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Effects of a prudent diet containing either lean beef and mutton or fish and skinless chicken on the plasma lipoproteins and fatty acid composition of triacylglycerol and cholesteryl ester of hypercholesterolemic subjects

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In this two-phase crossover study, 39 hypercholesterolemic subjects followed a prudent diet with either lean red meat or fish and skinless chicken (treatment groups), and 13 subjects (reference group) followed their habitual diet. Fasting blood samples were analyzed for plasma total cholesterol, triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein one- and two-cholesterol, apolipoprotein-B, very low density lipoprotein cholesterol, and very low density lipoprotein TAG, and fatty acid composition of plasma TAG and cholesteryl ester (CE). Body mass and blood pressure were determined. Seven-day dietary records were kept once at baseline and twice during the treatment periods. Significant differences were observed in dietary intake between the baseline and treatment diets and between the two treatment diets. HDL-C ($P < 0.05$*) and diastolic blood pressure (*$P < 0.01$*) were higher in patients on the red meat diet than in those on the chicken-fish diet. No other significant differences in lipoproteins were observed between the effects of the two treatment diets. The linoleic acid (%), eicosapentaenoic acid (%), and the eicosapentaenoic acid/arachidonic acid ratios in TAG and CE were higher (*$P < 0.01$*) in subjects on the chicken-fish diet than in those on the red meat diet. In conclusion, this study showed that the effect of two lipid-lowering diets containing either lean red meat or skinless chicken and fish on the atherogenic lipoproteins did not differ significantly. A prudent diet with skinless chicken and fish, however, had a more favorable effect on the fatty acid composition of the plasma TAG and the CE than did the lean red meat diet.* (J. Nutr. Biochem. 10:598–608, 1999)

Keywords: hypercholesterolemia; lipoproteins; prudent diet; lean red meat; chicken; fish

Introduction

Hypercholesterolemia is a major risk factor for the development of coronary heart disease (CHD), and dietary factors such as a high fat, especially saturated fatty acid intake, increase plasma cholesterol concentrations.[1–4] Dietary intervention should be the first step in the treatment of hypercholesterolemia, with the emphasis on the reduction of total fat and saturated fatty acid intake.[1–4]

The recommendation to lower saturated fat intake with a lipid-lowering diet implies that the intake of animal fat from foods such as red meat, milk, and milk products should be restricted. Red meat contributed substantially to the total fat, saturated fat, and dietary cholesterol intake of South Afri-

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cans consuming a Western diet[5] and known to have a very high prevalence of hypercholesterolemia.[6] In vegetarians, the intake of meat seems to be associated with increased plasma total cholesterol (TC) concentrations,[7] but the incidence of CHD may be 24% lower in lifelong vegetarians than in meat eaters.[8]

The perception still exists among many healthcare workers and members of the public that red meat should be excluded from the diet of hypercholesterolemic subjects. However, O'Dea et al.[9] showed that the fat, and not the meat itself, had a cholesterol elevating effect. When diets containing beef and pork were compared with diets of poultry and fish, no difference in the TC could be shown in free-living normolipidemic subjects.[10,11] O'Brien and Reiser[12] also did not find a difference in the effect of fish and poultry or red meat (beef, pork, lamb) on the plasma TC concentrations of free-living normolipidemic subjects. A study of 15 hyperlipidemic free-living men showed that the plasma TC concentration of hyperlipidemic patients could be lowered significantly by lipid-lowering diets that included a daily 180-g portion of very lean meat and meat products.[13] Scott et al.[14] also showed that a diet with lean beef or chicken and fish had similar effects on serum lipoproteins of 47 men with borderline hypercholesterolemia.

Although the above-mentioned studies compared the effect of red meat (mainly beef) with that of chicken and fish on plasma lipids and lipoproteins, they did not investigate the effect of these diets on the plasma fatty acid composition. It is known that the type of fat in the diet also influences the fatty acid composition of plasma[15,16] and that a positive association between the stearic acid and palmitic acid content of serum cholesteryl ester (CE) and CHD has been shown.[17]

This study was designed to compare the effect of two prescribed prudent diets containing either lean beef and mutton or skinless chicken and fish on plasma lipids and lipoproteins as well as on the fatty acid composition of plasma triacylglycerol (TAG) and CE of free-living subjects with elevated plasma cholesterol concentrations.

Subjects and methods

Study population

Seventy subjects between the ages of 20 to 53 years with age-related elevated blood cholesterol concentrations[18] were recruited from the male staff of the South African Medical Research Council (MRC) and from the male and female staff of an insurance company. Only one of the subjects was slightly below the low risk cut-off point. Baseline data for the subjects are summarized in *Table 1*.

Exclusion criteria for the study were: diabetes mellitus; plasma TAG concentrations above 4 mmol/L; lipid-lowering, anticoagulant, or antihypertensive treatment; intake of beta-blockers; and body mass index (BMI) of more than 30. Subjects were clinically evaluated by a medical doctor prior to the baseline period of the study to exclude those who did not meet the inclusion criteria.

The study was approved by the Ethics Committee of the MRC. Informed written consent was obtained from the subjects.

Table 1 Age, anthropometric data, plasma total cholesterol, and blood pressure measurements at baseline

Variables		Men (n = 36)	Women (n = 34)
Age (yr)	Mean	35.1	31.5
	SD	7.8	9.6
Height (m)	Mean	1.795	1.669
	SD	0.057	0.068
Body mass (kg)	Mean	81.0	65.7
	SD	8.9	9.0
Body mass index	Mean	25.1	23.6
	SD	2.2	3.1
Plasma total cholesterol (mmol/L)	Mean	5.75	5.65
	SD	0.97	1.25
Systolic blood pressure (mmHg)	Mean	121.4	114.4
	SD	8.8	7.5
Diastolic blood pressure (mmHg)	Mean	76.6	70.8
	SD	11.2	9.5

SD–standard deviation.

Study design

The study had a crossover design with two phases, each with a baseline period, a 6-week treatment period, and a washout period of approximately 3.5 months between phases (*Figure 1*). Subjects were matched for age, BMI, and TC and randomly allocated to either Treatment Group 1 (14 men, 14 women), Treatment Group 2 (15 men, 13 women), or the Reference Group (7 men, 7 women). Measurements taken during the study and the time intervals of the measurements are shown in *Figure 1*.

Dietary intake during the study

The treatment groups followed their habitual diet during the baseline and washout periods, and the treatment diet during the 6-week treatment periods.

A series of prudent diets ranging from 7,500 to 14,000 kilojoules (kJ) and differing in approximately 500 kJ was calculated to meet the energy needs of the subjects. Calculation of the number of food exchanges (i.e., milk, meat, bread, fat, fruit, and vegetables) allowed on the diet was based on the American Exchange List system[19] and the method described in the *Handbook of Clinical Dietetics*.[20] The portions of cooked lean red meat, skinless chicken, and fish varied between 120 g and 210 g per day, depending on the energy allowance.

Treatment Group 1 received a prudent diet with lean red meat (RMD) in Phase 1 and a prudent diet with skinless chicken and fish (CFD) in Phase 2. Treatment Group 2 received a CFD during Phase 1 and a RMD during Phase 2. Subjects consumed lean beef (5 times per week) and lean mutton (2 times per week) on the RMD and skinless chicken (5 times per week) and fish (hake 1 time per week and pilchards or tuna 1 time per week) on the CFD. The intake of eggs was restricted to a maximum of two per week. Alcohol intake was restricted to two drinks per day. Subjects were asked to avoid hard cheeses. The beef, mutton, chicken, and fish portions were provided free of charge during the treatment periods but subjects were responsible for providing the rest of the food exchanges. Every subject in Treatment Groups 1 and 2 received a personalized dietary prescription book containing information regarding the treatment diet.

The Reference Group followed their habitual diet throughout the study.

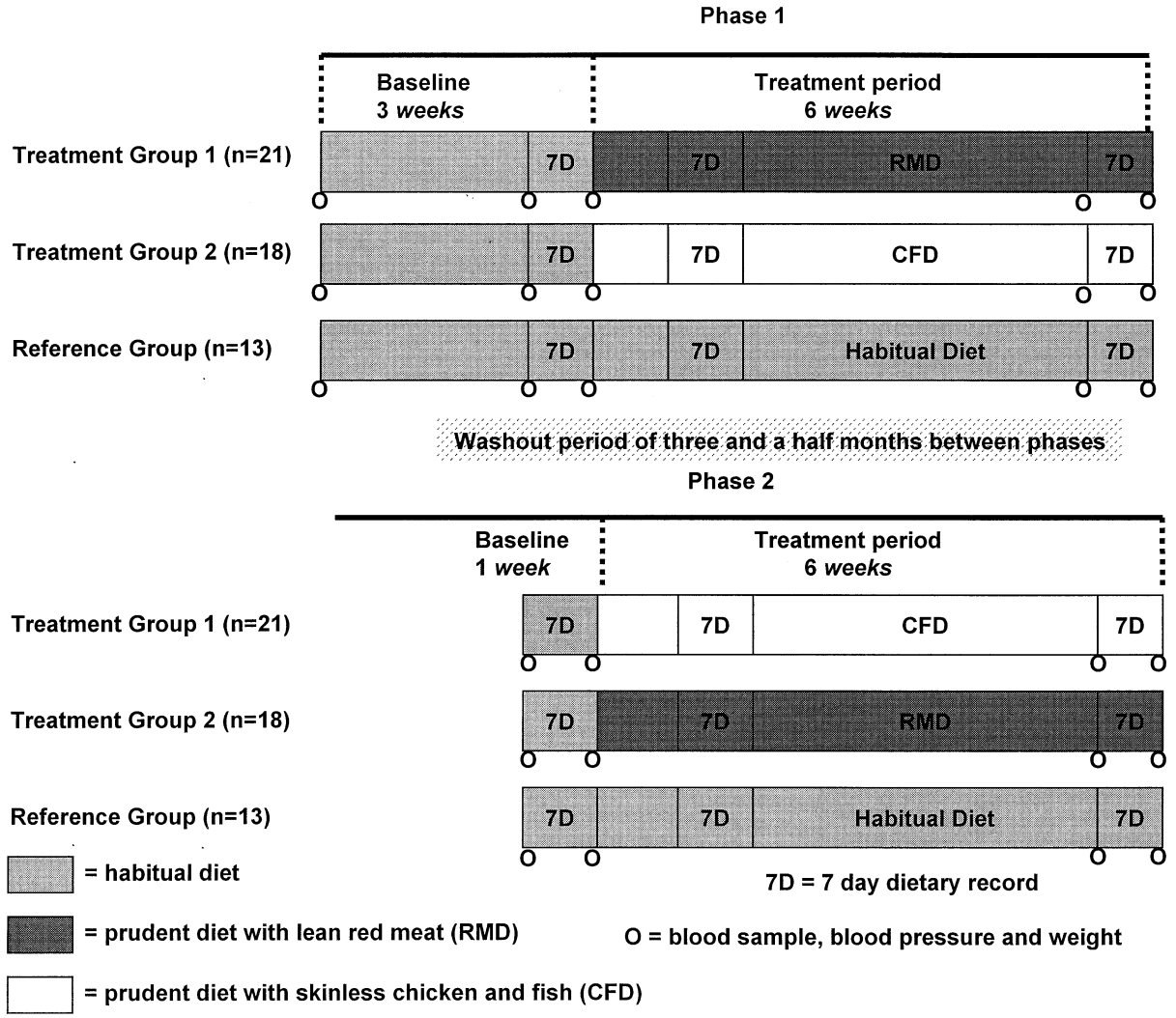

Figure 1 Study design

Measurements

Dietary data. Dietary information was collected by means of 7-day weighed and estimated dietary records, during the last week of the baseline period and during the second and sixth weeks of the treatment periods of Phases 1 and 2. Subjects received verbal and written instructions on record keeping with a "Weigh less" scale and on how to use household measures for quantification. Dietary records were checked after 2 days and again after completion of the 7-day dietary records to control for completeness of the records and for compliance with the dietary prescription.

The National Research Institute for Nutritional Diseases (NRIND) Food Composition Tables[21] were used for encoding the type of food eaten. If the food was not weighed, the NRIND Food Quantities Manual[22] was used to convert the amount of food reported in household measures into grams of food eaten. Dietary data were analyzed for energy, macronutrient, and fatty acid intake.

Evaluation of dietary compliance. Dietary compliance was determined by questionnaire at the end of Phase 2 of the crossover study.

Anthropometric data. Body mass in light clothing without shoes was measured with an ordinary bathroom scale to the nearest 0.5 kg. Height without shoes was measured to the nearest 0.1 cm with a special tape measure device fitted to a wall.

Blood pressure. Blood pressure was measured on the left arm with a vertical mercury manometer (Model 300 Baumanometer, WA Baum, NY USA) from subjects who were seated. The measurements were taken by the same three observers who were standardized against each other before the start of the study.

Blood sampling and analyses. Stasis-free fasting (12 hours) blood samples were collected in 8 mL evacuated glass tubes (1 mg $EDTA[K]_3$/mL blood).

TC, high density lipoprotein cholesterol (HDL-C), HDL_3-C, low density lipoprotein one and two cholesterol (LDL_1-C and LDL_2-C), and very low density lipoprotein cholesterol (VLDL-C) concentrations were determined enzymatically (Boehringer Mannheim, Cat. No. 237574). Precinorm® L (Boehringer Mannheim, Mannheim, Germany) was used as the external control for accuracy. Pooled serum was used as the internal control. A Boehringer Mannheim calibrator for automated systems 759350 was used to calibrate the RA1000 auto-analyzer.

The HDL were separated from the plasma by precipitation of the apoprotein B (apo B) containing lipoproteins with manganese

chloride/heparin.[23] The HDL_2 was precipitated with dextran sulphate (MW = 15,000) to separate HDL_3.[23] The HDL_2-C was calculated using the formula:

$$HDL_2\text{-}C = HDL\text{-}C - HDL_3\text{-}C^{23}$$

The VLDL fraction and intermediate density lipoprotein (IDL) fractions were isolated together as one fraction by preparative ultracentrifugation at a density of $d = 1.019$ in a titanium Beckman (Beckman, Palo Alto, CA USA) 40.3 rotor at 40,000 rpm and 10°C for 20 hours. LDL_1 (d = 1.019–1.030 g/mL) and LDL_2 (d = 1.030–1.063 g/mL) were isolated under the same conditions. Plasma TAG and VLDL-TAG were determined by an enzymatic colorimetric method (Boehringer Mannheim, Cat. No. 701904). A nephelometer was used for the turbidimetric determination of apo B in LDL_1 and LDL_2 using Boehringer Mannheim's anti-human-apo B antiserum (Cat. No. 726494).

Plasma TAG and CE fatty acid composition. For the analysis of the fatty acid composition of plasma TAG and CE, 300 μL plasma was extracted according to the method of Folch et al.[24] with chloroform-methanol (2:1 v/v). Butylated hydroxytoluene was used as anti-oxidant. TAG and CE were separated by thin-layer chromatography and the spots containing CE and TAG were scraped off and transmethylated by heating with 2.5 mL methanol/18 mol/L sulphuric acid (95:5; v/v) for 2 hours at 70°C.[25]

A Varian 3700 gas chromatograph (GC) (Varian Associates, Sunnyvale, CA USA) equipped with a flame ionization detector and a Varian CDS 402 data system were used to analyze fatty acid methyl esters, which were extracted with hexane. Separation was done with the GC using 30-m fused silica megabore DB-225 columns of 0.53 mm internal diameter (J & W Scientific, Folsom, CA USA, Cat. No. 125-2232). The following conditions were used: gas flow rates for hydrogen (carrier gas) were 5 to 8 mL/min; for medical air 250 mL/min; and for hydrogen 25 mL/min. The injector temperature was 240°C and the detector temperature 250°C. A mixture prepared from individual fatty acids (Sigma, St. Louis, MO USA) was used as reference standard. Fatty acid methyl esters of CE and TAG were identified by comparison with the retention times of those of the standard mixture.[26]

Statistical analysis

The analysis took the experimental design of the study into account, which consisted of two treatment groups in a two period crossover design plus a reference group which was also followed-up over two periods.

The three groups were compared at each baseline, and for the two active treatments, direct treatment-by-period interactions were tested. Under the assumption of no baseline differences and no treatment-by-period interaction, the direct treatment effect of the two active treatments was compared.[27] Only analysis for the comparisons between the treatment groups will be reported and discussed. Results of the reference group are available from the first author.

The standardized range test of Tukey was used for the baseline comparisons and the Student's *t*-test was used for all the other two sample comparisons.

The change from each baseline to the treatment period was computed for each individual and the mean changes were estimated. The data were analyzed with SAS (SAS Institute Inc., Cary, NC USA).

Results

Study population

Fifty-two subjects (74.3%) completed the crossover study: 21 subjects from Treatment Group 1, 18 from Treatment Group 2, and 13 from the Reference Group. The quality of the dietary data from two of the subjects was unacceptable and they were excluded from the analysis. Patients did not complete the study if they did not adhere to the dietary prescription (nine subjects); moved out of town (three); or had personal problems (four). In addition, there was incomplete data on one patient and one patient was excluded because she lost too much weight during the study.

Only results on the comparison between the RMD and the CFD will be reported below.

Compliance with dietary prescription

Energy intake, which was determined by means of the 7-day dietary records, was lower ($P < 0.001$) during the treatment periods than the energy that was prescribed. Subjective evaluation by the subjects indicated that 94.7% were of the opinion that their compliance with the dietary prescription was between 70% and 100%. Qualitative evaluation also showed that compliance with the dietary prescriptions was better in Phase 1 than in Phase 2. Red meat was consumed by 9 subjects while on the CFD, and 14 subjects on the RMD consumed chicken and 9 consumed fish. The frequency of transgression varied between one and three times during a 6-week treatment period. One subject consumed fish once per week on the RMD. The majority of subjects had a mean intake of one to two eggs per week during the treatment periods. Only three (7.9%) of the subjects indicated that they violated the prescription for alcohol consumption regularly (defined as more than three times per week).

Dietary intake

Baseline versus treatment periods. Significant differences between the baseline periods and the treatment periods were found for the RMD and the CFD. Energy intake (kJ), total fat as a percentage of total energy intake (%E), saturated fat (%E), monounsaturated fat (%E), dietary cholesterol intake, and the Keys dietary score were lower ($P = 0.0001$) on the RMD and the CFD than on the baseline diet (*Table 2*). Compared with the baseline diet, the intake of eicosapentaenoic acid (EPA) was lower on the RMD ($P = 0.0001$) but higher on the CFD ($P = 0.0006$). Protein (%E) and carbohydrate (%E) intake, as well as the polyunsaturated to saturated fatty acid (P/S) ratio of the diet, was higher ($P = 0.0001$) on the RMD and the CFD than on the baseline diet. Fiber and polyunsaturated fat intake did not differ significantly between the RMD and the baseline diet, but was higher on the CFD than on the baseline diet ($P = 0.0121$; $P = 0.0051$). Alcohol intake did not differ between the treatment and the baseline diets (*Table 2*).

Crossover analysis. No significant differences for the baseline comparisons of energy and macronutrient intake were observed, which indicated no first-order carryover

Table 2 Difference in energy, macronutrient and fatty acid intake, polyunsaturated to saturated fatty acid ratio, and Keys dietary score between the baseline period and treatment period

	Red meat diet (n = 37)					Chicken and fish diet (n = 37)				
			Difference*					Difference		
Dietary variables	Baseline Mean	Treatment Mean	Mean	SD	P-value	Baseline Mean	Treatment Mean	Mean	SD	P-value
Energy (kJ)	9,036.34	7,495.5	1,540.9†	1,567.6	0.0001	8,912.1	6,926.7	1,985.4†	1,788.0	0.0001
Protein (%E)	15.3	17.2	1.8	2.9	0.0002	15.0	17.7	2.7	3.2	0.0001
Carbohydrate (%E)	46.4	51.4	5.0	8.2	0.0003	46.9	54.7	7.8	5.9	0.0001
Fibre (g)	19.5	20.7	1.3	7.3	0.1394	17.9	20.5	2.6	6.1	0.0121
Total fat (%E)	36.5	29.8	6.7†	6.3	0.0001	36.8	26.9	9.9†	4.2	0.0001
Saturated fat (%E)	12.4	8.8	3.6†	3.4	0.0001	12.2	6.5	5.8†	2.0	0.0001
Myristic acid (g)	2.4	1.2	1.2†	1.4	0.0001	2.2	0.7	1.5†	0.8	0.0001
Palmitic acid (g)	14.5	8.9	5.6†	5.3	0.0001	14.0	6.2	7.9†	4.0	0.0001
Stearic acid (g)	7.4	4.7	2.7†	3.3	0.0001	7.0	2.8	4.2†	2.0	0.0001
Monounsaturated fat (%E)	13.1	10.2	2.8†	2.6	0.0001	13.2	7.9	5.3†	2.1	0.0001
Oleic acid (g)	26.4	16.6	9.8†	9.7	0.0001	26.2	11.8	14.4†	8.9	0.0001
Polyunsaturated fat (%E)	8.0	8.1	0.1	2.7	0.6411	8.3	9.6	1.3	2.5	0.0051
Linoleic acid (g)	13.3	12.9	0.4†	5.4	0.6517	15.1	13.5	1.6†	7.0	0.2408
Eicosapentaenoic acid (g)	0.05	0.00	0.04†	0.07	0.0001	0.05	0.10	0.06	0.12	0.0006
P/S ratio	0.68	0.94	0.26	0.36	0.0001	0.70	1.50	0.80	0.53	0.0001
Dietary cholesterol (mg)	298.6	219.0	79.7†	99.1	0.0001	280.0	187.3	92.7†	73.8	0.0001
Alcohol (%E)	3.6	3.3	0.3†	2.5	0.8551	3.2	2.8	0.4†	2.3	0.4194
Keys dietary score (28)	38.8	29.1	9.7†	10.7	0.0001	37.4	20.6	16.8†	8.8	0.0001

*Difference between treatment and baseline period.
†Negative result, indicating that baseline value was higher than treatment value.
SD–standard deviation. %E–percentage of total energy intake; P/S–polyunsaturated to saturated fatty acid ratio.

effect at the time of the second baseline period. There was also no difference in treatment carryover at the time of the second order treatment measurement, which is the direct-by-period interaction effect.

Direct treatment effect (RMD versus CFD). The direct treatment effect refers to the effects of the RMD and the CFD, within the crossover design, on dietary intake. The intake of energy, total fat (%E), total saturated fatty acid (SFA; %E) and the SFAs myristic (g), palmitic (g), and stearic acids (g), total monounsaturated fatty acids (MUFA; %E) and oleic acid (g), dietary cholesterol, and the Keys dietary score were significantly higher on the RMD than on the CFD. Carbohydrate intake (%E), polyunsaturated fatty acid (PUFA) intake (%E), and the long chain PUFAs EPA (g), docosapentaenoic acid (DPA; g) and docosahexaenoic acid (DHA; g), and the P/S ratio of the diet were significantly higher on the CFD than on the RMD. Protein, fiber and linoleic acid intake did not differ between the two diets (*Table 3*).

Anthropometric, plasma lipid and lipoprotein, and blood pressure data

Baseline versus treatment periods. In *Table 4* mean values for the baseline and treatment periods as well as differences in body mass, plasma lipids and lipoproteins, and blood pressure measurements between the baseline and the treatment periods are shown. TC, LDL_1-C, LDL_2-C, and LDL_1-apo B and LDL_2-apo B were significantly lower on the RMD and the CFD than on the baseline diet. Body mass was lower (P = 0.0001) on the CFD than on the baseline diet. The HDL-C:TC ratio was higher on both treatment diets than on the baseline diet. Diastolic blood pressure was higher (P = 0.0286) on the RMD than on the baseline diet.

The percentage of stearic acid and oleic acid in plasma TAG and CE tended to be lower on the treatment diets than on the baseline diet (*Tables 5 and 6*). In contrast, the percentage of linoleic acid (LA; 18:2ω6) in plasma TAG was higher on the CFD (P = 0.0001) than on the baseline diet (*Table 5*). There was no significant difference in the percentage of LA in plasma CE between the baseline and treatment diets (*Table 6*).

Crossover analysis. Analysis of the plasma lipoproteins showed a significant carryover effect for VLDL-TAG. No other significant differences for the baseline comparisons or for the direct-by-period interaction effect were observed. Significant carryover effects (baseline comparisons) for palmitoleic acid (16:1; P = 0.0159) and linolenic acid (18:3ω3; P = 0.0127) in the plasma TAGs were observed. No other significant differences for the baseline comparisons or for the direct-by-period interaction effect were observed for the fatty acid composition of the plasma TAGs. No significant first-order carryover effects (baseline comparisons) or second-order carryover effects (direct-by-period interaction effects) were observed for the fatty acid composition of the plasma CE.

Direct treatment effect (RMD versus CFD). In *Table 7* the direct treatment effect of the RMD and the CFD on the plasma lipids and lipoproteins and blood pressure is given. There was a direct treatment effect for HDL-C (P = 0.0498), which was higher on the RMD than on the CFD. The effect was, however, very small (0.03 mmol/L). A

Table 3 Direct treatment effect between red meat diet and chicken-fish diet: Energy, macronutrient and fatty acid intake, polyunsaturated to saturated fatty acid ratio, and Keys dietary score (n = 37)*

Dietary variable	Difference between means†	95% Confidence interval	*P*-value
Energy (kJ)	284.0‡	−504.0– −64.0	0.0131
Protein (%E)	0.3	−0.3–0.8	0.3286
Carbohydrate (%E)	1.6	0.7–2.5	0.0008
Fibre (g)	0.1‡	−0.9–0.7	0.8087
Total fat (%E)	1.5‡	−2.0– −1.0	0.0001
Saturated fat (%E)	1.2‡	−1.4– −1.0	0.0001
Myristic acid (g)	0.27‡	−0.35– −0.18	0.0001
Palmitic acid (g)	1.39‡	−1.84– −0.94	0.0001
Stearic acid (g)	0.98‡	−1.23– −0.73	0.0001
Monounsaturated fat (%E)	1.2‡	−1.5– −0.9	0.0001
Oleic acid (g)	2.44‡	−3.35– −1.54	0.0001
Polyunsaturated fat (%E)	0.8	0.4–1.1	0.0001
Linoleic acid (g)	0.30	−0.47–1.07	0.4308
Eicosapentaenoic acid (g)	0.05	0.04–0.07	0.0001
Docosapentaenoic acid (g)	0.02	0.02–0.02	0.0001
Docosahexaenoic acid (g)	0.12	0.08–0.16	0.0001
P/S ratio	0.28	0.22–0.35	0.0001
Dietary cholesterol (mg)	15.8‡	−25.0– −6.7	0.0012
Keys dietary score (28)	4.25‡	−5.51– −2.98	0.0001

*Treatment Group 1 = Red meat Phase 1, Chicken-fish Phase 2; Treatment Group 2 = Chicken-fish Phase 1, Red meat Phase 2.
†Estimated treatment effect: Treatment Group 1–Treatment Group 2.
‡Negative result, indicating that the value on the red meat diet was higher than the value on the chicken-fish diet.
%E–percentage of total energy intake; P/S ratio–polyunsaturated to saturated fatty acid ratio.

direct treatment effect (P = 0.0027) between the RMD and the CFD was also observed for diastolic blood pressure. Diastolic blood pressure was significantly lower on the CFD than on the RMD but the difference was small, approximately 1.6 mmHg. Significant differences between the RMD and the CFD were observed for the fatty acid composition of plasma TAG and CE (*Table 8*). In TAG the percentage of stearic acid and oleic acid was significantly higher on the RMD than on the CFD whereas LA, EPA, DPA, and DHA were significantly higher on the CFD than on the RMD. A similar picture was observed for CE, but the percentages of palmitoleic acid and arachidonic acid (AA; 20:4ω6) were also higher (P = 0.0093) on the RMD than on the CFD. No difference between the two treatment diets was found for DPA. The EPA/AA ratio (P = 0.0003) and the P/S ratio (P = 0.0027) of plasma TAG and the EPA/AA

Table 4 Difference in body mass, plasma lipids and lipoproteins, and blood pressure between the baseline period and the treatment period

Body mass, lipids and lipoproteins, and blood pressure	Red meat diet (n = 39)					Chicken-fish diet (n = 39)				
	Baseline Mean	Treatment Mean	Difference* Mean	SD	*P*-value	Baseline Mean	Treatment Mean	Difference Mean	SD	*P*-value
Body mass (kg)	73.0	72.3	0.7†	2.0	0.0651	73.5	72.3	1.2†	1.6	0.0001
TC (mmol/L)	5.41	5.12	0.29†	0.63	0.0084	5.48	5.03	0.45†	0.55	0.0001
HDL-C (mmol/L)	1.34	1.35	0.004	0.13	0.8965	1.31	1.29	0.03†	0.14	0.2354
HDL-C:TC ratio	0.26	0.27	0.01	0.03	0.0170	0.25	0.26	0.02	0.04	0.0025
HDL_2-C (mmol/L)	0.24	0.21	0.03†	0.09	0.0362	0.20	0.20	0.004	0.11	0.7448
HDL_3-C (mmol/L)	1.10	1.14	0.04	0.15	0.1371	1.11	1.08	0.03†	0.13	0.0267
LDL_1-C (mmol/L)	0.42	0.34	0.08†	0.13	0.0003	0.44	0.35	0.09†	0.14	0.0003
LDL_2-C (mmol/L)	3.10	2.84	0.27†	0.49	0.0014	3.17	2.73	0.44†	0.55	0.0001
LDL_1-apo B (mg/dL)	6.44	5.26	1.18†	2.13	0.0014	6.56	5.67	0.89†	2.18	0.0133
LDL_2-apo B (mg/dL)	60.54	52.23	8.32†	10.90	0.0001	63.50	52.04	11.46†	9.69	0.0001
VLDL-C (mmol/L)	0.49	0.49	0.002	0.22	0.8537	0.49	0.47	0.02†	0.15	0.3111
TAG (mmol/L)	1.03	1.06	0.03	0.39	0.5353	1.14	1.06	0.08†	0.32	0.3199
VLDL-TAG (mmol/L)	0.55	0.57	0.02	0.26	0.5370	0.63	0.58	0.05†	0.23	0.3079
Blood pressure										
Systolic (mmHg)	117.2	117.6	0.44	6.84	0.8935	117.1	115.8	1.23†	8.59	0.4242
Diastolic (mmHg)	73.4	75.5	2.18	5.91	0.0286	73.3	72.2	1.18†	6.91	0.3489

*Difference between treatment and baseline period.
†Negative result, indicating that baseline value was higher than treatment value.
SD–standard deviation. TC–total cholesterol. HDL-C–high density lipoprotein cholesterol. LDL-C–low density lipoprotein cholesterol. apo B–apolipoprotein B. VLDL-C–very low density lipoprotein cholesterol. TAG–triacylglycerols.

Table 5 Difference in mean fatty acid composition of plasma triacylglycerols of subjects between the baseline and treatment periods

Triacylglycerol†	Red meat diet (n = 39)					Chicken-fish diet (n - 39)				
	Baseline Mean	Treatment Mean	Difference* Mean	SD	P-value	Baseline Mean	Treatment Mean	Difference Mean	SD	P-value
Palmitic acid (16:0)	23.10	22.99	0.11‡	3.46	0.4254	24.03	22.29	1.75‡	2.19	0.0001
Palmitoleic acid (16:1ω-9)	3.07	3.73	0.66	1.31	0.0037	3.64	3.66	0.02	1.23	0.8803
Stearic acid (18:0)	4.04	3.43	0.61‡	1.36	0.0040	3.74	2.99	0.75‡	1.13	0.0001
Oleic acid (18:1ω-9)	40.12	39.14	0.98‡	4.39	0.1957	39.75	36.73	3.01‡	4.48	0.0001
Linoleic acid (18:2ω-6)	25.07	26.94	1.88	6.99	0.0547	24.76	29.55	4.79	6.08	0.0001
Linolenic acid (18:3ω-3)	0.48	0.38	0.09‡	0.19	0.0020	0.40	0.39	0.01‡	0.19	0.5190
Eicosatrienoic acid (20:3ω-6)	0.33	0.34	0.01	0.15	0.8701	0.35	0.33	0.02‡	0.18	0.6265
Arachidonic acid (20:4ω-6)	1.91	1.62	0.28‡	0.96	0.0646	1.62	1.66	0.03	0.46	0.6810
Eicosapentaenoic acid (20:5ω-3)	0.32	0.18	0.15‡	0.37	0.0009	0.21	0.34	0.13	0.27	0.0006
Docosapentaenoic acid (22:5ω-3)	0.36	0.30	0.07‡	0.24	0.0929	0.33	0.42	0.09	0.15	0.0003
Docosahexaenoic acid (22:6ω-3)	1.20	0.95	0.25‡	0.54	0.0051	1.16	1.64	0.48	0.78	0.0001
EPA/AA ratio	0.16	0.11	0.05‡	0.12	0.0027	0.14	0.22	0.08	0.18	0.0003
P/S ratio	1.13	1.19	0.06	0.47	0.1490	1.05	1.38	0.33	0.39	0.0001

*Difference between treatment and baseline period.
†Fatty acids %.
‡Negative result, indicating that baseline value was higher than treatment value.
SD–standard deviation. EPA/AA ratio–eicosapentaenoic acid to arachidonic acid ratio; P/S ratio–polyunsaturated to saturated fatty acid ratio.

ratio (P = 0.0001) of plasma CE were higher on the CFD than on the RMD.

Discussion

Baseline versus treatment diets

The major impact of dietary intervention on lowering the risk of CHD in terms of plasma lipoproteins was clearly demonstrated by this study. TC, LDL-C, and apo B are associated with CHD[1,29] and in this study TC, LDL_1-C, LDL_2-C, LDL_1-apo B, and LDL_2-apo B were significantly lower during the prudent diet with lean red meat and the prudent diet with skinless chicken and fish than during the respective baseline periods. This confirms the beneficial effect of changing from a Western-type diet to a prudent diet and also that dietary intervention should be the first step in the treatment of hypercholesterolemia. The study further showed that significant changes could be observed within a period of 6 weeks. The treatment period of 6 weeks was probably more than adequate to achieve a maximum change in lipoprotein concentrations with dietary intervention. According to Morgan et al.,[30] serum lipoprotein concentrations seem to stabilize within 10 to 14 days.

TAGs reflect recent dietary intakes of LA and the high P/S ratio of the treatment diets was reflected by a significantly higher percentage of LA in plasma TAG on the CFD compared with the baseline period. In contrast with TAG, the difference in the percentage of LA in CE between the

Table 6 Difference in mean fatty acid composition of plasma triacylglycerols (TAG) of subjects between the baseline and treatment periods

Cholesteryl ester†	Red meat diet (n = 39)					Chicken-fish diet (n = 39)				
	Baseline Mean	Treatment Mean	Difference* Mean	SD	P-value	Baseline Mean	Treatment Mean	Difference Mean	SD	P-value
Palmitic acid	11.27	11.74	0.48	2.06	0.1657	11.14	11.81	0.67	1.97	0.0668
Palmitoleic acid	1.95	2.35	0.40	0.95	0.0047	2.01	2.11	0.10	0.88	0.4403
Stearic acid	1.25	1.05	0.20‡	0.27	0.0001	1.24	0.91	0.33‡	0.40	0.0001
Oleic acid	20.31	19.08	1.22‡	2.44	0.0027	19.66	17.95	1.71‡	2.95	0.0007
Linoleic acid	58.22	58.87	0.65	3.87	0.2758	58.95	60.51	1.56	4.76	0.0547
Linolenic acid	0.23	0.13	0.10‡	0.12	0.0001	0.23	0.13	0.10‡	0.12	0.0001
Eicosatrienoic acid	0.47	0.46	0.01‡	0.22	0.7884	0.51	0.45	0.06‡	0.17	0.0840
Arachidonic acid	5.38	5.63	0.24	1.22	0.3282	5.38	5.14	0.24‡	0.97	0.0713
Eicosapentaenoic acid	0.46	0.29	0.17‡	0.29	0.0004	0.39	0.48	0.08	0.26	0.0194
Docosapentaenoic acid	0.00	0.00	0.00	0.00	—	traces	traces	<0.00	0.008	—
Docosahexaenoic acid	0.46	0.40	0.06‡	0.18	0.0316	0.48	0.51	0.03	0.19	0.3297
EPA/AA ratio	0.09	0.05	0.04‡	0.05	0.0001	0.07	0.09	0.02	0.04	0.0052
P/S ratio	5.33	5.23	0.10‡	1.21	0.8373	5.43	5.36	0.07‡	1.28	0.7115

*Difference between treatment and baseline period.
†Fatty acids %.
‡Negative results, indicating that baseline value was higher than treatment value.
SD–standard deviation. EPA/AA ratio–eicosapentaenoic acid to arachidonic acid ratio; P/S ratio–polyunsaturated to saturated fatty acid ratio.

Table 7 Direct treatment effect between red meat diet and chicken-fish diet: Body mass, plasma lipids and lipoproteins, and blood pressure (n = 39)*

Variables	Difference between means†	95% Confidence interval	*P*-value
Body mass (kg)	0.03‡	−0.30–0.25	0.8509
TC (mmol/L)	0.05‡	−0.12–0.03	0.2261
HDL-C (mmol/L)	0.03‡	−0.06– <−0.001	0.0498
HDL_2-C (mmol/L)	0.003‡	−0.02–0.02	0.7759
HDL_3-C (mmol/L)	0.03‡	−0.06– <0.001	0.0555
LDL_1-C (mmol/L)	0.002	−0.02–0.02	0.8562
LDL_2-C (mmol/L)	0.05‡	−0.13–0.03	0.1924
LDL_1-apo B	0.19	−0.13–0.52	0.2390
LDL_2-apo B	0.26‡	−1.75–1.21	0.7193
VLDL-C (mmol/L)	0.01‡	−0.04–0.02	0.4413
TAG (mmol/L)	0.002‡	−0.05–0.04	0.9088
VLDL-TAG (mmol/L)	0.01	−0.02–0.03	0.4644
Blood pressure			
Systolic (mmHg)	0.77‡	−2.06–0.51	0.2295
Diastolic (mmHg)	1.64‡	−2.67–−0.61	0.0027

*Treatment group 1 = Red meat Phase 1, Chicken-fish Phase 2; Treatment Group 2 = Chicken-fish Phase 1, Red meat Phase 2.
†Estimated treatment effect: Treatment group 1–Treatment group 2.
‡Negative result, indicating that the value on the red meat diet is higher than the value on the chicken-fish diet.
TC–total cholesterol. HDL-C–high density lipoprotein cholesterol. LDL-C–low density lipoprotein cholesterol. apo B–apolipoprotein-B. VLDL-C–very low density lipoprotein cholesterol. TAG–triacylglycerols.

baseline periods and the treatment periods was not significant. This might indicate that the intervention period of 6 weeks was too short or that the differences between the P/S ratio of the baseline and treatment diets were not large enough to increase the percentage of LA in the CE. In this study, the P/S ratio of the diet at baseline varied between 0.68 and 0.70, whereas it was 0.94 on the RMD and 1.5 on the CFD, which reflected the differences in the P/S ratio of red meat versus that of the chicken-fish diet used in the study. Tremoli et al.[31] found significant increases in the LA levels of TAG and CE after 6 weeks, but in their study there was a sharp increase in the P/S ratio of the diet when the dietary P/S ratio was increased from 0.2 to 2.0. In our study subjects already had a relatively high percentage of LA in their CE at baseline (57.79–59.4%) compared with a value of slightly more than 60% on the CFD and approximately 59% on the RMD.[32] In a study of patients with hyperlipoproteinemia, Vessby et al.[33] showed that the LA content of CE was approximately 63% on a diet with a P/S ratio of 1.98, whereas a high percentage of LA (more than 60%) in CE has also been observed with vegetarian diets.[34]

Red meat diet versus chicken and fish diet

In free-living normolipidemic subjects, TC did not differ when diets containing beef and pork were compared with diets of poultry and fish.[10,11] In the present study the TC also did not differ significantly, but HDL-C was 0.03

Table 8 Direct treatment effect between red meat and chicken-fish diet: Fatty acid composition of plasma triacylglycerol and cholesteryl ester of subjects (n = 39)*

Fatty acid (%)	Difference between means†	95% Confidence interval	*P*-value
Triacylglycerol			
Palmitic acid (16:0)	0.3645‡	−0.8414–0.1125	0.1301
Palmitoleic acid (16:1ω-9)	0.0546‡	−0.2765–0.1673	0.6210
Stearic acid (18:0)	0.2255‡	−0.3653– −0.0856	0.0023
Oleic acid (18:1ω-9)	1.1963‡	−1.8619– −0.5308	0.0008
Linoleic acid (18:2ω-6)	1.3332	0.4771–2.1892	0.0032
Linolenic acid (18:3ω-3)	0.0005‡	−0.0292–0.0281	0.9700
Eicosatrienoic acid (20:3ω-6)	0.0056‡	−0.0316–0.0204	0.6632
Arachidonic acid (20:4ω-6)	0.0300	−0.0475–0.1075	0.4379
Eicosapentaenoic acid (20:5ω-3)	0.0802	0.0450–0.1155	0.0001
Docosapentaenoic acid (22:5ω-3)	0.0594	0.0354–0.0833	0.0001
Docosahexaenoic acid (22:6ω-3)	0.3457	0.2198–0.4716	0.0001
EPA/AA ratio	0.0515	0.0251–0.0779	0.0003
P/S ratio	0.0986	0.0365–0.1607	0.0027
Cholesteryl ester			
Palmitic acid	0.0361	−0.2442–0.3163	0.7958
Palmitoleic acid	0.1165‡	−0.2200–−0.0131	0.0283
Stearic acid	0.0712‡	−0.1192– −0.0233	0.0047
Oleic acid	0.5544‡	−0.9046–−0.2042	0.0028
Linoleic acid	0.7923	0.2500–1.3345	0.0053
Linolenic acid	0.0030‡	−0.0196–0.0135	0.7142
Eicosatrienoic acid	0.0061‡	−0.0347–0.0226	0.6714
Arachidonic acid	0.2302‡	−0.4001–−0.0603	0.0093
Eicosapentaenoic acid	0.0953	0.0524–0.1382	0.0001
Docosapentaenoic acid	0.0005	−0.0006–0.0015	0.3615
Docosahexaenoic acid	0.0579	0.0268–0.0890	0.0006
EPA/AA ratio	0.0216	0.0136–0.0295	0.0001
P/S ratio	0.0623	−0.0997–0.2244	0.4408

*Treatment Group 1 (TG 1) = Red meat Phase 1, Chicken-fish Phase 2; Treatment Group 2 (TG 2) = Chicken-fish Phase 1, Red meat Phase 2.
†Estimated treatment effect: TG 1–TG 2.
‡Negative result, indicating that the value on the red meat diet is higher than on the chicken-fish diet.
EPA/AA ratio–eicosapentaenoic acid to arachidonic acid ratio; P/S ratio–polyunsaturated to saturated fatty acid ratio.

mmol/L higher ($P < 0.05$) on the RMD than on the CFD. This is in agreement with the finding of Scott et al.,[14] but not with that of Flynn et al.,[11] who showed both increases and decreases in HDL-C with three diets of either beef, or poultry and fish, or pork. The higher HDL-C on the RMD could be explained by the higher intake of total fat and cholesterol on the RMD than on the CFD, as well as the lower P/S ratio of the RMD. Total fat[35] and dietary cholesterol intake[36] are positively associated with HDL-C. Very low fat diets (<10%E) tend to lower LDL-C as well as HDL-C,[30,37] but a moderate fat diet (30%E) that is low in saturated fat seems to lower only LDL-C.[30] The difference in HDL-C (0.03 mmol/L) between the RMD and the CFD was very small and therefore of questionable biological significance.

SFAs, MUFAs, and PUFAs influence risk factors for CHD differently. Stearic acid in the TAG and CE was significantly higher on the RMD than on the CFD. It is known that SFAs with a carbon number of 12 to 16 increase plasma cholesterol,[2–4] whereas stearic acid does not.[38,39] In vitro studies, however, suggested that stearic acid might be the most thrombotic of the SFAs.[40–42] Watts et al.[43] showed that the intake of stearic acid remained significantly related to the progression of coronary artery disease even after adjustment for plasma cholesterol concentrations and other risk factors.

Oleic acid intake was significantly higher on the RMD than on the CFD and it has been shown that MUFAs lower plasma TC when they replace SFAs in the diet.[35] Although the dietary intake of oleic acid is not necessarily reflected in the plasma lipid fractions, the percentage of oleic acid in the TAG and CE was significantly higher ($P < 0.01$) on the RMD than on the CFD. Stearic acid can be converted to oleic acid by the human body, but Emken[44] estimated that less than 10% can be converted and the contribution of the higher stearic acid intake on the RMD than on the CFD to the higher percentage of oleic acid in the TAG and the CE was therefore probably very small if at all. The progression of CHD was directly associated with the intake of oleic acid.[43] Therefore, the higher intake of oleic acid on the RMD than on the CFD could be of concern.

LA is an essential fatty acid and the LA content of the plasma lipids therefore reflects the dietary intake of LA. Results showed that LA in TAG and CE was significantly higher on the CFD than on the RMD, but although the intake of LA was higher on the CFD than on the RMD, the difference was not significant. There are indications in the literature of a negative relationship between TC, as well as LDL-C, and the percentage of LA in CE.[45] An inverse relationship between LA and CHD has been shown,[46] and significantly lower levels of LA in platelets and adipose tissue of subjects with angina or first acute myocardial infarction than in controls were reported by Wood et al.[47] The possible role of LA in the development of atherosclerosis is, however, also of concern.[48,49] In humans, a positive association between LA in the serum and the aortic plaque was observed by Felton et al.,[49] who suggested that this implies a direct influence of dietary PUFAs on aortic plaque formation. Other researchers,[50,51] however, did not agree with them. LA is the major PUFA in aortic plaque[51] and it is suggested that fatty acids are highly correlated among various lipid pools in the body.[50] The higher percentage of LA in the TAG and CE on the CFD than on the RMD is probably more beneficial than detrimental in terms of the prevention of CHD.

Although diastolic blood pressure was lower on the CFD than on the RMD, the difference was very small (1.6 mmHg). The literature indicates that the intake of ω-3 PUFAs lowers blood pressure.[52] In this study the intake of EPA, an ω-3 PUFA, differed significantly (mean difference 0.05 g) between the CFD and the RMD and the percentage of EPA in TAG and CE was significantly higher on the CFD than on the RMD.

Fish was included twice per week on the CFD and even this limited number of fish meals resulted in a significant difference in the EPA content of the plasma lipids between the two treatment diets. The study of Bønaa et al.[53] showed that the fatty acid concentration of phospholipids may be modified by an intake of one portion (or less) of fish per week. Therefore, small amounts of fish in the diet seem to have biological effects. Kromhout et al.[54] showed that men who consumed two or more fish meals per week had a lower mortality rate from CHD than those who did not eat fish. EPA has an anti-aggregatory effect compared with AA[55,56] but there are still many unanswered questions about the beneficial effect of ω-3 PUFAs on cardiovascular disease.[57] A study of two separate, but genetically comparable Icelandic populations indicated that ω-3 PUFA may be cardioprotective, in spite of an otherwise atherogenic diet.[58]

The EPA/AA ratio in plasma free fatty acids may be a coronary risk indicator. It was shown to be significantly lower in effort angina patients than in controls.[59] Although plasma free fatty acids were not investigated in the present study, the ratio of EPA/AA in plasma TAG and CE was higher on the CFD than on the RMD, which might indicate a more beneficial effect of the CFD.

Dietary compliance

Qualitative evaluation by questionnaire of dietary compliance showed that subjects followed the dietary prescriptions for the CFD slightly better than for the RMD. This may have concealed significant differences between the effect of the two lipid-lowering diets. However, noncompliance on the RMD was of a mild nature. Dietary prescriptions were violated more often on the RMD than on the CFD.

Another way of testing dietary compliance is to use a biomarker. In this study EPA was used as such a biomarker for dietary compliance because fish, which is the main source of EPA in the Western-type diet, was excluded from the RMD. Small percentages of EPA were observed in the plasma TAG (0.16–0.19%) and CE (0.26–0.32%) of subjects who consumed the RMD, compared with those who consumed the CFD (0.29–0.38% and 0.43–0.53%, respectively). Although the main source of EPA in the Western-type diet is fish,[56,60] EPA can be synthesized by the human body from α-linolenic acid through a slow process of desaturation and elongation.[60] The decrease of EPA in plasma CE and TAG on the RMD, and the concomitant increase on the CFD, indicated that the dietary instructions were followed. In addition, the percentage of EPA in TAG and CE was significantly higher on the CFD than on the RMD.

Dietary compliance was the most important requirement for this study on a free-living population. Subjects who completed the study could be regarded as "good" compliers because the majority of the subjects who withdrew from the study did so because they did not want to comply with the dietary prescriptions. Noncompliance of those who followed the treatment diets was of a mild nature. The crossover design of the study compensated for the high dropout rate of 25%. Treatment effects were estimated within subjects rather than between subjects and in this case the crossover design was statistically more powerful and optimal.

In conclusion, the first step in the treatment of the individual with hypercholesterolemia requires changes in dietary intake. This study was undertaken on free-living individuals with hypercholesterolemia to compare the effect of two prudent diets on their plasma lipoproteins, as well as on the fatty acid composition of plasma TAG and CE. Free-living individuals were chosen as the study population to reflect the real-life situation when dietary guidelines are prescribed for the individual with hypercholesterolemia. The study confirmed that changing the Western-type diet of free-living subjects to a prudent diet can have beneficial effects on plasma lipid concentrations within 6 weeks. It also showed that there was no difference in the effect of the two treatment diets on the atherogenic lipids and lipoproteins but the CFD seemed to have a more favorable effect than the RMD on the fatty acid composition of the plasma TAG and CE. Depending on energy needs (7,500 kJ to 14,000 kJ) and against the background of the requirements of a prudent diet, a daily portion of 120 g to 210 g cooked lean red meat could be included in a lipid-lowering diet. However, even as little as two fish meals per week resulted in a significantly higher EPA content in the plasma TAG and CE on the CFD than on the RMD. Therefore, the effect of the two diets on CHD risk could potentially differ if risk factors other than plasma lipids and lipoproteins are investigated. The difference in the effect of the two diets on the plasma TAG and CE fatty acid composition may result in different effects on thrombogenesis.

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