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Bioanalysis of PUFA metabolism and lipid peroxidation in coronary atherosclerosis

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Abstract

Twenty eight men (age 34–77 years) who underwent an elective coronary angiography for coronary artery disease (CAD), were studied. They were divided into group A (luminal narrowing $< 50\%$; $n = 11$) and group B (luminal narrowing $> 50\%$; $n = 17$). Capillary gas chromatography was used for determination of fatty acids. Retinol and alpha-tocopherol were analyzed by reversed-phase high-performance liquid chromatography (HPLC), other parameters were determinated spectrofluorometrically and spectrophotometrically. Severe coronary atherosclerosis in group B was associated with higher serum low density lipoprotein/high density lipoprotein (LDL/HDL) cholesterol ratio, triacylglycerols, and phospholipids ($P < 0.05$). Erythrocyte membrane fatty acids C14:0, C16:1 and C22:6n3 were significantly higher in group B ($P < 0.05$). We found significantly higher plasma polyunsaturated fatty acids (PUFA) C18:3n6 in group B, whereas plasma linoleic acid was not changed significantly. There was a significant increase of IDL–C18:0, LDL–C14:0 and HDL–C22:6n3 PUFA in group B. We conclude that disturbances in saturated fatty acids (SUFA) and PUFA metabolism are associated with coronary atherogenesis. Such abnormalities may include enhanced extrahepatic transport of C14:0 SUFA via LDL and its incorporation into cell membranes, and enhanced clearance of anti atherosclerotic C22:6n3 PUFA via serum HDL.

Keywords: Capillary gas chromatography; Reversed-phase high-performance liquid chromatography; Coronary artery disease; Lipoproteins; Fatty acid metabolism

1. Introduction

Atherosclerosis with resulting coronary artery disease (CAD) has been associated with abnormal metabolism of lipoproteins and fatty acids [1]. Considerable data suggest that the influence of fat quantity and saturation on atherosclerosis and mortality due to cardiovascular disease is mediated in part by affects on plasma cholesterol [2,3] and low density lipoprotein–cholesterol (LDL–C) concentrations [2,4]. However, effects on plasma lipids and lipoproteins can explain only part of the increased progression of CAD associated with

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higher amounts of dietary total and saturated fat [4]. This suggests that fatty acids may contribute to atherosclerosis in other ways. Such mechanism(s) may include alterations in cellular functions [5], which may be related to changes in cellular membrane fatty acid composition [6]. Dietary fatty acids and saturation may also influence cellular cholesterol balance via effects on (1) delivery of cholesterol to arterial cells by metabolism of LDL–C secondary to influencing interaction of LDL–C of altered fatty acid composition [7] with arterial proteoglycans [8], and (2) high density lipoprotein (HDL) fatty acid composition, which alters the ability of HDL to remove cholesterol from arterial cells [9]. To prevent CAD, substitution of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) for saturated fatty acids (SUFA) is generally recommended [10].

SUFA increase the risk for the development of CAD [11]. Underlying atherogenic mechanism of SUFA is their effect on lipid metabolism. Despite different effects on HDL– and LDL–cholesterol, all saturated fatty acids increase the LDL:HDL ratio to a comparable degree [1]. Resulting cholesterol-raising activity [12] appears to be limited to lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0). In addition, SUFA affect other processes that determine atherogenesis, such as hemostatic variables (composition of platelet membranes, coagulation and fibrinolytic factors) [13]. Saturates with different chain length may, however, differ in their cholesterol-raising effects. For example, fats rich lauric (C12:0) plus myristic acids (C14:0) increase serum total, low density cholesterol and high density cholesterol compared with fats rich in stearic acid (C18:0) [14]. Recently, it has bee suggested that the cholesterol-raising effect of palmitic acid (C16:0) seems to be neutral in normocholesterolemic subjects provided their diet contains little cholesterol and the linoleic acid intake is adequate [15].

There are two series of essential of PUFAs, alpha-linolenic acid (C18:3n3) is the parent compound of (n3) family, and linoleic acid (C18:2n6) of (n6) fatty acids. Long-chain (n3) fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as (n6) fatty acids [(mainly linoleic acid and its derivative gamma-linolenic acid (C18:3n6)], have been reported to possess lipid-lowering properties [16]. An increase in (n3) fatty acids mainly decreases serum triglycerides and increases high density lipoprotein cholesterol [17], whereas an increase in linoleic acid and gamma-linolenic acid mainly decreases total cholesterol and low density cholesterol [18].

Although atherogenic effects of the individual dietary fatty acids and dietary cholesterol on fasting serum lipids and lipoproteins have been studied extensively, possible interactions among fatty acids or with lipoproteins, as well as lipoperoxidation processes, are only poorly understood. This should be investigated more thoroughly using recently available bioanalytical techniques. Therefore, the aim of this study was to apply a combination of capillary gas chromatography, reversed-phase high-performance liquid chromatography (HPLC), as well as spectrophotometrical, spectrofluorometrical and enzymatic techniques (i) to study fatty acid composition of plasma, erythrocyte membrane and serum lipoproteins in hyperlipidaemic subjects, (ii) to investigate association of fatty acid composition with lipoperoxidation factors known to be pro-atherogenic (thiobarbituric acid reactive substance activity) or anti atherogenic (alpha-tocopherol), and (iii) to ascertain the role of fatty acids in CAD as assessed by coronary angiography.

2. Materials and methods

2.1. Study group

Twenty eight hyperlipidaemic men (age 34–77 years) who underwent an elective coronary angiography for CAD, were studied. Hyperlipidaemia was treated conventionally using statins, resins and/or fibrates in standard doses. Patients were counseled to follow the National Institute of Health (NIH) Cholesterol Education Program (NCEP) step I diet which limits dietary cholesterol to < 300 mg/day, saturated fats $< 10\%$ of total calories, and total fats to $< 30\%$ of total calories [19]. The patients were divided in group A (luminal narrowing $< 50\%$; $n = 11$) and group B (luminal narrowing $> 50\%$; $n = 17$). Exclusion

criteria were, acute myocardial infarction within 3 months of blood sampling, obesity (body mass index over 30), thyreopathy (i.e. hyper-or hypothyreosis), treatment with thiazide diuretics or steroid hormones (including hormonal replacement therapy during menopause), alcoholism, and diabetes mellitus treated by insulin.

Selective coronary angiography was performed according the standard procedures, provided that indicated by a cardiologist, and was performed and analyzed according to the clinical routine of the hospital. The results obtained were within standard selectivity, specificity and sensitivity [20]. The patients with lesions of less than 50% luminal narrowing were referred to group A. A luminal narrowing of 50% or more was defined as a significant lesion, and the patients were referred to group B. All assessments of the degree of CAD were performed by a single radiologist in order to limit errors due to inter-observer variability. The radiologist was blinded in terms of lipid and lipoprotein data.

2.2. Bioanalysis

Blood samples were drowned from the peripheral vein between 6:00 and 8:00 h after 12-h overnight fasting, and were collected in Na-EDTA tubes. The samples were centrifuged immediately. Plasma was separated and red blood cells were washed in a standard manner [21].

Serum lipoprotein fractions were prepared by density gradient ultracentrifugation (Beckman TL 100, Palo Alto, CA) [22]. The lipoprotein fractions were distinguished in the following density ranges, very low density lipoprotein (VLDL) $<$ 1.006 g/ml; intermediate density lipoprotein (IDL) $<$ 1.019 g/ml; LDL $<$ 1.063 g/ml; HDL $>$ 1.063 g/ml.

The fractions were than analyzed for content of vitamin E (alpha-tocopherol) and vitamin A (retinol) by reversed-phase HPLC technique (LC 200, Perkin Elmer, Norwalk, USA). The mobile phase 100% methanol, flow rate 1.2 ml/min, column C_{18} RP, 4.6×150 mm, 5 μm, (Perkin Elmer, Norwalk, USA) were used. The vitamins were simultaneously detected with diode-array detector at 325 and 290 nm for vitamin A and vitamin E, respectively (Perkin Elmer; Norwalk, USA) [23,24].

Total concentration and/or lipoprotein fraction concentration of cholesterol [25] and triacylglycerols [26] were assessed enzymatically by conventional diagnostic kits (Lachema, Brno, Czech Republic) and spectrophotometric analysis (ULTROSPECT III, Pharmacia LKB Biotechnology, Uppsala, Sweden).

Free fatty acids (FFA) in serum, fatty acid composition of erythrocyte cell membranes, including SUFA, MUFA and PUFA, and FA of lipoprotein fractions were measured using capillary gas chromatography.

The red blood cells were haemolysed by the addition of an equivalent volume of water. Lipids were extracted according to the procedure described by Dodge and Phillipis [27]. Plasma FFA were extracted after addition of internal standard (heptadecanoic acid) by modified Dole method [28]. Lipids from lipoprotein fractions were extracted following Bligh and Dyer [29]. The fatty acid methyl esters were than formed by heating for 30 min with BF3/methanol. Profiling of fatty acid methyl esters (FAME) was performed by Hewlett-Packard gas chromatograph 5890 II (Palo Alto, MA) equipped with flame ionization detector and HP 3396 A integrator. FAME were separated on a SP-2330 Fused Silica Capillary Column 30 m $\times$ 0.25 mm i.d., 0.20 μm film (Supelco Inc., Bellafonte, PA). Injection port and FID temperatures were both 250°C. Oven temperature was programmed to change from 120 to 230°C at 4°C/min. Helium was used as the carrier gas, splitting ratio was 1:100. Individual fatty acids were identified by relative retention times determined with known standards.

Thiobarbituric acid-reactive substances (TBARS), which are products of lipid peroxidation, were determinated after reaction with thiobarbituric acid spectrofluorometrically (LS-5 spectrofluorimeter, Perkin Elmer, Norwalk, USA) [30].

Phospholipids in serum and erythrocyte membranes were analyzed spectrofotometrically using Specol 11, Carl Zeiss Jena, Germany [31].

2.3. Statistical analysis

The fatty acids, TBARS, retinol, alpha-tocopherol, cholesterol, triacylglycerols, phospholipids in serum and lipoprotein fractions and fatty acids and phospholipids in erythrocyte membrane were determined in two groups of patients who were divided according to their angiography (groups A and/or B). Statistics was performed using software NCSS 6.0.1 (Kaysville, UT, 1995). Statistical analysis was calculated using two sample test. Results were expressed as mean ± SEM. Significance was established if the P value was less than 0.05.

The study protocol had been accepted by local ethical committee, Charles University, Faculty Hospital, Hradec Králové, Czech Republic.

3. Results

3.1. Serum lipoproteins

Severe coronary atherosclerosis in group B was associated with significantly higher serum triacylglycerols and IDL–triacylglycerols. LDL cholesterol did not differ significantly. A ratio of LDL/HDL cholesterol was significantly higher in group B ($1.85 + 0.13$ in group A; $2.66 + 0.19$ in group B; $P < 0.01$) (Table 1).

3.2. Vitamin E, vitamin A, TBARS

The increase of vitamin E in group B was associated with increase of vitamin E in fractions of VLDL, IDL and LDL, but not HDL (Table 2). Serum vitamin A did not differ significantly (2.67 ± 0.67 μmol/l in group A; 2.39 ± 0.44 μmol/l in group B; n.s.). Serum TBARS did not differ significantly (3.15 ± 1.19 μmol/l in group A; 3.38 ± 0.98 μmol/l in group B; n.s.).

3.3. Phospholipids

There were significantly higher plasma phospholipids in group B (2.18 ± 0.41 mmol/l in group A; 2.65 ± 0.30 mmol/l in group B; $P < 0.01$). Erythrocyte membrane phospholipids did not differ significantly ($3.02 + 0.45$ mmol/l in group A; $2.97 + 0.50$ mmol/l in group B; n.s.).

Table 1
Cholesterol and triacylglycerols in serum and lipoproteins[a]

Variable	Unit of measurement	Group A		Group B		P value
		N	Mean (± SEM)	n	Mean (± SEM)	
Total cholesterol	mmol/l	11	5.31 (0.34)	15	5.85 (0.97)	n.s.
VLDL–cholesterol	mmol/l	11	1.20 (0.12)	15	1.50 (0.10)	n.s.
IDL–cholesterol	mmol/l	11	0.88 (0.06)	15	1.07 (0.07)	n.s.
LDL–cholesterol	mmol/l	11	1.97 (0.17)	15	2.31 (0.14)	n.s.
HDL–cholesterol	mmol/l	11	1.07 (0.07)	15	0.90 (0.06)	n.s.
LDL/HDL–cholesterol		11	1.85 (0.13)	15	2.66 (0.19)	0.004
Triacylglycerols	mmol/l	11	1.5 (0.15)	15	2.12 (0.21)	0.034
VLDL–triacylglycerols	mmol/l	11	0.97 (0.13)	15	1.45 (0.18)	0.056
IDL–triacylglycerols	mmol/l	11	0.24 (0.02)	15	0.34 (0.03)	0.008
LDL–triacylglycerols	mmol/l	11	0.14 (0.03)	15	0.20 (0.03)	n.s.
HDL–triacylglycerols	mmol/l	11	0.05 (0.01)	15	0.11 (0.06)	n.s.

[a] Cholesterol and triacylglycerols in serum and lipoproteins (VLDL, IDL, LDL, HDL), LDL/HDL cholesterol ratio in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $< 50\%$; $n = 11$) and group B (luminal narrowing $> 50\%$; $n = 17$). Results were expressed as mean ± SEM. Significance was established if the P value was less than 0.05; n.s., not significant.

Table 2
Total vitamin E in serum and lipoprotein fractions[a]

Variable	Unit of measurement	Group A		Group B		*P* value
		N	Mean (±SEM)	*n*	Mean (±SEM)	
Total vitamin E	umol/l	11	20.9 (1.3)	17	26.6 (1.2)	0.005
VLDL–vitamin E	umol/l	11	6.5 (0.9)	17	10.0 (0.8)	0.006
IDL–vitamin E	umol/l	11	3.5 (0.4)	17	4.7 (0.3)	0.01
LDL–vitamin E	umol/l	10	5.5 (0.3)	17	7.9 (0.4)	0.0008
HDL–vitamin E	umol/l	11	4.9 (0.2)	17	5.0 (0.5)	n.s.

[a] Serum total vitamin E, and vitamin E in lipoprotein fractions, VLDL–vitamin E; IDL–vitamin E; LDL–vitamin E; HDL–vitamin E in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established if the *P* value was less than 0.05; n.s., not significant.

3.4. Fatty acids in plasma

There was significantly higher plasma gama-linolenic PUFA (C18:3n6) in group B (Fig. 1), whereas plasma linoleic acid (C18:2n6; 73.72 ± 1.24 μmol/l in group A; 78.65 ± 23.76 μmol/l in group B; n.s.) was not changed in this group. Other plasma fatty acids did not differ significantly.

3.5. Fatty acids in erythrocyte membranes

There was significantly higher erythrocyte membrane myristic acid (C14:0) (Fig. 2), palmitoleic acid (C16:1) (Fig. 3) and docosahexaenoic PUFA (C22:6n3) (Fig. 4). Other fatty acids of erythrocyte membrane did not differ significantly.

3.6. Fatty acids in lipoprotein fractions (Tables 3–6)

There was a significant increase of IDL stearic fatty acid (C18:0) (Table 4), LDL C14:0 (myristic acid) (Table 5) and HDL-docosahexaenoic PUFA (C22:6n3) (Table 6). Other fatty acids in lipoprotein fraction did not differ significantly.

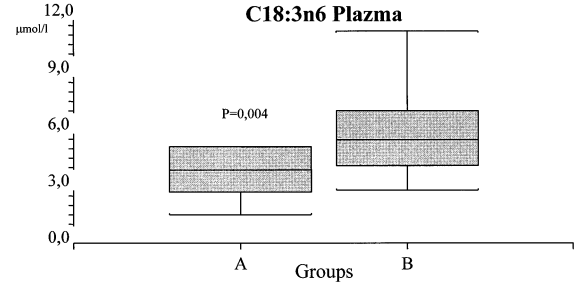

Fig. 1. Plasma gama-linolenic acid (C18:3n6) in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established as $P<0.01$.

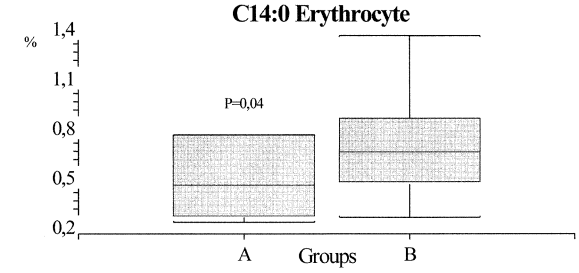

Fig. 2. Myristic acid (C14:0) in erythrocyte membrane of patients who underwent an elective coronary angiography for coronary artery disease. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established as $P<0.05$.

4. Discussion

Previous studies demonstrated that PUFA and MUFA over SUFA generally prevent against

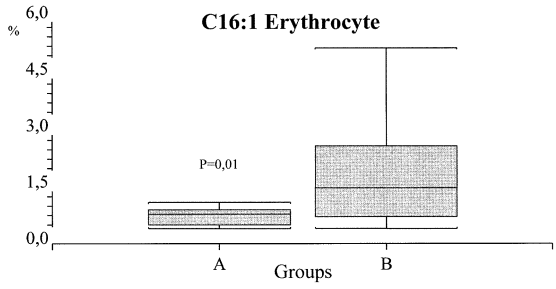

Fig. 3. Palmitic acid (C16:1) in erythrocyte membrane of patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $< 50\%$; $n = 11$) and group B (luminal narrowing $> 50\%$; $n = 17$). Results were expressed as mean $\pm$ SEM. Significance was established as $P < 0.05$.

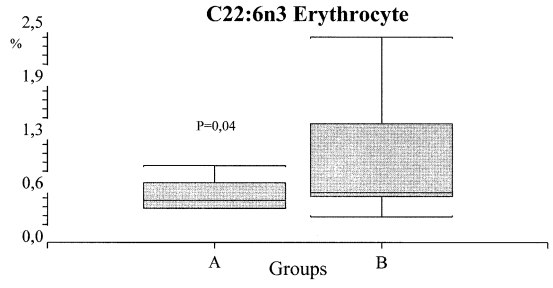

Fig. 4. Docosahexaenoic acid (C22:6n3) in erythrocyte membrane of patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $< 50\%$; $n = 11$) and group B (luminal narrowing $> 50\%$; $n = 17$). Results were expressed as mean $\pm$ SEM. Significance was established as $P < 0.05$.

CAD [10–13,15,16,18]. The combined use of several bioanalytical techniques to measure fatty acids in plasma, erythrocyte membranes and lipoproteins in man with CAD provided further information about the possible interactions among fatty acids or with lipoproteins, (1) Severe coronary atherosclerosis in group B was associated with higher serum LDL/HDL cholesterol ratio, triacylglycerols, and phospholipids. (2) There were significantly higher plasma PUFA 18:3n6 in group B, whereas plasma linoleic acid was not changed significantly. (3) Erythrocyte membrane fatty acids 14:0, 16:1 and 22:6n3 were significantly higher in group B. (4) There was a significant increase of IDL-18:0, LDL-14:0 and HDL-22:6n3 PUFA in group B.

Half of the saturated fatty acids in the western diet consists of palmitic acid (C16:0), thereby the major saturated fatty acid. A smaller proportion of saturated fat intake is provided by stearic acid (C18:0), followed by myristic acid (C14:0), lauric acid (C12:0) and short and medium chain fatty acids (MCFA; $\leq$C10:0) [32]. The subjects in this study were instructed to be homogenous in terms of their dietary fat intake. Accordingly to that, there were not any significant differences in their plasma SUFA. Significant increase of LDL–C14:0 in man with CAD in group B was associated with significant increase of erythrocyte membrane C14:0. It may be speculated that the severity of CAD was associated with enhanced extrahepatic transport of C14:0 via LDL and its incorporation into cell membranes. Because the hypercholesterolemic effect of myristic acid has been mostly associated with its effect via lower plasma cholesterol ester transfer protein activity and thus increase of HDL [33], increase of LDL–C14:0 and of erythrocyte membrane C14:0 may be another CAD promoting mechanism. Several analyses clearly show that, in contrast with other saturated fatty acids, stearic acid (C18:0) affects neither serum LDL– nor HDL–cholesterol levels [1]. It is therefore of particular interest to find significant increase of IDL–C18:0 in man with more severe CAD, which was not associated with changes of C18:0 content in plasma or erythrocyte membranes. To evaluate the definitive role of C18:0 in CAD would need analysis of fatty acids metabolism in atherosclerotic coronary lesions.

Gama-linolenic acid (C18:3n6) has been reported to possess lipid-lowering properties and negative correlation with CAD [16]. The present study found an increase of plasma C18:3n6 in more severe CAD patients in group B. Because this was not associated with changes of C18:3n6 content in plasma lipoproteins or erythrocyte membranes, the higher dietary intake would the most probable explanation of this change. Docosahexaenoic acid (DHA, C22:6n3) possesses antiatherogenic properties [16]. HDL fatty acid composition has been also shown to alter the ability of HDL to remove cholesterol from arterial cells [9]. As plasma DHA was not changed in the present study, a significant increase of HDL-

bound and erythrocyte membrane-bound C22:6n3 PUFA in association with severe coronary atherosclerosis in group B may reflect enhanced clearance of anti atherosclerotic DHA via plasma HDL.

Vitamin E as antioxidative agent and TBARS as markers of lipoperoxidation have been shown to counteract with coronary arterosclerosis. TBARS were similar in both experimental group, although some increase in CAD patients would be

Table 3
Fatty acids in VLDL[a]

Variable	Unit of measurement	Group A		Group B		*P* value
		n	Mean (±SEM)	*n*	Mean (±SEM)	
C12:0	%	11	0.55 (0.10)	17	0.44 (0.05)	n.s.
C14:0	%	11	0.98 (0.18)	17	0.80 (0.62)	n.s.
C16:0	%	11	25.82 (1.60)	17	29.29 (5.36)	n.s.
C16:1	%	11	2.29 (0.32)	17	2.38 (0.26)	n.s.
C18:0	%	11	7.72 (2.84)	17	8.31 (0.97)	n.s.
C18:1n9	%	11	30.73 (1.84)	17	32.31 (0.87)	n.s.
C18:1n7	%	11	2.49 (0.19)	17	2.73 (0.11)	n.s.
C18:2n6	%	11	21.96 (1.91)	17	21.62 (1.19)	n.s.
C18:3n6	%	11	0.42 (0.06)	17	0.36 (0.04)	n.s.
C20:3n6	%	11	0.59 (0.11)	17	0.54 (0.11)	n.s.
C20:4n6	%	11	5.12 (0.43)	17	5.06 (0.28)	n.s.
C22:4n3	%	11	0.85 (0.21)	17	0.59 (0.10)	n.s.
C22:6n3	%	11	0.66 (0.09)	17	0.83 (0.02)	n.s.

[a] Fatty acids in serum VLDL in patients who underwent an elective coronary angiography for CAD. They were divided inyo group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established if the *P* value was less than 0.05; n.s., not significant.

Table 4
Fatty acids in IDL[a]

Variable	Unit of measurement	Group A		Group B		*P* value
		n	Mean (±S.D.)	*n*	Mean (±S.D.)	
C12:0	%	10	0.64 (0.17)	17	0.91 (0.14)	n.s.
C14:0	%	10	0.82 (0.11)	17	1.04 (0.18)	n.s.
C16:0	%	10	23.33 (0.65)	17	25.25 (1.31)	n.s.
C16:1	%	10	1.88 (0.35)	17	2.24 (0.24)	n.s.
C18:0	%	10	7.84 (0.36)	17	10.39 (1.03)	0.03
C18:1n9	%	10	28.03 (1.18)	17	25.77 (0.88)	n.s.
C18:1n7	%	10	2.56 (0.11)	17	2.34 (0.14)	n.s.
C18:2n6	%	10	24.96 (0.75)	17	22.22 (1.71)	n.s.
C18:3n6	%	10	0.53 (0.08)	17	0.59 (0.06)	n.s.
C20:3n6	%	10	0.85 (0.16)	17	0.72 (0.09)	n.s.
C20:4n6	%	10	6.74 (0.45)	17	6.47 (0.41)	n.s.
C22:4n3	%	10	0.94 (0.15)	17	1.09 (0.11)	n.s.
C22:6n3	%	10	0.87 (0.10)	17	0.95 (0.09)	n.s.

[a] Fatty acids in serum IDL in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established if the *P* value was less than 0.05; n.s., not significant.

Table 5
Fatty acids in LDL[a]

Variable	Unit of measurement	Group A		Group B		*P* value
		n	Mean (±S.D.)	*n*	Mean (±S.D.)	
C12:0	%	10	0.48 (0.12)	16	0.83 (0.13)	n.s.
C14:0	%	10	0.53 (0.06)	16	0.83 (0.11)	0.03
C16:0	%	10	22.02 (0.39)	16	21.22 (1.33)	n.s.
C16:1	%	10	2.30 (0.17)	16	1.98 (0.21)	n.s.
C18:0	%	10	8.23 (0.43)	16	9.64 (0.74)	n.s.
C18:1n9	%	10	25.30 (1.80)	16	22.24 (0.74)	n.s.
C18:1n7	%	10	2.20 (0.20)	16	2.04 (0.08)	n.s.
C18:2n6	%	10	27.68 (1.26)	16	27.54 (1.55)	n.s.
C18:3n6	%	10	0.44 (0.05)	16	0.52 (0.05)	n.s.
C20:3n6	%	10	0.84 (0.15)	16	0.76 (0.09)	n.s.
C20:4n6	%	10	7.90 (0.76)	16	9.29 (0.67)	n.s.
C22:4n3	%	10	1.08 (0.24)	16	1.20 (0.20)	n.s.
C22:6n3	%	10	0.96 (0.17)	16	1.12 (0.16)	n.s.

[a] Fatty acids in serum LDL in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established if the *P* value was less than 0.05; n.s., not significant.

Table 6
Fatty acids in HDL[a]

Variable	Unit of measurement	Group A		Group B		*P* value
		n	Mean (±SEM)	*n*	Mean (±SEM)	
C12:0	%	9	0.45 (0.07)	16	0.83 (0.18)	n.s.
C14:0	%	9	0.65 (0.13)	16	0.76 (0.08)	n.s.
C16:0	%	9	23.85 (1.98)	16	23.58 (0.87)	n.s.
C16:1	%	9	1.89 (0.18)	16	2.05 (0.29)	n.s.
C18:0	%	9	12.42 (1.31)	16	12.40 (0.92)	n.s.
C18:1n9	%	9	21.05 (1.97)	16	21.12 (0.95)	n.s.
C18:1n7	%	9	2.20 (0.14)	16	2.04 (0.11)	n.s.
C18:2n6	%	9	23.01 (2.08)	16	22.18 (1.18)	n.s.
C18:3n6	%	9	0.52 (0.07)	16	0.52 (0.06)	n.s.
C20:3n6	%	9	0.78 (0.40)	16	1.05 (0.21)	n.s.
C20:4n6	%	9	11.26 (1.58)	16	10.10 (0.66)	n.s.
C22:4n3	%	9	1.07 (0.24)	16	1.68 (0.27)	n.s.
C22:6n3	%	9	0.85 (0.21)	16	1.72 (0.28)	0.005

[a] Fatty acids in serum HDL in patients who underwent an elective coronary angiography for CAD. They were divided into group A (luminal narrowing $<50\%$; $n=11$) and group B (luminal narrowing $>50\%$; $n=17$). Results were expressed as mean ± SEM. Significance was established if the *P* value was less than 0.05; n.s., not significant.

expected. The definitive comparison would need to compare the data of CAD patients versus a group of healthy controls undergoing coronary angiography, which was not done in present study. Vitamin E decreases lipid peroxidation [34], decreases the mitogenic effects of oxidized LDL [35] and platelet-derived growth factor [36] on smooth muscle cells. As we found in our previous studies patients with coronary artery disease had lower serum vitamin E as compared to healthy controls (group A, 20.9 ± 4.5 μmol/l; 26.6 ± 4.9 μmol/l in group B; 35.3 ± 8.9 μmol/l in healthy

controls). The increase of vitamin E in group B was associated with increase of vitamin E in fractions of VLDL, IDL and LDL, but not HDL. However, there were not any significant differences in the relative content of vitamin E in corresponding cholesterol fractions (results not shown), thus such changes should be associated with the increased lipid carrier of plasma vitamin E in hyperlipidemic subjects.

5. Conclusions

The present data indicate that besides of high serum LDL/HDL cholesterol ratio, triacylglycerols, phospholipids and enhanced lipid peroxidation, disturbances in SUFA and PUFA metabolism are associated with coronary atherogenesis. Such abnormalities may include enhanced extrahepatic transport of C14:0 SUFA via LDL and its incorporation into cell membranes, and enhanced clearance of anti atherosclerotic C22:6n3 PUFA via serum HDL.

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