol/g)	4.7 ± 0.8	4.8 ± 0.5	+0.3 ± 0.6 (+ 6)	+0.3 ± 0.8 (+ 6)	-0.1 ± 0.7 (- 2)	-0.2 ± 0.6 (- 4)
VLDL						
Cholesterol (mmol/l)	0.31 ± 0.15	0.21 ± 0.10	0.00 ± 0.10 (0)	+0.11 ± 0.13 (+52)	+0.06 ± 0.21 (+19)	+0.15 ± 0.12 (+71)
Triglycerides (mmol/l)	0.44 ± 0.22	0.30 ± 0.17	+0.06 ± 0.22 (+14)	+0.22 ± 0.22 (+73)	+0.04 ± 0.31 (+ 9)	+0.22 ± 0.18 (+73)
Apolipoprotein-B (mg/l)	63 ± 30	46 ± 23	-10 ± 25 (-16)	+25 ± 19 (+54)	-8 ± 41 (-13)	+8 ± 15 (+17)
Apolipoprotein-C_{II}/C_{III} total	0.32 ± 0.10	0.31 ± 0.07	0.00 ± 0.06 (0)	0.00 ± 0.05 (0)	-0.02 ± 0.05 (- 6)	-0.02 ± 0.03 (- 6)
Triglycerides/apo-B (mmol/g)	7.7 ± 2.5	6.8 ± 1.4	+2.3 ± 3.7 (+30)	+0.7 ± 1.9 (+10)	+3.6 ± 6.7 (+47)	+3.2 ± 2.5 (+47)

a) Isolated after Mn-heparin precipitation. b) Isolated by density gradient ultracentrifugation.
c) Assuming total serum apolipoprotein-A_I is located in this fraction. In the control period apo-A_I decreased by-170 ± 156 mg/l in group MOD and by-92 ± 122 mg/l in group LO (not significantly different from each other).
d) *Calculated as total serum apo-B minus VLDL apo-B. In the control period total serum apolipoprotein B decreased by-137 ± 106 mg/l* in group LO and by-126 ± 84 mg/l in group MOD (not significantly different from each other).
e) Significantly different from change in control group ($p < 0.01$, t-test). Conversion factors from SI to traditional units: - cholesterol, 1 mmol/l = 39 mg/dl; - triglyceride, 1 mmol/l = 89 mg/dl)

apolipoprotein-B in VLDL increased strongly compared with the group which continued on the control diet MOD.
However, the effects did not reach statistical significance, because the standard deviations were also large. The ratio of apolipoprotein-C_{II} to C_{III} was unchanged until 5 weeks to test period but decreased slightly thereafter both in the LO group and in the MOD control group (-5.2 and -7% respectively). The ratio of triglycerides to apo-B in VLDL increased in both groups, indicating an increase in VLDL particle size on both diets.

DISCUSSION

The present experiment was set up to evaluate the effects of a low fat diet on lipoprotein concentration and composition as compared with a moderate fat, high PUFA diet on a more long-term basis then in our previous experiment (4). Thirty-five volunteers took part in the study, which lasted 16 weeks.
Dietary intake was strictly controlled and independent checks on food intake (composition of cholesterolester fatty acids, intestinal transit time and fecal wet weight) confirmed dietary adherence.

Effect of changing from the habitual diet to the high-PUFA control diet

The habitual fat and cholesterol consumption in our group of participants (Table 1) is lower than the average intake in western countries, as described earlier (4, 7, 23). As a consequence the main difference between the habitual diet and the MOD diet of the control period was a higher P/S ratio of the latter. As might be expected (24, 25) this resulted in a decrease in serum cholesterol and triglyceride concentration. The decrease in serum cholesterol was even larger than predicted by the formulas of Keys and Hegsted, which was also the case in our previous experiment (4).
As for individual lipoproteins, the decrease in serum cholesterol during the control period was located mainly in VLDL plus LDL; it was accompanied by a decrease in apo-B (footnote Table 3).
HDL-cholesterol did not change during this period (fig. 4) but apolipoprotein-A_I concentrations fell markedly (footnote Table 3).
This lack of an effect of dietary fat modification on HDL cholesterol concentrations in the control period cannot be ascribed to simultaneous changes in other factors which influence HDL levels: our subjects' level of exercise, body fatness, oral contraceptive use and cigarette smoking probably changed very

little upon entering the experiment, while their alcohol consumption decreased (Table 1), which only could have lowered HDL cholesterol levels.
We have previously observed (4) that test diets that differed in P/S ratio but contained the same amount of fat and cholesterol caused differences in VLDL and LDL, but not in HDL cholesterol concentrations.
Conventional lipid-lowering diets cause a decrease of HDL cholesterol (26, 27); we suggest that this is due not to the high P/S ratio but to the low total fat and cholesterol content of such diets.

Changes during the test period

Effect of the carbohydrate-rich diet on HDL

The high-carbohydrate, low fat LO diet caused a clear-cut depression of HDL-cholesterol compared with the control MOD diet. The difference was due mainly to the cholesterol-rich HDL_2 fraction. The latter was also reflected in the HDL cholesterol/apo A_I ratio, which decreased. These results agree with observations of Blum *et al.* (28), Schonfeld *et al.* (29) and Levy *et al.* (30), who report a lowering of HDL cholesterol in short-term controlled studies on high-carbohydrate diets containing less than 2% of energy as fat. Our results now suggest that these observations can be extrapolated to the normal range of fat consumption and to long-term intakes, because we find a permanent decrease of HDL cholesterol on lowering fat intake from 30 to 20% of energy.
Epidemiological studies agree with this conclusion. Thus, Ernst *et al.* (31) using data from the Lipid Research Clinics Program Prevalence Study, report a negative association between total carbohydrate, sucrose and starch intake and HDL cholesterol levels. We have recently reported (32) that among schoolboys from several countries low levels of total serum cholesterol were accompanied by low levels of HDL cholesterol and suggested that this might be due to a low consumption of fat-rich animal products, which would agree with the experimental results reported here.

The difference in HDL cholesterol between the moderate- and low-fat diet (0.16 mmol/l or 6 mg/dl) may seem modest, but it is in the same range as the difference between CHD victims and healthy controls (33) and it would predict a sizeable difference in coronary risk (34).

As for the mechanism of the effect of fat intake on HDL, high density lipoproteins are intimately involved in fat catabolism, both as activators and as side products of the catabolism of fat-rich particles. The main protein component of HDL, apo-A_I, is absent on nascent HDL particles emerging from the

liver. It is probably synthesized originally in intestinal cells as part of chylomicrons during fat absorption. As the chylomicrons are broken down they lose apo-A_I and other surface materials, which are transferred to the HDL density range and are probably taken up by HDL_3 under the formation of a less dense HDL_2 like particle (35) through the action of the cholesterol-esterifying enzyme LCAT. On the other hand the chylomicrons obtain from HDL the apo-C_{II} protein which is essential for the breakdown of triglyceride-rich particles by lipoprotein lipase. Thus it should not come as a surprise that the healthy human organism will have a higher HDL-level when it has to handle higher daily fat loads and lower HDL-levels on low-fat diets.

Variations in HDL cholesterol are often located in HDL_2. It was found, for example, that the higher level of HDL cholesterol in long-distance runners was mainly due to HDL_2 (36). The higher level of HDL in females than in males was also found to be due to HDL_2 (37). Blum *et al.* (28) also found a relative decrease in HDL_2 on a fat-free carbohydrate-rich diet. Thus it seems reasonable to expect that HDL_2 is more sensitive to changes in diet than HDL as a whole.

Effects of the carbohydrate-rich test-diet on total and LDL cholesterol

Our previous experiment showed a slight increase in both total and LDL cholesterol and an increase in total serum apolipoprotein-B on the LO diet relative to the MOD diet, while the present study showed a decrease (Fig. 2 and Table 3). The difference with the previous experiment cannot be explained by dietary composition or dietary adherence (Tables 1 and 2), and we have to assume that it is due to chance fluctuations. Neither the change in total nor the change in LDL cholesterol differed significantly from the respective changes in the control group.
In agreement with our previous experiment the ratio of LDL cholesterol to apolipoprotein-B showed only minimal changes.

Carbohydrate-induced hypertriglyceridemia and VLDL

In agreement with our previous experiment total serum triglyceride levels rose rapidly after the change-over from the control diet to the low fat, high carbohydrate diet and remained stable afterwards: there was no sign of a decrease of serum triglycerides up until 13 weeks on this diet.
Several authors (38, 39) have reported that carbohydrate-induced hypertriglyceridemia reaches a maximum within a few weeks and then starts to diminish again. However, none of these studies lasted long enough to establish whether

subjects' triglyceride values actually returned to baseline levels.

An exception is formed by the experiment of Antonis and Bersohn (5). They reported that when fat intake was lowered from 40 energy% to 15 energy%, triglyceride concentrations increased and it took between 20 and 32 weeks for subjects' triglyceride values to return to baseline levels. Analysis of unpublished details of this study (Fig. 5) shows that the white prisoners who had initially received the high fat butter diet did indeed reach base triglyceride levels.

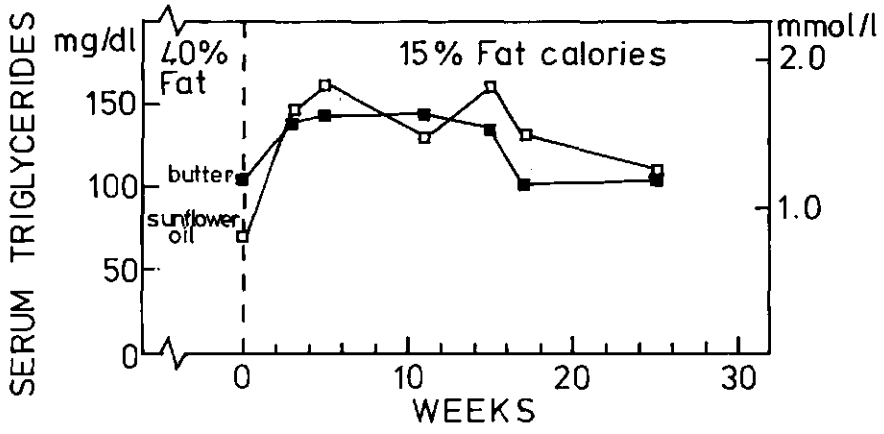

Fig. 5. Influence on fasting serum triglyceride levels of reducing the proportion of fat calories in white South-African prisoners. ■——■, data for 12 subjects who had received the high-fat butter diet; □——□, data for 13 subjects who had received the high-fat sunflower oil diet. No points are given beyond 25 weeks, because most subjects were removed from the study by then. (After unpublished data of Antonis & Bersohn; Kotze, J.P. personal communication).

However, those who had started out on the high fat sunflower oil diet were still above their baseline triglyceride levels after 25 weeks on the high-carbohydrate low-fat diet, when most of them were taken off the study. This suggests that total serum triglycerides on a low-fat diet will not return to baseline-level if the baseline control diet has been high in PUFA, in agreement with the findings reported here.

Carlson and Ballantyne (40) (in contrast with Dewailly *et al.* (41)) reported low levels of the apolipoprotein-C_{II}/C_{III} ratio in the VLDL of hyper-

triglyceridemic patients compared with controls, suggesting that changes in this ratio might play a role in the causation of hypertriglyceridemia. Schonfeld *et al.* (29) found increases in apo-C_{II}/C_{III} ratios after 5 days of high carbohydrate liquid formula diets. In our experiment, however, we found no changes in apo-C_{II}/C_{III} ratio on the low-fat diet as compared with the moderate-fat, high-PUFA diet. Perhaps this is due to the small difference in carbohydrate content between our control and test diet, which was only 10 energy% carbohydrates as opposed to a difference of 40 energy% carbohydrates between free diet and test diet in the study of Schonfeld *et al.* (29).

Evaluation of the low-fat diet in comparison with the moderate-fat, high-PUFA diet

Our data do not offer a definitive answer to the question which type of cholesterol-lowering diet should be preferred. Results for total serum or LDL-cholesterol were equivocal, with the low-fat LO diet causing higher LDL levels in our previous experiment (4) and lower LDL levels in the present experiment. Both types of diet are probably quite effective in lowering total serum cholesterol levels compared with what is found on habitual diets of affluent societies. There were, however, clear-cut differences in the effects on HDL and VLDL: HDL is depressed and fasting VLDL (and total serum triglycerides) are elevated by the low-fat high-carbohydrate LO diet relative to the high-PUFA moderate-fat MOD diet. This is also reflected in the ratios of HDL to total cholesterol and of apolipoprotein-A_I to apolipoprotein-B (Table 4), which were both lowered by the LO diet.

The epidemiological and biochemical evidence suggests that high levels of HDL protect against coronary heart disease (33-35). However, there is as yet no evidence from controlled intervention trials that manipulation of HDL, be it by dietary or other means, will actually shift a person to another risk category.

Thus, the importance of the effects described here with respect to atherogenic risk remain to be established.

ACKNOWLEDGEMENTS

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The help of all those who through their assistance and skill made this study possible is gratefully acknowledged.

TABLE 4. THE EFFECT OF TYPE AND AMOUNT OF DIETARY FAT ON THE HDL- TO TOTAL CHOLESTEROL AND APO-A_I/APO-B RATIOS

Values expressed as mean ± SD

	End of control		Change over test period (percent)			
			5 weeks		12 weeks	
	MOD	LO	MOD	LO	MOD	LO
HDL-/total cholesterol (mol/mol)	0.34±0.05	0.36±0.09	0.00±0.03 *(0)*	-0.03±0.05 *(-8)*	+0.01±0.03 *(+3)*	-0.02±0.05 *(-5.5)*
apo-A_I/apo-B (g/g)	2.59±0.54	2.88±1.46	-0.05±0.45 *(-2)*	-0.13±0.39 *(-4.5)*	0.00±0.50 *(0)*	-0.17±0.50 *(-6)*

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4. Fecal excretion of bile acids and neutral steroids on diets differing in type and amount of dietary fat in young healthy persons.

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ABSTRACT

The effect of different amounts and types of dietary fat on fecal steroid excretion in healthy volunteers was examined in the course of two controlled experiments. The first experiment lasted for 8 weeks and sixty volunteers took part in it. During a control period of 2.5 weeks all subjects received the control diet (MOD), containing 30% of daily energy as total fat, 11% of energy as polyunsaturated fatty acids (PUFA). Then four subgroups were formed. One group continued on the control diet, one group (LO) received 22% of energy as total fat, 3% as PUFA, one group (HIPUF) received 40% of energy as total fat, 19% as PUFA and one group (HISAT) received 39% of energy as total fat, 3% as PUFA. Feces were collected during the last seven days of the control period and again during the last seven days of the test period, and analysed individually. In the second experiment with 35 volunteers, the low fat diet containing 21% of energy as total fat (4% as PUFA) was again compared with the control diet (31% as total fat, 11% as PUFA) but this time the test period lasted 13 weeks. Feces were collected during the first seven days of the test period and during 7 consecutive days near the end of the test period and pooled per dietary group. Throughout the whole experimental period all foodstuffs were weighed out individually and supplied according to each person's energy needs. Intake of cholesterol was approx. 250 mg per day; intake of dietary fiber was approx. 24 g per day in the first experiment and approx. 41 g per day in the second. Intake of cholesterol and dietary fiber was equal in all diet groups within each experiment. The latter was confirmed by measurement of fecal mass, percentage dry matter, mean transit time and frequency of stools, which showed no differences between the diet groups.

Fecal excretion of neutral steroids increased by 0.42, 0.30, 0.34 and 0.14 mmol/24 h in the first experiment in groups LO, MOD, HIPUF and HISAT respectively and by 0.12 and 0.23 mmol/24 h in the second experiment in groups LO and MOD respectively. (1 mmol of coprostanol = 389 mg).
Bile acid excretion changed by +0.10, +0.01, +0.02 and -0.03 mmol/24 h in the first experiment in groups LO, MOD, HIPUF and HISAT respectively and by +0.18 and -0.01 mmol/24 h in the second experiment in groups LO and MOD respectively. In neither experiment were the changes in the groups significantly different from each other.

Neither the concentration of steroids in stools nor the degree of bacterial degradation was influenced by the diets.

INTRODUCTION

The effect of diet on fecal steroid excretion is of interest because of its possible relationship with changes in the level of serum cholesterol. In addition a relation has been suggested between intestinal steroids and colon cancer.

Although the effects of dietary fat on serum cholesterol concentrations have been known for 25 years (1, 2) the mode of action of the diet in changing serum cholesterol levels remains somewhat controversial. One of the proposed mechanisms is an increased excretion of fecal steroids, caused by decreased resorption of cholesterol or increased conversion of hepatic cholesterol into bile acids or both of these mechanisms.

Dietary fat has been implicated in the etiology of cancer of the colon (3, 4) and it has been suggested (3, 5) that dietary fat acts by affecting the concentration of certain steroids in the gut.

Up until now conflicting results have been published on the effect of increasing the amount of dietary fat on fecal steroids. Increased excretion of fecal steroids was found by Antonis and Bersohn (6) and increased bile acid excretion by Cummings *et al.* (7). However, a decrease of bile acid excretion on a high fat diet has also been reported (8).

Replacement of saturated fatty acids by polyunsaturated fatty acids in the diet has been found to increase the fecal excretion of bile acids and neutral steroids (6, 9, 10). Others found that this effect was dependent on the amount of cholesterol (9, 11, 12) and the amount of plant sterols (13) in the diet.

Replacement of carbohydrates by polyunsaturated fatty acids has also been found to increase fecal steroid excretion (6).

In the course of two controlled experiments on the effect of diet on serum lipoprotein concentration and composition we have re-examined the effect of different amounts and types of fat on fecal steroid excretion at constant cholesterol intake. Other results of these studies have been published elsewhere (14-16).

METHODS

The design of the first experiment was as follows (Fig. 1).

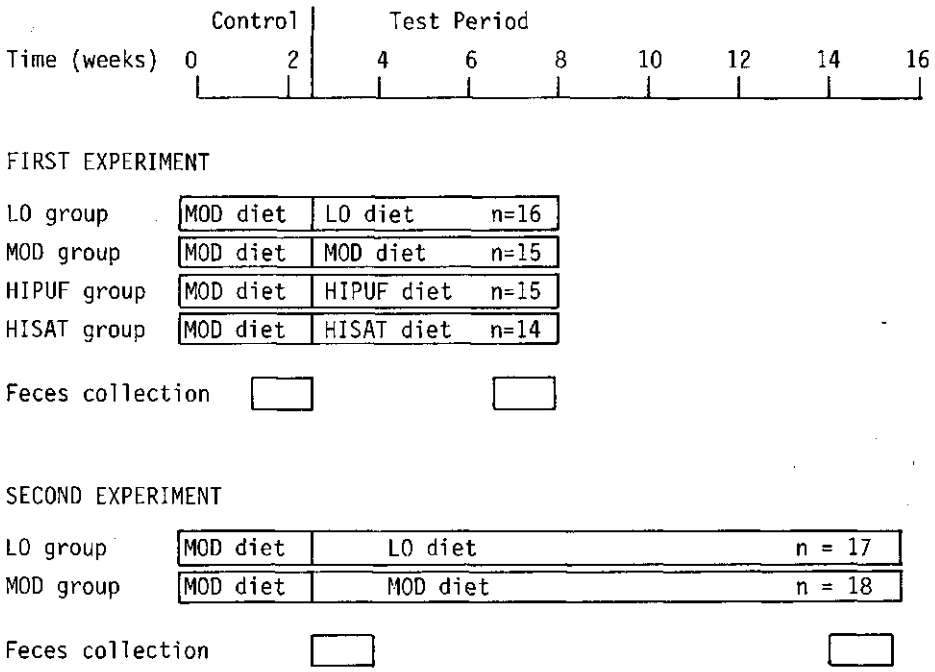

Figure 1. Experimental design.
LO = low fat, high carbohydrate diet. 22% of daily energy as fat, 3% of daily energy as polyunsaturated fat.
MOD = moderate fat, high polyunsaturated fat diet. 30% of daily energy as fat, 11% of daily energy as polyunsaturated fat.
HIPUF = high fat, high polyunsaturated fat diet. 40% of daily energy as fat, 19% of daily energy as polyunsaturated fat.
HISAT = high fat, low polyunsaturated fat diet. 39% of daily energy as fat, 3% of daily energy as polyunsaturated fat.

Sixty young healthy student volunteers received a control diet for 2.5 weeks.

They were then randomized into four subgroups which received test diets as indicated in Fig. 1. The subgroups were stratified for initial serum cholesterol levels, male/female ratio and energy intake. The test period lasted 5 weeks. The second experiment with 35 volunteers differed from the first in that only two diets were tested, namely the low fat LO diet and the "Dietary Goals" diet MOD, and that the test period was extended to 13 weeks.
In each experiment diets differed between groups in one component only; one group always received the control diet throughout the test period as a check against baseline drift. Throughout the whole experimental period all foodstuffs were weighed out individually and supplied according to each person's energy needs. Actual nutrient intake was measured by 7-day weighed food records using Dutch food composition tables (17) and by analysis of double portions (18) for one imaginary person of average energy intake on each diet.

Diets

The moderate fat control diet MOD in the first experiment contained 11% of total daily energy as polyunsaturated fatty acids (PUFA); in total, fat provided 30% of daily energy (P/S 1.1). The low-fat, low PUFA diet LO provided 22% of energy as total fat (3% of daily energy as PUFA; P/S 0.4) the high-fat, high PUFA diet HIPUF provided 40% of daily energy intake as total fat (19% of daily energy as PUFA; P/S 1.7) and the high fat, low PUFA HISAT diet provided 39% of daily energy as total fat (3% of daily energy as PUFA; P/S 0.2). In all diets protein provided 13% of daily energy and mono- and disaccharides 23%, while starch made up the total energy balance. Cholesterol intake was 244, 275, 255 and 210 mg per day for groups LO, MOD, HIPUF and HISAT respectively.
Intake of plant sterols was 247, 395, 530 and 270 mg per day and dietary fiber intake was 24, 24, 24 and 25 g per day for groups LO, MOD, HIPUF and HISAT respectively[1)].

In the second experiment the control diet MOD and the low fat, low PUFA diet LO were tested again, but this time the test period lasted 13 weeks instead of 5 weeks. Total fat intake and dietary fatty acid composition were equal to the first experiment. Cholesterol intake was 260 and 267 mg per day, dietary fiber intake was 46 and 39 g per day and intake of plant sterols was 269 and 384 mg per day for groups LO and MOD respectively.

[1)] In our earlier publication (14) the values for the plant sterol content of diets HIPUF and HISAT were accidentally interchanged.

Feces collection and analysis

In the first experiment feces were collected during the last seven days of the control period and again during the last seven days of the test period, and analysed individually (Fig. 1). In the second experiment feces were collected during the first seven days of the test period and during 7 consecutive days near the end of the test period. In this experiment a fixed proportion of each subject's feces was pooled per dietary group and only pools were analyzed. The stools were usually frozen within 12 h of being passed and stored at -40°C. Mean transit time through the gut (MTT) was measured using radio-opaque markers (19). Fecal primary (cholic acid and chenodeoxycholic acid) and secondary (deoxycholic acid and lithocholic acid) bile acids were extracted and determined by gas-liquid chromatography according to Evrard (20). Cholesterol and the secondary neutral steroids (coprostanol, (epi)coprostanol and coprostanone) were measured according to Miettinen *et al.* (21) but without prior thin-layer chromatography. Repeated determinations on a control pool of freeze-dried feces revealed an inter-assay variability of 6.9% (coefficient of variation) for cholesterol and 6.5% for coprostanol and 5% for total bile acids. Fecal fat was measured in the first experiment as fatty acids after saponification (22).

Statistical evaluation

For statistical evaluation of results mean changes over the test period were compared between groups, using a two-tailed Student's t-test. For the second experiment, where only pooled samples were analyzed, standard deviations were estimated from previous experiments with comparable subjects (18, 23) and experiment 1.

RESULTS

Fecal mass and intestinal transit time

Results for mass of feces passed per day, percentage dry matter, frequency of stools and mean transit time are given in Table 1.
There were no differences between the groups in the changes over the test period, whether measured from end of control to end of test period (experiment 1) or from start to end of test period (experiment 2).

As reported previously (18, 23) there was a large variation in fecal output between subjects; in the control period, when all subjects consumed the same

TABLE 1. THE OUTPUT OF FECES AND THE MEAN FECAL TRANSIT TIME DURING THE TWO EXPERIMENTS (mean ± SD)

	EXPERIMENT 1[a] Diet group				EXPERIMENT 2[b] Diet group	
	LO (n=16)	MOD (n=15)	HIPUF (n=15)	HISAT (n=14)	LO (n=17)	MOD (n=18)
Wet weight (g/24 h)						
Control period	107 ± 45	118 ± 53	109 ± 49	122 ± 70	--------[c]	--------[c]
Start test period	--------[c]	--------[c]	--------[c]	--------[c]	151 ± 71	140 ± 56
End test period	113 ± 43	117 ± 43	123 ± 65	113 ± 62	164 ± 82	160 ± 74
Dry matter (g/100 g wet weight)						
Control period	24 ± 4	24 ± 4	25 ± 5	25 ± 6	--------[c]	--------[c]
Start period	--------[c]	--------[c]	--------[c]	--------[c]	24 ± 4	24 ± 3
End test period	26 ± 5	25 ± 4	26 ± 5	27 ± 6	24 ± 3	24 ± 4
Frequency of stools (per 24 h)						
Control period	0.84 ± 0.28	1.08 ± 0.36	1.04 ± 0.62	1.06 ± 0.47	--------[c]	--------[c]
Start test period	--------[c]	--------[c]	--------[c]	--------[c]	1.0 ± 0.3	0.9 ± 0.4
End test period	0.94 ± 0.29	1.03 ± 0.37	1.16 ± 0.54	1.01 ± 0.45	1.1 ± 0.4	1.0 ± 0.3
Mean transit time (h)						
Control period	51 ± 20	53 ± 34	62 ± 45	56 ± 24	--------[c]	--------[c]
Start test period	--------[c]	--------[c]	--------[c]	--------[c]	59 ± 28	57 ± 26
End test period	61 ± 27	59 ± 33	67 ± 41	57 ± 36	50 ± 20	46 ± 26

[a] Test period 5 weeks

[b] Test period 12 weeks

[c] Not measured

diet, fecal wet weight, averaged over a seven-day period ranged from 28 to 246 g per day and the percentage dry matter from 18 to 38%. Mean transit time through the gut ranged from 13 to 172 h.

Fecal steroids and fat

Fecal excretion of neutral and acidic steroids and of fecal fat is given in Table 2. There was an increase in the excretion of neutral steroids from the end of the control period to the end of the test period in all four groups in the first experiment. However, the changes in groups LO, HIPUF and HISAT were not significantly different from the change in the control group MOD. In the second experiment fecal excretion of neutral steroids increased from the first week to the end of the test period but again the change in group LO was not significantly different from the change in group MOD, if we assume that the standard deviations in this experiment were comparable to those in previous experiments (18, 23) and experiment 1.

Bile acid excretion (Table 2) was rather constant on all 4 diets in the first experiment. There was a slight increase on diet LO, a slight decrease on diet HISAT and no change on diets MOD and HIPUF. The changes in groups LO, HIPUF and HISAT were not significantly different from those in the control group MOD. In the second experiment fecal bile acid excretion was again unchanged on diet MOD, but on diet LO it was clearly higher at the end of the test period compared with the start of the test period. However, this change was not significantly different from the change in the control group MOD, again provided that we made the right estimations of the standard deviations.

Fecal excretion of fat showed an increase in all four groups in the first experiment. The increase in the high-fat diet groups HIPUF and HISAT was significantly greater than the change in the control group MOD. However, the differences were small in absolute terms. In the second experiment fecal fat was not measured.

Changes in steroid and fat excretion related to changes in total serum cholesterol concentration

In Table 3 the correlations between the changes in steroid excretion and the changes in total serum cholesterol concentration are given.
If total serum cholesterol changes are related to changes in steroid excretion one would expect a negative correlation between the two: if serum cholesterol goes up, excretion should go down. This is not what we observed. Mean serum

TABLE 2. THE EXCRETION OF NEUTRAL STEROIDS AND BILE ACIDS AND OF FAT DURING THE TWO EXPERIMENTS (mean ± SD)[a]

	EXPERIMENT 1[b] Diet group				EXPERIMENT 2[c] Diet group	
	LO (n=16)	MOD (n=15)	HIPUF (n=15)	HISAT (n=14)	LO (n=17)	MOD (n=18)
			(mmol/24h)			
Neutral steroids						
Control period (MOD diet)	1.42 ± 0.54	1.67 ± 0.61	1.48 ± 0.45	1.36 ± 0.64	-----[d]	-----[d]
Start test period	---------[d]	---------[d]	---------[d]	---------[d]	1.34	1.60
End test period	1.84 ± 0.60	1.96 ± 0.63	1.82 ± 0.78	1.50 ± 0.74	1.46	1.83
Change over test period	+0.42 ± 0.44	+0.30 ± 0.44	+0.34 ± 0.75	+0.14 ± 0.89	+0.12	+0.23
Bile acids						
Control period (MOD diet)	0.67 ± 0.32	0.76 ± 0.35	0.76 ± 0.36	0.67 ± 0.28	-----[d]	-----[d]
Start test period	---------[d]	---------[d]	---------[d]	---------[d]	0.73	0.78
End test period	0.77 ± 0.29	0.77 ± 0.30	0.77 ± 0.34	0.64 ± 0.26	0.91	0.77
Change over test period	+0.10 ± 0.21	+0.01 ± 0.15	+0.02 ± 0.16	-0.03 ± 0.19	+0.18	-0.01
Fatty acids						
Control period (MOD diet)	5.6 ± 2.4	6.2 ± 2.7	6.1 ± 2.1	4.8 ± 1.8	-----[d]	-----[d]
Start period	---------[d]	---------[d]	---------[d]	---------[d]	-----[d]	-----[d]
End test period	6.0 ± 2.8	6.5 ± 2.5	7.3 ± 1.8	7.2 ± 3.3	-----[d]	-----[d]
Change over test period	+0.4 ± 2.8	+0.3 ± 1.9	+1.2 ± 1.4[e]	+2.4 ± 2.7[e]		

a) Conversion from SI to traditional units: 1 mmol of neutral steroids (as coprostanol) = 389 mg; 1 mmol of bile acids (as deoxycholic acid) = 393 mg; 1 mmol of fatty acids (as oleic acid) = 283 mg.

b) Test period 5 weeks. Start test period not measured. Results of individually analysed feces.

c) Test period 12 weeks. Control period not measured. Results of analysis of pooled feces. Estimated standard deviations (18, 23 and experiment 1): 0.33 mmol/24 h for bile acids and 0.59 mmol/24 h for neutral steroids.
Estimated standard deviations for change over test period 0.23 mmol/24 h for bile acids and 0.60 mmol/24 h for neutral steroids.

d) Not measured.

e) Change over test period significantly different from change in control group MOD ($p < 0.01$, t-test).

TABLE 3. CORRELATION COEFFICIENT (r) BETWEEN THE CHANGE IN SERUM CHOLESTEROL LEVEL AND IN FECAL STEROID EXCRETION PER PERSON WITHIN THE DIET GROUPS IN EXPERIMENT 1[a].

	Diet group			
	LO	MOD	HIPUF	HISAT
	Correlation coefficient with Δ serum cholesterol concentration over the test period			
Δ bile acid excretion	-0.52[b]	-0.04	-0.25	+0.18
Δ neutral steroid excretion	-0.07	+0.10	-0.44[b]	-0.07

a) No individual data are available for experiment 2

b) Significantly different from 0, $p < 0.05$

cholesterol concentrations remained stable in groups MOD and HIPUF and increased in groups LO and HISAT, by 0.25 and 0.38 mmol/l respectively (14). However, as shown in Table 2, group means of fecal neutral steroid excretion increased in all four groups and bile acid excretion was almost unchanged. Within each diet group, different individuals reacted differently to the diet. As shown in Table 3 there was a negative correlation between a subject's change in serum cholesterol and his or her change in excretion of bile acids within group LO. In general, however, the correlation between individual changes in serum cholesterol level and fecal steroid excretion within a group was weak and inconsistent.

Fecal concentration of steroids and percentage secondary steroids

The fecal concentration of neutral steroids at the end of the control period in the first experiment was 15 mmol per kg wet feces; the fecal concentration of bile acids was 7 mmol per kg wet feces. The fecal concentration of neutral steroids and bile acids at the start of the test period in the second experiment was 10 mmol and 6 mmol per kg wet feces respectively. The changes in fecal steroid concentration were small in both experiments and there were no statistically significant differences between the diet groups.

At the end of the control period of the first experiment, 91% of the neutral steroids and 73% of the bile acids in the feces consisted of secondary steroids, formed from primary steroids by bacterial activity. Changes over the test period in the proportion of secondary products on the various test diets ranged on average from -2 to +4 percentage points for neutral steroids and from -8 to +5 percentage points for bile acids. The changes were not significantly different from changes in the control group MOD.
The results for the second experiment were similar.

DISCUSSION

The present experiments were set up to evaluate the effects of type and amount of dietary fat on serum lipoprotein composition and concentration.
One of the possible mechanisms by which dietary fat influences serum cholesterol concentration is by increasing the excretion of fecal steroids. However, we found neither an acute nor a chronic effect of type and amount of dietary fat on fecal excretion of neutral steroids and bile acids in two carefully controlled experiments with large groups of participants and using natural foodstuffs.

Replacement of saturated by polyunsaturated fatty acids

In the first experiment we found no difference in excretion of bile acids and neutral steroids in the feces between subjects on diets HIPUF and HISAT. These diets contained the same amount of fat and cholesterol, but had P/S ratios of 1.7 and 0.2 respectively.

Table 4, part I, lists studies on the effect on fecal steroid excretion of replacing saturated by polyunsaturated fat. Only controlled studies with healthy subjects consuming natural solid diets are included. Evaluation of research on the effect of diet on fecal steroid excretion is hampered by the great differences in experimental design, type of diet and type of participants between the various studies. Therefore, studies with liquid formula diets were excluded because they usually have a very low fiber content, which could interfere with the results. Studies of patients with disorders of lipid metabolism were also excluded because such patients are known to have a fecal steroid excretion different from healthy controls (24). The intake of cholesterol and plant sterols are given because these components influence fecal steroid excretion (11-13).

As can be seen from Table 4, replacement of saturated by polyunsaturated fatty acids led to increased bile acid excretion in the experiment of Antonis and Bersohn (6). Although sterol consumption was not listed, the intake of cholesterol was probably lower and the intake of plant sterols higher on the polyunsaturated diet. Moore *et al.* (10) found modest increases in bile acid excretion on a high safflower oil diet compared with a butter diet, but the intake of cholesterol and plant sterols was not constant; Nestel *et al.* found no effect of polyunsaturated fatty acids in one experiment (9) and in another experiment they found an increase in bile acid excretion only when the cholesterol content of the diets was high (11).

Replacement of saturated fat by polyunsaturated fat led to a higher excretion of neutral steroids in the above-mentioned experiments, although it cannot be excluded that the analysis of fecal neutral steroids in the experiment of Antonis and Bersohn included some plant sterols. Eneroth *et al.* (25) also found modest increases in fecal neutral steroid excretion on a high corn oil diet compared with a butter diet. One might speculate that this was at least partly due to the higher intake of plant sterols on diets rich in polyunsaturated fat. However, in our experiment we did not find a higher excretion of neutral steroids on diet HIPUF than on diet HISAT, despite a higher intake of plant sterols on diet HIPUF.

In our first experiment the intake of cholesterol was almost identical in all four groups, and uniformly low. According to the study of Nestel *et al.* (11) this might have obscured an effect on bile acid excretion. However, it is not likely that this also explains the absence of a difference in neutral steroid excretion between diets HIPUF and HISAT.

Replacement of carbohydrates by fat

Studies on the effect of the proportion of fat in the diet on steroid excretion are rare. The few controlled studies with healthy subjects and regular diets that we are aware of are listed in the second part of table 4. Replacement of carbohydrates by butter led to a decrease of bile acid excretion and a small increase in neutral steroid excretion in the experiment of Antonis and Bersohn. Surprisingly, in another phase of the same experiment replacement of butter by carbohydrates gave the same results. Cummings *et al.* found an increase in bile acid excretion when the proportion of saturated fat in the diet was increased. However, the intake of cholesterol was also increased.

Antonis and Bersohn found increased bile acid and neutral steroid excretions when carbohydrates were replaced by polyunsaturated fat, while the reverse was found when polyunsaturated fat was replaced by carbohydrates. In our experiment we found no differences in fecal steroid excretion on diets with different amounts of polyunsaturated fat.

It has been suggested that the transition to a diet high in polyunsaturated fat causes a temporary increase in fecal steroid excretion which subsides when steroid metabolism in the body has reached a new equilibrium. However, in experiment 2 we saw no significant difference between the excretion on the low fat LO diet in the first and in the thirteenth week after the change of diet.

It would appear logical that an increase of fat in the diet leads to the excretion of more bile acids in the gut. However, the effect on total excretion has not been shown convincingly. The relation between changes in fecal steroid excretion and changes in serum cholesterol concentration within one diet group was also weak in our experiments.

Thus, differences at the intestinal level apparently do not explain the different ways in which serum cholesterol levels of individuals reacted to a given diet.

Fecal steroid concentration and composition

Populations consuming low-fat diets were reported to have lower concentra-

TABLE 4. DAILY FECAL STEROID EXCRETION IN SUBJECTS ON DIETS WITH DIFFERENT AMOUNTS OR TYPES OF FAT OR BOTH. DATA ARE GIVEN ONLY FOR CONTROLLED STUDIES OF SUBJECTS WITH NORMAL LIPID METABOLISM CONSUMING NATURAL SOLID DIETS.

Authors	Number of participants	Duration	Diet				Fecal excretion	
			Amount of fat (% of energy)	Type of fat	Cholesterol (mg/day)	Plant sterols (mg/day)	Bile acids (mg/day)	Neutral steroids (mg/day)
Part I. Studies on the type of dietary fat.								
Antonis and Bersohn (6)	9	29 weeks	40	butter	-----[a]	-----[a]	286	169
	6	8 weeks	40	sunflower seed oil	-----[a]	-----[a]	524	399
	8	29 weeks	40	sunflower seed oil	-----[a]	-----[a]	640	463
	5	8 weeks	40	butter	-----[a]	-----[a]	427	261
Eneroth *et al.*(25)	6	4 weeks	40-50	butter	271	0	-----[a]	527
	6	4 weeks	40-50	corn oil	141	447	-----[a]	614
Moore *et al.* (10)	5	16 days	40	butter	347	420	473	486
	5	16 days	40	safflower oil	197	720	564	580
Nestel *et al.* (9)	5	4 weeks	45-50	ruminant saturated	±500	-----[a]	196	607
	5	4 weeks	45-50	ruminant polyunsaturated	±500	-----[a]	271	681
				Dairy fat, beef, lamb				
Nestel *et al.* (11)	5	19 days	42-44	high cholesterol, saturated	789	-----[a]	281	806
	5	23 days	42-44	high cholesterol, polyunsaturated	796	-----[a]	381	1039
	5	23 days	42-44	low cholesterol, saturated	493	-----[a]	297	674
	5	23 days	42-44	low cholesterol, polyunsaturated	520	-----[a]	314	847
Nestel *et al.* (12)	3	28 days	44	pork saturated	549	-----[a]	231	830
	3	28 days	44	pork polyunsaturated	535	-----[a]	239	1096

[a] Data not given

TABLE 4. Continued

Authors	Number of participants	Duration	Diet: Amount of fat (% of energy)	Diet: Type of fat	Diet: Cholesterol (mg/day)	Diet: Plant sterols (mg/day)	Fecal excretion: Bile acids (mg/day)	Fecal excretion: Neutral steroids (mg/day)
Part II. Studies on the amount of fat.								
Antonis and Bersohn (6)	9	39 weeks	15	prison diet	-----a)	-----a)	450	184
	9	22 weeks	40	butter	-----a)	-----a)	353	294
	5	8 weeks	40	butter	-----a)	-----a)	427	261
	5	17 weeks	15	prison diet	-----a)	-----a)	351	379
	8	39 weeks	15	prison diet	-----a)	-----a)	539	223
	8	22 weeks	40	sunflower seed oil	-----a)	-----a)	577	636
	6	8 weeks	40	sunflower seed oil	-----a)	-----a)	524	399
	6	17 weeks	15	prison diet	-----a)	-----a)	321	270
Whyte *et al.* (8)	2	2 weeks	68	safflower oil	513	423	397	859
	2	2 weeks	10	high sucrose diet	502	-----a)	513	848
	2	2 weeks	69	safflower oil	219	383	266	555
	2	2 weeks	5	high sucrose diet	233	375	301	336
	2	2 weeks	10	high sucrose diet	652	20	425	690
	2	2 weeks	68	saturated fat (lard)	652	20	238	761
Cummings *et al.* (7)	6	4 weeks	21	saturated fat	466	-----a)	140	-----a)
	6	4 weeks	48	saturated fat	732	-----a)	320	-----a)

a) Data not given

tions of steroids in their feces than affluent populations (3, 26). This is explained most easily by assuming that these low-fat diets have a high content of cereal fiber. This is known to increase fecal bulk, thus diluting the steroid concentration. In our experiment fiber intake was kept constant over the whole range of fat intake; as a result, intestinal transit time, fecal mass and fecal steroid concentration also remained constant.

Populations consuming different diets have been found to differ in the extent of bacterial degradation of their fecal steroids (27). However, in controlled experiments consistent effects of diet on the ratio of primary to secondary bile acids or neutral steroids have not been observed (28). This agrees with our own results. It could be that much longer periods are necessary to establish a new equilibrium in the gut, or alternatively, that the differences observed between different groups of people are caused by differences other than their dietary habits.

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5. Fasting and postprandial plasma insulin and glucose levels in young healthy persons on a high-carbohydrate diet and on high-fat diets of different polyunsaturated fat content.

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ABSTRACT

The effects of type and amount of dietary fat on fasting and postprandial plasma glucose and insulin were investigated. In the first experiment 60 young healthy volunteers received a moderate fat control diet rich in polyunsaturated fat (30% of daily energy as total fat, 11% as polyunsaturated fat) for a control period of 2½ week, after which they were randomized into 4 subgroups for a test period of 5 weeks. One group continued on the control diet. Three other diets were investigated: one low in total fat and low in polyunsaturated fat (21% of daily energy as total fat, 3% as polyunsaturated fat); one high in total fat and low in polyunsaturated fat (39% of daily energy as total fat, 3% as polyunsaturated fat) and one high in total fat and high in polyunsaturated fat (40% of daily energy as total fat, 19% as polyunsaturated fat). In the second experiment the moderate fat, high polyunsaturated fat diet and the low fat, low polyunsaturated fat diet were compared again, but this time the test period lasted 13 weeks. Serum insulin and plasma glucose levels were measured in fasting blood and one hour after the beginning of a test meal, the composition of which was similar to that of the respective diet as a whole. We found no effect of type or amount of dietary fat on fasting plasma glucose and insulin levels nor on the postprandial rise in plasma glucose and insulin.

INTRODUCTION

Diabetes and hypercholesterolemia are both risk factors for cardiovascular disease. The effect of various cholesterol-lowering diets on carbohydrate metabolism are thus of interest, because a diet that is optimal for serum

cholesterol concentrations does not necessarily improve the handling of glucose. As far as dietary fat is concerned, two types of dietary changes have been advocated for the prevention and/or treatment of hypercholesterolemia, namely replacement of saturated by polyunsaturated fatty acids and reduction of total fat intake, accompanied by an increase in carbohydrate consumption. Favorable effects on glucose tolerance have been described for both high-carbohydrate diets (1-3) and for high linoleic acid diets (4). However, long-term controlled studies of the effects of such diets on plasma glucose and insulin levels in healthy subjects are quite rare, and the diets tested have often been artificial and not composed of regularly used foodstuffs.

We have recently completed two studies on the effect on cardiovascular risk factors of a high-starch, low-fat diet compared with diets higher in fat and having various degrees of polyunsaturation. Data on the effects of these diets on composition and concentration of serum lipoproteins (5, 6) and on blood pressure (7) have been reported. This report deals with effects on plasma glucose and insulin levels, both fasting and after a test meal of the same composition as the diet under study.

METHODS

Subjects

The subjects were all unpaid volunteers, aged 18-30 years. They were apparently healthy as judged by a detailed questionnaire and had diastolic blood pressures below 90 mm Hg. None of the volunteers in the seond experiment had glucosuria or proteinuria; in the first experiment this was not measured. Two of the females in the first experiment and four in the second experiment used hormonal anticonception.

Mean body weights were 71.1 kg for males and 59.6 kg for females in the first experiment and 73.8 kg and 62.7 kg respectively, in the second experiment. Mean body fat percentage (8, 9) for males was 13% and 14% and for females 27% and 26% in the first and second experiment respectively. Body fat percentage never exceeded 23% for males and 30% for females.

The average fasting serum triglyceride concentration at the start of the experiments was 0.69 ± 0.44 (range: 0.14 - 2.18) and 0.96 ± 0.43 (range: 0.35 - 2.32) mmol/l and fasting serum cholesterol was 4.25 ± 0.72 (range: 2.95 - 5.82) and 4.60 ± 0.75 (range: 3.50 - 6.33) mmol/l for participants in the first and second experiment respectively.

TABLE 1. NUTRIENT COMPOSITION OF THE OVERALL TEST DIETS AND OF THE TEST LUNCHES, CALCULATED FROM WEIGHED FOOD RECORDS. (Mean per diet group).

	Experiment 1[a]						Experiment 2			
	Overall diet			Test lunch			Overall diet		Test lunch	
	LO	HIPUF	HISAT	LO	HIPUF	HISAT	LO	MOD	LO	MOD
	(% of daily energy)			(g)			(% of daily energy)		(g)	
Total carbohydrates	64	44	46	110	75	76	60	50	125	101
mono- and disaccharides	21	21	23	42	41	46	20	20	53	57
Total fat	22	40	39	7	28	27	21	31	8	20
saturated fatty acids	8	11	18	2	5	12	7	9	4	4
polyunsaturated fatty acids	3	19	3	1	16	2	4	11	1	5
Protein	13	14	13	23	20	22	16	15	24	20
Dietary fiber (g[b])	25	25	26	13.5	11.7	13.3	46	39	12.1	7.3
Total energy (kcal[b])	2517	2552	2455	626	656	678	2460	2470	668	661

a) Group MOD was not studied in this part of the experiment

b) Per day for the overall diets, per meal for the lunches

The habitual diet of our participants contained on average 33% of daily energy as total fat, 5% of daily energy as polyunsaturated fat, 13% as protein, 40% as total carbohydrates and 24% of daily energy as oligosaccharides. Alcohol yielded 5% of daily energy on average.
Their habitual cholesterol intake was low (290 mg per day) and the intake of dietary fiber high (39 g per day).

Experimental design

Experiment I was carried out from October 9 till December 1, 1978 and experiment II from August 27 till December 14, 1979.
During the first 2.5 week of the first experiment 60 young, healthy volunteers (37 men and 23 women) received a moderate fat control diet, rich in polyunsaturated fatty acids (PUFA) (30% of daily energy as total fat, 11% of daily energy as PUFA) in agreement with the recommendations of the Netherlands Nutrition Council, the Dietary Goals for the US and other official recommendations. They were then randomized into 4 subgroups, which received test diets for the next five weeks as indicated in Fig. 1. The subgroups were stratified for initial serum cholesterol level, male/female ratio and energy intake.
In the second experiment 35 volunteers (23 men and 12 women) took part. The design differed from the first in that the test period was prolonged to 13 weeks and only two diets were tested, namely the low fat LO diet and the "recommended" diet MOD.
In each experiment diets differed between groups in one component only; one group always received the "recommended" control diet throughout the test period as a check against baseline drift. Throughout the whole experimental period all foodstuffs were weighed out individually and supplied according to each person's energy needs. Actual nutrient intake was measured by 7-day weighed food records using Dutch food composition tables (10) and by analysis of double portions (11) for one imaginary person of average energy intake on each diet.

The detailed composition of the diet is given in Table 1. In both experiments feces were collected for two 7-day periods. Intestinal transit time, fecal wet and dry weight and frequency of stools were equal between diet groups. This confirms the absence of physiologically significant differences in dietary fibre intake.

Blood sampling and analysis

Blood samples for insulin and glucose measurements were collected before

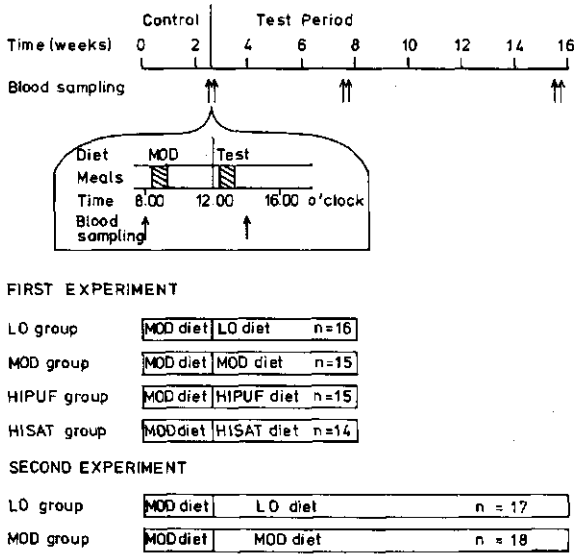

Figure 1. Experimental design. The inset shows the blood sampling scheme on the first day of the test period.

LO = low fat, high carbohydrate diet (22% of daily energy as fat, 3% of daily energy as polyunsaturated fatty acids).

MOD = moderate fat, high polyunsaturated fat diet (30% of daily energy as fat, 11% of daily energy as polyunsaturated fatty acids).

HIPUF = high fat, high polyunsaturated fat diet (40% of daily energy as fat, 19% of daily energy as polyunsaturated fatty acids).

HISAT = high fat, low polyunsaturated fat diet (39% of daily energy as fat, 3% of daily energy as polyunsaturated fatty acids).

breakfast at the end of the control period and again one hour after the beginning of a test-lunch on the same day (Fig. 1). Breakfast on this day was the last meal of the control period and was still the same for all participants; the test-lunch constituted the subjects' first exposure to the various test diets. Blood samples were collected again after 5 weeks of test period in both experiments and for a third time after 13 weeks of test period in the second experiment. On each occasion, blood was sampled before breakfast and one hour after the subject had started to eat lunch. On these days the composition of both breakfast and test lunch corresponded to the test diet to which the subject had been assigned and both breakfast and test lunch were thus different for each diet group. Subjects were asked to refrain from smoking from the moment they rose until the postprandial blood sample had been collected.

The lunch consisted of cooked vegetables, potatoes, gravy, a salad with egg and dessert and contained 25% of the day's calories. The lunch was weighed out and its composition recorded for each subject.

Blood for glucose analysis was collected into evacuated tubes containing 0.2 mg NaF and 1.0 mg dipotassium EDTA per ml blood, and stored in ice. Plasma glucose was measured by the ferricyanide method of Hoffman (12) within 24 hours after blood sampling. Reproducibility for blind controls was 2.6% (coefficient of variation).

Insulin was measured in serum by radio-immunoassay according to the double antibody solid phase technique (13) within 5 months after completion of the experiment. Serum was stored at -20^{o}C until analysis, and all samples of one subject were analysed in one run.

Statistical evaluation

For statistical evaluation mean changes per group over the test period were compared between groups using a 2-tailed Student's t-test.

RESULTS

Results of insulin and glucose measurements in fasting and postprandial blood are given in Tables 2 and 3.

Fasting plasma glucose levels at the end of the control period were low in both experiments and equal in all dietary groups. Fasting serum insulin levels at the end of the control period were on the whole somewhat lower in the second experiment than in the first, but differed little between the groups in each

TABLE 2. SERUM INSULIN CONCENTRATIONS BEFORE BREAKFAST AND ONE HOUR AFTER THE BEGINNING OF THE LUNCH ON THE SAME DAY ON DIETS DIFFERING IN TYPE AND AMOUNT OF DIETARY FAT (mean ± SD).

	Serum insulin concentration (mU/l)					
	Diet group - Experiment 1				Diet group - Experiment 2	
	LO	MOD	HIPUF	HISAT	LO	MOD
First day of test period						
Fasting (control level)	12.6 ± 3.7	12.7 ± 4.7	10.5 ± 3.4	8.4 ± 1.7	7.9 ± 3.3	7.8 ± 2.1
Postprandial	50.8 ± 18.2	a)	25.2 ± 15.1	19.1 ± 7.7	41.1 ± 20.7	42.8 ± 21.6
Response to meal	+38.2 ± 16.0	a)	+14.7 ± 14.0	+10.7 ± 7.2	+33.2 ± 21.1	+35.0 ± 21.8
After 5 weeks test period						
Fasting	11.9 ± 3.2	11.1 ± 3.6	9.3 ± 2.7	9.6 ± 3.3	8.1 ± 2.6	8.4 ± 2.7
Postprandial	60.7 ± 24.5	a)	32.1 ± 19.8	32.8 ± 16.7	43.4 ± 26.8	44.9 ± 16.1
Response to meal	+48.8 ± 23.7	a)	+22.9 ± 18.5	+23.1 ± 16.3	+35.4 ± 26.0	+36.5 ± 16.1
After 13 weeks test period						
Fasting					8.3 ± 2.6	9.2 ± 2.9
Postprandial					50.1 ± 21.5	47.2 ± 19.6
Response to meal					+41.9 ± 20.7	+38.0 ± 19.0

a) not measured

TABLE 3. PLASMA GLUCOSE CONCENTRATIONS BEFORE BREAKFAST AND ONE HOUR AFTER THE START OF THE LUNCH ON THE SAME DAY ON DIETS DIFFERING IN TYPE AND AMOUNT OF DIETARY FAT (mean ± SD).

	Plasma glucose concentration (mmol/l)					
	Diet group - Experiment 1				Diet group - Experiment 2	
	LO	MOD	HIPUF	HISAT	LO	MOD
First day of test period						
Fasting (control level)	5.3 ± 0.6	5.1 ± 0.5	4.9 ± 0.4	5.0 ± 0.3	5.1 ± 0.6	5.1 ± 0.3
Postprandial	5.5 ± 0.9	a)	4.6 ± 1.0	4.6 ± 0.6	5.8 ± 1.4	5.5 ± 0.5
Response to meal	+0.2 ± 0.9	a)	-0.3 ± 1.0	-0.4 ± 0.7	+0.7 ± 1.7	+0.4 ± 0.6
After 5 weeks test period						
Fasting	4.8 ± 0.6	4.9 ± 0.3	4.7 ± 0.3	4.8 ± 0.5	4.8 ± 0.4	5.0 ± 0.5
Postprandial	5.2 ± 1.0	a)	4.7 ± 0.7	4.7 ± 0.6	5.8 ± 1.7	4.9 ± 0.8
Response to meal	+0.4 ± 1.0	a)	0.0 ± 0.6	-0.1 ± 0.8	+1.0 ± 1.9 b)	-0.1 ± 1.2
After 13 weeks test period						
Fasting					4.9 ± 0.4	5.1 ± 0.5
Postprandial					5.1 ± 0.8	4.8 ± 0.9
Response to meal					+0.2 ± 1.0	-0.3 ± 1.2

a) not measured

b) significantly different from control diet ($p < 0.05$)

Conversion factor from SI to traditional units: 1 mmol/l = 18 mg/dl.

experiment.
The fasting plasma glucose and serum insulin levels showed little change over the course of the test period on any of the diets.

Carbohydrate-tolerance is usually evaluated by means of a uniform glucose load. In the present experiment, however, we measured the glucose and insulin response to lunches of a composition that differed for the different diet groups. The composition of the test lunches is given in Table 1, together with the overall composition of the test diets.
The differences between the groups in fatty acid composition of the test lunches were greater then the differences between the diets as a whole. The mean difference in fat content between the high fat meals (groups HIPUF and HISAT) and the low fat meals (group LO) in the first experiment was on average 20 g; the difference between the moderate and the low fat meal in the second experiment was 12 g of fat on average. In both cases this was mainly compensated for by starch, at a rather constant level of dietary fiber intake.
Little or no rise in plasma glucose was observed after any of the test-lunches. The only significant postprandial change in plasma glucose was the rise after the high-carbohydrate LO lunch in the fifth week of the second experiment. No such effect was observed on this diet, however, in week one or week 13 in the same experiment.

The serum insulin levels one hour after the test lunches were twice as high in the high carbohydrate group LO as in the high fat groups HIPUF and HISAT in the first experiment. In the second experiment the insulin response in group LO was equal to that in the moderate fat group MOD.
The insulin response to the test lunch was greater at the end of the test period than at the start of the test period in all 3 groups examined in the first experiment; the differences between the groups, however, were not statistically significant. The same was found for the two diet groups examined in the second experiment.

DISCUSSION

Epidemiological studies have revealed that the prevalence of diabetes is lower in countries with a high starch consumption than in countries with a high fat or high sugar intake (14, 15). A low incidence of diabetes was also found among Eskimos, who consume a diet, low in carbohydrate but very high in polyunsaturated fat (16, 17). Other studies suggest that diabetes becomes more

prevalent as the intake of dietary fiber decreases (18). Of course, such results cannot be accepted as evidence that high-starch, high-fiber or high-polyunsaturate diets can prevent disturbances of carbohydrate metabolism. The observed differences in the prevalence of disease might have been caused by other, confounding factors, such as obesity or total energy intake, which are both known to influence carbohydrate metabolism (14, 19). Aspects of life style other than diet could also be important. Therefore, controlled experiments are necessary to establish the influence of single dietary factors.

In our experiments, we did not find lower fasting or postprandial glucose or insulin levels on a high-carbohydrate, low-fat diet, compared with diets moderate or high in fat. This appears to contradict certain earlier studies (1-3). However, the design of our studies was different in several respects. Firts, carbohydrate tolerance is usually measured by means of an oral glucose tolerance test. In contrast, we measured carbohydrate tolerance by means of test-lunches of the same general composition as the test diet as a whole, and thus different for each dietary group. Second, most of the dietary research on carbohydrate metabolism in man has been carried out with diseased subjects, especially diabetic and/or obese patients. In contrast, our subjects were in general lean and they had normal fasting glucose and insulin levels. They exhibited practically no elevation of plasma glucose concentrations after a test-lunch, whatever its carbohydrate content. This indicates that their carbohydrate tolerance was excellent. Hence, comparisons of our results with those obtained in patient populations are probably not justified. We will therefore limit the following discussion to studies with healthy subjects.

One of the few studies on carbohydrate metabolism that utilized regular foodstuffs is the experiment of Cohen (20) with 15 healthy adult human volunteers. He reported a lowering of fasting glucose levels (-10 mg/dl) and a lowering of glucose curves after an oral glucose load when fat in the diet was replaced by starch, mainly from bread, but not when fat was replaced by sugar. No data on dietary fiber consumption were given. It is conceivable that the lower glucose levels on the bread diet were due to the high fiber content of such diets rather than to their starch content. High-fiber diets have been reported to reduce plasma glucose and insulin concentrations after a meal (21). Brodribb and Humphreys reported that long term consumption of bran also lowered fasting blood glucose curves in non-diabetic patients after a standard oral glucose load (22).

Differences in fiber intake probably do not play a role when test-diets are given as liquid formulas. In an experiment of Reaven and Olefsky (23) with

liquid formula diets, fat was replaced by carbohydrates, while at the same time the proportion of polyunsaturated fat was increased. This led to a small increase in postprandial glucose concentrations and a more striking increase in postprandial insulin concentrations. No change in preprandial glucose concentrations was observed, but preprandial insulin levels were ca. 15 µU/ml higher on the high carbohydrate diet. However, the test period lasted only four days.

Other studies with liquid formula diets did not measure actual postprandial insulin or glucose levels, but instead employed a standard glucose load. Such tests did show a more favorable effect of replacing fat by carbohydrate. Thus Brunzell *et al.* (24) and Wigand *et al.* (3), found lower insulin and glucose curves after oral glucose load on very high carbohydrate diets than on very high fat diets. In these studies fasting glucose levels were lower on the high carbohydrate diets, but the difference was small (4-6 mg/dl) and the effects on fasting insulin levels in the two experiments were in opposite directions. Anderson *et al.* (25), who exchanged glucose for corn oil in liquid formula diets, also found lower glucose curves after an oral glucose load if the glucose content of the diets was higher. Fasting glucose concentrations, however, were not different on the various diets. In an experiment of Hales *et al.* (2) high carbohydrate diets caused lower fasting insulin and glucose concentrations and lower glucose curves after an oral glucose load than diets containing more fat and protein. Insulin curves, however, did not differ. Wilkerson *et al.* (1) also reported lower fasting glucose levels on high carbohydrate diets in males, but not in females, and lower glucose curves after an oral glucose load in both males and females. In both experiments carbohydrates were replaced by fat plus protein. This makes it difficult to evaluate the results, because fat and protein may interact in determining blood glucose concentrations (26).

As far as the influence of the type of dietary fat on carbohydrate tolerance is concerned, we could not detect a favorable effect of polyunsaturated fatty acids compared with saturated fatty acids. This is in agreement with the results of Nestel (27).

The discrepancies between our results for high-carbohydrate diets and those discussed above might be explained by our use of normal foodstuffs instead of liquid formula diets, the use of a 'lunch tolerance test' that differed in carbohydrate content between the diets under study instead of a uniform glucose load and by the type of subjects. Liquid formula diets are usually very low in dietary fiber, and glucose tolerance tests are hardly representative for a normal physiological stimulus. Therefore, it is questionable to what extent the

results of such diets can be extrapolated to populations consuming regular diets. Our results seem to indicate that neither the type nor the amount of dietary fat has a strong influence on carbohydrate tolerance in young healthy subjects. However, our results might not be valid for older people, or obese or hyperlipidaemic patients whose glucose tolerance may be impaired. Also, our results do not exclude beneficial effects of changes in those dietary components that were forcibly held constant in our experiments, such as dietary fiber.

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6. Blood pressure and diet in normotensive volunteers: absence of an effect of dietary fiber, protein or fat.

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ABSTRACT

In the course of four controlled experiments on the effect of specific dietary components on cardiovascular risk factors, the effects on blood pressure of various sources of dietary fiber, of type and amount of dietary fat, and of animal versus plant protein were measured in young normotensive volunteers. In each of the four experiments a group of 50-75 healthy student volunteers received a control diet for 1½ to 2½ weeks. They were then randomized into subgroups which received various test diets for periods ranging from 4-12 weeks. In each experiment one group received the control diet throughout the whole experimental period. Diets differed between groups in one dietary component only. All foodstuffs were weighed out individually according to each person's energy needs. Body weights and Na intake were controlled. In the fiber experiment the control diet had a relatively low fiber content. Test diets consisted of the low fiber control diet supplemented with coarse wheat bran, isolated citrus pectin or a large amount of vegetables and fruits. In the protein experiment sixty-five percent of total protein was replaced by casein or by soy protein. In fat experiment 1 the control diet was moderate in total fat but contained 11% of daily energy as polyunsaturated fatty acids (PUFA). The test diets were a low fat diet containing 3% of daily energy as PUFA; a high fat diet containing 3% of daily energy as PUFA and a high fat diet in which half of the total fat was polyunsaturated. In fat experiment 2 the moderate fat control diet and the low fat diet were tested again for a longer period. Initial blood

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pressures were about 120 mm Hg systolic and 70 mm Hg diastolic. Both systolic and diastolic blood pressure decreased during the test period in all four experiments on almost every diet, including the control diets, by about 0-5 mm Hg. However, changes in blood pressure over the test period were never significantly different between the test groups and the control groups. Thus, none of the investigated dietary factors had a demonstrable effect on blood pressure in young normotensive persons.

INTRODUCTION

Hypertension is one of the most important risk factors for the development of cerebrovascular, coronary and aortic atherosclerosis (1, 2) and is present in about 15% of the adult American population (3). It has been clearly established that antihypertensive drug treatment reduces not only the morbid complications of hypertension but also total mortality (3, 4). For obvious reasons, however, primary prevention of the disease would be preferable to widespread drug treatment.

In many primitive communities, hypertension is virtually absent, because the rise of blood pressure with age which is common to Western societies does not occur (5, 6, 7). Among the many determinants of life style, several authors have stressed the importance of diet in the emergence of widespread hypertension in affluent societies. There is good evidence for a role for dietary sodium in the development of high blood pressure (for a review see: 8); it has also been suggested that a high intake of potassium might be protective (8, 9). In addition to sodium and potassium intake other dietary factors may be important. There is evidence that dietary linoleic acid will lower blood pressure in hypertensive animals (10) while epidemiological studies and controlled trials in man also suggest a slight beneficial effect (11-14). Other studies suggest a beneficial effect on blood pressure of dietary fiber (15), or a deleterious effect of sugar (16) and animal products (17). However, controlled studies in man on the effect of fiber or animal products on blood pressure are rare or absent.

In the course of four controlled trials on the effect of specific dietary components on cardiovascular risk factors we have measured the effects of various sources of dietary fiber, type and amount of dietary fat and type of protein on blood pressure in young, normotensive volunteers. It was found that none of these factors had a measurable effect on blood pressure.

METHODS

The primary aim of the four experiments was to test the effect of various dietary components on cholesterol metabolism. The details of each trial have been described elsewhere (18-22; Brussaard *et al.*, in preparation). The general design of the experiments was as follows (Table 1).

TABLE 1. GENERAL DESIGN OF EXPERIMENTS

Group	Control period (1½ - 2½ wk)	Test period (4 - 13 wk)
Control group	control diet	control diet
Diet group A	control diet	test diet A
Diet group B	control diet	test diet B
Diet group C	control diet	test diet C

The subjects were young healthy student volunteers; their age ranged from 18 to 30 years (median: 21 years).
In each of the four trials a group of 50-75 volunteers received a control diet for 1½ to 2½ weeks. They were then randomized into subgroups stratifying for initial serum cholesterol level, male/female ratio and energy intake. The subgroups received various test diets for periods ranging from 4 to 12 weeks. Diets differed between groups in one dietary component only; one group always received the control diet throughout the test period as a check against baseline drift. Throughout each experiment all foodstuffs were individually weighed out and supplied according to each person's energy needs.

Dietary composition

Fiber experiment (18). In the fiber experiment the control diet was a relatively low-fiber diet with a mean intake of 18 g total dietary fiber per day. During the test period of five weeks one group continued on the control diet. A second group received 43 g dietary fiber per day on the average, which came mainly from vegetables and fruit. A third group received the low fiber diet plus on the average 9 g isolated citrus pectin NF (degree of esterification 78%) per day so that the total dietary fiber intake was 28 g per day on average.

A fourth group received the low-fiber control diet enriched with on the average 38 g coarse wheat bran per day (dietary fiber content 50 g/100 g fresh weight); mean intake of total dietary fiber in this group was 37 g/day. Consumption of polygalacturonic acid (pectin) amounted to 1.7, 7.5, 8.8 and 1.7 g per day in the control, vegetables/fruits, citrus pectin and bran group respectively. The diets provided 37% of total energy intake as fat (4% of total energy as lonoleic acid), 13% as protein, 48% as carbohydrate (23% of total energy as oligosaccharides) and 142 mg cholesterol/4.2 MJ.

Protein experiment (19, 20). In the protein experiment all diets contained 38% of total energy as fat (8% of total energy as linoleic acid), 47% as carbohydrates (23% of total energy as oligosaccharides) and 12-13% as protein. Cholesterol intake was 146 mg/4.2 MJ per day. Sixty five percent of the protein in the diets consisted of casein or soy protein isolate or a 2:1 mixture of casein and soy protein isolate (control diet). The test period lasted 4 weeks.

Fat experiment 1 (21). The control diet in this experiment contained 11% of total energy as polyunsaturated fat (PUFA); in total, fat provided 30% of daily energy. A low fat, low PUFA diet provided 22% of energy as total fat (3% of daily energy as PUFA); a high fat, high PUFA diet provided 40% of daily energy as total fat (19% of daily energy as PUFA) and a high fat, low PUFA diet provided 39% of daily energy as total fat (3% of daily energy as PUFA). The test period lasted 5 weeks. In all diets protein provided 13% of daily energy, while carbohydrates made up the total energy balance. Intake of oligosaccharides was 23% of daily energy in all groups; cholesterol intake was 110 mg/4.2 MJ.

Fat experiment 2 (22). In the second fat experiment the control diet and the low fat, low PUFA diet of fat experiment 1 were tested again, but this time the test period lasted 12 weeks instead of 5 weeks.

Intake of Na and K

Customary amounts of salt were added to dishes by us during preparation. No further addition of salt was permitted. Na en K content of the diets were measured by analysis of double portions (18). Diets provided on the average 170, 168, 171 and 190 mmol Na per day and 98, 58, 83 and 90 mmol K per day in the fiber, protein fat 1 and fat 2 experiment respectively. There were only minor differences between groups and between control and test period in each experiment, with the exception of the fiber experiment: the vegetables/fruits diet contained more Na and K and the bran diet more K than the control diet,

resulting in Na/K ratios of 0.90, 0.95, 1.11 and 1.22 in the vegetables/fruits, bran, control and pectin diet respectively.
As an additional check on Na and K consumption, 24 hour urinary excretion of Na and K was measured in the protein and fat 1 experiment. The results confirm the dietary analysis data (Table 2).

TABLE 2. EXCRETION OF Na AND K IN URINE DURING THE CONTROL AND TEST PERIOD OF THE VARIOUS EXPERIMENTS (mean ± SD).

Group	n	Na		K	
		Control[+] period	Change over test period[‡]	Control[+] period	Change over test period[‡]
		(mmol/day)			
Fiber experiment	62	*)	*)	*)	*)
Protein experiment					
Cassoy (control) group	20	195 ± 60	-23 ± 67	55 ± 18	21 ± 23
Casein group	25	188 ± 51	-21 ± 58	55 ± 12	19 ± 17
Soy group	24	183 ± 48	-14 ± 69	52 ± 9	17 ± 20
Fat experiment 1					
Moderate fat, high PUFA (control) group	15	165 ± 51	11 ± 64	78 ± 18	3 ± 17
Low fat, low PUFA group	16	156 ± 57	40 ± 49	79 ± 21	8 ± 14
High fat, high PUFA group	15	153 ± 82	8 ± 77	76 ± 19	-9 ± 23
High fat, low PUFA group	14	148 ± 53	6 ± 30	76 ± 22	-8 ± 20
Fat experiment 2	35	*)	*)	*)	*)

*) not measured

+) duration of control period: fiber, fat 1, fat 2: 2½ week; protein: 1½ week.

‡) duration of test period: fiber 3 weeks; protein 4 weeks; fat 1: 4 weeks; fat 2: 13 weeks.

At the end of the test period in the fat experiment 1, the excretion of Na was slightly higher in the low fat low PUFA group than in the other groups. However, the excretion of K was also higher, so that the Na/K ratio was not different from the other groups. In the protein experiment there were only minor differences between groups.

Body weights

None of the participants had body fat percentages above 23% (male) or 30% (female). From the 60-100 subjects who volunteered per experiment 2-6 persons had to be rejected on this criterium

For the combined population the P5, P50 and P95 for body weights for females were 50, 60 and 73 kg; for males these values were 60, 71 and 84 kg.
For males these values are slightly lower than the body weights of male North American subjects (23); for females the values agree well.
In each experiment body weight was recorded weekly and energy intake was adapted when necessary to maintain stable weight. Mean change in body weights per diet-group from start to finish of the test periods was -0.2 to +0.3 kg in the fiber experiment; -1.0 to -1.6 kg in the protein experiment; -0.4 to -0.8 in fat experiment 1 and -1.3 to -1.6 kg in fat experiment 2.

Measurement of blood pressures

Blood pressures were measured before the start of each experiment for familiarization of the participants with the measurement and elimination of hypertensives. Measurement was repeated at the end of the control period and in the test period; after 3 weeks in the fiber experiment, after 4 weeks in the protein experiment and fat experiment 1, and after 13 weeks in fat experiment 2. From all subjects who volunteered for these experiments only one or two in each trial exceeded our criteria for participation (150 mm Hg systolic and/or 95 mm Hg diastolic in the protein experiment and fat experiment 2; 90 mm Hg diastolic in the fiber experiment and fat experiment 1). No restrictions were made as to performing physical activity, eating or smoking prior to the blood pressure determinations. (Forty-three out of the 139 males and 14 of the 86 females smoked cigarettes; however, only 5 subjects, all males, smoked more than 15 cigarettes per day).

The measurement was performed in a quiet room in the department after a 5-10 minute rest. During the measurement the subjects were sitting (fiber experiment) or lying down (protein, fat experiments 1 and 2).

In the fiber experiment the mean of two consecutive measurements on one day was recorded. In the protein and fat experiments blood pressure was measured twice on two consecutive days and the mean of these four measurements was used. Care was taken that each person was measured at the same time of day during all measurements and that the subjects in different diet groups were measured in random order. All measurements in each trial were performed by the same person.

An automatic recording sphygmomanometer type BE 207S (Elag, Köln, Germany) which employs phase IV (Korotkoff) as diastolic blood pressure was used. This instrument uses an inflated cell inside the cuff to detect movements of the arterial wall. The pressure waves are converted into electronic signals by means of a microphone. The instrument was recalibrated periodically against a mercury column manometer.

For all subjects combined, the median blood pressure prior to the experiments was 115/68 mm Hg (systolic/diastolic) for females and 129/67 mm Hg for males. The 5th-95th percentile ranges were 93-132/50-85 (systolic/diastolic) for females and 107-156/46-88 for males. These values are in excellent agreement with values for the general Dutch population in this age group (24) and are similar to what has been reported for North-American populations (23). The subjects in fat experiment 2 had somewhat higher mean systolic and diastolic blood pressures than in the other groups, but were still well within normal range.

For statistical evaluation mean changes per group over the test period were compared between groups using 2-tailed Student's t-test.

RESULTS

Mean blood pressures per diet group at the end of the control periods and mean changes per group over the various test periods are given in Table 3.

In all four trials systolic blood pressures decreased in all test groups as well as in the control group. In almost all cases these changes were significantly different from zero. However, the differences in blood pressure changes over the test period between test groups and the control group were not significant in any experiment.

Diastolic blood pressures decreased also in all test groups as well as in the control group, with the exception of the change in the vegetables/fruits group in the fiber experiment (+1 mm Hg). But again the differences in blood pressure changes over the test period between test groups and the control group

TABLE 3. EFFECT ON BLOOD PRESSURE IN NORMOTENSIVE SUBJECTS OF DIETARY FIBER FROM VARIOUS SOURCES (Stasse-Wolthuis *et al.*, 1980), CASEIN AND SOY PROTEIN (van Raaij *et al.*, 1980) OR DIFFERENT TYPES AND AMOUNTS OF DIETARY FAT (Brussaard *et al.*, 1980) (mean ± SD)

		Systolic		Diastolic	
Group	n	Control period[1] (mean ±SD)	Change over test period[2] (mean ± SD)	Control period[1] (mean ± SD)	Change over test period[2] (mean ± SD)
			(mm Hg)		
Fiber experiment					
Low fiber (control)	16	124 ± 12	-4.6 ± 9.3	63 ± 11	-4.4 ± 10.1
Vegetables/fruits	15	117 ± 15	-2.0 ± 7.4	58 ± 14	+1.2 ± 11.4
Citrus pectin	15	125 ± 15	-1.1 ± 7.0	59 ± 11	-0.5 ± 5.8
Bran	16	128 ± 18	-1.0 ± 10.1	65 ± 8	-3.4 ± 11.0
Protein experiment					
Cassoy (control)	20	127 ± 12	-3.0 ± 6.0	73 ± 8	-5.8 ± 7.6
Casein	25	123 ± 11	-3.1 ± 8.9	69 ± 10	-2.7 ± 6.6
Soy	24	124 ± 15	-2.5 ± 5.9	69 ± 11	-2.5 ± 6.1
Fat experiment 1					
Moderate fat, high PUFA (control)	15	119 ± 12	-4.8 ± 10.3	67 ± 6	-3.4 ± 6.7
Low fat, low PUFA	16	122 ± 11	-5.0 ± 6.0	64 ± 9	-4.4 ± 4.9
High fat, high PUFA	15	116 ± 15	-5.1 ± 5.4	65 ± 7	-2.7 ± 8.1
High fat, low PUFA	14	115 ± 17	-3.3 ± 6.4	65 ± 9	-3.1 ± 5.7
Fat experiment 2					
Moderate fat, high PUFA (control)	17	130 ± 10	-7.1 ± 6.4	80 ± 10	-8.3 ± 9.2
Low fat, low PUFA	18	131 ± 6	-5.5 ± 5.9	79 ± 8	-10.5 ± 11.6

[1] Duration of control period: fiber, fat 1, fat 2: 2½ week; protein 1½ week.

[2] Duration of test period: fiber 3 weeks; protein 4 weeks; fat 1: 4 weeks; fat 2: 13 weeks.

never attained statistical significance.

DISCUSSION

Because of the importance of primary prevention of hypertension attention has been focussed on dietary changes to lower blood pressure. Kempner (25) has shown that a rice-fruit diet low in sodium can lower blood pressure in hypertensive patients by as much as 30 mm Hg. There is also evidence from recent controlled trials (26, 27) that a modest reduction in salt intake can produce a modest reduction of blood pressure in hypertensive patients. The effect of changes in other dietary components has not been satisfactorily tested. In our experiments we found no effect of changes in intake of dietary fiber, type of protein or type or amount of fat on blood pressure in young normotensive men and women.

Fiber

It has been suggested that a high-fiber diet could lower blood pressure through its high content of potassium (9, 28). A lowering of blood pressure by 4-8 mm Hg systolic and 3-4 mm Hg diastolic on diets rich in dietary fiber (mainly from cereals) was reported by Wright *et al.* (15). Kelsay *et al.* (29) reported a lower diastolic blood pressure during a high fiber diet than during a low fiber diet. Their conclusion however, was due to misinterpretation of results that could be completely accounted for by regression towards the mean. In our experiment with dietary fiber the effect on blood pressure of the vegetables/fruits diet and the bran diet was not different from the low-fiber control diet, despite the lower Na/K ratio of these test diets. The intake of K-rich foodstuffs on these diets was quite high: the subjects consumed on average 600 g apples plus 400 g cooked vegetables plus 170 g tomatoes or 38 g bran per day. We think it would be very difficult to increase the potassium intake further if only natural foodstuffs are to be employed.

Protein

Armstrong *et al.* (30) and Sacks *et al.* (17) suggested from their studies with vegetarians that a higher consumption of animal protein or animal products is accompanied by higher blood pressures. To our knowledge no controlled experiments on the effect of type of protein on blood pressure in humans have

been published. We found no difference in effect on blood pressure of a diet rich in animal protein (casein) or plant protein (soy).
Body weights decreased to some extent in this experiment and this is known to affect blood pressure (31). However, it seems unlikely that this could have interfered with the results because the decrease in body weight was small and was very similar in all 3 diet groups.

Fat

A lowering of blood pressure on diets rich in linoleic acid has been reported by several authors (12-14, 32). Iacono (13) reported that a diet containing 25% of total energy as fat and 6.5% as linoleic acid lowered blood pressure by 13 mm Hg systolic and 7 mm Hg diastolic in normotensive persons compared with their habitual diet; systolic blood pressure remained low after switching to a diet with 35% of total energy as fat and 9% as linoleic acid, but diastolic blood pressure increased by 3 mm Hg. It was concluded that lowering the intake of dietary fat and raising the intake of polyunsaturated fat had a beneficial effect on blood pressure. Although blood pressures were within the normal range they were higher than in our participants, which may partially account for the discrepancy with our results.

Judd *et al.* compared the effect of diets containing 43 and 25% of total energy as fat, each with P/S ratios of 0.3 and 1. They conclude that a lowering of total fat intake as well as a rise in linoleic acid consumption can lower blood pressure. Maximum effects were observed in the group with the highest initial blood pressure level.

In the experiment of Stern *et al.* (14) blood pressure was lowered in overweight hypertensives on a linoleic acid rich diet. However, the interpretation of this experiment is hampered by the absence of details on diet composition and control of nutrient intake. There was no control group in this experiment; thus it is possible that the experimental conditions themselves caused a lowering of blood pressure, independent of dietary composition.

Oster *et al.* (12) reported a small tendency towards lower blood pressures (2-4 mm Hg) on a diet containing 70 g cornoil per day in subjects with blood pressures comparable to our participants.

Weak although significant negative correlations between C 18:2 content of adipose tissue and blood pressure ($r = -0.11$ for systolic and -0.07 for diastolic blood pressure) were found in an epidemiological study (11). In contrast to these observations we found no effect on blood pressure of diets rich in

linoleic acid, nor did we find an effect of the amount of dietary fat. As changes in body weight in the fat experiments were slight and very similar in the different diet groups, it does not seem likely that this could have disturbed our results. Also a confounding influence of Na consumption is unlikely, because Na-intake was controlled in all four trials.

Although we did not find an effect of dietary fiber, fat or protein on blood pressure, our results do not rule out the possibility that these dietary components can indeed lower blood pressure.

Firstly, the small number of participants in this type of controlled experiment and the large range of individual changes in blood pressure, resulting in a poor estimate of the change difference between groups, render a considerable risk of committing a type II error (*i.e.* not detecting an actually existing difference between dietary effects). When testing the difference of two group means, two-sided on for example 5% level, the power of the test (*i.e.* the probability of rejecting the null hypothesis of no effect in case of a real effect) depends on the magnitude of the true effect and the standard error of its estimate. In experiments like ours, a typical value of the standard deviation of blood pressure change is SD=7. Kelsay (29), Morgan (26) and Iacono (13) found lower SD's, but similar values are reported by Parijs (27), Oster (12) and Stern (14). With about 16 persons per group, the probability of detecting a difference between diet groups of 2 mm Hg is only about 11%; for 4 mm Hg this is 31% and for 7 mm Hg it is 75%.

It should be noted that a lowering of blood pressure by only 4 mm Hg was found to cause a marked reduction in mortality from all causes in the Hypertension Detection and Follow-up Program (3). To increase the power of the test up to 80% for a difference of 2 mm Hg between the diet groups, each group has to comprise about 200 persons. In our experiment the probability that we missed a difference of 7 mm Hg is only 25%, but almost 70% that we missed a 4 mm Hg difference. Thus we must face the possibility that we missed an effect of this magnitude purely by chance.

Secondly, our participants were young healthy men and women, who were not obese and had low to normal blood pressures. This makes it perhaps more difficult to lower blood pressures. The investigated dietary components might be effective in lowering blood pressure in older persons with higher blood pressure levels, as was also indicated in the experiment of Judd *et al.* (32).

Thirdly, the relatively short duration of our experiments could obscure an effect of diet on blood pressure in the long run.

In summary, we have not been able to show an effect of dietary fiber from different sources, type of protein or type and amount of dietary fat on blood pressure in normotensive subjects. However, the possibility that these dietary components can influence blood pressure in persons with higher initial levels of blood pressure or to an extent of less than 5-7 mm Hg in young normotensive people cannot be ruled out.

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Bijlage. Voeding en hart- en vaatziekten, een verzekering met slechts gedeeltelijke dekking.

Dit proefschrift gaat over het verband tussen voeding en hart- en vaatziekten. Dat onderwerp houdt veel mensen bezig en er is de laatste jaren dan ook veel over te doen geweest. In krante-artikelen, t.v.-uitzendingen en reclame worden nogal eens tegenstrijdige adviezen de wereld ingestuurd, variërend van: "allemaal onzin, eet maar gewoon wat je lekker vindt" tot reclame voor een bekende dieetmargarine, ronduit "goed voor hart- en bloedvaten".

In dit hoofdstuk wil ik proberen enige duidelijkheid te scheppen over de vraag óf de voeding een rol kan spelen bij het verkleinen van de kans op een hartinfarkt, en zoja hoe belangrijk die rol is.

Hart en bloedvaten

Het menselijk lichaam heeft de bloedsomloop nodig om het bloed bij alle delen van het lichaam te brengen. Dit heeft o.a. tot doel om voedsel en zuurstof naar de lichaamscellen te brengen en afvalstoffen af te voeren.
Het hart is de motor van de bloedsomloop. Via de slagaders wordt het bloed door het lichaam verspreid en via de aderen wordt het weer teruggevoerd naar het hart.

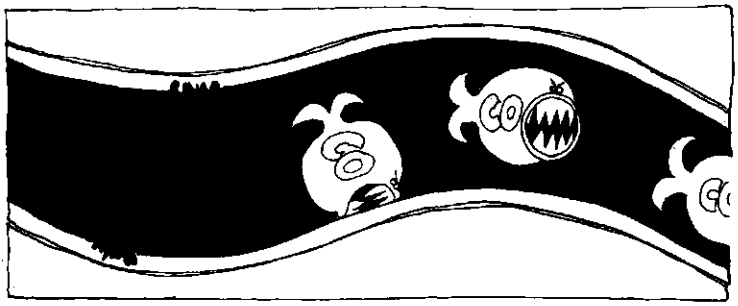
Ziekten van hart en bloedvaten

Net als alle andere lichaamsdelen kunnen hart en bloedvaten door verschillende ziekten aangetast worden, b.v. ontstekingen of rheuma, of er kunnen al bij de geboorte bepaalde afwijkingen aanwezig zijn. Deze ziektes kunnen al dan niet fatale gevolgen hebben. De meeste hart- en vaataandoeningen, waarbij gevaar voor het leven dreigt, zijn het gevolg van atherosclerose (populair gezegd: aderverkalking) in de slagaders die de spierwand van het hart zelf van voedsel en zuurstof voorzien of van atherosclerose in de slagaders van de hersenen.

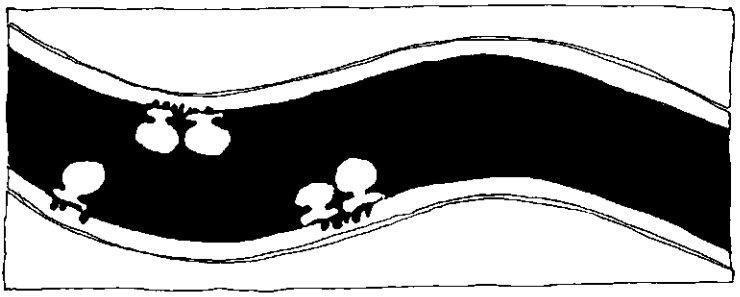
Wat is atherosclerose?

Atherosclerose is het verschijnsel dat in de wand van bloedvaten vetachtige stoffen -o.a. cholesterol- zich ophopen, waardoor de doorstroming van het bloed wordt belemmerd. (Er kan ook afzetting van kalkachtige stoffen plaatsvinden; vandaar de minder juiste naam aderverkalking). Het proces van atherosclerose

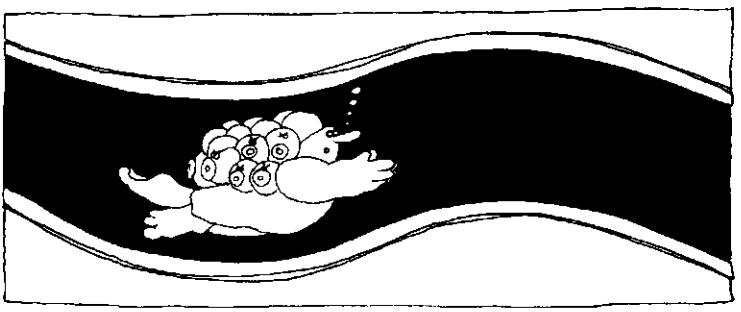
ATHEROSCLEROSE (ADERVERKALKING) BEGINT MET KLEINE BESCHADIGINGEN VAN DE VAATWAND DOOR CO (ROKEN) EN HOGE BLOEDDRUK.



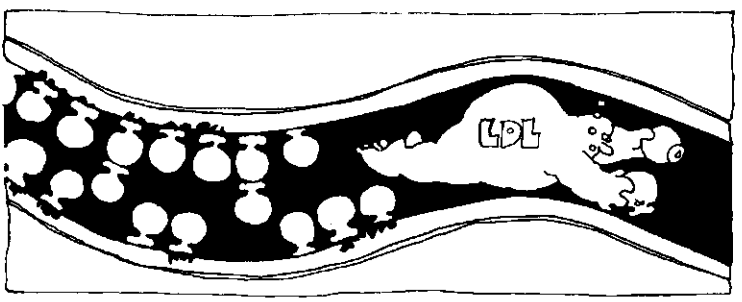
OP DE PLAATS VAN DIE BESCHADIGINGEN KUNNEN ZICH MAKKELIJK KALK- OF VETACHTIGE STOFFEN (ZOALS CHOLESTEROL) AFZETTEN.



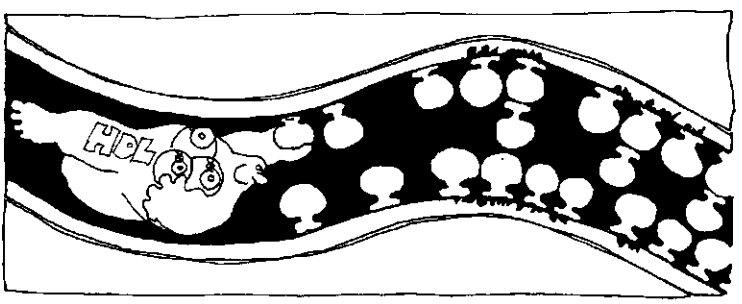
CHOLESTEROL WORDT IN HET BLOED GETRANSPORTEERD DOOR LIPOPROTEINEN.



DE 'LOW DENSITY LIPOPROTEINEN' (LDL) ZETTEN CHOLESTEROL AF, WAARDOOR DE VATEN VERSTOPT RAKEN.



DE 'HIGH DENSITY LIPOPROTEINEN' (HDL) VOEREN CHOLESTEROL JUIST WEER AF.



begint met kleine beschadigingen in de vaatwand. Deze beschadigingen kunnen b.v. veroorzaakt worden door koolmonoxide (een gevolg van sigaretten roken) of door hoge bloeddruk. Op de plaats van beschadiging kunnen zich gemakkelijk stoffen uit het bloed ophopen (b.v. cholesterol). Ook kan zich op zo'n aangetast plekje een bloedstolsel vastzetten. Daardoor raakt de doorstroming van het bloed nog meer belemmerd. In ernstige gevallen kan een bloedvat zelfs helemaal verstopt raken. Wanneer een slagader van het hart (of de hersenen) geheel verstopt is, krijgt een deel van het hart (of de hersenen) geen voedingsstoffen en zuurstof meer en houdt op te funktioneren. Als dit in het hart gebeurt spreken we van een hartinfarkt; in de hersenen van een beroerte. Van de plaats en grootte van het betrokken gebied hangt het af, of het infarkt dodelijk is of niet.
Wanneer een slagader van het hart gedeeltelijk verstopt is, kan dit in bepaalde gevallen ook al leiden tot pijn en benauwdheid (of wanneer het in de hersenen is tot bepaalde verlammingsverschijnselen). Vooral bij inspanning kan de hartspier dan meer zuurstof nodig hebben dan er met het bloed door de vernauwde slagader kan worden aangevoerd. Dit komt echter niet altijd duidelijk tot uiting; vandaar dat een hartinfarkt soms volkomen onverwacht optreedt.
Overigens kunnen ook nieren en andere organen afwijkingen vertonen ten gevolge van bloedvatvernauwingen.

Hoeveel mensen hebben eigenlijk hart- en vaatziekten?

Uit rapporten van het Ministerie van Volksgezondheid en Milieuhygiëne en van het Centraal Bureau voor de Statistiek blijkt, dat bij 19% van alle personen tussen 20 en 65 jaar die per jaar blijvend arbeidsongeschikt worden verklaard, een hart-/vaatziekte hiervan de oorzaak is. Vijfenveertig procent van alle sterfgevallen per jaar in Nederland wordt veroorzaakt door hart- en vaatziekten. Van de mensen die aan hart- en vaatziekten overlijden is 13% jonger dan 60 jaar en 8% is tussen de 60 en 65 jaar.
De sterfte ten gevolge van hart- en vaatziekten is bij mannen jonger dan 60 jaar 4 à 6 maal zo hoog als bij vrouwen van dezelfde leeftijd.

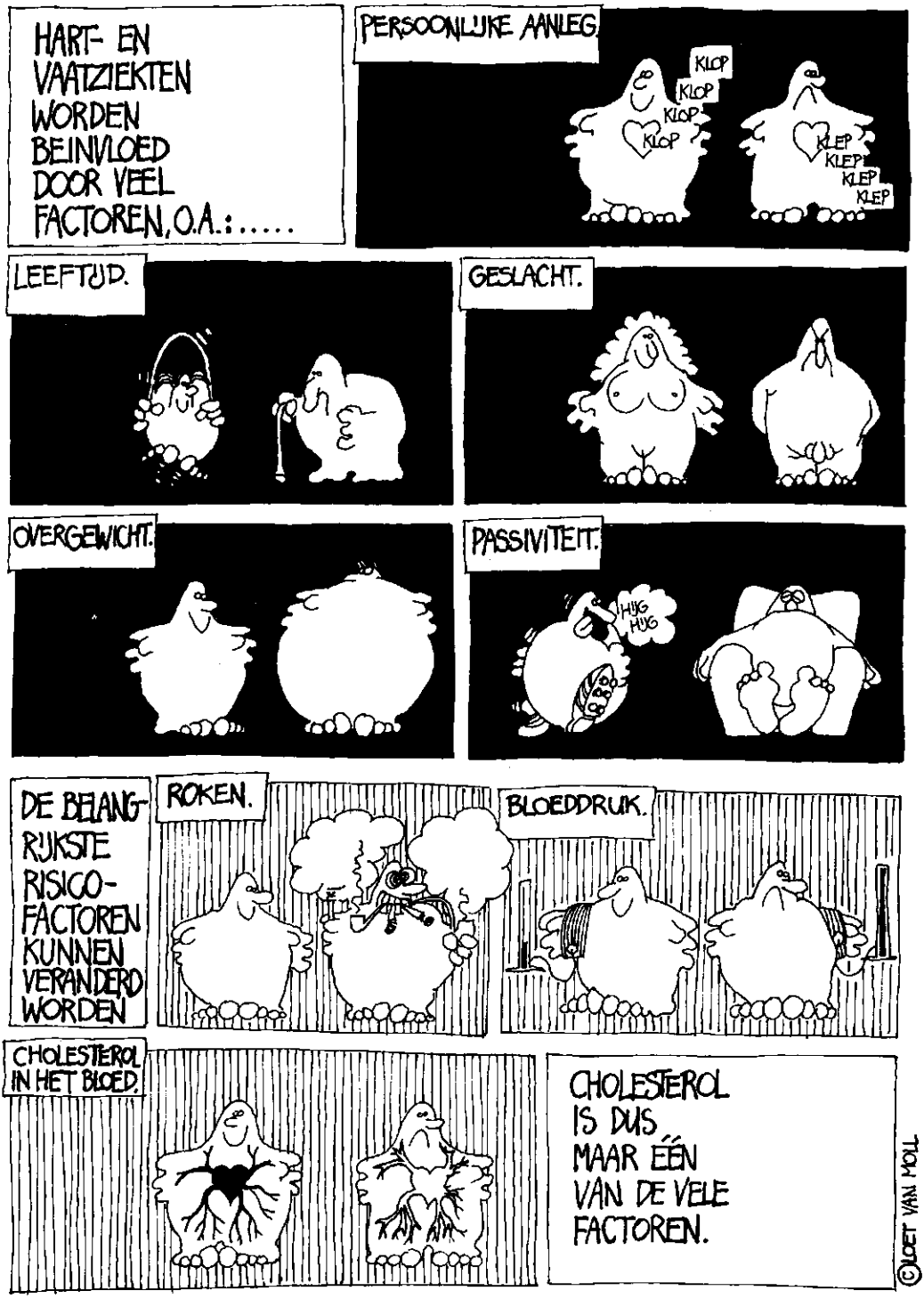
Risikofaktoren voor hart- en vaatziekten

Er is veel onderzoek verricht om na te gaan of mensen die een hartinfarkt krijgen in bepaalde opzichten verschillen van mensen die geen hartinfarkt krijgen. Daarbij bleek, dat er zeer veel faktoren zijn die samengaan met een

verhoogd risiko voor hart- en vaatziekten, b.v. hoge bloeddruk, verhoogd cholesterolgehalte van het bloed, sigaretten roken, oudere leeftijd, mannelijk geslacht, bepaalde erfelijke aanleg, suikerziekte, misschien ook overgewicht en geringe lichamelijke activiteit, enz. enz. Dergelijke faktoren worden risikofaktoren voor hart- en vaatziekten genoemd. Het is niet bekend of al deze faktoren ook werkelijk het proces van atherosclerose beïnvloeden. Er kunnen ook faktoren bij zijn, die werken als het rode lampje voor het oliepeil in een auto, dat gaat branden als het oliepeil te laag is.
Als we het lampje losdraaien zodat het niet meer brandt, betekent dat nog niet dat het oliepeil weer in orde is. Met andere woorden: het is niet zonder meer zo, dat als we een risikofaktor verlagen dan ook de kans op een hartinfarkt afneemt.
De risikofaktoren die het belangrijkste zijn voor de kans op een hartinfarct zijn het roken van sigaretten, hoge bloeddruk en een verhoogd gehalte cholesterol in het bloed. Het is zeer waarschijnlijk dat deze faktoren ook echt het proces van atherosclerose beïnvloeden: naarmate het cholesterolgehalte in het bloed hoger is, kan meer cholesterol in de vaatwand afgezet worden. Door hoge bloeddruk en door koolmonoxide en nicotine, die door het roken van sigaretten in het bloed komen, wordt de wand van een bloedvat steeds een beetje beschadigd. Daardoor kunnen weer gemakkelijker stoffen in de vaatwand afgezet worden en bloedstolsels ontstaan met de hierboven beschreven gevolgen.
Wanneer we nu willen zorgen dat er minder hart- en vaatziekten voorkomen lijkt het dan ook logisch om deze risikofaktoren te veranderen. Inderdaad is ook gebleken dat wanneer iemand stopt met roken, of wanneer de bloeddruk door b.v. medicijngebruik daalt, de kans om een hartinfarkt te krijgen of om aan een hartinfarkt te overlijden afneemt. Datzelfde geldt voor verlaging van het cholesterolgehalte in het bloed.

Iets meer over cholesterol in het bloed

De laatste jaren is duidelijk geworden, dat niet alleen het totale cholesterolgehalte in het bloed belangrijk is voor het mogelijke risiko.
Cholesterol wordt in het bloed getransporteerd in de vorm van lipoproteinen. Dat zijn deeltjes, die bestaan uit cholesterol, andere vetachtige stoffen en eiwitten. Die deeltjes zijn echter niet allemaal eender; er zijn zware (high density lipoproteins; HDL), lichte (low density lipoproteins; LDL) en zeer lichte lipoproteinen (very low density lipoproteins; VLDL). Volgens recente


HART- EN VAATZIEKTEN WORDEN BEINVLOED DOOR VEEL FACTOREN, O.A.:.....
PERSOONLIJKE AANLEG.
KLOP KLOP KLOP KLOP
KLEP KLEP KLEP KLEP
LEEFTIJD.
GESLACHT.
OVERGEWICHT.
PASSIVITEIT.
HUG HUG
DE BELANGRIJKSTE RISICO-FACTOREN KUNNEN VERANDERD WORDEN
ROKEN.
BLOEDDRUK.
CHOLESTEROL IN HET BLOED.
CHOLESTEROL IS DUS MAAR ÉÉN VAN DE VELE FACTOREN.
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theorieën gaat een hoog gehalte cholesterol in LDL gepaard met een hoog risiko voor hart- en vaatziekten en een hoog gehalte cholesterol in HDL juist met een laag risiko voor hart- en vaatziekten. Het zou b.v. kunnen zijn, dat het cholesterol in LDL het kwade werk doet en het dichtslibben van de bloedvaten bevordert terwijl de HDL juist cholesterol vervoert dat uit het lichaam wordt verwijderd. Een hoog gehalte cholesterol in LDL is dus ongunstig, een hoog gehalte cholesterol in HDL gunstig. VLDL bevat heel weinig cholesterol en is vooral belangrijk voor het transport van vetten.
Het HDL-cholesterol maakt maar 25 à 35% van het totale cholesterol uit. Het totale cholesterolgehalte weerspiegelt dus vooral het cholesterol in LDL.

Omdat het in dit proefschrift vooral gaat over voeding en het cholesterolgehalte van het bloed zal daar in het nu volgende iets dieper op worden ingegaan.

Het verband tussen de voeding, het cholesterolgehalte van het bloed en hart- en vaatziekten

De hoogte van het cholesterolgehalte in het bloed wordt behalve door erfelijke aanleg voor een belangrijk deel bepaald door de samenstelling van de voeding. Voedingsproeven met mensen hebben aangetoond dat verhoging van het gehalte linolzuur (zie kader) in de voeding ten koste van het gehalte verzadigd vet het totale cholesterolgehalte in het bloed verlaagt. Waar het natuurlijk om gaat is, of dat dan ook daadwerkelijk het ontstaan van hart- en vaatziekten vermindert. En daarover is nogal wat diskussie.

Iets meer over verzadigd en meervoudig onverzadigd vet

Vetten en oliën zijn naar hun scheikundige samenstelling te verdelen in verzadigde, enkelvoudig onverzadigde en meervoudig onverzadigde. Meervoudig onverzadigd vet is eigenlijk ook weer een verzamelnaam; het belangrijkste bestanddeel ervan is linolzuur. Alle vetten en oliën bevatten deze drie soorten, maar de verhoudingen verschillen nogal.

In dierlijke vetten (roomboter, rundvet, varkensvet) en in de plantaardige vetten palmpitvet en kokosvet overheerst het verzadigd vet.

In de meeste andere plantaardige oliën (sojaolie, zonnebloemolie) en de traansoorten (levertraan) overheerst het meervoudig onverzadigde vet.

Olijfolie bestaat voor het grootste deel uit enkelvoudig onverzadigd vet.

De verzadigde vetten verhogen het cholesterolgehalte van het bloed, de meervoudig onverzadigde vetten verlagen het cholesterolgehalte van het bloed en enkelvoudig onverzadigd vet is in dit opzicht neutraal.

Plantaardige margarines worden samengesteld uit plantaardige oliën die echter vaak gehard worden. Het harden is een chemisch proces waarbij een deel van het meervoudig onverzadigd vet wordt omgezet in verzadigd vet. Deze margarines bevatten dus minder meervoudig onverzadigd vet dan b.v. zonnebloemolie, maar wel meer dan b.v. roomboter. Dieetmargarines worden in het algemeen bereid uit ongeharde oliën, zodat ze een hoog gehalte meervoudig onverzadigd vet (ofwel linolzuur) bevatten.

Betrouwbare studies naar het effekt van voedingsveranderingen op het cholesterolgehalte van het bloed kunnen al goed uitgevoerd worden met enkele tientallen proefpersonen en de effekten zijn meestal in enige weken tot maanden zichtbaar. Gedegen studies naar het effekt van voedingsveranderingen op ziekte en sterfte vereisen een grotere omvang en moeten langer duren. Dat komt omdat zelfs in een hoog-risiko groep het aantal nieuwe gevallen van hartaandoeningen hoogstens één op de honderd per jaar bedraagt. Na een grondige studie in 1962 in Amerika werd berekend dat voor een definitieve uitspraak over de kwestie van voeding en hart- en vaatziekten een gekontroleerd experiment nodig zou zijn met 100.000 proefpersonen gedurende 4-5 jaar. Aan deze eisen heeft tot nu toe geen enkel onderzoek voldaan en men kan dus, een beetje akademisch, stellen, dat het bewijs niet echt geleverd is.
In voedingsproeven met mensen, waar behalve naar veranderingen in het bloed ook is gekeken naar het aantal mensen dat hart- en vaatziekten kreeg, bleek echter in bijna alle gevallen, dat hart- en vaatziekten minder voorkwamen op de cholesterolverlagende voeding.

In vele welvarende landen, waaronder Nederland, wordt door de officiële instanties dan ook een vermindering van het vetgebruik, vooral verzadigd vet, aanbevolen en een verhoging van het gebruik van linolzuur. Een aantal wetenschappelijke onderzoekers maakt hiertegen bezwaar, omdat er geen bevolkings-

groepen bekend zijn, die van nature gewend zijn zoveel linolzuur te eten. Men vreest voor mogelijk nadelige effekten van zo'n hoge linolzuur consumptie op de lange termijn. De dierproeven die over mogelijk nadelige effekten van linolzuur zijn gedaan hebben tot nog toe niet duidelijk aangetoond of deze vrees gerechtvaardigd of overbodig is. Als alternatief wordt een sterkere beperking aanbevolen van het totale vet en cholesterolgebruik, en een verhoging van de hoeveelheid zetmeel in de voeding.
De vraag naar mogelijk nadelige effekten op de lange termijn is echter niet de enige reden dat het hele thema zo in diskussie blijft. De verschillende belangen die een rol spelen zijn hier mede de oorzaak van.

Welke belangen zijn er in het spel?

Ekonomische belangen spelen zeker een rol. Dat de producenten van dieetmargarines en plantaardige oliën grote belangen hebben bij de huidige officiële aanbevelingen over voeding en hart- en vaatziekten is duidelijk. Wat echter ook duidelijk is, is dat een massaal opvolgen van de diverse voedingsadviezen zou kunnen leiden tot ernstige gevolgen voor een groot deel van de rest van de levensmiddelenindustrie, waaronder vooral de zuivel-, maar ook de eieren- en de vlees- en vleeswarenproducenten. Ook deze belangengroepen roeren zich duchtig in de diskussie.

Er ontstaan ook moeilijkheden als we willen schatten wat het belang voor het individu is van het opvolgen van de voedingsadviezen. Bij alle preventieve maatregelen die er worden genomen zijn er van de velen op wie die maatregel wordt toegepast uiteindelijk maar enkelen die ook werkelijk ervan zullen profiteren. Van alle kinderen die tegen kinderverlamming worden ingeënt zouden maar enkelen die ziekte gekregen hebben als er niemand werd ingeënt. Maar wél is het zo, dat 3-maal een prik volledige bescherming tegen de ziekte biedt. In het geval van voeding en hart- en vaatziekten ligt de kosten-baten balans veel ongunstiger. Men moet er veel meer voor doen (en laten) en de geboden bescherming blijft desondanks onvolledig. Vragen die meespelen zijn ook b.v.: wat zijn de kosten van voedingsveranderingen in termen van persoonlijk leefplezier, sociale kontakten e.d. en wat zijn de baten met betrekking tot het voorkómen van hart- en vaatziekten? Ook moet rekening gehouden worden met mogelijk nadelige gevolgen van het niet opvolgen van voedingsadviezen: meer hartoperaties, meer medicijngebruik, invaliditeit etc. Deze vragen gelden niet alleen op individueel niveau maar ook op bevolkingsniveau.

In het nu volgende wordt op de 'kosten-baten' balans wat meer ingegaan.

Omdat individuen onderling sterk kunnen verschillen kan een dergelijke afweging hier alleen gemaakt worden voor groepen mensen.

De kosten: welke voedingsveranderingen zijn nodig om een daling van het cholesterolgehalte in het bloed te bereiken?

Doordat er veel onderzoek naar is gedaan kan het effekt van veranderingen in de voeding op het cholesterolgehalte in het bloed redelijk worden geschat. Daarbij moet wel bedacht worden dat de daling van het cholesterolgehalte in het bloed groter is naarmate iemands uitgangsniveau van cholesterol in het bloed hoger is en kleiner naarmate dat uitgangsniveau lager is. Zoals al eerder gezegd gelden dergelijke schattingen gemiddeld voor groepen mensen; individuen kunnen onderling sterk verschillen in hun reaktie op veranderingen in de voeding.

De invloed van vet en cholesterol

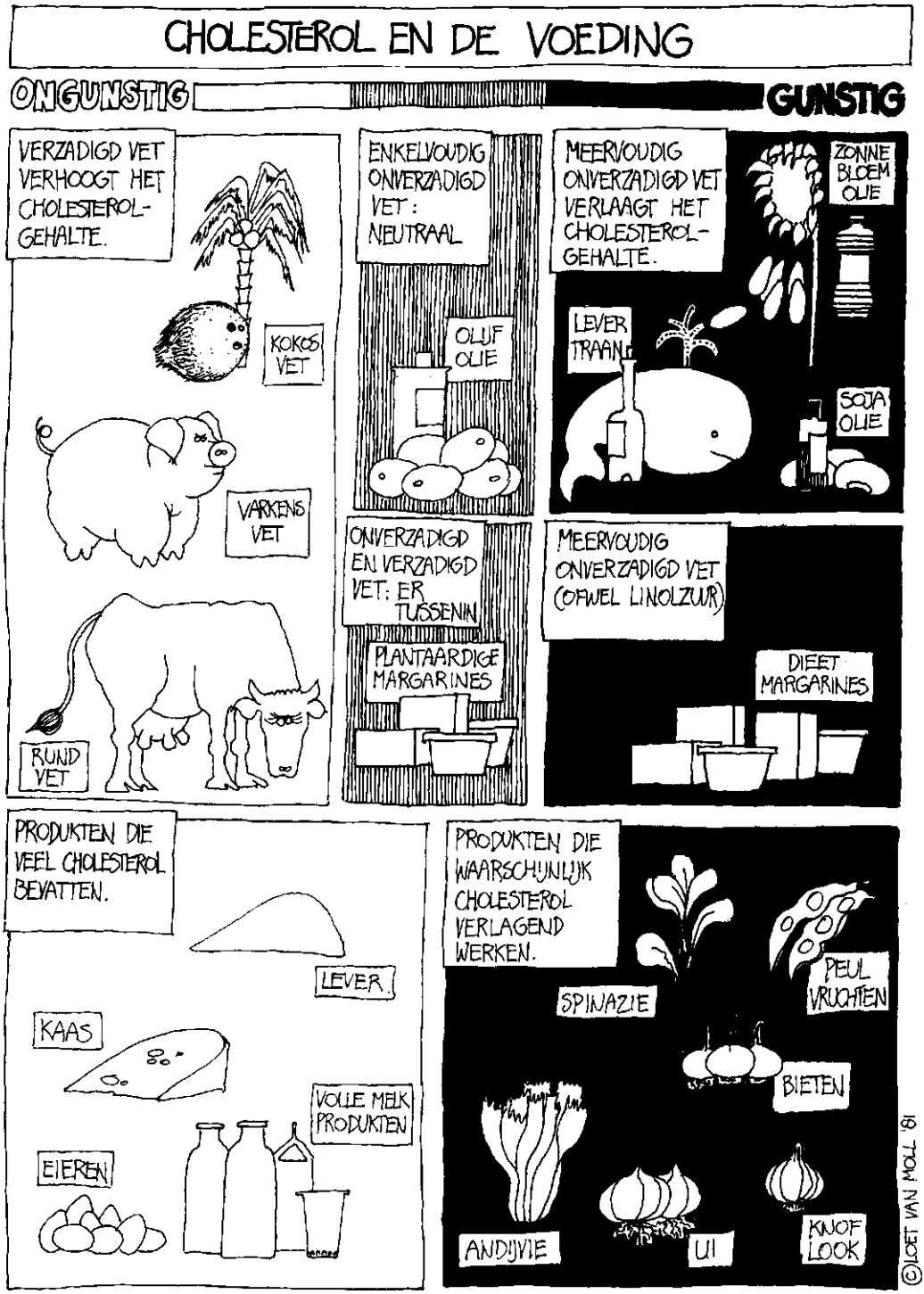
Naar de invloed van vet op het totale cholesterolgehalte in het bloed is veel onderzoek gedaan.
Gemiddeld betrekt een Nederlander 40% van de dagelijkse energieopname uit vet. Ruim 1/6 hiervan valt in de kategorie meervoudig onverzadigd vet.
De Nederlandse voedingsraad beveelt aan de totale vetconsumptie te beperken tot ongeveer 1/3 van de totale energiehoeveelheid die we per dag opnemen. Daarbij zouden verzadigd, enkelvoudig onverzadigd en meervoudig onverzadigd vet elk 1/3 deel van het geconsumeerde vet moeten uitmaken (elk dus 11% van de dagelijkse energieopname). Wanneer een groep mensen overeenkomstig dit advies het energiepercentage vet in de voeding verlaagt van 40 naar 33% van de dagelijkse energieopname heeft dit een gemiddelde daling van het cholesterolgehalte in het bloed met ca. 0,25 mmol per liter bloed tot gevolg. Verhoging van het gehalte meervoudig onverzadigd vet van 6 naar 11% van de dagelijkse energieopname betekent een daling met nog eens 0,15 mmol/l bloed. Totaal dus 0,4 mmol/l bloed. Als we bedenken dat het cholesterolgehalte voor mensen van 30-40 jaar gemiddeld 5,8 mmol/l bloed is betekent dit een daling van 7%.

Onderzoek van een alternatief

Het onderzoek dat in dit proefschrift beschreven is, ging om de vraag of het hierboven beschreven effekt ook bereikt kan worden door alleen maar heel weinig vet te eten (20% van de dagelijkse energieopname) en niet de consumptie van meervoudig

onverzadigd vet op te voeren, maar wel die van zetmeel.
Er werden twee proeven uitgevoerd. De eerste proef, met 64 proefpersonen, duurde 8 weken. De deelnemers werden ingedeeld in 4 groepen die elke een andere voeding kregen. De voedingen verschilden alleen in de soort vet of in de hoeveelheid vet en zetmeel. De tweede proef, met 40 deelnemers, duurde 16 weken. In die proef werden slechts twee voedingen vergeleken: de aanbevolen voeding van de Voedingsraad en de laag-vet voeding. Tijdens de proefperiode aten de deelnemers de warme maaltijd op de afdeling Voeding van de Landbouwhogeschool en kregen ze een voedselpakket mee voor de rest van de dag. Op die manier kon de samenstelling van de voeding goed onder controle gehouden worden. Tijdens de proef werd op verschillende tijdstippen een klein beetje bloed afgenomen voor laboratoriumbepalingen; ook urine en ontlasting werden op bepaalde tijdstippen verzameld.
Het bleek dat de aanbevolen voeding en de laag-vet voeding allebei het totale cholesterolgehalte in het bloed verlaagden als we vergelijken met de voeding zoals die in welvarende landen gebruikelijk is. Maar de laag-vet voeding had als nadeel dat ze ook het HDL-cholesterol verlaagde en de hoeveelheid triglyceriden (neutraal vet) in het bloed verhoogde en dat deed de aanbevolen voeding niet. Enige voorzichtigheid lijkt dus geboden.

Ook het eten van cholesterolrijke produkten (vooral eieren, lever; maar ook vlees, kaas, roomboter en volle melkprodukten) is van belang. Dit is echter alleen belangrijk beneden een bepaalde hoeveelheid gegeten cholesterol. Veranderingen in de hoeveelheid gegeten cholesterol boven een niveau van 500 mg per dag hebben zeer waarschijnlijk nauwelijks effekt op het cholesterolgehalte in het bloed. Met andere woorden het maakt waarschijnlijk niet uit, of je nu 15 of 20 eieren per week eet, maat wel of je van 10 naar 5 eieren gaat.
Een verlaging van de hoeveelheid cholesterol in de voeding van 450 naar 250 mg per dag zal, als we zeer voorzichtig schatten, een daling van ca. 0,10 mmol/l bloed teweeg brengen.
Veranderingen in de soort en hoeveelheid gegeten vet en in de hoeveelheid cholesterol kunnen samen zorgen voor een daling in het cholesterolgehalte in het bloed van 0,5 mmol/l bloed. In de praktijk betekent dit: minder vlees eten en bij voorkeur mager vlees met magere jus, groentes afgemaakt zonder margarine of boter, halfvolle en magere melkprodukten, dieetmargarine i.p.v. 'gewone'


CHOLESTEROL EN DE VOEDING
ONGUNSTIG
GUNSTIG
VERZADIGD VET VERHOOGT HET CHOLESTEROL-GEHALTE.
KOKOS VET
VARKENS VET
RUND VET
ENKELVOUDIG ONVERZADIGD VET: NEUTRAAL
OLIJF OLIE
ONVERZADIGD EN VERZADIGD VET: ER TUSSENIN
PLANTAARDIGE MARGARINES
MEERVOUDIG ONVERZADIGD VET VERLAAGT HET CHOLESTEROL-GEHALTE.
ZONNE BLOEM OLIE
LEVER TRAAN
SOJA OLIE
MEERVOUDIG ONVERZADIGD VET (OFWEL LINOLZUUR)
DIEET MARGARINES
PRODUKTEN DIE VEEL CHOLESTEROL BEVATTEN.
LEVER
KAAS
VOLLE MELK PRODUKTEN
EIEREN
PRODUKTEN DIE WAARSCHIJNLIJK CHOLESTEROL VERLAGEND WERKEN.
SPINAZIE
PEUL VRUCHTEN
BIETEN
ANDIJVIE
UI
KNOF LOOK
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margarine en minder eieren. En meer aardappelen, groente, brood etc.

De invloed van andere voedingsmiddelen op het cholesterolgehalte in het bloed

Ook van andere voedingsmiddelen dan vet en cholesterol kan een cholesterolverlagende werking uitgaan. Door veel fruit en vezelrijke groenten (wel 3 à 4 ons per dag) te eten kan het cholesterol in het bloed ook nog wat dalen. (Vezelrijke groenten zijn groenten rijk aan onverteerbare bestanddelen, zoals spinazie, andijvie, bieten). Ook peulvruchten, knoflook en ui zouden een cholesterolverlagende werking kunnen hebben. Omdat daar vrij weinig onderzoek naar is gedaan kunnen we slechts gokken hoe groot het effekt daarvan is. Door het eten van heel veel groenten en fruit, plus gemiddeld ca. 35 g (ongeweekt gewicht) peulvruchten per dag plus regelmatig gebruik van knoflook en ui zou het cholesterolgehalte met nog eens 0,25 mmol/l bloed kunnen dalen.
De totale daling van het cholesterolgehalte in het bloed die met de voeding bereikt zou kunnen worden bedraagt dus gemiddeld 0,75 mmol/l bloed. Dit zou gemiddeld gelden voor een groep mensen met een gehalte cholesterol in het bloed van 5,7 mmol/l. Voor mensen met een cholesterolgehalte van 4 mmol/l zou deze daling ca. 0,35 mmol/l bloed bedragen en voor mensen met een cholesterolgehalte van 8 mmol/l bloed zou deze daling ca. 1,35 mmol/l bloed bedragen.
Het gezamenlijke effekt van al deze voedingsveranderingen tegelijk is ook wel eens in een voedingsproef aangetoond en waarschijnlijk zijn dergelijke voedingsveranderingen in de praktijk haalbaar.

De baten: Wat levert verandering van voedselkeuze op wat betreft de kans op een hartinfarkt?

De grote vraag is nu natuurlijk wat alle zorg, die men eventueel op de boven beschreven wijze aan de voeding besteedt, oplevert. Met behulp van gegevens van een groot bevolkingsonderzoek in het Amerikaanse stadje Framingham is geschat hoe groot het effekt van verlaging van het cholesterolgehalte in het bloed op het aantal hartinfarkten zou kunnen zijn. Dit voorbeeld dient om een indruk te geven van de orde van grootte van het effekt.
Uit deze berekeningen bleek, dat, als 100 mannen (niet-rokers, met normale bloeddruk en geen hartafwijkingen) beginnend op 35-jarige leeftijd, hun cholesterolgehalte verlagen van 8 naar 6,7 mmol/l bloed, 6 van hen hiermee een hartinfarkt in de komende 20 jaar voorkomen, 8 zouden zelfs ondanks alles toch een hartinfarkt krijgen vóór hun 55e jaar en de overige 86 zouden er geen voor-

deel uit halen. Het risiko daalt dus van 14% naar 8%; een vermindering met bijna de helft. Het mogelijke voordeel is kleiner voor vrouwen en voor degenen die op latere leeftijd beginnen, maar groter als het cholesterolgehalte nog verder verlaagd wordt en ook groter als er tegelijkertijd ook andere risikofaktoren aanwezig zijn. Bijvoorbeeld: als 100 mannen, die sigaretten roken en matig verhoogde bloeddruk hebben en een kleine hartafwijking, hun cholesterolgehalte in het bloed verlagen van 8 naar 5,4 mmol/l bloed, zou hun risiko voor een hartinfarkt vóór het 55e jaar afnemen van 57 naar 29%.

Konklusie

Op grond van bovenstaande overwegingen zou men een cholesterolverlagende voeding kunnen beschouwen als een soort verzekeringspremie, waarbij de risikodekking voor verschillende situaties verschilt, maar nooit 100% is.
Op bevolkingsniveau zou verandering van de voeding zeer waarschijnlijk leiden tot een daling van het gemiddelde cholesterolgehalte in het bloed en tot daling van de sterfte. Per individu kan geen voorspelling gedaan worden of voedingsveranderingen een hartinfarkt zullen voorkómen. Dat komt enerzijds doordat er grote onderlinge verschillen tussen mensen zijn in de mate waarin hun cholesterolgehalte reageert op veranderingen in de voeding. Anderzijds blijft er om onbekende redenen altijd een kans bestaan dat iemand ondanks verlaging van het cholesterolgehalte in het bloed tóch een hartinfarkt krijgt, zoals we in bovenstaande voorbeelden hebben willen laten zien.
Bij het beoordelen van de wenselijkheid van voedingsveranderingen zijn dus de 'kosten' (in termen van voedingsveranderingen) bekend, terwijl de 'baten' (in termen van de kans op hart- en vaatziekten) per individu onvoorspelbaar zijn.

Dit hoofdstuk is voor het grootste deel gewijd aan voeding en hart- en vaatziekten. Zoals in het begin al is gezegd kunnen ook vermindering van sigarettengebruik en verlaging van de bloeddruk bijdragen aan het verminderen van de kans op een hartinfarkt. Deze faktoren zouden in de voorlichting dan ook minstens evenveel nadruk moeten krijgen als de faktor voeding. Voeding zelf speelt bovendien een rol bij veel meer ziekteprocessen. Denk alleen maar aan alkohol en leverziekten, suikers en tandbederf, zout en hoge bloeddruk. Voeding dient niet beschouwd te worden als specifiek middel ter voorkoming van ziekten of zelfs als medicijn. Voeding heeft zijn plaats in een geheel van aktiviteiten en gedragingen onder het hoofd gezondheidsbescherming en -bevordering. Niet meer, maar ook niet minder.

Curriculum vitae

Tineke Brussaard werd in 1953 geboren te Bergschenhoek. In 1971 behaalde zij het diploma gymnasium-β aan de Chr.Scholengemeenschap Melanchton te Rotterdam. In hetzelfde jaar begon zij de studie aan de Landbouwhogeschool te Wageningen. In 1978 slaagde zij voor het doctoraal examen met als hoofdvak humane voedingsleer en als keuzevakken dierfysiologie en wiskundige statistiek. Van juli 1978 tot juli 1981 verrichtte zij met financiële steun van de Nederlandse Hartstichting het in dit proefschrift beschreven onderzoek bij de vakgroep Humane Voeding van de Landbouwhogeschool. Vanaf februari 1981 is zij in deeltijd werkzaam als secretaris van de Commissie Adipositas van de Gezondheidsraad.