Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat

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Abstract A 12-week dietary intervention was carried out among 40 families from North Karelia, a county in Finland with an exceptionally high rate of coronary heart disease and high serum cholesterol values. The proportion of dietary energy derived from fat was reduced during the 12-week intervention period from about 39% to 23% in all families. The families were randomly allocated into two groups. Twenty families consumed a diet with a polyunsaturated to saturated fat (P/S) ratio of 0.9 (group I), while the other 20 families had a diet with a P/S ratio of 0.4 (group II). Total serum cholesterol decreased by 16% and 9% in men of groups I and II, respectively, and by 16% in women of both groups. These changes were due to a decrease in both low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol. LDL cholesterol and phospholipid reached minimum values after 6 weeks on both intervention diets, but LDL protein responded more slowly. Thus, after 6 weeks LDL had an altered composition containing less cholesterol and phospholipids and more protein and triglycerides than during the baseline diet. During the intervention, the linoleic acid content in the serum cholesteryl ester fraction increased, and the magnitude of this change correlated negatively with the changes in total and LDL cholesterol. The decrease in HDL cholesterol during the two intervention diets was due to a fall in the HDL_2 cholesterol (29% and 24% in men, and 26% and 25% in women in groups I and II, respectively). In conclusion our results indicate that the effects of a low-fat diet on serum LDL and HDL concentrations are not augmented by an increase of the P/S ratio from 0.4 to 0.9.—**Kuusi, T., C. Ehnholm, J. K. Huttunen, E. Kostiainen, P. Pietinen, U. Leino, U. Uusitalo, T. Nikkari, J. M. Iacono, and P. Puska.** Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat. *J. Lipid Res.* 1985. **26:** 360–367.

Supplementary key words diet intervention • P/S ratio • LDL • HDL • cholesterol

Epidemiological studies have demonstrated a positive association between serum total cholesterol and the incidence of coronary heart disease (CHD) (1). The atherogenicity of serum cholesterol depends on its distribution among lipoproteins of low and high density (LDL and HDL, respectively). Thus, serum LDL cholesterol has been shown to be the component responsible for the positive association between total cholesterol and CHD incidence, whereas the concentration of HDL cholesterol is inversely related to the risk of CHD.

Serum total and LDL cholesterol can be influenced by diet modification (2). It is well established that populations with high intake of saturated fat and cholesterol and low intake of polyunsaturated fats have higher serum cholesterol concentrations and also run a higher risk of CHD than those with a high polyunsaturated/saturated fat (P/S) ratio in their diets (3). However, the specific mechanisms by which saturated fats raise and polyunsaturated fats lower serum cholesterol concentration are not known.

Dietary factors may also influence serum HDL cholesterol concentration. Thus, HDL cholesterol has been reported to decrease in response to reduced fat intake (4–7). Furthermore, a positive correlation has been described between HDL cholesterol and total cholesterol in population comparisons (8). On the other hand, little is known about the effect of the dietary P/S ratio on the plasma HDL cholesterol level.

We have recently reported on the effects of a modified diet on serum lipoproteins and blood pressure in the population of North Karelia, a county in Finland with an exceptionally high incidence of CHD (6, 7, 9). A reduction in the fat intake with a concomitant increase of the P/S ratio from 0.2 to 1.2 resulted in a prominent fall of serum total cholesterol, LDL cholesterol, and HDL cholesterol concentrations. These changes were reversed after the return to the original diet. As a continuation of these studies we have now investigated the effect on serum lipids and lipoproteins of two low-fat diets, one with a polyun-

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; P/S ratio, ratio of polyunsaturated to saturated fatty acids; CHD, coronary heart disease.

saturated to saturated fat ratio of 0.9 and the other with a ratio of 0.4.

METHODS

Subjects

This study, conducted in spring 1983 in two semi-rural communities (Kitee and Tohmajärvi) in North Karelia, Finland, was carried out as part of a larger investigation on the effects of dietary modification on blood pressure. Subjects with known borderline or mild hypertension and their spouses were invited to take part in the study. After giving their written informed consents, they filled in a questionnaire. Forty-four couples who fulfilled the following criteria were included in the study: age between 35 and 49 years, no long-term use of medication including antihypertensives, and no specific treatment of hyperlipoproteinemia. Eighty subjects were randomly selected for the lipoprotein study because of ultracentrifugal rotor capacity. Two subjects were excluded from the calculations, one because of major health problems and one because of alcohol abuse. Thus, 78 persons completed the investigation.

Study design

The families were randomly allocated into two groups. Both groups underwent a base-line period of 2 weeks, a 12-week intervention period, and a 5-week switch-back period. During the intervention period the families in group I were advised to consume a diet containing about 25% of energy as fat and a P/S ratio of 1.0–1.2. Group II was instructed to follow a diet with a similar reduction in fat (to 25%), but a P/S ratio between 0.4 and 0.5. During the base-line and switch-back periods, all families were asked to consume their usual diets. Otherwise, the families were requested to make no other changes in their habits (e.g., in smoking, exercise, or caloric intake) throughout the study.

Diets

The changes in the diets during the intervention period were accomplished by substituting low fat items for high fat foods typically consumed in North Karelia and by partially substituting polyunsaturated fats for saturated fats. Strategic food items in the experimental diets were skim milk, lean meat, low fat sausage, and low fat cheese. The use of vegetables, including beets, and berries and fruit was strongly encouraged. The difference between the two groups in the dietary P/S ratio was adjusted by providing the families with different fat spreads on a single-blind basis. Group I received margarine high in polyunsaturated fats, whereas group II received a mixture of butter and margarine, prepared specially for this study. Factors in the diet such as salt, alcohol, and coffee were kept unchanged.

Dietitians visited the families at least twice a week to supervise their adherence to the diet regimens and to other study requirements. During these visits, the dietitians brought the food items of strategic importance, advised the families in the practical management of their diet, and checked the food consumption records.

Methods

The families kept a food consumption record for 47 predetermined days, and those of 7 base-line, 12 intervention, and 10 switch-back period days were coded and used for further analysis. The weights and volumes of all food items were measured and recorded. The records were coded by the dietitians and the data was processed using Finnish food composition tables and a computer program developed for this purpose (10). A venous blood sample was obtained after an overnight fast for lipid and lipoprotein analysis at the end of each period and in the middle of the intervention period. Lipoproteins were separated using a Beckman L7-70 ultracentrifuge (Beckman Instruments, Palo Alto, CA) operated at 4°C (11). A Kontron TFT 45.6 rotor for forty tubes was used (Kontron AG, Basel, Switzerland). A running time of 18 hr at a density of 1.006 g/ml and at 38,000 rpm was used for the isolation of VLDL; 24 hr at d 1.063 g/ml and at 42,000 rpm for the isolation of LDL; and finally, 64 hr at d 1.125 g/ml and 42,000 rpm for the isolation of HDL_2. The bottom fraction of the 1.125 g/ml ultracentrifugation was taken to represent the lipids of HDL_3. The recovery of cholesterol in the fractions was always greater than 92% with a mean of 95.5%. No corrections were, therefore, considered to be necessary.

Cholesterol and triglyceride concentrations in serum and lipoprotein fractions were determined using Boehringer Kits No. 263 691 and 297 771 (Boehringer GmbH, Mannheim, FRG), respectively, in a Kone Olli-C Discrete Analyzer (Kone Ltd, Espoo, Finland). Phospholipids were determined enzymatically using a Wako B-Phospholipids kit No 279-54009 (Wako Pure Chemical Industries Ltd., Osaka, Japan). Protein was measured according to Lowry et al. (12). The fatty acid composition of serum lipid fractions was determined by gas–liquid chromatography in an open tubular fused silica column coated with OV.351 and using a splitless injection technique. The methods used for isolation of the lipids and quantification of the methyl esters in a flame ionization detector have been described earlier (13). The detailed results of the fatty acid analysis will be presented elsewhere, and only the percentage of linoleic acid in the fractions is included in the present report. The internal laboratory control was arranged according to good laboratory practice by including both commercial lyophilized control samples and

samples from our own serum pools (stored at −20°C) into each set of analyses.

Statistical analysis

The results are expressed as means ± SEM. Differences in the mean values were tested using Student's *t*-test or *t*-test for paired data.

RESULTS

The compositions of the diets in the two groups at different stages of the study are shown in **Table 1**. The proportion of energy obtained as fat decreased from 37% to 23% in Group I and from 38% to 24% in Group II. The decrease in fat intake was due to approximately 50% reductions in the percentage of daily energy derived from saturated fats and mono-unsaturated fats. The P/S ratio increased to 0.93 and 0.41 in groups I and II, respectively, during the intervention period. During the switch-back period, it returned to the pre-intervention level (0.21–0.22). The estimated caloric intake decreased during the intervention in both groups. However, only slight changes occurred in the mean body weight (72.6 ± 1.9, 71.1 ± 1.9, and 70.9 ± 1.9 kg in Group I and 76.9 ± 2.2, 75.3 ± 2.2, and 75.0 ± 2.2 kg in Group II at the end of the three study periods, respectively).

The intervention resulted in highly significant decreases in the serum concentrations of total, LDL, and HDL cholesterol in both men and women (**Table 2**), whereas the changes in serum and VLDL triglycerides were not statistically significant. The reductions in total, LDL, and HDL cholesterol were similar in magnitude in the two groups despite different dietary P/S ratios during the intervention, and were evident already in the middle of the intervention period. The decrease in HDL cholesterol was due to a fall in the concentration of HDL_2 cholesterol (Table 2). Thus, HDL_2 cholesterol decreased by 29% and 24% in men and by 26% and 25% in women of groups I and II, respectively. The changes in the serum level of HDL_3 cholesterol were not consistent. HDL and HDL_2 cholesterol returned to their initial level during the switch-back, whereas total and LDL cholesterol remained at a level somewhat lower than that observed during the baseline period. No correlation existed between the changes in LDL cholesterol and HDL_2 cholesterol during the study.

The dietary intervention influenced both the apoprotein and lipid components of LDL but the rates of the changes were not similar (**Table 3**). LDL cholesterol and phospholipids decreased during the first 6 weeks, whereas the decrease of LDL-protein was evident only after the whole 12 weeks intervention. Thus LDL contained less cholesterol and phospholipids and more triglycerides and protein after 6 weeks on the low-fat, high P/S ratio diet than during the basal diet. No difference in LDL composition was observed between the two intervention diets with the different P/S ratios (**Table 4**).

The effect of the diets on serum lipids and lipoproteins was also evaluated by observing the changes in their concentrations within the families. During the baseline period there was no correlation between spouses in any serum or lipoprotein lipid concentration (**Table 5**). The changes in LDL cholesterol occurring during the first 6

TABLE 1. Composition of diet and mean daily nutrient uptakes, calculated on the basis of food consumption records for the participants in Groups I and II

			Intervention		
	Group	Baseline	6 Weeks	12 Weeks	Switch-Back
Protein[a]	I	15 ± 0.2	20 ± 0.4	19 ± 0.4	15 ± 0.3
	II	15 ± 0.2	19 ± 0.4	19 ± 0.3	15 ± 0.3
Carbohydrate[a]	I	46 ± 0.9	56 ± 0.9	58 ± 1.0	47 ± 0.8
	II	47 ± 0.9	55 ± 1.1	56 ± 1.1	46 ± 0.9
Fat[a]	I	38 ± 0.9	24 ± 0.5	22 ± 0.7	37 ± 0.7
	II	38 ± 0.9	25 ± 0.8	23 ± 0.7	37 ± 0.6
Saturated fat[a]	I	20 ± 0.7	9 ± 0.3	8 ± 0.3	19 ± 0.5
	II	20 ± 0.5	11 ± 0.4	10 ± 0.4	19 ± 0.5
Monounsaturated[a]	I	13 ± 0.3	6 ± 0.2	6 ± 0.2	12 ± 0.3
	II	12 ± 0.4	8 ± 0.3	7 ± 0.2	12 ± 0.2
Polyunsaturated[a]	I	4 ± 0.3	8 ± 0.3	7 ± 0.3	4 ± 0.2
	II	4 ± 0.1	5 ± 0.2	4 ± 0.2	4 ± 0.2
P/S ratio	I	0.2 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.2 ± 0.0
	II	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
Ethanol[a]	I	1 ± 0.2	1 ± 0.3	1 ± 0.3	1 ± 0.3
	II	1 ± 0.4	1 ± 0.6	1 ± 0.5	1 ± 0.5
Cholesterol (mg/day)	I	466 ± 28	277 ± 17	283 ± 14	480 ± 27
	II	533 ± 29	368 ± 19	351 ± 21	535 ± 25
Energy (kcal)	I	2556 ± 118	2169 ± 89	2302 ± 90	2744 ± 119
	II	2659 ± 110	2250 ± 94	2329 ± 91	2788 ± 118

[a]Results are expressed as the mean percentage of daily energy derived from each nutrient ± SEM.

TABLE 2. Serum and lipoprotein triglyceride and cholesterol concentrations before and after 6 weeks of a low-fat diet with a P/S ratio of 0.9 (Group I) or 0.4 (Group II), and after 5 weeks of switch-back period

	Group		Baseline Period	Intervention Period 6 Weeks	Intervention Period 12 Weeks	Switch-Back Period
			mg/dl ± SEM			
Men						
Serum triglycerides	I	(19)[a]	119 ± 15	125 ± 15	120 ± 13	110 ± 13
	II	(19)	132 ± 25	134 ± 18	134 ± 17	119 ± 15
VLDL triglycerides	I	(19)	61 ± 10	68 ± 10	68 ± 10	59 ± 11
	II	(19)	73 ± 9	77 ± 14	78 ± 14	66 ± 12
Serum cholesterol	I	(19)	250 ± 13	205 ± 9***	210 ± 8	232 ± 8***
	II	(19)	263 ± 12	229 ± 8***	238 ± 11	252 ± 11**
LDL cholesterol	I	(19)	163 ± 9	133 ± 6***	135 ± 6	148 ± 6*
	II	(19)	175 ± 11	150 ± 9***	156 ± 11	160 ± 10
HDL cholesterol	I	(19)	56 ± 5	47 ± 3***	46 ± 3	54 ± 4***
	II	(19)	56 ± 5	50 ± 2**	50 ± 2	56 ± 2***
HDL_2 cholesterol	I	(19)	29 ± 3	23 ± 2**	20 ± 2	29 ± 3***
	II	(19)	29 ± 3	24 ± 2*	22 ± 2	31 ± 2***
HDL_3 cholesterol	I	(19)	27 ± 1	24 ± 2**	26 ± 1*	26 ± 1
	II	(19)	28 ± 1	26 ± 1*	28 ± 1*	25 ± 1*
Women						
Serum triglycerides	I	(20)	76 ± 3	91 ± 8*	73 ± 7***	80 ± 6
	II	(20)	85 ± 8	94 ± 11	93 ± 9	83 ± 6
VLDL triglycerides	I	(19)	28 ± 2	40 ± 7*	30 ± 5**	32 ± 5
	II	(20)	36 ± 5	44 ± 8	41 ± 5	34 ± 4
Serum cholesterol	I	(20)	229 ± 9	187 ± 7***	194 ± 8	214 ± 7***
	II	(20)	232 ± 8	196 ± 5***	202 ± 7	219 ± 7***
LDL cholesterol	I	(19)	142 ± 8	111 ± 6***	118 ± 8	124 ± 7
	II	(20)	145 ± 6	122 ± 5***	123 ± 5	131 ± 6*
HDL cholesterol	I	(19)	67 ± 3	54 ± 2***	56 ± 2	67 ± 3***
	II	(20)	66 ± 3	56 ± 3***	56 ± 2	65 ± 3***
HDL_2 cholesterol	I	(19)	44 ± 3	33 ± 2***	32 ± 2	46 ± 3***
	II	(20)	42 ± 3	34 ± 2***	32 ± 2	43 ± 3***
HDL_3 cholesterol	I	(19)	23 ± 1	21 ± 1*	23 ± 1**	21 ± 1**
	II	(20)	24 ± 1	22 ± 1**	24 ± 1*	22 ± 1*

[a]Number of subjects is in parentheses.
*Significantly different from the previous value ($P < 0.05$ by paired comparison test).
**Significantly different from the previous value ($P < 0.01$ by paired comparison test).
***Significantly different from the previous value ($P < 0.001$ by paired comparison test).

weeks of the intervention were significantly related within the couples, but such a relationship was no longer present when the 12-week intervention period was taken as a whole. On the other hand, the HDL_2 cholesterol changes both during the first 6 weeks and during the entire 12-week intervention period were significantly interrelated within the couples (Table 5). No correlation was observed between the changes in LDL protein within the family at any stage of the study (data not shown).

The fatty acid composition of triglycerides, cholesteryl esters, and phospholipids changed during the study so that their linoleic acid content increased during the dietary intervention and decreased during the switch-back period (**Table 6**). The linoleic acid content of the cholesteryl ester fraction, the main cholesterol-containing lipid in serum, was significantly related to total ($r = -0.36$, $P < 0.01$) and LDL ($r = -0.03$, $P < 0.01$) but not to HDL or HDL_2 cholesterol concentration during the baseline period. During the intervention period, the percentage of linoleic acid in the cholesteryl ester fraction increased in both groups ($P < 0.001$). The changes in serum and LDL cholesterol occurring during the first 6 weeks were significantly related to the change of linoleic acid in the cholesteryl ester fraction ($r = 0.44$, $P < 0.001$ and $r = 0.39$, $P < 0.01$, respectively). However, during the whole 12-week intervention the changes in these variables were no longer interrelated. No correlation was present between the changes in HDL and HDL_2 cholesterol level and those in the linoleic acid content of the cholesteryl ester fraction.

DISCUSSION

The results of this study confirm and extend our earlier observations on the effects of a low-fat diet on plasma lipoproteins in free-living subjects (6, 7). Thus, highly significant reductions in total cholesterol, LDL cholesterol, and

TABLE 3. Low density lipoprotein, cholesterol, triglyceride, phospholipid, and protein concentrations before and after 6 and 12 weeks of a low-fat diet with a P/S ratio of 0.9 (Group I) or 0.4 (Group II), and after 5 weeks switch-back period

	Group		Baseline Period	Intervention Period		Switch-Back Period
				6 Weeks	12 Weeks	
			mg/dl ± SEM			
Men						
Cholesterol	I	(19)[a]	163 ± 9	133 ± 6***	135 ± 6	148 ± 6*
	II	(19)	175 ± 11	150 ± 9	156 ± 11	160 ± 10
Triglyceride	I	(19)	33 ± 3	33 ± 3	32 ± 3	29 ± 2
	II	(19)	33 ± 3	35 ± 3	32 ± 2	29 ± 3
Phospholipid	I	(19)	103 ± 6	81 ± 4***	82 ± 3	94 ± 4***
	II	(19)	115 ± 6	92 ± 5***	95 ± 6	105 ± 7
Protein	I	(19)	103 ± 7	110 ± 7	95 ± 5***	94 ± 5
	II	(19)	113 ± 6	121 ± 6	104 ± 7*	95 ± 6
Women						
Cholesterol	I	(19)	142 ± 8	111 ± 6***	118 ± 8	124 ± 7
	II	(20)	146 ± 6	122 ± 5***	123 ± 5	131 ± 6*
Triglyceride	I	(19)	27 ± 1	28 ± 1	26 ± 1	23 ± 1**
	II	(20)	27 ± 2	31 ± 2*	33 ± 4	25 ± 2**
Phospholipid	I	(19)	92 ± 5	69 ± 3***	72 ± 5	76 ± 4
	II	(20)	91 ± 5	75 ± 3**	75 ± 4	84 ± 5**
Protein	I	(19)	94 ± 5	89 ± 7	79 ± 4	79 ± 4
	II	(20)	96 ± 4	95 ± 4	82 ± 4***	81 ± 4

[a]Number of subjects is in parentheses.
*Significantly different from the previous value ($P < 0.05$ by paired comparison test).
**Significantly different from the previous value ($P < 0.01$ by paired comparison test).
***Significantly different from the previous value ($P < 0.001$ by paired comparison test).

TABLE 4. Composition of low density lipoprotein fractions before and after 6 and 12 weeks of a low-fat diet with a P/S ratio of 0.9 (Group I) or 0.4 (Group II), and after 5 weeks switch-back period

	Group		Baseline Period	Intervention Period		Switch-Back Period
				6 Weeks	12 Weeks	
			% of LDL mass ± SEM			
Men						
Cholesterol	I	(19)[a]	40.6 ± 0.8	37.6 ± 0.6**	39.3 ± 0.4*	40.6 ± 0.5***
	II	(19)	39.8 ± 0.7	37.5 ± 0.7**	40.2 ± 0.4**	41.1 ± 0.9
Triglyceride	I	(19)	8.3 ± 0.6	9.1 ± 0.4	9.1 ± 0.5	8.0 ± 0.5***
	II	(19)	7.6 ± 0.6	8.9 ± 0.5***	8.4 ± 0.5	7.5 ± 0.5
Phospholipid	I	(19)	25.6 ± 0.6	22.8 ± 0.2***	23.9 ± 0.2**	25.8 ± 0.4***
	II	(19)	26.6 ± 0.6	23.1 ± 0.2***	24.5 ± 0.3***	27.0 ± 0.8*
Protein	I	(19)	25.5 ± 1.3	30.5 ± 0.5**	27.7 ± 0.3**	25.5 ± 0.5***
	II	(19)	26.0 ± 0.4	30.5 ± 0.8***	26.8 ± 0.3***	24.4 ± 0.8**
Women						
Cholesterol	I	(19)	39.7 ± 0.4	36.6 ± 0.6***	39.9 ± 0.4***	40.8 ± 0.6
	II	(20)	40.6 ± 0.5	37.8 ± 0.4***	39.3 ± 0.5*	41.0 ± 0.7*
Triglycerides	I	(19)	7.8 ± 0.3	9.4 ± 0.3***	9.2 ± 0.4	7.6 ± 0.3***
	II	(20)	7.5 ± 0.3	9.3 ± 0.5***	10.5 ± 0.9	7.6 ± 0.4**
Phospholipid	I	(19)	26.0 ± 0.7	23.1 ± 0.3**	24.0 ± 0.3*	25.6 ± 0.3**
	II	(20)	25.0 ± 0.6	23.3 ± 0.2*	24.1 ± 0.4	25.8 ± 0.5*
Protein	I	(19)	26.4 ± 0.4	31.0 ± 0.8***	26.8 ± 0.3***	26.1 ± 0.8
	II	(20)	26.9 ± 0.4	29.5 ± 0.3***	26.2 ± 0.4***	25.6 ± 0.6

[a]Number of subjects is in parentheses. The respective serum concentrations of LDL lipids are given in Table 3.
*Significantly different from the previous value ($P < 0.05$ by paired comparison test).
**Significantly different from the previous value ($P < 0.01$ by paired comparison test).
***Significantly different from the previous value ($P < 0.001$ by paired comparison test).

TABLE 5. Within family correlations between the baseline lipids, lipoproteins, and linoleic acid concentrations, and their respective changes during the study

Serum Lipid	Correlation Coefficient between the Spouses with Respect to: Baseline Concentration	Change in 6 Weeks	Change in 12 Weeks
Cholesterol	0.11	0.57***	0.13
Triglycerides	0.12	0.30	0.55***
VLDL-cholesterol	0.26	0.30	0.53***
VLDL-triglycerides	0.12	0.25	0.53***
LDL-cholesterol	0.06	0.55***	0.12
HDL_2-cholesterol	0.29	0.50**	0.68***
HDL_3-cholesterol	0.24	0.49**	0.60***
Linoleate of			
Triglycerides	0.39*	0.48**	0.19
Cholesteryl esters	0.57***	0.53***	0.39*
Phospholipids	0.52**	0.62***	0.51**

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

HDL_2 cholesterol, but not in HDL_3 cholesterol, were seen in 40 married couples when the fat content of their diet was lowered from 38% to 23% and the P/S ratio increased simultaneously from 0.2 to 0.4 or to 0.9. On the other hand, no difference was observed in the lipid and lipoprotein responses between the groups with different dietary P/S ratios and slightly different cholesterol intakes during the low-fat diet. These results indicate that a maximal decrease in serum cholesterol occurs already at a P/S ratio of 0.4 when the fat content of the diet is as low as it was (23%) in this study during the intervention period.

The mechanism by which dietary polyunsaturated fatty acids influence the metabolism of LDL is not clear. It has been suggested that polyunsaturates lower LDL cholesterol by expanding the space occupied by fatty acids in the cholesteryl esters and phospholipids of LDL (14). This hypothesis is based on studies (14–18) reporting a decrease in LDL cholesterol and phospholipids but no change in LDL protein during diets rich in polyunsaturated fatty acids. However, in more recent reports (19, 20) these observations have been disputed. In the present study a short-term (i.e., 6 weeks) dietary modification influenced LDL cholesterol and phospholipid but not protein. After 12 weeks of intervention, a decrease was evident also in LDL protein and the composition of LDL no longer was different from that observed during the base-line period. Similar observations have also been reported by Vega et al. (19), who found a decrease of LDL protein and no changes in the cholesterol to protein ratio in LDL after 8 weeks. It is thus possible that the reason for the conflicting results on the effect of diet on LDL composition is the length of the intervention period.

The present results are in accordance with the hypothesis of Spritz and Mischkel (14) on the space occupation in LDL by unsaturated cholesteryl esters also, in that there were significant correlations between the changes in cholesteryl ester linoleic acid and serum total and LDL cholesterol concentrations during the first phase of the study. However, during the second half of the intervention, this mechanism was no longer operating, since the LDL protein concentration also decreased.

A significant decrease in HDL_2 cholesterol was observed during the diet modifications. The change in HDL was, however, not correlated with the changes in the linoleate content of serum lipids suggesting that other

TABLE 6. Linoleic acid content of various serum lipid fractions as per cent of total fatty acids before and after 6 and 12 weeks of a low-fat diet with a P/S ratio of 0.9 (Group I) or 0.4 (Group II), and after 5 weeks switch-back period[a]

	Group		Baseline Period	Intervention Period: 6 Weeks	Intervention Period: 12 Weeks	Switch-Back Period
Men						
Triglyceride	I	(19)[b]	9.8 ± 0.6	20.0 ± 1.5***	20.3 ± 1.4	11.8 ± 0.7***
	II	(19)	10.7 ± 0.8	16.0 ± 0.8***	16.0 ± 1.3	11.1 ± 0.5***
Cholesteryl esters	I	(19)	45.9 ± 1.2	55.4 ± 1.8***	55.6 ± 1.2	49.6 ± 1.5***
	II	(19)	48.1 ± 0.8	52.0 ± 1.2**	53.1 ± 1.3	50.1 ± 0.9*
Phospholipids	I	(19)	19.9 ± 0.8	24.4 ± 1.0***	24.0 ± 0.8	21.9 ± 0.8*
	II	(19)	21.7 ± 0.5	23.0 ± 0.6	23.3 ± 0.7	22.1 ± 0.5
Women						
Triglycerides	I	(20)	10.7 ± 0.7	19.3 ± 1.0***	20.0 ± 1.4	13.0 ± 0.7***
	II	(20)	11.3 ± 0.5	15.6 ± 0.7***	17.3 ± 0.7	12.8 ± 0.6***
Cholesteryl esters	I	(20)	48.2 ± 0.8	55.2 ± 1.2***	56.2 ± 1.0	51.5 ± 0.9***
	II	(20)	49.6 ± 0.9	52.4 ± 0.8**	54.3 ± 1.0	51.7 ± 1.0*
Phospholipids	I	(20)	20.7 ± 0.5	23.9 ± 0.7***	24.9 ± 0.7	22.9 ± 0.5**
	II	(20)	21.8 ± 0.5	22.5 ± 0.5	23.6 ± 0.7	22.3 ± 0.6

[a]Data are presented as means ± SEM.
[b]Number of subjects is in parentheses.
*Significantly different from the previous value ($P < 0.05$ by paired comparison test).
**Significantly different from the previous value ($P < 0.01$ by paired comparison test).
***Significantly different from the previous value ($P < 0.001$ by paired comparison test).

factors than the degree of fatty acid unsaturation causes the decrease of HDL_2. Synthesis of apoA-I, the main protein constituent of HDL, occurs in the intestinal wall (21) and is regulated by the quantity of dietary fats (5, 22). The total fat intake decreased during both diet modifications, and this could be a reason for the decrease in HDL cholesterol concentration observed in the present study, as suggested by Vessby et al. (16).

No relationship was present between the LDL cholesterol concentrations or between the HDL cholesterol concentrations of the spouses in the baseline measurements. On the other hand, significant positive correlations were observed within families between *the changes* in LDL cholesterol level and between *the changes* in HDL cholesterol level during the first 6 weeks of the study diet. This indicates a similar response of the two spouses to the same dietary modification. During the second half of the intervention, the correlation between the LDL changes disappeared, suggesting that the subjects had adapted to the new diet in an individual way and through mechanisms that take longer than 6 weeks. In contrast, the correlation between the changes in HDL cholesterol further improved, suggesting that HDL cholesterol is more sensitive to environmental factors than LDL cholesterol, although full adaptation may require a relatively long time period (i.e., more than 6 weeks).

Little is known about how diet-induced changes in HDL and HDL subfractions affect the risk of coronary heart disease. It should be noted, however, that populations with low intake of dietary fats have lower LDL and HDL cholesterol concentrations and lower risk of CHD than those with higher fat intake (8, 23). On the other hand, the decrease in HDL observed during the low-fat high-P/S diet may be transient, as a dietary intervation similar to the one reported here after 4 years resulted in an increase in HDL cholesterol levels (24).

It should be emphasized that the dietary intervention in the present study included a decrease in total fat, saturated fat, and cholesterol, and an increase in the P/S ratio and in the intake of total carbohydrate. Thus, it is not possible to point out the exact reason for the observed changes in the concentrations and composition of serum lipoproteins. However, our data strongly suggest that the effects of a low-fat diet on serum LDL and HDL concentration and composition are not augmented by an increase in the P/S ratio.

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