Milk-Derived Fatty Acids Are Associated with a More Favorable LDL Particle Size Distribution in Healthy Men[1,2]

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ABSTRACT A predominance of small dense LDL (sdLDL) particles is a well-established component of the atherogenic lipoprotein phenotype associated with insulin resistance and increased risk for coronary heart disease. However, the influence of diet on LDL particle size distribution is not clear. We investigated (cross-sectionally) the relations between LDL profile and dietary fatty acids (FAs) in 291 healthy men (62–64 y old) with a range of insulin sensitivities. Individuals completed a 7-d dietary record, and fasting plasma insulin, lipid, and lipoprotein concentrations as well as serum and adipose tissue FA composition were determined. The LDL profile was examined by polyacrylamide gradient gel electrophoresis, protein-staining, and quantitative scanning, giving LDL peak particle size and the percentage distribution of LDL in 4 subfractions. The men were divided into tertiles of percentage distribution of sdLDL. Small dense LDL was positively related to plasma triacylglycerol and fasting insulin concentrations (both $P < 0.0001$) and inversely related to HDL cholesterol ($P < 0.0001$). No strong relations were found between sdLDL and the reported intake of SFA, monounsaturated fatty acids, or PUFA. However, individual FAs typically found in milk products were associated with a more favorable LDL profile (i.e., fewer sdLDL particles). This was shown for 4:0–10:0 and 14:0 in the diet (both $P < 0.05$), 15:0 and 17:0 in serum phospholipids (both $P < 0.05$), and 15:0 in serum nonesterified FAs ($P < 0.01$). Furthermore, 20:3(n-6) in adipose tissue and serum phospholipids was positively related to sdLDL. Therefore, LDL particle size distribution appears to be modified by dietary factors with an apparently beneficial effect of milk products. J. Nutr. 134: 1729–1735, 2004.

KEY WORDS: • *small dense LDL* • *diet* • *milk fatty acids* • *fatty acid composition* • *healthy men*

Circulating LDL cholesterol (LDL-C)[4] concentrations are a well-established risk factor for coronary heart disease (CHD), but many individuals with CHD have normal or only slightly elevated LDL-C (1). However, the distribution pattern of LDL particles according to size and density was shown to be important in numerous studies, with an increased occurrence of small dense LDL particles (sdLDL) in individuals suffering from CHD (2,3) and insulin resistance–related dyslipidemia (4). Although the independent contribution of sdLDL to CHD has been questioned (5), a strong relation between plasma concentrations of sdLDL and carotid intima media thickness has been shown (6). The link between LDL size and CHD may be explained by sdLDL being particularly atherogenic due to increased plasma residence time, susceptibility to oxidation, and binding affinity for proteoglycans in the subendothelial space (7).

Genetic factors are suggested to explain as much as half of the variation in LDL size distribution with the remainder due to environmental factors such as adiposity, hormonal status, and diet. The influence of dietary habits on LDL-C concentrations is well documented, but its effect on LDL size distribution has been insufficiently investigated. A diverse response to a low-fat, high-carbohydrate diet with a beneficial effect on LDL size distribution was seen only in individuals starting with a predominance of sdLDL, whereas adverse effects could occur in individuals with large buoyant LDL (8,9). High SFA intake increases LDL-C and may play a role in the development of insulin resistance (10). In turn, high unsaturated fat intake has a more favorable effect on LDL-C (11) and insulin sensitivity (10). However, high SFA intake seems to be associated with larger LDL particles (12). Moreover, a diet high in myristic

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[4] Abbreviations used: apoB, apolipoprotein B; CHD, coronary heart disease; FA, fatty acid; HDL-C, HDL cholesterol; HOMA, homeostasis model assessment of insulin sensitivity; LDL-C, LDL cholesterol; MUFA, monounsaturated fatty acids; NEFA, nonesterified fatty acid; PL, phospholipid; SAD, sagittal abdominal diameter; sdLDL, small dense LDL; TAG, triacylglycerol; WC, waist circumference; WHR, waist-to-hip ratio.

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(14:0) and palmitic (16:0) acids shifted the LDL size distribution toward larger particles (13). Furthermore, an inverse relation was shown between serum cholesterol concentrations and dietary intake of SFAs of chain length 4–15 carbon atoms, derived mainly from milk fat (14). A beneficial effect on hyperinsulinemia was also suggested for food patterns dominated by milk products (15), as well as a decreased relative risk for death from all causes, including CHD (16).

Together, these studies suggest that a dietary pattern dominated by certain fatty acids (FAs) might have a beneficial effect on some cardiovascular risk factors. Therefore, in this study, we investigated the associations of LDL size distribution with different FAs, as determined in the diet, adipose tissue, serum phospholipids (PLs), and nonesterified fatty acids (NEFAs) in a population of 62- to 64-y-old healthy Swedish men.

SUBJECTS AND METHODS

Subjects. A total of 303 healthy 62- to 64-y-old men were randomly recruited from a larger population-based cohort of 2039 men living in Stockholm county (17). Exclusion criteria were non-Swedish descent, manifest diabetes, cardiovascular disease, cancer or chronic degenerative disease, treatment with antihypertensive or lipid-lowering agents, and BMI outside the range 19–35 kg/m^2. Of the original 2039 men, 995 fulfilled the criteria and were divided into tertiles of fasting plasma insulin concentration. Approximately 100 men from each tertile (participation rate 71%) were included in the current study. Further exclusions were incomplete 7-d dietary record ($n = 2$), thyroid stimulating hormone > 5.0 mmol/L ($n = 7$), and particularly low "area under the curve" for LDL after scanning (see below) ($n = 3$), leaving 291 subjects for the present study. The Ethics Committee of Karolinska Institutet approved the study and all subjects gave informed consent to participate.

Clinical procedure. Participants visited Karolinska Hospital in the morning, having fasted since 2200 h the night before and refrained from smoking during the morning hours. BMI, waist circumference (WC), waist-to-hip ratio (WHR), sagittal abdominal diameter (SAD), and systolic and diastolic blood pressures were determined as described (18,19). A needle biopsy of subcutaneous adipose tissue was taken from the left upper buttock (20). Information about medications, smoking, and physical activity was recorded during a structured interview. Participants were told not to change their eating habits during the study and were given written and oral instructions by a nutritionist on how to fill in a 7-d dietary record and how to collect a 24-h urine sample. After ~1 wk, the participant returned to the hospital with the completed food record and urine sample. The food record was examined and ambiguities resolved.

Dietary assessment. The 7-d record described by Rosell et al. (18) was a slightly modified, preprinted, optically readable version of a questionnaire used by the Swedish National Food Administration and Statistics Sweden in a national dietary survey in 1989 (21). It contained preprinted alternatives for foods and dishes commonly eaten and also space for recording foods and snacks not preprinted. The intake of foods and nutrients was calculated using the food-composition database of the Swedish National Food Administration, version 1/99 (22) and the software program SAS (SAS Institute). Intake of dairy fat was calculated as the sum of fat from milk, yogurt, cream, cheese, ice cream, and butter. Individuals identified as underreporting their energy intake were done so according to the Goldberg cutoff value (23), which compares reported energy intake with energy expenditure (level of physical activity included), both expressed as multiples of the basal metabolic rate (18). Identification of underreporters was validated on a group level by measuring sodium, potassium, and nitrogen collected in the 24-h urine samples.

Laboratory procedures. Plasma glucose was measured by a glucose oxidase method (Kodak Ektachem), insulin and proinsulin by commercial ELISAs (DAKO Diagnostics), serum NEFAs by an enzymatic colorimetric method (WAKO Chemicals), and plasma apolipoprotein B (apoB) by immunoprecipitation (Beckman Coulter). Plasma cholesterol and triacylglycerol (TAG) in VLDL, LDL, and HDL were determined by a combination of preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid determinations (ABX Diagnostics and Roche Diagnostics) (24). For determination of LDL particle size distribution, a lipoprotein-rich fraction (containing VLDL to LDL) was isolated from freshly thawed EDTA-plasma by adjusting the density to 1.070 kg/L and subsequent ultracentrifugation (142,500 $\times$ *g* for 22 h, 4°C). Recovery of total plasma apoB was 77 ± 12% ($n = 8$). The lipoprotein-rich fraction was applied to a 3–7.5% polyacrylamide gel together with standard lipoproteins [isolated human lipoprotein (a) and LDL] and proteins (thyroglobulin mono- and dimer, Pharmacia, LKB) of known size (6). Electrophoresis was conducted at 45 V for 30 min, 60 V for 30 min, followed by 80 V for 20 h, using Tris (270 mmol/L), boric acid (240 mmol/L), and EDTA (4.3 mmol/L, pH 8.35) as the running buffer. Gels were stained for protein (0.04% Coomassie Brilliant Blue, Serva) and analyzed using a Fuji LAS-1000 system and Image Gauge software to give peak particle size of LDL and relative distribution of LDL in predefined subfractions with the following cutoff values: LDL-I (27.0–25.0 nm), LDL-II (25.0–23.5 nm), LDL-III (23.5–22.5 nm), and LDL-IV (22.5–21.0 nm), corresponding to densities of 1.006–1.030, 1.030–1.040, 1.040–1.050, and 1.050–1.063 kg/L, respectively (6). A density of 1.040 kg/L is considered a classic boundary for dividing LDL into large and small particles (6). Validation of this modified method against the original method, which was designed to determine LDL profiles using isolated LDL samples (6), yielded significant correlations for LDL peak particle size and LDL size distribution ($r = 0.94$ and $r = 0.99$, respectively, both $P < 0.0001$, $n = 10$). Mean deviations of 24 duplicate runs using samples with a wide range of LDL distribution were 0.63% (95% CI, 0.41–0.84) for LDL peak size and 16% (95% CI, 12.7–25.7) for LDL-III (%). FAs in adipose tissue and serum PLs and NEFAs were separated by GLC (25) and expressed as the relative molar percentage of the sum of FAs analyzed. The FAs quantified in diet, adipose tissue, and serum were 14:0, 16:0, 18:0, 16:1, 18:1, (n-6) FAs, 18:2, 20:4, and (n-3) FAs 18:3, 20:5, and 22:6. In addition, dietary 4:0–10:0, 12:0, and 20:0 FAs and adipose/serum 15:0, 17:0, 20:3(n-6), and 22:5(n-3) FAs were quantified.

Statistical procedures. Statview (SAS Institute) and Stata (Stata Corporation) software was used for analysis. Skewed data were transformed logarithmically, but arithmetic means ± SD are presented for ease of understanding. ANOVA was used to test for differences between means. Outliers, determined as exceeding mean ± 4 SD, were excluded. Differences with $P < 0.05$ were considered significant. The homeostasis model assessment (HOMA) of insulin sensitivity was derived as described (26). Three multivariate regression models were generated by multiple stepwise linear regression analysis to identify variables independently related to LDL-III (%). A forward approach was used with significance levels set to <0.05. Models 1 and 2 examined dietary data and included only individuals identified as non-underreporters; the models were identical except for the dietary FA included. The 2 models were generated because of the strong correlation between the dietary FAs, preventing them from being included in the same model. Model 3 included all individuals, and correlation coefficients between variables were <0.7.

RESULTS

Basic characteristics. The 291 healthy men had normal plasma and VLDL TAG concentrations, but slightly elevated plasma and HDL cholesterol (HDL-C) concentrations **(Table 1)**. They were slightly obese, but had plasma insulin concentrations within the normal range. The majority of LDL was in LDL subfraction II (~50%), a minor part in subfraction IV (~4%) with the remainder distributed almost equally between subfractions I and III (both ~20%). This distribution pattern, as well as the mean LDL peak particle size, was similar to that seen in a previous study of 50-y-old healthy Swedish men (27). Men were divided into tertiles of percentage distribution of LDL-III (mean ± SD in each tertile, high: 29.0 ± 7.3%, medium: 15.9 ± 1.6%, and low 10.7 ± 1.8%). LDL subfraction III was chosen because of its strong correlation with carotid intima media thickness, a surrogate marker of early

TABLE 1

Basic characteristics of healthy Swedish men aged 62–64 y[1]

Age, *y*	63 ± 0.6
BMI, *kg/m*^{2}	25.8 ± 3.1
WHR	0.94 ± 0.06
SAD, *cm*	21.2 ± 2.3
WC, *cm*	96.2 ± 8.9
SBP, *mmHg*	135 ± 17
DBP, *mmHg*	80.9 ± 8.6
Plasma glucose, *mmol/L*	5.1 ± 1.0
Plasma insulin, *pmol/L*	40.8 ± 23.7
Plasma proinsulin, *pmol/L*	4.1 ± 4.1
HOMA	1.6 ± 1.2
Smoking, %	15 ± 0.4
Alcohol, *g/d*	20.1 ± 17.4
Plasma apoB, *g/L*	1.16 ± 0.28
Serum NEFA, *mmol/L*	0.52 ± 0.18
Plasma triacylglycerol, *mmol/L*	
Total	1.19 ± 0.61
VLDL	0.85 ± 0.58
LDL	0.24 ± 0.08
HDL	0.16 ± 0.04
Plasma cholesterol, *mmol/L*	
Total	5.91 ± 1.01
VLDL	0.40 ± 0.33
LDL	3.68 ± 0.94
HDL	1.66 ± 0.39
LDL peak particle size, *nm*	23.9 ± 4.5
Relative distribution of LDL subfractions,[2] %	
LDL-I	21.4 ± 6.8
LDL-II	53.4 ± 8.9
LDL-III	18.5 ± 8.9
LDL-IV	4.2 ± 1.8

[1] Values are means ± SD, $n = 291$ except for alcohol, $n = 192$.
[2] Represents the proportions of LDL-I, 27.0–25.0 nm; LDL-II, 25.0–23.5 nm; LDL-III, 23.5–22.5 nm; LDL-IV, 22.5–21.0 nm.

atherosclerosis (6). In contrast, subfraction IV correlates weakly with atherosclerosis and exhibits little variation, making it a poor marker of sdLDL. Individuals in the highest LDL-III (%) tertile had the greatest proportion of sdLDL and could therefore be considered to have a more atherogenic LDL-profile. Analysis was performed with subjects divided into tertiles of LDL-III (%) (giving reasonably large subject groups) so that the magnitude of the differences in the variables associated with changes in LDL-III (%) distribution could be assessed and not simply the strength of the relation, as would be the case if correlation analysis had been performed.

Metabolic variables. Plasma and VLDL TAG concentrations were positively and significantly related to the proportion of LDL-III, whereas an inverse relation was seen for HDL-C **(Table 2)**. The proportion of LDL-III was also positively related to concentrations of glucose, insulin and proinsulin, HOMA-index, and the various anthropometric measurements (BMI, WHR, SAD, and WC). Plasma LDL-C, serum NEFA concentrations, and blood pressure did not differ among the different LDL-III (%) tertiles. LDL size distribution and the variables in Table 2 did not differ when the cohort was divided into tertiles of LDL peak particle size (data not shown), except for plasma cholesterol and LDL-C, which had negative relations to LDL peak size (both $P < 0.001$).

Dietary effect. Relations between reported dietary fat intake (expressed as the percentage of total energy intake) and LDL size distribution were analyzed only in those subjects identified as non-underreporters of their energy intake ($n = 207$), divided into tertiles of LDL-III (%) **(Table 3)** [mean ± SD for LDL-III (%) in each tertile, high: 28.7 ± 7.2%, medium: 15.7 ± 1.6%, and low 10.7 ± 1.7%]. The tertiles did not differ for reported intake of total fat, SFAs, monounsaturated fatty acids (MUFAs), or PUFAs, although SFA intake

TABLE 2

Characteristics of healthy Swedish men aged 62–64 y grouped according to tertiles of LDL-III (%)[1]

	LDL-III (%) tertile			
	Low	Medium	High	*P*-value[2]
BMI, *kg/m*^{2}	25.2 ± 2.9	26.0 ± 3.1	26.3 ± 3.0	0.022
WHR	0.93 ± 0.05	0.94 ± 0.06	0.96 ± 0.05	0.0014
SAD, *cm*	20.5 ± 2.1	21.3 ± 2.3	21.9 ± 2.3	<0.0001
WC, *cm*	94.0 ± 9.0	96.7 ± 8.8	98.0 ± 8.4	0.0064
SBP, *mmHg*	136 ± 18	135 ± 17	135 ± 16	0.89
DBP, *mmHg*	82 ± 7	80 ± 9	81 ± 9	0.29
Plasma glucose, *mmol/L*	4.9 ± 0.5	5.1 ± 1.0	5.3 ± 1.3	0.0036
Plasma insulin, *pmol/L*	35.5 ± 21.0	39.5 ± 21.1	47.5 ± 27.1	0.0010
Plasma proinsulin, *pmol/L*	3.3 ± 1.9	4.0 ± 4.0	5.0 ± 5.4	0.0068
HOMA	1.3 ± 0.8	1.5 ± 1.0	2.0 ± 1.5	0.0002
Cigarettes, *n/d*	14.3 ± 9.5	11.8 ± 7.8	19.6 ± 11.6	0.13
Alcohol, *g/d*	17.4 ± 13.6	22.1 ± 19.0	20.4 ± 18.7	0.58
Plasma apoB, *g/L*	1.15 ± 0.26	1.09 ± 0.25	1.25 ± 0.30	0.0002
Serum NEFA, *mmol/L*	0.52 ± 0.16	0.52 ± 0.18	0.52 ± 0.19	0.99
Plasma triacylglycerol, *mmol/L*				
Total	0.95 ± 0.33	1.03 ± 0.41	1.62 ± 0.78	<0.0001
VLDL	0.60 ± 0.29	0.71 ± 0.38	1.24 ± 0.75	<0.0001
LDL	0.23 ± 0.08	0.22 ± 0.08	0.26 ± 0.09	0.0003
HDL	0.16 ± 0.03	0.16 ± 0.04	0.17 ± 0.04	0.0009
Cholesterol, *mmol/L*				
Total	5.99 ± 1.02	5.69 ± 0.89	6.04 ± 1.10	0.038
VLDL	0.26 ± 0.15	0.30 ± 0.19	0.64 ± 0.43	<0.0001
LDL	3.77 ± 0.95	3.52 ± 0.89	3.74 ± 0.96	0.13
HDL	1.79 ± 0.40	1.70 ± 0.39	1.48 ± 0.33	<0.0001

[1] Values are means ± SD, $n = 99$ (low), 95 (medium), 97 (high) except for apoB, $n = 99/95/96$; glucose, $n = 99/94/97$; and alcohol, $n = 60/68/64$.
[2] *P*-values are calculated by ANOVA.

TABLE 3

Fatty acid composition of diet and adipose tissue in healthy Swedish men aged 62–64 y grouped according to tertiles of LDL-III (%)[1]

	LDL-III (%) tertiles			
	Low	Medium	High	*P*-value[2]
Dietary intake,[3] *% energy*				
Total fat	35.6 ± 5.4	34.7 ± 5.8	34.3 ± 5.5	0.38
SFA	16.0 ± 3.3	15.1 ± 3.3	14.9 ± 2.9	0.098
MUFA	12.8 ± 1.9	12.7 ± 2.2	12.5 ± 2.1	0.81
PUFA	4.3 ± 0.8	4.6 ± 0.9	4.4 ± 0.9	0.18
Milk FA	12.8 ± 6.5	10.4 ± 6.2	10.3 ± 5.1	0.036
4:0–10:0	1.37 ± 0.50	1.21 ± 0.44	1.18 ± 0.41	0.025
12:0	0.91 ± 0.27	0.93 ± 0.33	0.85 ± 0.31	0.29
14:0	1.90 ± 0.54	1.73 ± 0.50	1.70 ± 0.45	0.043
16:0	8.11 ± 1.53	7.66 ± 1.48	7.64 ± 1.38	0.11
18:0	3.22 ± 0.62	3.13 ± 0.69	3.11 ± 0.61	0.53
18:1	11.5 ± 1.8	11.3 ± 2.1	11.3 ± 1.9	0.85
18:2(n-6)	3.2 ± 0.6	3.4 ± 0.8	3.3 ± 0.7	0.18
18:3(n-3)	0.59 ± 0.15	0.61 ± 0.18	0.59 ± 0.18	0.56
Adipose tissue,[4] *mol%*				
SFA	30.5 ± 3.1	30.2 ± 2.9	30.5 ± 2.9	0.66
MUFA	57.4 ± 2.6	57.6 ± 2.7	57.3 ± 2.9	0.80
PUFA	12.1 ± 1.5	12.3 ± 1.4	12.2 ± 1.7	0.72
14:0	4.1 ± 0.8	4.0 ± 0.8	3.9 ± 0.8	0.19
15:0	0.40 ± 0.09	0.38 ± 0.08	0.38 ± 0.08	0.30
16:0	22.1 ± 2.0	22.1 ± 1.9	22.6 ± 1.9	0.19
17:0	0.24 ± 0.04	0.23 ± 0.04	0.22 ± 0.04	0.061
18:0	3.6 ± 0.8	3.5 ± 0.8	3.4 ± 0.8	0.16
18:1	49.4 ± 1.8	49.6 ± 2.0	49.4 ± 1.9	0.72
18:2(n-6)	9.5 ± 1.3	9.7 ± 1.2	9.5 ± 1.5	0.59
18:3(n-3)	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	0.60
20:3(n-6)	0.15 ± 0.04	0.16 ± 0.04	0.17 ± 0.05	0.0036

[1] Values are means ± SD.
[2] *P*-values are calculated by ANOVA.
[3] Only individuals identified as non-underreporters are included, $n = 67$ (low), 70 (medium), 70 (high). Milk FA, fatty acids from milk, yogurt, cream, cheese, ice-cream, and butter.
[4] $n = 96$ (low), 95 (medium), 96 (high) except for 20:3(n-6), $n = 94/95/96$.

was significantly lower in the high compared with the low LDL-III (%) tertile ($P = 0.04$, t test). Reported intake of fat from dairy products as well as specific FAs (4:0–10:0 and 14:0), found abundantly in dairy products, was negatively related to the LDL-III (%) proportion. Similar results were seen for reported dietary intake of FAs in absolute amounts (g/d) (data not shown).

Serum and adipose tissue FA composition. Relations between tertiles of LDL-III (%) in the whole cohort ($n = 291$) and relative proportions of FAs in adipose tissue, serum PLs, and serum NEFAs are shown in **Tables** 3 and **4**. Total amounts of SFAs, MUFAs, and PUFAs in adipose tissue and PLs were not related to the proportion of LDL-III (%). There was a significant difference between the tertiles for total SFAs in serum NEFAs, although this was not a graded response, whereas no relations were found for MUFAs or PUFAs. However, individual FAs, typical of dairy products, were significantly related to LDL-III (%) tertiles. Relative amounts of pentadecanoic acid (15:0) in serum PLs and serum NEFAs as well as heptadecanoic acid (17:0) in serum PLs were inversely related to the proportion of LDL-III. The same trend was seen for 17:0 in adipose tissue ($P = 0.06$). In addition, differences between the tertiles were detected for myristic (14:0), palmitic (16:0), and oleic acids (18:0) in serum PLs, although these differences were not graded across the tertiles. Among the other FAs, the relative amount of dihomo-γ-linolenic acid [20:3(n-6)] in both adipose tissue and serum PLs was positively related to the proportion of LDL-III (levels in serum NEFAs were too low to be quantified in most individuals). The other long-chain FAs analyzed were not related significantly to sdLDL (data not shown).

Data were also analyzed after grouping of subjects into tertiles of LDL-II (%) (the predominant large, buoyant LDL subfraction). These results largely confirmed those from analysis with LDL-III (%) in that increased amounts of FAs typical of milk products were associated with a greater proportion of larger LDL particles (data not shown).

Multivariate analysis. Because of strong relations between TAG concentrations and sdLDL, multiple stepwise regression analysis was performed in which individual FAs were included in the model, as well as plasma TAG, apoB, HDL-C, and insulin and BMI **(Table 5)**. Two separate models were generated for dietary FAs 4:0–10:0 (model 1) and for 14:0 (model 2) because these variables were strongly correlated with each other ($r > 0.80$). In both models, plasma TAG explained the majority of the variation in LDL-III (%) (30%), but FAs 4:0–10:0 and 14:0 entered each model with an increase in adjusted R^2 of 0.01 for each FA. Model 3 included all individuals and a selection of FAs in adipose tissue, PLs, and

TABLE 4

Fatty acid composition of serum phospholipids and serum NEFAs in healthy Swedish men aged 62–64 y grouped according to tertiles of LDL-III (%)[1]

	LDL-III (%) tertiles			
	Low	Medium	High	*P*-value[2]
	mol%			
Phospholipids[3]				
SFA	46.4 ± 1.0	46.3 ± 0.9	46.5 ± 1.1	0.48
MUFA	13.4 ± 1.1	13.6 ± 1.1	13.3 ± 1.5	0.29
PUFA	40.2 ± 1.4	40.1 ± 1.3	40.2 ± 1.8	0.98
14:0	0.50 ± 0.09	0.46 ± 0.08	0.48 ± 0.09	0.035
15:0	0.23 ± 0.04	0.22 ± 0.04	0.21 ± 0.04	0.0098
16:0	31.8 ± 1.2	32.0 ± 1.1	31.5 ± 1.2	0.017
17:0	0.41 ± 0.06	0.40 ± 0.06	0.38 ± 0.07	0.031
18:0	13.5 ± 0.9	13.3 ± 0.9	13.9 ± 1.0	<0.0001
18:1	12.8 ± 1.0	12.9 ± 1.0	12.6 ± 1.2	0.23
18:2(n-6)	19.4 ± 2.1	19.4 ± 2.1	19.0 ± 2.5	0.48
18:3(n-3)	0.33 ± 0.10	0.32 ± 0.10	0.33 ± 0.11	0.57
20:3(n-6)	2.82 ± 0.56	2.94 ± 0.64	3.06 ± 0.64	0.031
NEFA[4]				
SFA	41.0 ± 2.9	40.0 ± 2.5	40.6 ± 2.7	0.039
MUFA	45.1 ± 3.0	45.9 ± 2.9	45.2 ± 3.5	0.13
PUFA	13.6 ± 1.7	13.8 ± 1.9	13.9 ± 2.5	0.72
14:0	2.50 ± 0.48	2.37 ± 0.39	2.39 ± 0.42	0.080
15:0	0.34 ± 0.12	0.31 ± 0.07	0.30 ± 0.06	0.0020
16:0	26.7 ± 2.0	26.3 ± 1.7	26.8 ± 1.7	0.081
17:0	0.43 ± 0.07	0.41 ± 0.07	0.42 ± 0.08	0.054
18:0	11.0 ± 1.6	10.6 ± 1.6	10.6 ± 1.8	0.23
18:1	41.1 ± 2.6	42.0 ± 2.5	41.3 ± 3.1	0.050
18:2(n-6)	10.1 ± 1.2	10.3 ± 1.2	10.2 ± 1.6	0.64
18:3(n-3)	1.50 ± 0.32	1.56 ± 0.47	1.59 ± 0.64	0.72

[1] Values are means ± SD in molar percent of total fatty acids.
[2] *P*-values are calculated by ANOVA.
[3] $n = 97$ (low), 95 (medium), 97 (high) except for 15:0 $n = 97/95/96$.
[4] $n = 98$ (low), 95 (medium), 97 (high) except for 15:0 and 16:0, $n = 97/95/97$.

TABLE 5

Multiple stepwise regression analysis of the relations of plasma TAG, apoB, HDL-C, and insulin, BMI, and various FAs to LDL-III (%)[1]

	Coefficient ± SD	Standardized coefficient	Adjusted R^2
Non-underreporters (n = 207)			
Model 1[2]			0.30
Intercept	0.45 ± 0.30	0.45	
Plasma TAG	1.24 ± 0.14	0.53	
Dietary 4:0–10:0	−0.12 ± 0.06	−0.13	
Model 2[3]			0.30
Intercept	0.49 ± 0.30	0.49	
Plasma TAG	1.25 ± 0.14	0.53	
Dietary 14:0	−0.11 ± 0.05	−0.13	
Whole cohort (n = 291)			
Model 3[4]			0.31
Intercept	1.97 ± 0.66	1.97	
Plasma TAG	1.20 ± 0.11	0.52	
Serum PL 16:0	−0.72 ± 0.32	−0.12	
Serum NEFA 15:0	−0.04 ± 0.02	−0.11	

[1] Forward multiple stepwise regression analysis with the significance level set to <0.05.

[2] Model 1: Including plasma TAG, apoB, HDL-C, insulin, BMI, and dietary intake (energy%) of FAs 4:0–10:0 (non-underreporters only).

[3] Model 2: Including plasma TAG, apoB, HDL-C, insulin, BMI, and dietary intake (energy%) of FA 14:0 (non-underreporters only).

[4] Model 3: Including plasma TAG, apoB, HDL-C, insulin, BMI, proportion of FAs 14:0, 15:0, 18:0, 20:3(n-6) in adipose tissue, 14:0, 15:0, 16:0, 18:0, 20:3(n-6) in serum PLs and 14:0 and 15:0 in serum NEFAs (all individuals).

NEFAs. In this model, again plasma TAG explained the majority of the variation in LDL-III (%) (29%) but with 16:0 in serum PLs and 15:0 in serum NEFAs entering the model with an increase in adjusted R^2 of 0.01 for each FA.

DISCUSSION

This study in healthy 62- to 64-y-old men confirmed relations between sdLDL and several variables related to the insulin resistance syndrome.

Dietary fatty acids and sdLDL. In studies of twins, heritability and plasma TAG concentrations were estimated to account for 30–50% and 50–60% of the variability in LDL profile, respectively (28,29). However, dietary habits may also influence LDL size distribution. Cross-sectional and dietary intervention studies reported an increase in sdLDL with increasing carbohydrate intake (30) and a slight shift to smaller LDL particles when MUFAs or PUFAs replaced SFAs (31). The effect of the latter change was modest and might be explained by opposing effects of individual FAs in each class (SFA/MUFA/PUFA) influencing lipoprotein metabolism differently (see below).

Intervention studies showed decreasing sdLDL with increasing fat intake (8,9), but no significant relation was found in the present study. This might be due to the cross-sectional design, comprising individuals with low variation in dietary fat intake compared with the relatively large differences achieved in intervention studies. However, subjects in the high tertile of sdLDL reported a lower intake of SFAs than individuals in the low tertile, as in other cross-sectional studies (12). This seemed to be mediated via lower intake of certain SFAs (4:0–10:0 and 14:0) abundantly found in milk products, relations that remained significant in multivariate analysis that included plasma TAG. In contrast to the SFA-induced increase in larger LDL particles in healthy men (13), mildly hypercholesterolemic men responded with an increase in smaller LDL subfractions when substituting SFAs for MUFAs (32). These results mirror the heterogeneous responses to different diets of both LDL size distribution and overall lipoprotein concentrations, likely reflecting the different genetic backgrounds and metabolic status of the subjects. For example, when healthy men changed from a high-fat to a low-fat, high-carbohydrate diet, there was an increase in the smaller LDL subfractions in the cohort as a whole. The individual responses revealed that only individuals with a high proportion of sdLDL consuming a high-fat diet would benefit from this dietary change. However, a subset of the individuals with a less atherogenic LDL-profile consuming the high-fat diet developed a predominance of sdLDL when changing to the low-fat, high-carbohydrate diet (9). Furthermore, the magnitude of increase in smaller LDL subfractions was related to the apoE phenotype (33).

Adipose tissue and serum fatty acids, and sdLDL. Bias from underreporting in dietary records can be eliminated only to some extent (23). The FA composition of adipose tissue, serum PLs, and serum NEFAs was determined for a more objective assessment of dietary pattern (34). The FA profile of adipose tissue reflects dietary FA composition during the last 6–24 mo, whereas that of serum PLs reflects the short-term diet (weeks to months) (35,36). This is especially valid for essential FAs and FAs that cannot be synthesized endogenously (34), indicating that FA composition analysis in serum and adipose tissue is a good complement to dietary records.

In this study, results from FA compositional and dietary record analyses were in accordance, demonstrating a relation between a high percentage of FAs derived from milk products and a less atherogenic LDL-profile. Pentadecanoic (15:0) and heptadecanoic (17:0) acids are found almost exclusively in milk products, and the relative amount of sdLDL was inversely related to these FAs in serum and adipose tissue, although not significantly in adipose tissue (P = 0.30 and 0.06, respectively). Furthermore, myristic acid in serum differed across the tertiles of sdLDL, but with no linear gradient. This unexpected finding, as well as other such nonlinear relations observed between individual FAs and sdLDL, is hard to interpret, and it is questionable whether it is of biological importance. Surprisingly, no strong relations between sdLDL and typical milk FAs in adipose tissue were detected. This might be explained by desaturase/elongase activities within adipose tissue leading to preferential storage of MUFAs and/or by physical activity causing preferential release of shorter-chain FAs (37), both of which could potentially confound relations between sdLDL and milk-derived FAs. The relative amount of palmitic acid in serum PLs was significantly related to sdLDL, but with no linear gradient among the 3 groups. Interestingly, multivariate analysis revealed significant effects on sdLDL of both palmitic acid in serum PLs and pentadecanoic acid in serum NEFAs, suggesting a specific FA-mediated effect on LDL-particle size distribution above and beyond the influence of plasma TAG. The different multivariate analyses suggested a modest effect of milk-derived FAs on sdLDL. However, the biological relevance of these findings should be considered. The energy intake from milk-derived FAs was 2.5% higher in individuals in the low compared with the high tertile of sdLDL, corresponding to the consumption of ~200–400 mL pure milk (depending on milk type). Furthermore, the LDL peak size was 0.8 nm larger in the low vs. high tertile (24.2 ± 0.2 vs. 23.4 ± 0.4 nm, respectively), a difference associated with a 38%

decreased risk for myocardial infarction over 7 y in the Physicians' Health Study (38). Thus, the relatively small changes in consumption of milk-derived FAs in the present study are associated with potentially important changes in LDL size.

To our knowledge, no previous study showed a relation between milk FAs and LDL-profile, but several publications report a lack of association between milk consumption and CHD (16,39–42). These findings, together with reports associating a high intake of milk products with a decreased risk of developing insulin resistance (15,43), stand in contrast to the link between a high intake of palmitic acid (16:0) (the predominant FA in milk products) and increased risk of CHD and insulin resistance. However, the apparent adverse effects of certain saturated milk FAs (such as palmitic acid) could be counteracted by the different effects of other SFAs. For example, short- and medium-chain SFAs are more readily utilized as an energy source than their longer-chain counterparts (44). When the food pattern is dominated by milk products (with a predominance of short- and medium-chain SFAs) the oxidative preference for these SFAs might contribute to reduced fat accumulation (compared with other food patterns) with subsequent beneficial effects on the metabolic syndrome. Additionally, individual SFAs might differently influence hepatic lipoprotein secretion and hence LDL particle size distribution. Myristic acid (14:0), relative to stearic acid (18:0), inhibits apoB degradation and TAG secretion in rat hepatoma cells, and shifts the secretion pattern towards denser apoB-containing lipoproteins (45), thus potentially affecting the generation of sdLDL. Furthermore, an SFA-induced increase in large buoyant LDL was concomitant with decreased hepatic lipase activity (13), an important enzyme for the formation of sdLDL. Finally, milk products contain several bioactive substances in addition to FAs, and milk consumption might also be related to life-style factors. Both of these could mediate, either alone or in concert with milk-FAs, the observed associations with a less atherogenic LDL-profile.

A high proportion of sdLDL was related to higher relative amounts of dihomo-γ-linolenic acid [20:3(n-6)] in both adipose tissue and serum PLs, which might reflect increased Δ-6 desaturase activity, although it is unclear what importance this would have. Previous studies found positive associations between relative amounts of dihomo-γ-linolenic acid in serum cholesteryl-esters and both fasting plasma insulin concentrations and diastolic blood pressure (10), which was confirmed in serum PLs in this study (data not shown).

Although the cross-sectional design of this study prevents identification of causal relations, and the large number of variables analyzed increases the risk of identifying relations by chance, the consistency of relations between certain FAs and sdLDL cannot be ignored, supporting the view that dyslipidemic states are related to the FA composition of the diet, serum, and adipose tissue.

In conclusion, this study confirmed relations between sdLDL and variables related to insulin resistance. No relations were found between sdLDL and the major FA classes (SFA/MUFA/PUFA). However, specific FAs were related to LDL size distribution, suggesting that the physiologic effect of individual FAs might not be representative of the whole class. A consistent relation was found between a less atherogenic LDL-profile and a higher proportion of milk FAs in the diet, serum, and adipose tissue, which agrees with previous reports that did not detect an increased risk for CHD and overall mortality from a high consumption of milk products. This suggests that milk consumption might not be as hazardous to health as traditionally expected, given its high SFA content. However, the physiologic explanation behind this is unknown and the relation to a less atherogenic LDL-profile might not be mediated directly by the FAs but via some other factor(s), abundant in milk products, or related to milk consumption; this remains to be resolved.

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