Atherosclerosis, 42 (1982) 205–219
Elsevier/North-Holland Scientific Publishers, Ltd.

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

Jantine H. Brussaard [1], Martijn B. Katan [1], Pieter H.E. Groot [2], Louis M. Havekes [3] and Joseph G.A.J. Hautvast [1]

[1] *Department of Human Nutrition, Agricultural University, Wageningen;* [2] *Department of Biochemistry I, Erasmus University, Rotterdam; and* [3] *Gaubius Institute, TNO, Leiden (The Netherlands)*

(Received 15 April, 1981)
(Revised, received 17 September, 1981)
(Accepted 21 September, 1981)

Summary

In a controlled 16-week trial we have compared the effects on some risk factors for atherosclerosis of 2 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. All 35 volunteers were given diet MOD during a 2.5-week control period. This diet provided 31% of the daily energy intake (energy%) as total fat; one-third of the fatty acids were polyunsaturated (PUFA). VLDL plus LDL cholesterol decreased by 0.66 mmol/l during the control period while the HDL cholesterol (HDL-C) level was unchanged. Total serum triglycerides decreased by 0.33 mmol/l.

After the control period the subjects were randomized into 2 groups, one of which continued on diet MOD, while the other group received the low-fat diet LO, providing 21 energy% as total fat and 4 energy% as PUFA. All food was prepared daily and weighed out for each individual in amounts appropriate to the individual's energy needs. Nutrient intakes were checked by 7-day records and by chemical analysis of double portions. The diets contained the same amounts of cholesterol,

This work was supported by the Netherlands Heart Foundation, Grant 77.082.

Address for correspondence: M.B. Katan, Agricultural University, Department of Human Nutrition, De Dreijen 12, 6703 BC Wageningen, The Netherlands.

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

phytosterols, oligosaccharides and other nutrients known to affect serum lipid levels.

Total serum cholesterol increased by 0.21 ± 0.41 mmol/l on control diet MOD during the test period of 13 weeks. 0.09 ± 0.11 mmol/l of this was due to HDL-C. 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-C was lowered by -0.06 ± 0.20 mmol/l. The difference in HDL-C between the diet groups was mainly located in HDL_2. Total serum triglycerides increased by $+0.32 \pm 0.31$ mmol/l in group LO; the increase in VLDL triglycerides was $+0.22 \pm 0.18$ mmol/l.

The depression of HDL on diet LO was also reflected in the ratio of HDL to total cholesterol which decreased by -0.02 ± 0.05 on diet LO, and was unchanged on diet MOD, and in the ratio of apo A1 to apo B (6% decrease on diet LO and constant on diet MOD).

Combination of these data with those from a preceding study [Brussaard 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.

Key words: *Apolipoproteins – Carbohydrates – Dietary fat – Linoleic acid – Serum cholesterol – Serum triglycerides*

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. 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 lipoproteins in healthy volunteers over a period of 5 weeks. HDL cholesterol (HDL-C) was lowered and total serum triglycerides and VLDL concentration increased, when polyunsaturated fat was replaced by carbohydrates. However, Antonis and Bersohn [5] reported that the increase of serum triglycerides on carbohydrate-rich diets subsided after 3–4 months on this diet, and therefore both the changes in VLDL and HDL observed previously could have been temporary only. In order to verify our earlier findings on the effects on serum

replacing polyunsaturated fatty acids by starch, we have now carried out a similar experiment lasting 16 weeks. We found that the increase in VLDL and decrease in HDL caused by the high-carbohydrate diet relative to the high-polyunsaturated fat diet persisted throughout this period.

Subjects and Methods

Subjects

Twelve female and 23 male university students aged 19–30 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. Baseline data on body weights and serum lipoproteins are given in Table 1. None of the volunteers had glucosuria or proteinuria. Two of the females used hormonal contraception in each dietary group.

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 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 2 subgroups stratifying for initial serum total cholesterol and HDL-C 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 16-week period all foodstuffs were weighed out for each person in quantities appropriate to the individual's 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. All diets were composed of regular foodstuffs.

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

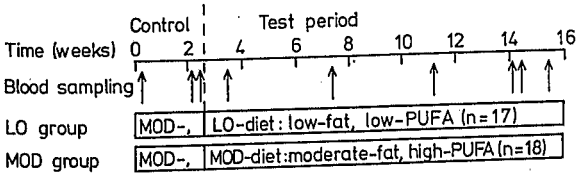

Fig. 1. Experimental design.

TABLE 1

BASELINE VALUES AND CHANGES DURING THE CONTROL PERIOD; BODY WEIGHTS, SERUM LIPIDS AND SERUM APOLIPOPROTEINS (mean±SD)

	Group LO (n=11 M, 6 F)		Group MOD (n=12 M, 6 F)	
	Start of control period	End of control period	Start of control period	End of control period
Body weight (kg)	68.4 ± 6.2	68.2 ± 6.6	71.6 ± 11.2	71.4 ± 12.0
Percentage body fat [a]				
Men	13 ± 4	[c]	14 ± 4	[c]
Women	26 ± 3	[c]	26 ± 4	[c]
Cholesterol (mmol/l)	4.65± 0.90	4.01± 0.90	4.63± 0.63	3.93± 0.61
HDL cholesterol (mmol/l) [b]	1.38± 0.23	1.39± 0.25	1.43± 0.26	1.34± 0.23
Triglycerides (mmol/l)	0.84± 0.37	0.54± 0.21	0.99± 0.49	0.76± 0.27
Apolipoprotein A1 (mg/l)	1489 ±216	1401 ±247	1542 ± 175	1390 ±140
Apolipoprotein B (mg/l)	685 ±211	515 ±197	682 ± 132	494 ±130

[a] From skinfold measurements according to [40,41].

[b] After Mn–heparin precipitation.

[c] Not measured.

TABLE 2

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

	Habitual intake before experiment [b]	Control [c] period (MOD diet)	Test period [d]	
			MOD diet	LO diet
Energy (MJ)	11.1	10.1	10.3	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 fibre (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 Dutch 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.

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 2. 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 2 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].

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 by 10.5 g/100 g fatty acid methylesters with compensatory significant increases in palmitic acid (+1.8), palmitoleic acid (+1.3) and oleic acid (+7.8).

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 7 days of the test period. There were no significant differences between groups in intestinal transit time [10], fecal 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 2).

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. 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 conventional value of 1.063 so as to reduce contamination of HDL by sinking pre-beta-lipoproteins. Lipoprotein fractionation was not done at the start of the control period, because we were mainly interested in the changes in lipoprotein composition and concentration during the test period.

Total serum cholesterol was measured with Liebermann–Burchard reagent [11] using serum calibrators calibrated according to Abell et al. [12]. The lipid laboratory was certified by the Center for Disease Control, Atlanta, GA, U.S.A., as meeting the WHO criteria. Reproducibility for blind control sera provided by the Center for Disease Control was $\pm 1.4\%$ (coefficient of variation) and the absolute level (accuracy) was within 1.2% of the "true" (target) values. Cholesterol concentrations in ultracentrifuged fractions (VLDL, LDL, HDL_2, HDL_3) 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%.

Total HDL-C 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 High Density Lipoprotein Cholesterol Measurements [15] a reproducibility of $\pm 2.2\%$ (coefficient of variation) was found. The bias with regard to the overall survey mean was on average 0.1%. The changes in HDL-C as measured after Mn–heparin precipitation were equal to the changes in cholesterol in total HDL as obtained by density gradient centrifugation. The percentage differences between HDL concentrations according to the 2

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.

Serum total, VLDL and LDL triglyceride concentrations were measured as described by Soloni [20].

Apolipoprotein B was measured in whole serum and in fresh ultracentrifugally isolated VLDL fractions by rocket immunoelectrophoresis [21] as described [4]. For apo A1 analysis, serum was first delipidated with redistilled tetramethylurea and diluted with 8 M urea, 10 mM Tris–HCl, pH 8.2 [22]. Apolipoprotein A1 was then measured by rocket immunoelectrophoresis as described [4] using rabbit anti-serum against apo A1. 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 A1 assays, respectively.

VLDL was isolated for analysis of VLDL-apolipoprotein C2/C3 ratios by selective precipitation with Mg–heparin according to [16] and apo C2/C3 ratios were analysed by polyacrylamide slab gel electrophoresis in urea-containing buffers [17]. Protein bands were stained with Fast Green FCF and quantified by densitometric scanning using a Shimadzu CS 910 dual wavelength densitometer with 640 and 750 nm as sample and reference wavelength, 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 2 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

The time courses of serum total cholesterol, total triglyceride and HDL cholesterol concentration are given in Figs. 2, 3 and 4, respectively. Baseline values and changes during the control period are given in Table 1. During the control period average serum total cholesterol for all subjects combined decreased significantly by -0.68 ± 0.44 mmol/l of which on average only 0.02 ± 0.13 mmol/l was located in HDL. For all parameters measured during the control period, the changes in those subjects who were later to consume the low-fat LO diet were not significantly different from those in the subjects randomized into the control diet group MOD.

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 ($P < 0.05$).

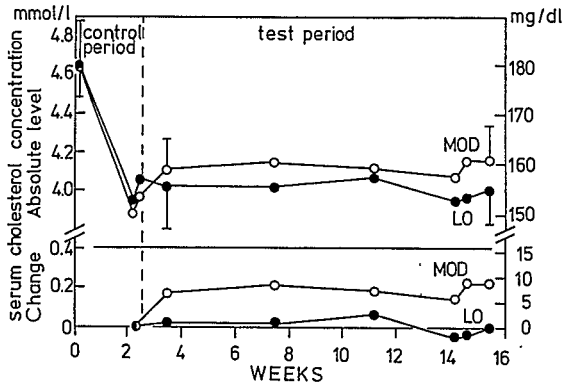

Fig. 2. Mean total serum cholesterol concentrations during the experiment and mean change in total serum cholesterol concentration during the test period. ●——●, LO; ○——○, MOD (control). Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P<0.05$ or 0.01) between the changes in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

Total serum cholesterol (Fig. 2) increased by $+0.21 \pm 0.41$ 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.

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

HDL-C (Fig. 4) increased in the control group by +7% and decreased on the low-fat diet by −4%. The changes in HDL-C in group MOD were mainly located in HDL_2. In group LO HDL_3-C had also changed, the change being smaller after 12 weeks than after 5 weeks. Apolipoprotein A1 concentrations were stable in both groups. The ratio of HDL-C to total serum apo A1 had increased in the control group by +5% after 12 weeks and was unchanged in group LO, after an initial decrease of −3%.

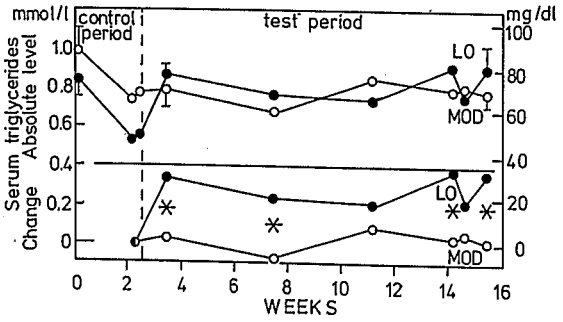

Fig. 3. Mean total serum triglyceride concentrations during the experiment and mean change in total serum triglyceride concentrations during the test period. ●——●, LO; ○——○, MOD (control). Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P<0.05$ or 0.01) between the changes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

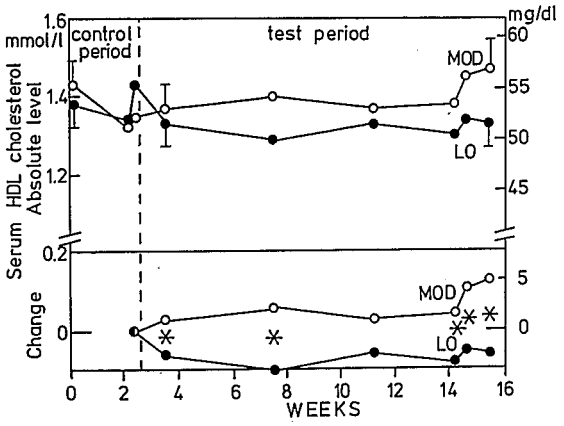

Fig. 4. Mean serum HDL cholesterol concentrations (after Mn–heparin precipitation) during the experiment and mean change in serum HDL cholesterol concentrations during the test period. ●———●, LO; ○———○, MOD (control). Vertical bars indicate 1 SEM; an asterisk denotes a significant difference ($P<0.05$ or 0.01) between the changes in the control group MOD and the LO group.

The concentration of LDL-C 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-C to apo B.

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 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 C2/C3 was unchanged after 5 weeks of 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.

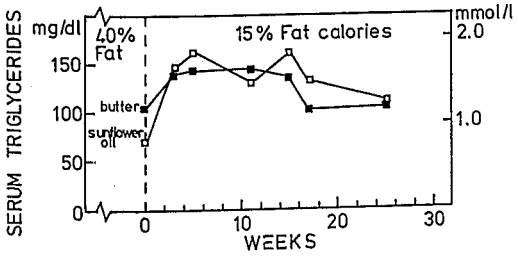

Fig. 5. Influence on fasting serum triglyceride levels of reducing the proportion of fat calories in white South-African prisoners. ■———■, data for 12 subjects who had received the high-fat butter diet; □———□, data for 13 subjects who had received the high-fat sunflower oil diet. No points are given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

TABLE 3

EFFECT OF A LOW-FAT DIET (LO) ON LIPOPROTEIN COMPOSITION AND CONCENTRATION AS COMPARED WITH A MODERATE-FAT DIET (MOD) [a] RICH IN LINOLEIC ACID

Values expressed as mean ± SD (Values in parentheses indicate changes as a percentage of control levels).

	Test period		
	End of control period		After 5 weeks
	MOD	LO	MOD
	Absolute level		Absolute change
Total serum			
Apolipoprotein A1 [d] (mg/l)	1390 ±140	1401 ±247	+20 ±121 (+1)
Cholesterol (mmol/l)	3.93± 0.61	4.01± 0.90	+0.22± 0.51 (+6)
Triglycerides (mmol/l)	0.76± 0.27	0.54± 0.21	−0.08± 0.31 (−11)
HDL			
Total HDL cholesterol [b] (mmol/l)	1.34± 0.24	1.35± 0.21	+0.06± 0.14 (+4)
HDL_2 cholesterol [c] (mmol/l)	0.73± 0.25	0.79± 0.25	+0.02± 0.15 (+3)
HDL_3 cholesterol [c] (mmol/l)	0.62± 0.11	0.61± 0.08	0.00± 0.11 (0)
LDL			
Cholesterol (mmol/l)	2.25± 0.53	2.44± 0.80	+0.31± 0.53 (+14)
Triglycerides (mmol/l)	0.20± 0.05	0.18± 0.05	0.00± 0.03 (0)
Apolipoprotein B [c] (mg/l)	494 ±130	515 ±197	+32 ± 93 (+6)
VLDL			
Cholesterol (mmol/l)	0.31± 0.15	0.21± 0.10	0.00± 0.10 (0)
Triglycerides (mmol/l)	0.44± 0.22	0.30± 0.17	+0.06± 0.22 (+14)
Apolipoprotein B (mg/l)	63 ± 30	46 ± 23	−10 ± 25 (−16)
Apolipoprotein C2/C3 (total)	0.32± 0.10	0.31± 0.07	0.00± 0.06 (0)

[a] Conversion factors from SI to traditional units: cholesterol, 1 mmol/l=39 mg/dl; triglyceride, 1 mmol/l=89 mg/dl.

[b] Isolated after Mn–heparin precipitation.

[c] Isolated by density gradient ultracentrifugation.

Discussion

The present study was set up to evaluate on a more long-term basis then in our previous study [4] the effects of a low-fat diet on lipoprotein concentration and composition as compared with a moderate-fat–high-PUFA diet. Dietary intake was strictly controlled and independent checks on food intake (composition of cholesterol ester fatty acids, intestinal transit time and fecal wet weight) confirmed dietary adherence.

Our data do not offer a definitive answer to the question of which type of cholesterol-lowering diet should be preferred. Both types of diet appear to be quite effective in lowering total serum cholesterol levels compared with what is found in habitual diets of affluent societies. There were, however, clear-cut differences in the effects on HDL and VLDL: HDL was depressed and fasting VLDL (and total

	After 12 weeks	
LO	MOD	LO
Over test period	(% of control level)	
−18 ±162 (−1)	+51 ±179 (+4)	−14 ±193 (−1)
+0.02± 0.29 (0)	+0.21± 0.39 (+5)	−0.03± 0.38 (0)
+0.23± 0.28[f] (+43)	+0.03± 0.34 (+4)	+0.32± 0.51[f] (+59)
−0.10± 0.20[f] (−7)	+0.09± 0.11 (+7)	−0.06± 0.20[f] (−4)
−0.07± 0.20 (−9)	+0.06± 0.16 (+8)	−0.11± 0.17[f] (−14)
−0.06± 0.12 (−10)	0.00± 0.14 (0)	−0.03± 0.09 (−5)
+0.06± 0.47 (+2)	+0.15± 0.54 (+7)	−0.04± 0.53 (−1)
+0.03± 0.05 (+17)	+0.04± 0.08 (+20)	+0.01± 0.03 (+6)
−1 ± 64 (0)	+42 ±102 (+9)	+13 ± 87 (+3)
+0.11± 0.13 (+52)	+0.06± 0.21 (+19)	+0.15± 0.12 (+71)
+0.22± 0.22 (+73)	+0.04± 0.31 (+9)	+0.22± 0.18 (+73)
+25 ± 19 (+54)	−8 ± 41 (−13)	+8 ± 15 (+17)
0.00± 0.05 (0)	−0.02± 0.05 (−6)	−0.02± 0.03 (−6)

[d] Total serum apolipoprotein A1 is mainly located in the HDL fraction.
[e] Calculated as total serum apo B minus VLDL apo B.
[f] Change in group LO significantly different from change in control group ($P<0.01$, t-test).

serum triglycerides) were elevated by the low-fat–high-carbohydrate LO diet relative to the moderate-fat–high-PUFA MOD 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.

In the next section changes in lipoprotein concentration and composition will be discussed in more detail.

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

As described earlier [4,7,23] the main difference between the habitual diet of our participants 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 located mainly in VLDL plus LDL; it was accompanied by a decrease in apo B. Although HDL-C did not change during this period, apolipoprotein A1 concentration fell markedly. This lack of an effect of dietary fat modification on HDL-C concentration 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-C 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-C concentrations. Conventional lipid-lowering diets cause a decrease of HDL-C [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-C compared with the control MOD diet. Although this difference between the moderate- and low-fat diet (0.16 mmol/l or 6 mg/dl) may seem modest, it is in the same range as the difference between coronary heart disease victims and healthy controls [33] and it would predict a sizeable difference in coronary risk [34].

Our results agree with the observations of Blum et al. [28], Schonfeld et al. [29] and Levy et al. [30], who report a lowering of HDL-C in short-term controlled studies of subjects consuming 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-C on lowering fat intake from 30 to 20% of energy.

The conclusion that replacement of fat by carbohydrates lowers HDL-C is in agreement with epidemiological studies. Thus Ernst et al. [31] report a negative association between total carbohydrate intake (either as sugar or as starch) and HDL-C 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-C and suggested that this might be due to a low consumption of fat-rich animal products. In view of what is known about the metabolism of HDL 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, because high density lipoproteins are intimately involved in fat catabolism, both as activators and as side products of the catabolism of fat-rich particles [35].

The difference between diet LO and MOD in HDL-C was due mainly to the cholesterol-rich HDL_2 fraction. This agrees with the observed decrease in the HDL-C/apo A1 ratio. Variations in HDL-C are often located in HDL_2 [36,37].

Blum et al. [28] also found a relative decrease in HDL_2 on a fat-free carbohydrate-rich diet. Thus it appears that HDL_2 is more sensitive to changes in dietary fat than HDL as a whole.

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

Our previous study showed a slight increase in both total and LDL-C and in serum apolipoprotein B on the LO diet relative to the MOD diet, while the present study showed a decrease. The difference with the previous experiment cannot be explained by dietary composition or dietary adherence and we 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.

Carbohydrate-induced hypertriglyceridemia and VLDL

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 the 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. 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.

Apolipoprotein C2 is an activator and apolipoprotein C3 an inhibitor of lipoprotein lipase, an enzyme essential for normal lipoprotein triglyceride metabolism. Thus, changes in the serum triglyceride concentration could be related to alterations in the relative proportions of apo C2 and apo C3. Indeed, Schonfeld et al. [29] report increased apo C2/C3 ratios after 5 days of high-carbohydrate liquid formula diets. This is in contrast with our own observations, as changes in serum triglyceride concentrations were not associated with changes in the apo C2/C3 ratio in VLDL. This discrepancy might be 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].

Acknowledgements

We thank the volunteers for their enthusiastic cooperation and we are grateful to Dr. J.P. Kotzé (Dept. of Health, Welfare and Pensions, Pretoria) for providing us with details of the study of Antonis and Bersohn.

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

References

1 Dayton, S., Pearce, M.L., Hashimoto, S., Dixon, W.J. and Tomiyasu, U., A Controlled Clinical Trial of a Diet High in Unsaturated Fat (American Heart Association Monograph, No. 25), New York, 1969.
2 Miettinen, M., Turpeinen, O., Karvonen, M.J., Elosuo, R. and Paavilainen, E., Effect of cholesterol-lowering diet on mortality from coronary heart-disease and other causes, Lancet, ii (1972) 835.
3 Connor, W.E. and Connor, S.L., The key role of nutritional factors in the prevention of coronary heart disease, Prev. Med., 1 (1972) 49.
4 Brussaard, J.H., Dallinga-Thie, G., Groot, P.H.E. and Katan, M.B., Effects of amount and type of dietary fat on serum lipids, lipoproteins and apolipoproteins in man—A controlled 8-week trial, Atherosclerosis, 36 (1980) 515.
5 Antonis, A. and Bersohn, I., The influence of diet on serum triglycerides in South African White and Bantu prisoners, Lancet,i (1961) 3.
6 Hautvast, J.G.A.J., Ontwikkeling van een systeem om voedingsenquêtes met behulp van de komputer te verwerken, Voeding, 36 (1975) 356.
7 Stasse-Wolthuis, M., Albers, H.F.F., Van Jeveren, J.G.C., De Jong, J.W., Hautvast, J.G.A.J., Hermus, R.J.J., Katan, M.B., Brydon, W.G. and Eastwood, M.A., Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids, and colonic function, Amer. J. Clin. Nutr., 33 (1980) 1745.
8 Rockerbie, R.A., Dobson, R.D. and Frohlich, J., Gas-chromatographic analysis of patterns of fatty acids of cholesteryl esters and phosphatidylcholine, Clin. Chem., 25 (1979) 1411.
9 Knuiman, J.T., West, C.E., Hermus, R.J.J. and Hautvast, J.G.A.J., Fatty acid composition of cholesteryl esters in serum in boys from 16 developing and developed countries, Atherosclerosis, 37 (1980) 617.
10 Cummings, J.H., Jenkins, D.J.A. and Wiggins, H.S., Measurement of the mean transit time of dietary residue through the human gut, Gut, 17 (1976) 210.
11 Huang, T.C., Chen, C.P., Wefler, V. and Raftery, A., A stable reagent for the Liebermann–Burchard reaction—Application to rapid serum-cholesterol determination, Anal. Chem., 33 (1961) 1405.
12 Abell, L.L., Levy, B.B., Brody, B.B. and Kendall, F.E., A simplified method for the estimation of total cholesterol in serum and a demonstration of its specificity, J. Biol. Chem., 195 (1952) 357.
13 Burstein, M. and Samaille, J., Sur un dosage rapide du cholestérol lié aux α- et β-lipoprotéines du sérum, Clin. Chim. Acta, 5 (1960) 609.
14 Van der Haar, F., Van Gent, C.M., Schouten, F.M. and Van der Voort, H.A., Methods for the estimation of high density cholesterol, comparison between two laboratories, Clin. Chim. Acta, 88 (1978) 469.
15 Hainline, Jr., A., Cooper, G.R., Olansky, A.S., Winn, C.L. and Miller, D.T., CDC Survey of High Density Lipoprotein Cholesterol Measurements—A Report, Center for Disease Control, Atlanta, GA, 1980.
16 Utermann, G., Albrecht, G. and Steinmetz, A., Polymorphism of apolipoprotein E, Part 1 (Methodological aspects and diagnosis of hyperlipoproteinemia type III without ultracentrifugation), Clin. Gen., 14 (1978) 351.

17 Kane, J.P., A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins, Anal. Biochem., 53 (1973) 350.
18 Redgrave, T.G., Roberts, D.C.K. and West, C.E., Separation of plasma lipoproteins by density gradient ultracentrifugation, Anal. Biochem., 65 (1975) 42.
19 Terpstra, A.H.M., Woodward, C.J.H. and Sanchez-Muñiz, F.J., Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation—Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum, Anal. Biochem. 111 (1981) 149.
20 Soloni, F.G., Simplified manual micromethod for determination of serum triglycerides, Clin. Chem., 17 (1971) 529.
21 Laurell, C.B., Electro-immuno assay, Scand. J. Clin. Lab. Invest., Suppl. 124 (1972) 21.
22 Albers, J.J., Whal, P.W., Cabana, V.G., Hazzard, W.R. and Hoover, J.J., Quantitation of apolipoprotein-A_I of human high density lipoproteins, Metabolism, 25 (1976) 633.
23 Stasse-Wolthuis, M., Hautvast, J.G.A.J., Hermus, R.J.J., Katan, M.B., Bausch, J.E., Rietberg-Brussaard, J.H., Velema, J.P., Zondervan, J.H., Eastwood, M.A. and Brydon, W.G., The effect of a natural high-fiber diet on serum lipids, fecal lipids and colonic function, Amer. J. Clin. Nutr., 32 (1979) 1881.
24 Keys, A., Anderson, J.T. and Grande, F., Serum cholesterol response to changes in the diet, Metabolism, 14 (1965) 747.
25 Hegsted, D.M., McGandy, R.B., Myers, M.L. and Stare, F.J., Quantitative effects of dietary fat on serum cholesterol in man, Amer. J. Clin. Nutr., 17 (1965) 281.
26 Ernst, N., Fischer, M., Bowen, P., Schaefer, E.J. and Levy, R.I., Changes in plasma lipids and lipoproteins after a modified fat diet, Lancet, ii (1980) 111.
27 Vessby, B., Boberg, J., Gustafsson, J.B., Karlström, B., Lithell, H. and Östlund-Lundquist, A.M., Reduction of high density lipoprotein cholesterol and apolipoprotein A-I concentrations by a lipid-lowering diet, Atherosclerosis, 35 (1980) 21.
28 Blum, C.B., Levy, R.I., Eisenberg, S., Hall, M., Goebel, R.H. and Berman, M., High density lipoprotein metabolism in man, J. Clin. Invest., 60 (1977) 795.
29 Schonfeld, G., Weidmann, S.W., Witztum, J.L. and Bowen, R.M., Alterations in levels and interrelations of plasma apolipoprotein induced by diet, Metabolism, 25 (1976) 261.
30 Levy, R.J., Lees, R.S. and Fredrickson, D.S., The nature of pre-beta (very low-density) lipoproteins, J. Clin. Invest., 45 (1966) 63.
31 Ernst, N., Fisher, M., Smith, W., Gordon, T., Rifkind, B.M., Little, J.A., Mishkel, M.A. and Williams, O.D., The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption, Circulation, 62 (1980) Suppl. IV, 41.
32 Knuiman, J.T., Hermus, R.J.J. and Hautvast, J.G.A.J., Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries, Atherosclerosis, 36 (1980) 529.
33 Albers, J.J., Cheung, M.C. and Hazzard, W.R., High-density lipoproteins in myocardial infarction survivors, Metabolism, 27 (1978) 479.
34 Gordon, T., Castelli, W.P., Hjørtland, M.C., Kannel, W.B. and Dawber, T.R., High density lipoprotein as a protective factor against coronary heart disease, Amer. J. Med., 62 (1977) 707.
35 Tall, A.R. and Small, D.M., Body cholesterol removal—Role of plasma high-density lipoprotein, Adv. Lipid Res., 17 (1980) 2.
36 Krauss, R.M., Lindgren, F.T., Wood, P.D., Haskell, W.L., Albers, J.J. and Cheung, M.C., Differential increases in plasma high density lipoprotein subfractions and apolipoproteins (apo-LP) in runners, Circulation, 56 (1977) III-4.
37 Anderson, D.W., HDL cholesterol–The variable components, Lancet, i (1978) 819.
38 Mancini, M., Mattock, M., Rabaya, E., Chait, A. and Lewis, B., Studies of the mechanisms of carbohydrate-induced lipaemia in normal man, Atherosclerosis, 17 (1973) 445.
39 Beveridge, J.M.R., Jagannathan, S.N. and Ford Connell, W., The effect of the type and amount of dietary fat on the level of plasma triglycerides in human subjects in the postabsorptive state, Canad. J. Biochem. Physiol., 42 (1964) 999.
40 Durnin, J.V.G.A. and Rahaman, M.M., The assessment of the amount of fat in the human body from measurements of skinfold thickness, Brit. J. Nutr., 21 (1967) 681.
41 Durnin, J.V.G.A. and Womersley, J., Body fat assessed from total body density and its estimation from skinfold thickness—Measurements on 481 men and women aged from 16 to 72 years, Brit. J. Nutr., 32 (1974) 77.