Eur J Nutr (2011) 50:71–79
DOI 10.1007/s00394-010-0116-9

ORIGINAL CONTRIBUTION

Effects of high-fat and low-fat diets rich in monounsaturated fatty acids on serum lipids, LDL size and indices of lipid peroxidation in healthy non-obese men and women when consumed under controlled conditions

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Received: 12 February 2010 / Accepted: 19 May 2010 / Published online: 3 June 2010

Abstract
Objective To study the effects of the dietary fat content on cardiovascular disease risk factors in humans when the fatty acid composition and types of carbohydrates are kept constant.
Methods A controlled dietary study in healthy volunteers with 2 dietary groups and a parallel design consisting of 2 dietary periods was conducted. First, participants received a 2-week wash-in diet rich in saturated fatty acids (SFA; 47% of total fatty acids) and were then randomly assigned to either a high-fat (40% of energy) or a low-fat diet (29% of energy) for 4 weeks. Both diets were isocaloric, rich in monounsaturated fatty acids (MUFA; 51% of total fatty acids) and had similar fatty acid and carbohydrate compositions.
Results Compared to the wash-in diet, the high-fat and low-fat diets significantly lowered LDL-cholesterol (−0.34 and −0.41 mmol/l, respectively; $P < 0.001$ for time effect in RM-ANOVA), and HDL-cholesterol (−0.13 and −0.18 mmol/l, respectively; $P < 0.001$ for time), without any differences between the high-fat and low-fat diets ($P = 0.112$ and $P = 0.085$ for time × group interaction in RM-ANOVA, respectively). The size of the major LDL fraction, the LDL susceptibility to oxidation and the plasma concentrations of oxidized LDL (ox-LDL) were significantly reduced by both the high-fat and low-fat diet, again without significant differences between the diets. The ratio of ox-LDL/LDL-cholesterol, serum triacylglycerols and urinary F2-isoprostanes were not significantly affected by the diets.
Conclusion A high-fat and a low-fat diet, both rich in MUFA, had similar effects on lipid-related cardiovascular disease risk factors in metabolically healthy men and women.

Keywords Monounsaturated fatty acids · LDL oxidation · Lipoproteins · Dietary fat content

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Introduction

Worldwide, cardiovascular diseases (CVD) are the primary cause of death [1]. A major underlying cause of cardiovascular mortality and morbidity is the atherosclerosis of the coronary arteries [2]. Important risk factors for atherosclerosis are increased serum concentrations of LDL-cholesterol (LDL-c), low serum concentrations of HDL-cholesterol (HDL-c), a high serum concentration of triacylglycerols (TAG) and a preponderance of smaller, denser LDL-particles. Furthermore, there is extensive evidence that oxidative and/or enzymatic modifications of LDL-particles play a decisive role

in all stages of atherogenesis. These have been assessed by measuring the LDL susceptibility to ex vivo oxidation, the concentration of oxidized LDL (ox-LDL) in plasma and the concentration of F2-isoprostanes in plasma or urine [3, 4].

Decades of research work have demonstrated that dietary fatty acids have various effects on atherosclerotic risk factors and several direct effects on atherogenesis. However, the appropriate amount and the distribution of dietary fatty acids required to achieve the most favorable impact on CVD risk have been a subject of discussion in recent years. The need to reduce dietary saturated fatty acids (SFA) from meats and dairy products as well as *trans* fatty acids from hydrogenated vegetable oils is now widely accepted. SFA and *trans* fatty acids are commonly judged to have a negative health impact as they lead to an increased serum LDL-c concentration. Elevated LDL-c levels are strongly associated with an increased risk for CVD and CHD [5–8]. Therefore, all dietary recommendation guidelines stress the importance to limit the intake of SFA and *trans* fatty acids (<7–10% of energy) [8–10]. Questions remain about the optimal levels of total fat and unsaturated fatty acids, specifically monounsaturated fatty acids (MUFA) and $(n-3)$ and $(n-6)$ polyunsaturated fatty acids (PUFA).

Low-fat diets are often advocated for weight reduction, to reduce serum total cholesterol (TC) and LDL-c, and to lower the risk of CVD. The replacement of SFA with carbohydrates has been the standard advice given for both weight loss and improvements in cardiovascular health. However, relatively recently this practice has been questioned, and the issue of low fat/high carbohydrate versus moderate fat has become quite controversial [11–14]. A low-fat, high-carbohydrate diet, compared with higher-fat diets, has been shown to induce atherogenic dyslipidemia characterized by low HDL-c and high TAG, effects that may be associated with increased risk for CVD [5, 15, 16]. Additionally, reduction in total fat intake and higher intake of carbohydrates, particularly refined, higher-glycemic index carbohydrates, may adversely affect glucose–insulin homeostasis, satiety and weight gain [17].

In the present study, we wanted to compare a high-fat diet with a low-fat diet with regard to its effects on fasting serum lipids and lipoproteins, LDL size, LDL fatty acid composition, LDL α-tocopherol and indices of lipid peroxidation in humans. Both diets were isocaloric, low in SFA and rich in unsaturated fatty acids, and the fatty acid and carbohydrate compositions were standardized across the diet arms. Our study was carried out with metabolically healthy volunteers under strictly controlled dietary conditions with natural-food diets.

Methods

Study participants

Of 700 students living under boarding school-like conditions in a third-level technical college, 88 volunteers were screened for participation. Inclusion criteria were non-smoking status, 19–40 years of age, a BMI < 27 kg/m^2, serum TC < 7.76 mmol/l and serum TAG < 3.39 mmol/l. Exclusion criteria were metabolic and endocrine diseases, malabsorption syndromes, alcohol abuse and restrictive dietary requirements. Each participant underwent a basic examination (including blood analyses) before the study. Forty-seven healthy volunteers were enrolled in the study. Of these, 10 ended the study prematurely because they were unwilling or unable to comply with the dietary regimen. Thus, the final analysis included 37 participants (12 men, 25 women, aged 18–34) (baseline characteristics presented in Table 1).

The participants did not take any medications or nutritional supplements before or during the study. Nineteen women took oral contraceptives and were instructed not to discontinue their use or change the form of contraception. All participants were asked to maintain their regular lifestyles and usual extent of physical activities throughout the study.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human volunteers were approved by the ethical committee of the University of Muenster, Germany. Written informed consent was obtained from all participants.

Study design and diets

The study was conducted in a parallel design and consisted of 2 consecutive dietary periods. First, all participants consumed a wash-in high-fat diet rich in SFA for 2 weeks and were then randomly divided into 2 groups. One group

Table 1 Baseline characteristics of participants ($n = 37$)

Age (years)	22.6 ± 4.2
Body height (cm)	173 ± 10
Body weight (kg)	64.9 ± 11.8
BMI (kg/m^2)	21.5 ± 2.4
Fasting serum TC (mmol/l)	4.49 ± 0.84
Fasting serum LDL-c (mmol/l)	2.37 ± 0.66
Fasting serum HDL-c (mmol/l)	1.63 ± 0.38
Fasting serum TAG (mmol/l)	1.08 ± 0.46

Values are expressed as mean ± SD

TC total cholesterol, *LDL-c* LDL-cholesterol, *HDL-c* HDL-cholesterol, *TAG* triacylglycerol

(6 men, 12 women) received a high-fat diet (40% of energy intake), while the other group (6 men, 13 women) received a low-fat diet (29% of energy intake) (Table 2). Both diets were rich in MUFA (51% of total fatty acids), and the relative proportion of the different fatty acids (SFA 26% of total fatty acids; PUFA 23% of total fatty acids) as well as the different types of carbohydrates (polysaccharides vs. mono- and disaccharides) was similar in both diets (Table 2). In addition, dietary contents of cholesterol, fiber and antioxidants were kept similar (Table 2). Wherever possible, we tried to use the same basic food items in both diets differing only in their daily amounts.

Before the study, all participants wrote a 3-day dietary record of all foods and beverages consumed. This was used to estimate each subject's habitual energy and nutrient intake. The dietary records and the study diets were calculated using the computer-based nutrient-calculation program EBISpro (University of Hohenheim, Stuttgart, Germany), based on the German Nutrient Data Base Bundeslebensmittelschlüssel (Max Rubner-Institute, Karlsruhe, Germany). The diets were calculated for 10 different energy levels, which ranged from 7.52 to 15.05 MJ/d and had a difference of 0.84 MJ between each energy group.

All participants were weighed twice a week while wearing light clothing, and energy intake was adjusted when necessary to maintain a stable body weight.

All study diets consisted of conventional mixed foods that were freshly prepared. Menus were changed daily. The kitchen and dining facilities were located in the school in which the students were trained and housed during the week. The participants were served breakfast, lunch and dinner from Monday morning to Friday noon. This food was immediately consumed in the school canteen under the direct supervision of 2 of the authors (SE, MK). On Friday afternoons, participants were given hampers containing their entire food supply for the weekend.

All foodstuffs were weighed to the nearest gram. Basic menus of the study diets were identical for all participants. Main components of both diets were low-fat foodstuffs, e.g., vegetable foods, bread and cereals, lean meat and fish, skimmed milk and low-fat dairy products. The principal sources of fat during the study period were rapeseed oil and high-oleic sunflower oil and a MUFA-rich commercial margarine (MUFA, 48% of total fatty acids; 64 g fat/100 g). The plant oils and the margarine were used for the preparation of all meals and snacks. They were incorporated in sauces, desserts, curd and salad dressings. Mean estimated daily intake of the plant oils were 25.7 and 42.5 g for the low-fat and high-fat diet group, respectively. Mean intake of the margarine was 24.1 g/d (low-fat group) and 32.7 g/d (high-fat group). In both diets, we also used specially baked oil-enriched

Table 2 Composition of the habitual diet and the study diets

	Habitual diet ($n = 37$)[a]	Wash-in diet (SFA-rich) ($n = 37$)	Low-fat diet (MUFA-rich) ($n = 19$)	High-fat diet (MUFA-rich) ($n = 18$)
Energy (MJ/day)	10.5 ± 3.2	10.1 ± 2.3	10.2 ± 2.3	9.7 ± 2.3
Protein (En%)	15.2 ± 2.6	15.7 ± 1.1	15.6 ± 0.4	15.6 ± 0.3
Carbohydrate (En%)	48.9 ± 5.4	42.6 ± 1.5	54.4 ± 1.9	43.1 ± 1.0
Mono- and Disaccharides (En%)	27.8 ± 6.2	21.0 ± 2.1	24.7 ± 2.1	20.3 ± 1.6
Polysaccharides (En%)	20.7 ± 4.1	20.9 ± 1.2	29.3 ± 1.1	21.9 ± 0.9
Ratio of polysaccharides to mono- and disaccharides	0.80 ± 0.31	1.01 ± 0.14	1.19 ± 0.14	1.10 ± 0.13
Fat (En%)[b]	33.6 ± 5.4	40.8 ± 0.8	28.7 ± 0.6	40.2 ± 0.8
SFA (En%)	15.0 ± 3.2	18.1 ± 0.4	7.2 ± 0.2	9.9 ± 0.4
MUFA (En%)	11.0 ± 1.8	13.1 ± 0.4	13.9 ± 0.4	19.8 ± 0.3
($n - 6$) PUFA (En%)	4.6 ± 1.7	6.6 ± 0.2	5.3 ± 0.1	7.0 ± 0.2
($n - 3$) PUFA (En%)	0.6 ± 0.6	1.1 ± 0.1	0.9 ± 0.0	1.6 ± 0.1
Cholesterol (mg/MJ)	25.6 ± 7.8	17.7 ± 1.2	15.4 ± 0.9	17.8 ± 0.9
Dietary fiber (g/MJ)	2.3 ± 0.5	2.5 ± 0.2	3.2 ± 0.2	3.0 ± 0.3
Vitamin C (mg/MJ)	17 ± 7.6	16 ± 4	20 ± 5	21 ± 4
Vitamin E (mg/MJ)[c]	1.27 ± 0.34	1.81 ± 0.10	2.87 ± 0.15	3.07 ± 0.20

Values are expressed as mean ± SD

En% % of energy intake, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids

[a] Calculated from 3-day dietary records

[b] Total fat contains ~95% fatty acids, the other ~5% is made up of glycerol and other lipids

[c] α-tocopherol equivalents

bread and cakes containing different amounts of the plant oils and margarine. A typical meal menu of the low-fat diet (energy group 10.04 MJ/d) e.g., contained 160 g bread (4 slices), 45 g bread rolls, 30 g cereals, cheese (1 slice), 50 g low-fat sausage and ham, 35 g marmalade and honey, 45 g curd, 150 g skimmed milk, 130 g fruits, 350 g vegetables, 200 g potatoes, 120 g lean meat and 50 g visible fat (plant oil and margarine). A typical meal menu of the high-fat diet group of the same energy group consisted of 120 g oil-enriched bread, 45 g bred roll, cheese (2 slices), 50 g low-fat sausage and ham, 20 g marmalade and honey, 35 g curd, 150 g skimmed milk, 130 g fruits, 200 g vegetables, 150 g potatoes, 150 g lean meat and 75 g visible fat (plant oil and margarine). Both diets were well tolerated.

To compensate for short-term differences in individual energy requirements, participants were provided on request with special bread rolls which were baked so as to contain the same nutrient composition as that person's study diet. By means of these rolls, energy balance was ensured without changing the composition of the diets.

Participants were directly supplied with enough food to meet 90% of their mean daily energy requirements. The remaining energy was provided in the form of free-choice foodstuffs such as beverages or fruit which contained only trace amounts of fat, protein or cholesterol. These were chosen from a given list and were recorded in diaries as was any food that was not consumed and deviations from the diets. Based on these diaries, adherence was found to be very high.

Blood sample processing and analysis

Venous blood samples were obtained at baseline (prewash-in; visit 1), after the wash-in period (week 0; start; visit 2), after 2 weeks (visit 3) and after 4 weeks (visit 4) of the study diets. All samples were drawn after an overnight fast of at least 10 h under standardized conditions. Blood was drawn into tubes containing EDTA or no additives (Sarstedt, Nümbrecht, Germany). Plasma and serum was obtained by centrifugation at $1{,}800\times g$; 10 min at 10 °C. After aliquotation in gas-tight cryovials, plasma and serum were immediately frozen and stored at −80 °C until analyses.

Serum TC, TAG and HDL-c were measured by enzymatic assays using commercially available kits (CHOD-PAP for TC, GPO-PAP for TAG and a precipitation method for HDL-c, Roche Diagnostics, Mannheim, Germany) on a Hitachi 747 autoanalyzer. LDL-c was calculated by use of the Friedewald equation.

The size of LDL was determined from plasma by the use of a commercially available polyacrylamide gradient gel electrophoresis kit (LFS Lipogel Assay Kit, LaboMed, Waldkirch, Germany) [18].

For analyses of LDL susceptibility to oxidation, LDL fatty acid composition and tocopherol content LDL was separated from EDTA plasma in a single run of 2 h by density gradient centrifugation [19].

Susceptibility of LDL to oxidation was measured by the method of Esterbauer et al. [20] as described previously [19]. CuSO4 was used as a pro-oxidant. The formation of conjugated dienes (CD) was monitored by measurement of the change in absorbance at 234 nm in an Uvikon 922 photometer (Kontron, Neufahrn, Germany), for 3 h, resulting in a curve. A tangent to this curve was drawn at the point of inflexion. The lag time was defined as the time from the addition of CuSO4 until the intersection of this tangent with the baseline. The rate of propagation was calculated from the slope of the tangent, and the maximum amount of CD formation was determined as the height of maximum absorbance above baseline.

The total fatty acid composition of LDL-particles was measured by GLC [19]. The concentration of α-tocopherol in LDL was determined by using reversed-phase HPLC and a UV diode array detector [19]. Circulating ox-LDL was determined using a commercial ELISA kit (Mercodia, Uppsala, Sweden) according to the manufacturer's protocol.

Morning urine samples were collected at each visit, 0.002% of butylated hydroxytoluene was added, and the urine was frozen to −80 °C until analysis. From these urine samples, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) was measured by the use of a commercially available competitive enzyme immunoassay (Cayman Chemicals, Ann Arbor, Michigan, USA) as described in [21]. Urinary creatinine was measured by an automated kinetic procedure on a Hitachi 747 autoanalyzer (Roche Diagnostics).

Statistical analyses

Statistical analyses were performed using the SPSS statistical software package (version 17, SPSS Inc., Chicago, IL, USA). All variables appeared to be approximately normally distributed as confirmed by looking at histograms and normal plots of the data, and by performing a Kolmogorov–Smirnov test, with the exception of serum TAG and urinary 8-iso-$PGF_{2\alpha}$, which were logarithmically transformed to normality. We then employed a repeated measures ANOVA (RM-ANOVA), with the data of visits 2, 3 and 4 as the 3 levels of the within-subjects factor (time), and group (high-fat vs. low-fat) as the between-subjects factor. In cases where the assumption of sphericity did not hold, we adjusted degrees of freedom according to Huynh–Feldt. All tests were two tailed, and $P < 0.05$ was considered significant. Parameters at the start of the

Table 3 Body weight, fasting serum lipid and lipoprotein concentrations and size of the predominant LDL fraction in healthy men and women throughout the study

	Treatment	Visit 1 (Prewash-in)	Visit 2 (Start)	Visit 3	Visit 4 (End)	RM-ANOVA	
						Time	Time × group
Body weight (kg)	High-fat diet ($n = 18$)	64.2 ± 13.6	63.9 ± 13.5	63.8 ± 13.5	63.7 ± 13.3	$P = 0.599$	$P = 0.064$
	Low-fat diet ($n = 19$)	65.0 ± 10.2	64.9 ± 10.1	64.8 ± 10.1	64.9 ± 10.1		
TC (mmol/l)	High-fat diet ($n = 18$)	4.76 ± 0.71	4.54 ± 0.75	4.12 ± 0.69	4.10 ± 0.68	$P < 0.001$	$P < 0.05$
	Low-fat diet ($n = 19$)	5.14 ± 0.86	4.93 ± 0.66	4.17 ± 0.69	4.38 ± 0.77		
LDL-c (mmol/l)	High-fat diet ($n = 18$)	2.50 ± 0.52	2.32 ± 0.53	1.96 ± 0.47	2.00 ± 0.51	$P < 0.001$	$P = 0.112$
	Low-fat diet ($n = 19$)	2.86 ± 0.70	2.73 ± 0.60	2.14 ± 0.49	2.33 ± 0.60		
HDL-c (mmol/l)	High-fat diet ($n = 18$)	1.72 ± 0.44	1.76 ± 0.52	1.68 ± 0.47	1.62 ± 0.41	$P < 0.001$	$P = 0.085$
	Low-fat diet ($n = 19$)	1.67 ± 0.38	1.72 ± 0.37	1.52 ± 0.33	1.55 ± 0.34		
LDL-c to HDL-c ratio	High-fat diet ($n = 18$)	1.54 ± 0.50	1.42 ± 0.51	1.25 ± 0.45	1.32 ± 0.51	$P < 0.001$	$P = 0.893$
	Low-fat diet ($n = 19$)	1.82 ± 0.65	1.69 ± 0.62	1.48 ± 0.49	1.58 ± 0.53		
TAG (mmol/l)	High-fat diet ($n = 18$)	1.18 ± 0.43	1.01 ± 0.33	1.05 ± 0.37	1.04 ± 0.30	$P = 0.682$	$P = 0.960$
	Low-fat diet ($n = 19$)	1.33 ± 0.53	1.05 ± 0.37	1.11 ± 0.43	1.09 ± 0.45		
LDL size (nm)	High-fat diet ($n = 18$)	26.4 ± 0.9	26.3 ± 0.9	25.7 ± 1.0	25.9 ± 0.9	$P < 0.001$	$P = 0.449$
	Low-fat diet ($n = 19$)	26.7 ± 1.2	26.7 ± 1.0	26.2 ± 1.1	26.2 ± 0.9		

Values are expressed as mean ± SD; The wash-in diet was consumed between visits 1 and 2, the high-fat or low-fat diets were administered between visits 2 and 4; the 2 groups did not differ in any of these variables at visit 2

TC total cholesterol, *LDL-c* LDL-cholesterol, *HDL-c* HDL-cholesterol, *TAG* triacylglycerol, *RM-ANOVA* repeated measures ANOVA

high-fat and low-fat diets (visit 2) were compared by using unpaired *t*-tests. All data are presented as means ± standard deviations (SD).

Results

Body weight

Mean body weight did not significantly change during the 6-week study (Table 3).

Serum lipids

In both groups, serum TC concentrations decreased significantly during the main diet phase ($P < 0.001$ for time) (Table 3). We also found a significant interaction between the within-subjects factor (time of measurement, visit 2 vs. visit 3 vs. visit 4) and the between-subjects factor (group, high-fat vs. low-fat; $P = 0.037$ for time × group), indicating that this decrease was different in the 2 groups. Compared to the wash-in diet, the high-fat and low-fat diets significantly lowered LDL-cholesterol (−0.34 and −0.41 mmol/l, respectively; $P < 0.001$ for time effect in RM-ANOVA), and HDL-cholesterol (−0.13 and −0.18 mmol/l, respectively; $P < 0.001$ for time), without any differences between the high-fat and low-fat diets ($P = 0.112$ and $P = 0.085$ for time × group interaction in RM-ANOVA, respectively). Serum TAG did not significantly change throughout the study and did not differ between diet groups (Table 3).

LDL size

The MUFA-rich diets significantly decreased the size of the predominant LDL fraction (−0.48 nm; $P < 0.001$ for time). This decrease was independent of the subjects' group affiliation (Table 3).

LDL susceptibility to oxidation

Both the high-fat and the low-fat diet reduced the ex vivo susceptibility of LDL to oxidation as indicated by a longer lag time ($P < 0.001$ for time), a reduced propagation rate ($P < 0.001$ for time) and a reduced maximum amount of CD formed during the ex vivo oxidation ($P < 0.001$ for time). Group affiliation did not affect these outcome measures (Table 4).

Plasma ox-LDL

Plasma concentrations of ox-LDL decreased significantly during both the high- and low-fat periods ($P < 0.001$ for time). This decrease was not significantly different between the diet groups (Table 4). Decreases in plasma ox-LDL levels significantly correlated with decreases in LDL-c (spearman correlation coefficient, $r = 0.456$, $P < 0.001$). Therefore, we adjusted ox-LDL levels for LDL-c and

Table 4 Parameters of LDL susceptibility to oxidation, fasting plasma oxidized LDL concentrations and urinary 8-iso-prostaglandin $F_{2\alpha}$ excretion in healthy men and women throughout the study

	Treatment	Visit 1 (Prewash-in)	Visit 2 (Start)	Visit 3	Visit 4 (End)	RM-ANOVA	
						Time	Time × group
Lag time (min)	High-fat diet ($n = 17$)	66.0 ± 4.9	65.0 ± 6.3	68.6 ± 6.3	66.8 ± 5.6	$P < 0.001$	$P = 0.498$
	Low-fat diet ($n = 18$)	67.5 ± 4.9	63.0 ± 6.9	67.7 ± 5.9	67.1 ± 6.4		
Propagation rate[a]	High-fat diet ($n = 17$)	20.0 ± 2.5	21.5 ± 2.9	20.6 ± 3.2	21.1 ± 3.2	$P < 0.001$	$P = 0.226$
	Low-fat diet ($n = 18$)	19.7 ± 2.2	22.2 ± 2.7	20.4 ± 2.1	20.8 ± 2.2		
Maximum amount of CD[b]	High-fat diet ($n = 17$)	922 ± 93	969 ± 83	940 ± 104	943 ± 109	$P < 0.001$	$P = 0.059$
	Low-fat diet ($n = 18$)	894 ± 54	979 ± 52	930 ± 52	922 ± 54		
Ox-LDL (mg/l)	High-fat diet ($n = 18$)	7.85 ± 2.10	7.35 ± 2.32	6.65 ± 2.24	6.52 ± 2.27	$P < 0.001$	$P = 0.302$
	Low-fat diet ($n = 19$)	8.49 ± 2.22	8.13 ± 1.97	6.75 ± 1.80	7.29 ± 2.07		
8-iso $PGF_{2\alpha}$ (ng/mmol creatinine)	High-fat diet ($n = 13$)	100.8 ± 31.6	97.8 ± 23.4	93.4 ± 36.6	124.8 ± 113.3	$P = 0.828$	$P = 0.685$
	Low-fat diet ($n = 14$)	103.8 ± 49.2	98.0 ± 42.0	109.0 ± 47.0	112.5 ± 61.6		

Values are expressed as mean ± SD; The wash-in diet was consumed between visits 1 and 2, the high-fat or low-fat diets were administered between visits 2 and 4; the 2 groups did not differ in any of these variables at visit 2

[a] nmol CD/(min mol LDL-c)

[b] nmol CD/mol LDL-c

CD conjugated dienes, *8-iso $PGF_{2\alpha}$* 8-iso-prostaglandin $F_{2\alpha}$, *ox-LDL* oxidized LDL, *RM-ANOVA* repeated measures ANOVA

reanalyzed the data. No significant change in the ox-LDL/LDL-c ratio in response to the high-fat or low-fat diets was noted ($P = 0.176$ for time, $P = 0.934$ for time × group; data not shown).

Urinary 8-iso-$PGF_{2\alpha}$

The full set of data was available for 27 participants. We observed rather large inter-individual differences in the urinary concentration of this metabolite of in vivo lipid oxidation, both in terms of their absolute levels and their responses to the study diets. Overall, the study diets did not significantly affect urinary 8-iso-$PGF_{2\alpha}$ concentrations (Table 4).

LDL fatty acid composition and LDL α-tocopherol

In the high-fat diet group, there was a significant increase in the LDL contents of oleic acid ($P < 0.001$ for time), α-linolenic acid ($P < 0.001$ for time) and arachidonic acid ($P < 0.05$ for time). The ingestion of the low-fat diet led to a significant enrichment of the LDL with palmitoleic acid ($P < 0.01$ for time), oleic acid ($P < 0.001$ for time), arachidonic acid ($P < 0.05$ for time) and eicosapentaenoic acid ($P < 0.05$ for time). The significant increase in the LDL content of α-linolenic acid during the high-fat diet was significantly different to the change during the low-fat diet ($P < 0.01$ for time × group) (Table 5). LDL α-tocopherol levels did not significantly change throughout the study (data not shown).

Discussion

The aim of the present strictly controlled dietary study in metabolically healthy volunteers was to investigate the effect of the dietary fat content on lipid-related risk factors for CVD. Our major finding was that, compared to the SFA-rich wash-in diet, the high-fat and low-fat diets both rich in MUFA had similar effects on serum lipid profiles, LDL size, LDL oxidizability and plasma ox-LDL. Several other similar studies have been conducted, but most of them have used diets with more extreme compositions, either a very low (~20–25% of energy) [22–25] or very high total fat content (~45–50% of energy) [24–26], or a very high content of MUFA (up to 30% of energy) [22, 27, 28]. These diets are hardly acceptable to long-term nutrition in European countries and the United States. In contrast to these studies, we used a more practical approach and nutrient relations that can be maintained over lifetime.

Substitution of high-fat or low-fat diets rich in MUFA for a diet rich in SFA significantly decreased TC and LDL-c concentrations. This has been confirmed in previous controlled human feeding studies [29–32]. In addition, in the present study HDL-c concentrations were significantly lowered on the high-fat as well as on the low-fat diet. However, on both diets, the simultaneous decrease in LDL-c was more pronounced, resulting in a decreased LDL-c/HDL-c ratio. Studies on the effects of the amount and type of fat on HDL-c concentrations have yielded inconsistent results [33–35]. The meta-analysis of Mensink et al. [33] concluded that the ratio of TC/HDL-c did not

Table 5 LDL fatty acid composition in healthy men and women throughout the study

Fatty acid (μmol/mmol LDL-c)	Treatment	Visit 1 (prewash-in)	Visit 2 (start)	Visit 3	Visit 4 (end)	RM-ANOVA	
						Time	Time × group
16:0	High-fat diet ($n = 18$)	260.8 ± 60.2	264.0 ± 61.9	255.5 ± 52.5	272.0 ± 89.3	$P = 0.415$	$P = 0.940$
	Low-fat diet ($n = 19$)	263.7 ± 61.5	250.7 ± 44.1	249.3 ± 58.0	260.5 ± 56.9		
18:0	High-fat diet ($n = 18$)	67.0 ± 10.0	64.0 ± 11.2	64.6 ± 10.2	68.2 ± 17.9	$P = 0.344$	$P = 0.898$
	Low-fat diet ($n = 19$)	66.2 ± 13.9	60.2 ± 11.6	59.9 ± 10.1	62.3 ± 9.0		
16:1 ($n - 7$)	High-fat diet ($n = 18$)	33.1 ± 13.4	25.6 ± 9.0	25.7 ± 9.3	26.5 ± 12.4	$P = 0.058$	$P = 0.142$
	Low-fat diet ($n = 19$)	35.3 ± 15.2	24.0 ± 7.3	29.4 ± 14.3	31.1 ± 12.6		
18:1 ($n - 9$)	High-fat diet ($n = 18$)	193.8 ± 45.1	187.9 ± 47.9	220.3 ± 40.5	241.9 ± 72.9	$P < 0.001$	$P = 0.519$
	Low-fat diet ($n = 19$)	196.2 ± 45.4	172.9 ± 31.6	219.1 ± 41.8	222.1 ± 41.5		
18:2 ($n - 6$)	High-fat diet ($n = 18$)	463.6 ± 67.4	524.2 ± 88.0	492.6 ± 74.2	547.1 ± 129.9	$P = 0.086$	$P = 0.373$
	Low-fat diet ($n = 19$)	438.7 ± 82.5	496.2 ± 89.6	456.3 ± 75.3	471.3 ± 58.0		
18:3 ($n - 3$)	High-fat diet ($n = 18$)	13.5 ± 4.2	11.7 ± 4.5	15.8 ± 4.2	17.6 ± 4.4	$P < 0.001$	$P < 0.01$
	Low-fat diet ($n = 19$)	12.1 ± 3.3	11.2 ± 3.2	13.1 ± 2.9	12.0 ± 5.4		
20:4 ($n - 6$)	High-fat diet ($n = 18$)	69.0 ± 15.8	68.3 ± 13.6	70.2 ± 13.3	75.2 ± 18.5	$P < 0.01$	$P = 0.468$
	Low-fat diet ($n = 19$)	71.9 ± 15.8	70.6 ± 9.6	78.6 ± 15.9	80.7 ± 17.5		
20:5 ($n - 3$)	High-fat diet ($n = 18$)	14.6 ± 6.9	11.1 ± 4.4	12.3 ± 5.3	12.7 ± 5.3	$P < 0.05$	$P = 0.401$
	Low-fat diet ($n = 19$)	13.0 ± 4.6	9.8 ± 3.0	10.6 ± 2.4	13.2 ± 6.0		
22:6 ($n - 3$)	High-fat diet ($n = 18$)	28.3 ± 7.7	27.0 ± 9.6	29.2 ± 8.3	28.9 ± 7.2	$P = 0.063$	$P = 0.424$
	Low-fat diet ($n = 19$)	25.9 ± 9.8	23.2 ± 5.3	25.7 ± 6.8	28.9 ± 11.4		

Values are expressed as mean ± SD; The wash-in diet was consumed between visits 1 and 2, the high-fat or low-fat diets were administered between visits 2 and 4; the 2 groups did not differ in any of these variables at visit 2

RM-ANOVA repeated measures ANOVA

change if carbohydrates replaced SFA, but it decreased if *cis* unsaturated fatty acids replaced SFA.

Furthermore, we found that serum TAG concentrations did not significantly change during the MUFA-rich high-fat and low-fat diets compared to the SFA-rich wash-in diet. SFA and MUFA appear to have a modest, if any, effect on fasting serum TAG concentrations [35]. However, low-fat high carbohydrate diets have been shown to increase TAG concentrations in healthy participants [32] and in patients with metabolic syndrome traits [36]. It has also been demonstrated that the effects of carbohydrates on TAG are less pronounced if the carbohydrates are provided in the form of low-glycemic-index, fiber-rich foods [36] used preferentially in both diets in this study. Thus, our finding of a lack of difference in TAG between the low-fat and high-fat diets suggests that the carbohydrate content is less important for plasma TAG concentrations than the carbohydrate composition, specifically the amount of sugars and fiber taken up.

In addition to their favorable effects on serum lipid profiles, both MUFA-rich diets lead to the formation of MUFA-rich LDL-particles and decreased ex vivo LDL susceptible to oxidation, as described by others during MUFA-rich diets [4, 31, 37, 38]. It is notable that this finding was consistent for all 3 parameters of ex vivo LDL oxidizability, lag time, propagation rate and maximum amount of CD. The measurement of the susceptibility to oxidation has been shown to correlate with the extent of atherosclerosis [39]. The decrease in LDL oxidizability was paralleled by a reduction in circulating concentrations of ox-LDL, which have been recognized as a risk factor for CVD [4]. However, ox-LDL levels, as measured by antibodies, correlated directly with LDL-c concentrations and after adjustment (ox-LDL/LDL-c), we found no significant change in circulating ox-LDL during the diet phase. Therefore, the decreases in ox-LDL were most likely mediated by decreases in LDL-c and not by an independent effect of the fat diets.

In contrast to the beneficial effects on parameters of LDL oxidation, we found no significant effect of the MUFA-rich study diets on urinary isoprostanoid excretion. Isoprostanes are members of a family of prostaglandin isomers that are produced from oxidative modification of PUFA via a free radical-catalyzed mechanism [40]. Thus, isoprostane levels in plasma or urine reflect general in vivo lipid peroxidation rather than oxidation of lipids associated with lipoproteins. Isoprostane levels in plasma or urine have been shown to be increased in association with a number of atherosclerotic risk factors, including hypercholesterolemia, diabetes mellitus and obesity, among others [40]. In addition, recent evidence suggests their quantification may represent an independent marker of atherosclerotic risk [40]. To the best of our

knowledge, no controlled study has investigated the effects of dietary fat content in healthy volunteers on this biomarker up to now. We have previously found that in patients with hypertriacylglycerolemia, low-fat or high-fat diets, both rich in MUFA and long-chain ($n-3$) PUFA, did not significantly affect urinary isoprostanoid excretion [21]. It should be noted that the rather large inter- and intraindividual variance of urinary isoprostane concentrations will have reduced our power to detect a change in this endpoint over time, as well as our ability to detect difference between the groups.

Surprisingly, we observed a small significant decrease in the LDL peak particle diameters during the high-fat and low-fat diets, with no significant difference between the fat diets. However, several studies have shown that low-fat diets not only increased TAG concentrations but also induced a shift toward smaller, denser LDL-particles compared to high-fat diets [24–26]. The predominance of the small, dense LDL subclass has been associated with an increased risk of CVD [41]. In addition, it is known from numerous studies that the small, dense LDL subspecies exhibit several properties that render them more atherogenic than large, buoyant LDL-particles [41]. We have previously shown that in normolipidemic participants, high-fat diets rich in MUFA (23.2% of energy) or PUFA (18.5% of energy) decreased LDL size by being incorporated into the LDL-particles [18]. The present study confirms these results. The precise underlying mechanism that may be responsible for this effect is unclear up to now. One factor might be the effect of individual unsaturated fatty acids on the expression or activity of involved enzymes such as cholesterol ester transfer protein and lecithin cholesterol acyltransferase, lipoprotein lipase or hepatic lipase. In agreement with this concept, plasma activities of cholesterol ester transfer protein and lecithin cholesterol acyltransferase have been reported to be influenced by MUFA-rich diets in healthy normolipidemic participants [42, 43] and in patients with hypertriacylglycerolemia [15]. In addition, the activities of these enzymes have been associated with the LDL subclass pattern [44]. However, because we have not measured enzyme activities, this hypothesis remains speculative. The significant decrease in LDL size in the present and in our previous study [18] observed in metabolically healthy, normolipidemic participants was very small. It is unclear whether this change is of clinical or physiological relevance, particularly as none of our participants fit into the classic small, dense LDL phenotype [i.e., LDL subclass phenotype B (peak particle diameter <25.5 nm) combined with high serum TAG and low HDL-c] [41].

In conclusion, a low-fat and a high-fat diet, both rich in MUFA, had similar effects on serum lipids and lipoproteins, LDL size and indices of lipid peroxidation in metabolically healthy, young and non-obese men and women. Our results show that across a wide range of fat intakes (between 29 and 40% of energy) and carbohydrate (between 43 and 54% of energy) intakes, lipid-related CVD risk factors do not differ when the fat and carbohydrate compositions are standardized. In addition, the present findings confirm that the moderate replacement of dietary SFA with either unsaturated fatty acids or carbohydrates leads to beneficial effects on LDL-c and on the LDL-c/HDL-c ratio provided that carbohydrates are ingested in the form of low-glycemic-index, fiber-rich foods. Our findings suggest that the fat-to-carbohydrate ratio is second to the fatty acid and carbohydrate composition in its effect on lipid-related risk factors for CVD.

Acknowledgments This study was financially supported by the German Union for the Promotion of Oil- and Protein-containing Plants (UFOP).

Conflict of interest statement None.

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