Serum and erythrocyte membrane phospholipids fatty acid composition in hyperlipidemia: effects of dietary intervention and combined diet and fibrate therapy

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Abstract. Hyperlipidemia is found to be associated with changes in fatty acid (FA) profiles. The aim of this study was to investigate the effects of AHA-Step-1 dietary treatment and combination of fibrates (gemfibrozil) with dietary intervention on serum and erythrocyte phospholipid FA composition in human hyperlipidemia. 78 study participants with hyperlipidemia were divided in two groups. In D group ($n = 41$) subjects followed AHA-Step-1 diet (<30% of total from fat, <10% of energy from saturated fat, and <300 mg cholesterol per day). D+F group ($n = 37$) followed Step-1 diet and were receiving gemfibrozil (300 mg/twice per day). Serum lipid levels and phospholipid serum and erythrocyte FA compositions were analyzed at the beginning and after 12 weeks of treatment. Alteration in serum and erythrocyte phospholipid FA profile were found in both groups. After both treatments we found significantly higher serum phospholipid percentages of n-3, n-6 and total polyunsaturated FA. Linoleic (LA, n-6) and docosahexaenoic acid (DHA, n-3) were higher in D group, but arachidonic (AA, n-6) and linolenic acid (LNA, n-3) in D+F group. In erythrocyte phospholipid levels of stearic, palmitoleic (16 : 1, n-7) and LA were significantly higher in D group, but palmitic acid, AA and eicosapentaenoic acid (EPA, n-3) in D+F group. Stronger correlation between serum triglycerides with EPA and DHA in erythrocyte membrane phospholipid was found in D+F group. Markedly increased percentage of AA in serum and erythrocyte membrane phospholipid in hyperlipidemic patients receiving gemfibrozil on Step-1 diet is especially important for physiological functions (inflammation, vascular tone, hemostasis etc.) in relation to cardiometabolic risk.

Key words: AHA-Step-1 diet — Gemfibrozil — Fatty acids — Phospholipids — Erythrocyte

Introduction

Dietary fat intake is a risk factor for coronary heart disease and the modification of dietary habits is important for the prevention of cardiovascular disease (CVD) (Grundy and Denke 1990; Lichtenstein et al. 2002). The assessment of dietary fat intake is a critically important first step in clinical decision-making regarding dietary and pharmacotherapeutic advice on coronary risk reduction. Large intakes of saturated fatty acids (SFA) and cholesterol together with low dietary levels of polyunsaturated fatty acid (PUFA), particularly long-chain n-3 PUFA, appear to have a great impact on the development of CVD (Hodson et al. 2001; Lichtenstein et al. 2002). A high dietary n-6/n-3 PUFA ratio is also considered as a marker of elevated risk of CVD, though little data on dietary intake is available (de Lorgeril and Salen 2003).

Biomarkers of essential fatty acid (FA) are among the best available biomarkers of previous dietary FA intakes (Sarkkinen et al. 1994; Bingham 2002). They offer an objective alternative to dietary assessment because they reflect actual, rather than reported intake, thus avoiding, for example, the particular problems in dietary assessment of under or over reporting food consumption or quantifying "hidden fats" in the diet (Bingham 2002; Arab 2003). Under homeostatic weight conditions and if

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properly sampled, collected and analyzed, the biomarkers can represent long-term intakes of individual fats, what appears to be a more objective marker for dietary intake than dietary questionnaire assessment (Rivellese et al. 2002). Concentration of essential n-6 and n-3 PUFA in cell membrane or in serum phospholipids are highly correlated with dietary intake (Ma et al. 1995; Arab 2003). Erythrocytes are a better long-term marker of dietary FA intake than platelet or plasma lipids because the turnover of erythrocytes (120-day lifespan) is much slower than that of platelets (10 days) (Arab 2003).

In addition, high serum cholesterol is a well-known primary risk factor for coronary heart disease (Wood et al. 1998; Yu-Poth et al. 1999). For this reason the US National Cholesterol Education Program recommendations for general population are as follows: total dietary fat should be reduced to 30% or less, saturated fat intake to 10% or less of total calories and daily dietary cholesterol intake has to be limited to 300 mg (Yu-Poth et al. 1999; NCEP Panel III 2001). These guidelines are consistent with the World Health Organization dietary recommendations for prevention of chronic diseases (see References:WHO 2003).

The attention of pharmacologists and clinicians to fibrates has been renewed in recent years in light of their multifaceted action on plasma lipids. Cardioprotective effects of fibrates such as gemfibrozil in the HHS and VA-HIT studies were substantially greater than those found with other fibrates in WHO, BIP and FIELD studies (Barter and Rye 2006; Asztalos et al. 2008). Fibrates are lipid lowering drugs that act as peroxisome proliferator-activated receptor-α (PPAR-α) agonist and are currently used in clinical settings to improve atherogenic lipid profiles (Formann et al. 1997; Schindler 2007). PPAR-α serves as a FA sensor (clear preference for PUFA) and as an important regulator of FA metabolism and energy homeostasis (see review article Krey et al. 1997; Kersten 2008). Strong evidence suggests that FA are major dietary constituents in the regulation of gene expression in response to food intake and qualitative nutritional changes (Kliewer et al. 1997; Kersten 2008). Only limited information exists on interaction between diet, fibrates and FA profile in human hyperlipidemia (Agheli and Jacotot 1991; Tavella et al. 1993; Nyuala et al. 2008).

The aim of this study was to investigate the effects of American Heart Association (AHA)-Step-1 dietary treatment and combined effects of fibrates (gemfibrozil) with dietary intervention on serum and erythrocyte phospholipid composition, as well as their interrelationship with plasma lipid concentration.

Materials and Methods

Study population

A total of 379 individuals were screened for enrolment in the study at the Department of Nutrition and Metabolism of the Institute for Medical Research in Belgrade. All participants received a full medical examination and standard laboratory blood tests were performed before enrolment. The participants were eligible for the study on the basis of the following criteria: 1. being between 45 and 65 years of age; 2. a body mass index (BMI) <32 and weight stable (±2 kg) for 6 months prior to the beginning of the study; 3. normal fasting glucose levels according to WHO criteria (fasting glucose <6.1 mmol/l); 4. mild hyperlipidemia as defined by total serum cholesterol 5.2–7.8 mmol/l, serum LDL-cholesterol 3.39–4.91 mmol/l and/or triglycerides levels 1.7–3.5 mmol/l. Fulfillment of these criteria was based on data obtained from two fasting serum lipid profiles taken one week apart.

Participants were not adhering to any special diet, were not on prescribed medication known to affect lipid metabolism (lipid lowering drugs, β-blockers, diuretics or hormones) nor did they take vitamin and/or mineral supplements in 12 weeks prior to the start of the study. Subjects that had evidence of any chronic disease, including hepatic, renal, cardiovascular and thyroid dysfunction, diabetes mellitus, uncontrolled hypertension, and known family lipid disorders (familiar combined hyperlipidemia or familiar hypercholesterolemia) were excluded. Individuals who smoked and those with an alcohol intake of more than 30 g per day were also excluded from the study.

78 free-living adults (25 man and 53 women) were selected fulfilling the criteria of the study design. The women were postmenopausal and were not taking hormone replacement therapy. All subjects consumed their habitual diet, typical for Serbian population, characterized by: low consumption of foods containing soy, fish intake once in two weeks and high animal fat intake (pork meat, eggs and whole milk products were consumed everyday) (Pavlovic et al. 2005). Study subjects did not take dietary supplementation of oils rich in long-chain FA (fish oil, sesame oil or linseed oil) at the time of recruitment, as it was determined by dietary assessment. Participants were either sedentary or moderately active as observed in a physical activity questionnaire.

All study participants signed an informed consent document and the whole study was approved by the Medical Ethics Committee (Institute for Medical Research, Belgrade) and conducted according to principles of the Declaration of Helsinki.

Study design

The participants were divided into two groups. In one group (D+F) were allocated subjects who agreed to take the prescribed lipid lowering drugs (fibrate gemfibrozil) from their cardiologist, in other group (D) were those who decided to try only the diet first. Participants were instructed to follow AHA-Step-1 diet (Yu-Poth et al. 1999).

Subjects in D group (n = 41) were on Step-1 diet for 12 weeks with no change in energy intake compared to their previous energy intake. In D+F group (n = 37) subjects were on Step-1 diet and additionally were taking gemfibrozil (300 mg/twice per day) for 12 weeks. Target intakes in NCEP AHA-Step-1 diet were less than 30% of energy gained from fat, 50–60% gained from carbohydrates and 20% from protein. Less than 10% of energy should come from saturated FA, 10% from PUFA and 12% of energy from monounsaturated FA (MUFA). Dietary cholesterol intake did not exceed 300 mg/day.

Information concerning dietary intake was obtained at the beginning of the study (the habitual diet) and at the end of the dietary intervention period using a semi-quantified food frequency questionnaire (FFQ). The FFQ contained 142 food items and beverages commonly consumed in Serbia. Dietary intake was prescribed individually according to data obtained from dietary questionnaires to maintain the initial caloric intake and nutrient proportions constant throughout the study. The main goal of the diet was to restrict both dietary total fat and daily cholesterol intake. The recommended food during the low-lipid diet included lean meat, fat-free or skimmed milk and low-fat dairy products, whole grain/high-fiber food, fruit and vegetables. Fish was suggested as a main meal twice a week. Restricted food included high-fat meat and dairy products, eggs, fried food, cream sauces, high-fat pastries and sweets. A registered dietitian provided assistance in adhering to the AHA-Step-1. Nutrient calculations were performed using US Department of Agriculture food composition tables (see References: USDA 2005) or Serbian food composition tables (Jokic et al. 1999) for certain local foodstuffs.

Body weight was measured weekly to determine whether subjects' weight remained stable, using a Tanita body composition analyzer (model TBF-300, Japan). Standing height was measured, while subjects were barefoot, to the nearest 0.5 cm using a wall-mounted statiometer. BMI was calculated by dividing the body mass in kg by the square of height in meters (kg/m^2). The subjects were encouraged to maintain their habitual level of physical activity through the intervention period. Two participants from group D left the study, for reasons unrelated to the study.

Biochemical Determination

Laboratory assays were determined on the day of the screening visit, on the day of randomization and after 12 weeks of treatment. Serum samples were prepared from venous blood collected after a 12–14 h fast. Blood was centrifuged on 4°C and serum was collected. Total cholesterol and triglyceride levels were measured in triplicate using a commercial colorimetric enzymatic reaction kits (EliTech Diagnostic, Sées, France). HDL-cholesterol was determined in supernatant liquid after precipitation with phosphotungstic acid and magnesium chloride (Lopes-Virela et al. 1977). LDL-cholesterol was estimated using Friedewald formula (Friedewald at al. 1972). Non-HDL cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol. Serum lipids were extracted according to method of Sperry and Brand (Matusik et al. 1984), which uses chloroform-methanol mixture (2 : 1 v/v) with 10 mg/100 ml 2,6-di-tert-buthyl-4-methylphenol (BHT) added as an antioxidant. Red blood-cell lipids were extracted by the method of Harth (Harth et al. 1978). The phospholipids fraction was isolated from the lipid extract using one-dimensional thin-layer chromatography (TLC) in a neutral lipid solvent system hexane-diethyl ether-acetic acid (87:12:1, v/v/v) using Silica Gel GF plates (C. Merck, Darmstadt, Germany).

FA determination

Methyl esters of phospholipids FA were prepared by methods that have already been reported (Ristić Medić et al. 2003). Methyl esters derivatives were analyzed by gas chromatography using Varian GC (model 3400, Varian Associates) equipped with DB-23 (30 m × 0.53 mm i.d., film thickness 0.5 μm; J&W Scientific Inc. Bellefonte, Folsona, CA, USA) fused silica capillary column. The flame ionization detector was set at 250°C, the injection port at 220°C and the oven temperature programmed from 130 to 190°C, at a heating rate of 3°C/min. Analysis was performed in duplicate for each sample. Individual FA methyl esters were identified by comparing peak retention times with authentic standards (Sigma Aldrich, Germany) and/or the PUFA-2 standard mixtures (Supelco Inc., Bellefonte). The results were expressed as the relative percentage of total identified FA.

Statistical analysis

All results were expressed as means ± SD. The differences between formed groups at the beginning of the study, as well as the differences before and after the treatment were analyzed using an unpaired Student's t-test, accepting an alpha level of significance, $p \leq 0.05$. The Pearson correlation coefficients were computed for examination of the relation between serum lipids and between serum and erythrocyte FA percentages. The SPSS 10.0 program for Windows (Chicago IL, USA) was used for statistical analysis.

Results

The mean clinical and metabolic parameters are shown in Table 1. No differences for lipid parameters, dietary intake and FA composition in serum and erythrocyte phospholipids

were found between groups at baseline (Tables 1–3). Both diet alone and diet combined with gemfibrozil induced significant decrease in serum triglyceride (TG), total cholesterol (TC), LDL and non-HDL cholesterol concentrations ($p < 0.001$) and also induced a slight, but significant increase in HDL cholesterol level (Table 2). Consequently, TC/HDL, LDL/HDL and non-HDL/HDL cholesterol ratios were significantly decreased in both groups. In Group D+F we found higher reduction in concentrations of all lipid parameters (but increased HDL) than in Group D, but only changes in TC level showed a significant difference ($p < 0.05$) between two groups.

Combination of gemfibrozil and diet and dietary intervention alone induced significant alteration in serum phospholipids FA profile (Table 3). Saturated 16 : 0, 18 : 0 and total SFA significantly decreased and total MUFA increased in serum phospholipids after both treatments. The differences in SFA and MUFA between D and D+F groups after treatments were not significant. Percentages of linoleic (LA; 18 : 2 n-6), dihomo-γ-linoleic (DGLA; 20 : 3 n-6), arachidonic (AA; 20 : 4 n-6), docosapentaenoic (22 : 5 n-3), alfa-linolenic (LNA; 18 : 3 n-3), eicosapentaenoic (EPA; n-3) and docosahexaenoic acids (DHA; 22 : 6 n-3) in serum phospholipids were significantly higher after 12 weeks of dietary intervention. Consistent with the previous were the increases in n-6 PUFA, n-3 PUFA, total PUFA and PUFA/SFA ratios as well.

Combined gemfibrozil-diet treatment also led to a significant increase in DGLA, AA, LNA, EPA and DHA, but percentages of docosapentaenoic and docosatetraenoic acids were not changed, while LA was even decreased after the intervention (Table 3). Comparing the two groups of patients, which were similar at the baseline, we found significantly higher LA and DHA in D group, but AA and LNA in D+F group. The n-6/n-3 ratio was significantly lower after the diet, while gemfibrozil-diet combination did not significantly affect this ratio. AA/DGLA ratio, as a measure of Δ-5-desaturase activity was significantly lower in D group, but higher levels were found in D+F group. Although DGLA/LA ratio, as a measure of Δ-6-desaturase activity, was significantly higher in both groups after 12 weeks of treatment, it was higher in gemfibrozil-diet combination than in diet alone.

Combined effects of gemfibrozil-diet treatment and dietary intervention alone induced significant alteration in erythrocyte membrane phospholipids FA profile (Table 4). Both treatments significantly decreased SFA, and increased MUFA, n-6 PUFA, n-3 PUFA, total PUFA and PUFA/SFA ratio. Comparing the two groups after treatments, we found significantly higher stearic acid (18 : 0), palmitoleic acid (16 : 1 n-7) and LA in D group and higher palmitic acid (16 : 0), AA, EPA, AA/DGLA, 18 : 1/18 : 0 and DGLA/LA ratios in D+F group.

Table 1. Baseline anthropometric and dietary characteristics of study participants

	D group	D+F group
Age (years)	56 ± 6	55 ± 5
Male/female	13/28	12/25
Weight (kg)	72 ± 14	71 ± 12
BMI (kg/m^2)	24.47 ± 3.07	24.57 ± 2.84
Energy (kcal/day)	2273 ± 102	2253 ± 91
Protein (% of energy)	17.30 ± 2.25	17.03 ± 3.67
Carbohydrates (% of energy)	47.84 ± 1.54	47.69 ± 2.86
Total fat (% of energy)	34.83 ± 1.58	35.28 ± 2.18
Saturated fat (% of energy)	15.15 ± 1.03	15.80 ± 1.16
Monounsaturated fat (% of energy)	11.05 ± 2.84	10.95 ± 2.47
Polyunsaturated fat (% of energy)	8.23 ± 0.96	7.98 ± 0.96
PUFA/SFA	0.55 ± 0.08	0.51 ± 0.03
Cholesterol (mg/day)	498 ± 76	507 ± 84

Table 2. Serum lipids at baseline and changes induced by dietary intervention alone (D group) or diet and fibrate combination treatments (D+F group)

Serum (mmol/l)	D group baseline	D group after 12 wk	D+F group baseline	D+F group after 12 wk
Triglycerides	2.10 ± 0.45	1.49 ± 0.43***	2.15 ± 0.44	1.35 ± 0.43***
Total cholesterol (TC)	6.82 ± 0.61	5.85 ± 0.65***	6.72 ± 0.57	5.57 ± 0.60***[a]
LDL cholesterol	4.62 ± 0.67	3.83 ± 0.66***	4.44 ± 0.70	3.72 ± 0.79***
HDL cholesterol	1.25 ± 0.09	1.35 ± 0.13**	1.30 ± 0.18	1.37 ± 0.12*
non HDL cholesterol	5.57 ± 0.64	4.51 ± 0.70***	5.42 ± 0.59	4.18 ± 0.80***
TC/HDL cholesterol	5.50 ± 0.71	4.51 ± 0.70***	5.25 ± 0.88	4.12 ± 0.56***
LDL/HDL cholesterol	3.37 ± 0.69	2.85 ± 0.68***	3.52 ± 0.86	2.76 ± 0.64***
non HDL/HDL cholesterol	4.50 ± 0.71	3.40 ± 0.75***	4.28 ± 0.88	3.09 ± 0.69***

All data represented as mean ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – intergroups compared baseline to after 12 weeks (wk) treatment; [a] $p < 0.05$ – compared groups after 12 wk treatment.

Table 3. Serum phospholipids fatty acid composition at baseline and changes induced by dietary intervention alone (D group) or diet and fibrate combination treatments (D+F group)

	D group baseline	D group after 12 wk	D+F group baseline	D+F group after 12 wk
16 : 0	30.30 ± 1.39	27.03 ± 0.68***	30.20 ± 1.27	27.29 ± 1.33***
18 : 0	15.74 ± 0.85	14.96 ± 0.85***	15.38 ± 1.30	14.84 ± 1.07*
SFA	46.03 ± 1.47	41.99 ± 0.34***	45.58 ± 1.51	42.12 ± 1.60***
16 : 1 n-7	0.39 ± 0.06	0.34 ± 0.08***	0.36 ± 0.09	0.32 ± 0.06*
18 : 1 n-9	11.24 ± 0.87	12.17 ± 0.96*	11.17 ± 1.08	12.36 ± 0.98***
MUFA	11.63 ± 0.86	12.56 ± 0.92***	11.53 ± 1.09	12.76 ± 0.85***
18 : 2 n-6 LA	23.13 ± 1.35	24.74 ± 0.96***	23.08 ± 1.52	22.27 ± 1.60*^{c}
20 : 3 n-6	3.01 ± 0.36	3.52 ± 0.26***	3.15 ± 0.66	3.60 ± 0.67**
20 : 4 n-6	11.37 ± 1.04	11.90 ± 0.89**	11.64 ± 1.16	14.01 ± 1.5***^{c}
22 : 4 n-6	0.44 ± 0.06	0.40 ± 0.06*	0.41 ± 0.10	0.41 ± 0.08
n-6	37.96 ± 1.73	40.56 ± 1.23***	38.27 ± 1.65	40.29 ± 1.46***
18 : 3 n-3 LNA	0.10 ± 0.02	0.12 ± 0.02***	0.10 ± 0.02	0.19 ± 0.13***^{b}
20 : 5 n-3 EPA	0.29 ± 0.05	0.38 ± 0.06***	0.30 ± 0.04	0.38 ± 0.06***
22 : 5 n-3	0.55 ± 0.08	0.60 ± 0.06**	0.57 ± 0.08	0.61 ± 0.32
22 : 6 n-3 DHA	3.10 ± 0.32	3.73 ± 0.33***	3.09 ± 0.38	3.39 ± 0.69*^{c}
n-3	4.05 ± 0.31	4.84 ± 0.34***	4.06 ± 0.42	4.57 ± 0.81**
PUFA	42.01 ± 1.69	45.40 ± 1.32***	42.33 ± 1.57	44.87 ± 1.63***
n-6/n-3	9.43 ± 0.94	8.43 ± 0.66***	9.55 ± 1.26	9.26 ± 2.98^{c}
20 : 4/20 : 3	3.81 ± 0.45	3.40 ± 0.36***	3.82 ± 0.72	4.02 ± 0.85
22 : 6/22 : 5	5.77 ± 1.22	6.27 ± 0.83*	5.47 ± 0.81	6.24 ± 1.00***
18 : 1/18 : 0	0.72 ± 0.07	0.82 ± 0.07***	0.73 ± 0.12	0.84 ± 0.08***
20 : 3/18 : 2	0.13 ± 0.02	0.14 ± 0.01***	0.14 ± 0.03	0.16 ± 0.04**^{b}
PUFA/SFA	0.91 ± 0.06	1.08 ± 0.05***	0.93 ± 0.06	1.07 ± 0.07***

All data are presented as a mean ± SE. Fatty acids concentrations are expressed in % of totally detected fatty acids. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – intergroups compared baseline to after 12 wk treatment; ^{b} $p < 0.01$, ^{c} $p < 0.001$ – compared groups after 12 wk treatment.

A negative correlation between serum lipids (TC, TG, LDL and non-HDL) and LA, LNA, EPA and DHA percentage in serum phospholipids were found in D group, while HDL-cholesterol was in positive correlation with LNA, EPA and DHA (Table 5). Also, serum lipids (TC, TG, and non-HDL) in gemfibrozil-diet treatment inversely correlated with AA, LNA and EPA percentages in serum phospholipids. TC and non-HDL negatively correlated with DHA, while LDL cholesterol negatively correlated with LNA, EPA and DHA serum phospholipids contents. HDL cholesterol was in a positive correlation only with EPA level (Table 6).

An inverse correlation between serum lipids (TC, TG, LDL and non-HDL) with LA and LNA percentage in erythrocyte membrane phospholipids was found in diet intervention group. TG was also in negative correlation with EPA, while HDL-cholesterol showed positive correlation with LA, AA and LNA (Table 7). Serum TC, TG, LDL and non-HDL in gemfibrozil-diet treatment negatively correlated with AA and LNA in erythrocyte membrane phospholipids. TC and non-HDL were also in inverse correlation with EPA, and TG with EPA and DHA (Table 8).

Discussion

In the present study we evaluated dietary AHA-Step-1 intervention alone and combined effects of fibrate (gemfibrozil) and dietary treatment on serum and erythrocyte membrane phospholipids FA composition in patients with hyperlipidemia. However, it is important to notice that Group D was allocated from patients who preferred not to take drugs at the beginning, and rather try to improve their lipid profile by the diet alone. Regarding different compliance of study participants to follow medical advice in term of lipid lowering drugs, the obtained differences between D and D+F groups might not originated only from fibrate therapy, but also from other factors related to the decision of patients to take no drugs. Although, there were a number of important features of this study. First, the effects of dietary treatment on

Table 4. Erythrocyte phospholipids fatty acid composition at baseline and changes induced by dietary intervention alone (D group) or diet and fibrate combination treatments (D+F group)

	D group baseline	D group after 12 wk	D+F group baseline	D+F group after 12 wk
16 : 0	23.64 ± 0.90	20.59 ± 0.70***	24.00 ± 0.95	21.30 ± 0.59***[c]
18 : 0	17.58 ± 0.94	16.88 ± 0.70***	17.20 ± 0.90	16.35 ± 0.55***[c]
SFA	41.23 ± 1.29	37.47 ± 0.70***	41.20 ± 1.33	37.64 ± 0.55***
16 : 1 n-7	0.25 ± 0.05	0.23 ± 0.05***	0.24 ± 0.06	0.20 ± 0.02***[b]
18 : 1 n-9	17.00 ± 1.47	18.20 ± 0.52***	17.09 ± 1.13	18.10 ± 0.80***
MUFA	17.26 ± 1.48	18.43 ± 0.53***	17.33 ± 1.13	18.31 ± 0.80***
18 : 2 n-6 LA	13.55 ± 1.11	15.27 ± 0.89***	13.43 ± 0.92	13.21 ± 1.36[c]
20 : 3 n-6	1.49 ± 0.20	1.59 ± 0.26*	1.49 ± 0.18	1.56 ± 0.11*
20 : 4 n-6	16.53 ± 1.47	17.07 ± 0.77*	16.65 ± 1.29	19.03 ± 1.04***[c]
22 : 4 n-6	3.65 ± 0.55	3.83 ± 0.56*	3.65 ± 0.47	3.87 ± 0.41*
n-6	35.23 ± 2.15	37.75 ± 0.95***	35.20 ± 1.16	37.68 ± 0.77***
18 : 3 n-3 LNA	0.10 ± 0.02	0.17 ± 0.03***	0.12 ± 0.02	0.18 ± 0.04***
20 : 5 n-3 EPA	0.33 ± 0.08	0.37 ± 0.05***	0.35 ± 0.07	0.40 ± 0.03***[a]
22 : 5 n-3	1.57 ± 0.30	1.57 ± 0.32	1.54 ± 0.24	1.55 ± 0.20
22 : 6 n-3 DHA	3.83 ± 0.56	4.12 ± 0.45	3.89 ± 0.51	4.06 ± 0.50
n-3	5.89 ± 0.63	6.22 ± 0.67*	5.89 ± 0.53	6.19 ± 0.54**
PUFA	41.12 ± 2.31	43.96 ± 0.88***	41.10 ± 1.08	43.86 ± 0.73***
n-6/n-3	6.05 ± 0.73	6.15 ± 0.77	6.03 ± 0.66	6.14 ± 0.60*
20 : 4/20 : 3	11.28 ± 1.90	11.02 ± 1.81*	11.42 ± 1.99	12.25 ± 1.04*[c]
22 : 6/22 : 5	2.52 ± 0.59	2.70 ± 0.46	2.58 ± 0.53	2.65 ± 0.44
18 : 1/18 : 0	0.97 ± 0.090	1.08 ± 0.06***	1.00 ± 0.10	1.11 ± 0.07***[b]
20 : 3/18 : 2	0.11 ± 0.02	0.10 ± 0.02	0.11 ± 0.01	0.12 ± 0.02[b]
PUFA/SFA	1.00 ± 0.08	1.17 ± 0.05***	1.0 ± 0.05	1.17 ± 0.04***

All data are presented as a mean ± SE. Fatty acids concentrations are expressed in % of totally detected fatty acids. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ – intergroups compared baseline to after 12 wk treatment; [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ – compared groups after 12 wk treatment.

Table 5. Pearson correlation coefficients between serum lipids and serum phospholipids fatty acids percentage in dietary intervention treatment

	TG		TC		HDL		LDL		non HDL	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LA	−0.364	0.001	−0.377	0.001	0.201	0.073	−0.305	0.006	−0.375	0.001
AA	−0.292	0.008	−0.206	0.066	0.185	0.101	−0.150	0.185	−0.225	0.045
LNA	−0.252	0.024	−0.245	0.028	0.299	0.007	−0.220	0.050	−0.268	0.016
EPA	−0.464	<0.001	−0.468	<0.001	0.285	0.01	−0.381	<0.001	−0.477	<0.001
DHA	−0.515	<0.001	−0.417	<0.001	0.325	0.003	−0.319	0.004	−0.436	<0.001

Table 6. Pearson correlation coefficients between serum lipid and serum phospholipids fatty acids percentage in diet and fibrate combination treatment

	TG		TC		HDL		LDL		non HDL	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LA	-0.222	0.054	−0.189	0.101	0.109	0.348	−0.186	0.109	−0.174	0.132
AA	−0.414	<0.001	−0.319	0.005	0.113	0.330	−0.188	0.103	−0.280	0.014
LNA	−0.254	0.027	−0.291	0.011	0.168	0.148	−0.255	0.026	−0.288	0.012
EPA	−0.512	<0.001	−0.374	0.001	0.291	0.011	−0.235	0.041	−0.404	<0.001
DHA	−0.031	0.793	−0.322	0.005	0.140	0.227	−0.248	0.031	−0.304	0.008

Table 7. Pearson correlation coefficients between serum lipids and erythrocyte membrane phospholipids fatty acids percentage in dietary intervention treatment

	TG		TC		HDL		LDL		non HDL	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LA	–0.316	0.004	–0.466	<0.001	0.277	0.013	–0.423	0.001	–0.472	<0.001
AA	–0.151	0.182	–0.090	0.429	0.052	0.047	–0.037	0.743	–0.075	0.511
LNA	–0.408	<0.001	–0.304	<0.001	0.187	0.096	–0.214	0.056	–0.308	0.005
EPA	-0.273	0.014	-0.170	0.131	0.158	0.161	-0.151	0.310	-0.185	0.100
DHA	-0.193	0.087	-0.146	0.196	0.100	0.375	-0.107	0.346	-0.154	0.173

Table 8. Pearson correlation coefficients between serum lipid and erythrocyte phospholipid fatty acids percentage in diet and fibrate treatment

	TG		TC		HDL		LDL		non HDL	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
LA	0.131	0.266	–0.024	0.839	0.182	0.121	–0,068	0.566	–0.042	0.725
AA	–0.494	<0.001	–0.444	<0.001	0.013	0.912	–0.231	0,048	–0.376	0.001
LNA	–0.507	<0.001	–0.556	<0.001	0.134	0.254	–0,316	0.006	–0.490	0.001
EPA	–0.371	<0.001	–0.286	<0.014	0.066	0.578	–0.131	0.267	–0,247	0.034
DHA	–0.272	0.019	0.007	0.954	0.015	0.897	–0.100	0.399	–0.040	0.735

serum and erythrocyte membrane phospholipids FA profile were consistent with published data and reflected changes in the FA composition of the diet (Kobayashi et al. 2001; King et al. 2006; Skeaff et al. 2006). Our results showed that dietary-induced changes in erythrocyte phospholipids FA composition were similar to plasma phospholipids. These results provide convincing, although indirect evidence, that the input of FA from plasma to erythrocytes is a major determinant of their membrane FA composition (King et al. 2006). Small but significant changes in PUFA/SFA ratio were recorded in erythrocyte membrane phospholipids after Step-1 diet, from 1.00 to 1.17. Therefore, it appears that red cell membrane changes parallel dietary intervention and hence are a potential marker for effects of dietary changes.

Higher proportions of LA and LNA, that form a potentially less atherogenic FA profile, were found in subjects after the Step-1 diet only, suggesting an additional benefit of the dietary intervention (Hodson et al. 2001; Lichtenstein et al. 2002). Increased formation of long-chain PUFA was important for membrane functions in several tissues, such as endothelium and thrombocytes. These functions may partly be mediated through eicosanoid metabolites of the membrane-bound 20-carbon FA: AA (the precursor of 2-series prostaglandins and thromboxanes and 4-series leukotrienes), DGLA (the precursor of 1-series prostaglandins), and EPA (the precursor of 3-series prostaglandins and thromboxanes and 5-series leukotrienes). A number of eicosanoids has been shown to activate PPAR. The strong preference of the PUFA for PPAR-α suggests that this receptor may play a specific role in situations of nutritional lipid overload (Kersten et al 2008). Recently, Calkin et al. (2007) have shown that both PPAR-α agonists – gemfibrozil and fenofibrate confer anti-atherosclerotic effects, partly independent of their metabolic effects.

Gemfibrozil is extremely effective in reducing cardiovascular risk in people with components of metabolic syndrome. Insulin sensitivity can be modulated by different lifestyle factors and the quality of dietary fat seems to be an important factor (Warensjö et al. 2006). Clifton et al. (1998) found an inverse relationship for fasting plasma insulin with erythrocyte membrane percentage of AA, total n-6 PUFA and a positive relationship with percentage of SFA in healthy men. Thus, the strong positive correlation between AA and the indexes of insulin sensitivity in previously reported studies may represent an effect on insulin action of eicosanoids specifically derived from this parent FA. In human obesity palmitoleic acid is reported to correlate with index of adiposity and insulin concentration (Okada et al. 2005). After combination of gemfibrozil and the dietary therapy decreased levels of palmitoleic acid were observed in our study. Our data showed a strong evidence of increasing levels of AA in serum and erythrocyte phospholipid after gemfibrozil-diet treatment in hyperlipidemic patients. Previous studies of coronary circulation suggested that endothelium-derived hyperpolarizing factor (EDHF), an important mediator of vasodilatation, especially in small arteries, may be a product of the non-cyclo-oxygenase-mediated metabolism of AA (Oltman et al. 2001). These observations lead one to

speculate that AA induced vasodilatation without the tonic inhibition. In this case a predominant regulatory pathway in arteriosclerosis could become the EDHF pathway (De Caterina et al. 2000).

An increased formation of DGLA, as observed here during the dietary intervention and in gemfibrozil-diet combination, may increase the formation of vasodilatory, anti-inflammatory and antiproliferative prostaglandin E1 (Mikhailidis et al. 1986). DGLA and its 15-OH derivate block the transformation of AA to proinflammatory 4-series leukotrienes (Iversen et al. 1992) and also increase the conversion of EPA to PGI-3, a vasodilatator and platelet anti-aggregator (Holman 1977). EPA inhibits the activity of the enzyme Δ-5-desaturase, disabling DGLA conversion to AA and increasing the tissue levels of DGLA (Das 1995). In previous cross-sectional studies in humans, reduced Δ-5-desaturase activity (AA/DGLA ratio) in membranes has been associated with insulin resistance (Borkman et al. 1993). According to animal studies, a high-cholesterol diet decreased the Δ-5- and Δ-6-desaturase steps, whereas a low-cholesterol diet had opposite effects (Garg et al. 1986; Leikin et al. 1988). These data show that AHA-Step-1 diet decreased levels of Δ-5-desaturase activity in serum and membrane phospholipid. The mode of action of fibrate on FA cascade remains speculative. In theory, fibrate may have direct or indirect stimulatory effects on FA desaturase and elongase enzyme activities. Thus, an increased Δ-5-desaturase activity, observed in our study after gemfibrozil-diet combination in serum and membrane phospholipid, may be at least partly influenced by gemfibrozil.

Nyalala et al. (2008) recently showed that gemfibrozil produced major modifications in FA composition of plasma lipids and red cell membrane in patients with hypertriglyceridemia. These changes were FA and lipid class specific, with generally decreased SFA and increased PUFA (mainly AA n-6). Results of this study are consistent with effect of gemfibrozil-diet combination from our data in patients with hyperlipidemia. Recently published article reported that the proportion of serum LA was inversely related, whereas serum FAs, associated with saturated fat intake (palmitic, palmitoleic, and DGLA), were directly related to total and cardiovascular mortality (Warensjö et al. 2008).

High proportion of n-3 FA in red blood cell membranes has been associated with a reduced risk of primary cardiac arrest (Siscovick et al. 1995). The cardioprotective action of n-3 PUFA can be attributed to their effect on lipids, hemostatic system and endothelial cell proliferation and function (Das 2000; De Caterina et al. 2000; Crowe et al. 2006). In agreement with Grimsgard et al. (2000), the present study shows strong correlation of decreased serum triglycerides with higher levels of EPA n-3. Lower levels of LDL-cholesterol after both treatments correlated with LNA, EPA and DHA n-3 in serum phospholipids.

We have demonstrated that both the dietary intervention, and combination of gemfibrozil and the diet resulted in significantly better serum lipid profile as well as serum and erythrocyte membrane phospholipid FA composition. This study also provides valuable data on the interrelationship of FA composition of lipids and plasma lipid concentration. Although the present study is unable to comment on the potential additional benefit of gemfibrozil over diet alone, due to a lack of random allocation of the subjects, it is evident that the combination of the diet and fibrate led to a more favorable lipid and FA profile in hyperlipidemic patients.

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References

Agheli N., Jacotot B. (1991): Effect of simvastatin and fenofibrate on the fatty acid composition of hypercholesterolaemic patients. Br. J. Clin. Pharmacol. **32**, 423–428

Arab L. (2003): Biomarkers of fat and fatty acid intake. J. Nutr. **133**, 925–932

Asztalos B. F, Collins D., Horvath K. V., Bloomfield H. E., Robins S. J., Schaefer E. J. (2008): Relation of gemfibrozil treatment and high-density lipoprotein subpopulation profile with cardiovascular events in the veterans affairs high-density lipoprotein intervention trial. Met. Clin. Exp. **57**, 77–83

Barter P. H. J., Rye K. A. (2006): Cardioprotective Properties of Fibrates which Fibrate, which patients, what mechanism? Circulation **113**, 1553–1555

Bingham S. (2002): Biomarkers in nutritional epidemiology. Public Health Nutr. **5**, 821–827

Borkman M., Storlien L. H., Pan D. A,. Jenkins A. B., Chrisholm D. J., Campbell L. V. (1993): The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N. Engl. J. Med. **328**, 238–244

Clifton P. M., Nestel P. J. (1998): Relationship between plasma insulin and erythrocyte fatty acid composition. Prostaglandins Leukot. Essent. Fatty Acids **59**, 191–194

Calkin A. C., Jandeleit-Dahm K. A., Sebekova E., Allen T. J., Mizrahi J., Cooper M. E., Tikellis C. (2007): PPARs and diabetes-associated atherosclerosis. Curr. Pharm. Des. **13**, 2736–2741

Crowe F. L., Skeaff C. M., Green T. J., Gray A. R. (2006): Serum fatty acids as biomarkers of fat intake predict serum cholesterol concentrations in a population-based survey of New Zealand adolescents and adults. Am. J. Clin. Nutr. **83**, 887–894

Das U. N. (1995): Essential fatty acid metabolism in patients with essential hypertension, diabetes mellitus and coronary heart disease. Prostaglandins Leukot. Essent. Fatty Acids **52**, 387–391

Das U. N. (2000): Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? Prostaglandins Leukot. Essen. Fatty Acids **63**, 351–362

De Caterina R., Liao J. K., Libby P. (2000): Fatty acid modulation of endothelial activation. Am. J. Clin. Nutr. **71**, S213–223

de Lorgeril M., Salen P. (2003): Dietary prevention of coronary heart disease: focus on omega-6/omega-3 essential fatty acid balance. World Rev. Nutr. Diet. **92**, 57–73

Iversen L., Fogh K., Kragballe K. (1992): Effect of dihomogammalinolenic acid and its 15-lipoxygenase metabolite on eicosanoid metabolism by human mononuclear leukocytes in vitro: selective inhibition of the 5-lipoxygenase pathway. Arch. Dermatol. Res. **284**, 222–226

Forman B. M., Chen J., Evans R. M. (1997): Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peoxisome proliferator-activated receptors alpha and delta. Proc. Natl. Acad. Sci. USA **94**, 4312–4317

Friedewald W. T., Levy R. I., Friedrickson D. S. (1972): Estimination of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. Clin. Chem. **18**, 449–502

Garg M. L., Snoswell A. M., Sabine J. R. (1986): Influence of dietary cholesterol on desaturase enzymes of rat liver microsomes. Prog. Lipid Res. **25**, 639–644

Grimsgaard S., Bonaa K. H., Bjerve K. S. (2000): Fatty acid chain length and degree of unsaturation are inversely associated with serum triglycerides. Lipids **35**, 1185–1193

Grundy S. M., Denke M. A. (1990): Dietary influences on serum lipids and lipoproteins. J. Lipid Res. **31**, 1149–1172

Harth S., Dreyfus H., Urban P. F., Mandel P. (1978): Direct thin-layer chomatography of gangliosides of total lipid extracts. Anal. Biochem. **86**, 543–551

Hodson L., Skeaff C., Chisholm W. A. (2001): The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. Eur. J. Clin. Nutr. **55**, 908–915

Holman R. T. (1977): Esential fatty acids in human nutrition. In: Function and biosynthesis of lipids. (Eds. N. G. Bazán, R. R. Brenner and M. N. Giusto), pp. 515–534, Plenum Press, New York

Jokic N., Dimic M., Pavlica M. (1999): Chemical composition tables of nutrition products. Kulin Art, Zavod za ekonomiku domacinstva Srbije, Belgrade (in Serbian)

Kersten S. (2008): Peroxisome proliferator activated receptors and lipoprotein metabolism. PPAR Res. **2008**, 132960

Kliewer S. A., Sundseth S. S., Jones S. A., Brown P. J., Wisely G. B., Koble C. S., Devchand P., Wahli W., Wilson T. M., Lenhard J. M., Lehmann J. M. (1997): Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and β. Proc. Natl. Acad. Sci. U.S.A. **94**, 4318–4323

Krey G., Braissant O., L'Horset F., Kalkhoven E., Perroud M., Parker M. G., Wahli W. (1997): Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol. Endocrinol. **11**, 779–791

King I. B., Lemaitre R. N., Kestin M. (2006): Effect of a low-fat diet on fatty acid composition in red cells, plasma phospholipids, and cholesterol esters: investigation of a biomarker of total fat intake. Am. J. Clin. Nutr. **83**, 227–236

Kobayashi M., Sasaki S., Kawabata T., Hasegawa K., Akabane M., Tsugane S. (2001): Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged Japanese men. Eur. J. Clin. Nutr. **55**, 643–645

Leikin A. I., Brenner R. R. (1988): *In vivo* cholesterol removal from liver microsomes induces changes in fatty acid desaturase activities. Biochim. Biophys. Acta **963**, 311–319

Lichtenstein A. H., Ausman L. M., Jalbert S. M., Vilella-Bach M., Jauhiainen M., McGladdery S., Erkkilä A. T., Ehnholm C., Frohlich J., Schaefer E. J. (2002): Efficacy of a therapeutic lifestyle change/step 2 diet in moderately hypercholesterolemic middle-aged elderly female and male subjects. J. Lipid Res. **43**, 264–273

Lopes-Virela M. F., Stone P., Ellis S., Colwell J. A. (1977): Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem. **23**, 882–884

Ma J., Folsom A. R., Sharar E., Eckfeldt J. H. (1995): Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. Am. J. Clin. Nutr. **62**, 564–571

Matusik E. J., Reeves V. B., Flanagan V. P. (1984): Determination of fatty acid methyl esters. Anal. Chim. Acta. **166**, 179–188

Mikhailidis D. P., Kirtland S. J., Barradas M. A., Mahadeviah S., Dandona P. (1986): The effect of dihomogammalinolenic acid on platelet aggregation and prostaglandin release, erythrocyte membrane fatty acids and serum lipids: evidence for defects in PGE1 synthesis and delta 5-desaturase activity in insulin-dependent diabetics. Diabetes Res. **3**, 7–12

National Cholesterol Education Program (2001): Third report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). NIH Pub. No. 01-3670. Bethesda, MD: National Heart, Lung and Blood Institute

Nyalala J. O., Wang J., Dang A., Faas F. H., Smith W. G. (2008): Hypertriglyceridemia and hypercholesterolemia: effects of drug treatment on fatty acid composition of plasma lipids and membranes**.** Prostaglandins Leukot. Essent. Fatty Acids **78**, 271–280

Oltman C. L., Kane N. L., Fudge J. L, Weintraub N. L., Dellsperger K. C. (2001): Endothelium-derived hyperpolarizing factor in coronary microcirculation: responses to arachidonic acid. Am. J. Physiol. Heart Circ. Physiol. **281**, H1553–1560

Okada T., Furuhashi N., Kuromori Y., Miyashita M., Iwata F., Harada K. (2005): Plasma palmitoleic acid content and obesity in children. Am. J. Clin. Nutr. **82**, 747–750

Pavlovic M., Grujic V., Oshaug A. (2005): Nutrition and physical activity of the population in Serbia. World Rev. Nutr. Diet. **94**, 51–59

Ristić Medić D., Ristić V., Tepšić V., Ranić M., Ristić G., Vrbaški S., Estelecki I. (2003): Effect of soybean leci-vita product on serum lipids and fatty acids composition in patients with elevated serum cholesterol and triglyceride levels. Nutr. Res. **23**, 465–477

Rivellese A., de Natale C., Lilli S. (2002): Type of dietary fat and insulin resistance. Ann. N. Y. Acad. Sci. **967**, 329–335

Sarkkinen E., Agren J., Ahola I., Ovaskainen M., Uusitupa M. (1994): Fatty acid composition of serum cholesterol esters, and erythrocyte and platelet membranes as indicators of long-term adherence to fat-modified diets. Am. J. Clin. Nutr. **59**, 364–370

Schindler C. (2007): The metabolic syndrome as an endocrine disease: is there an effective pharmacotherapeutic strategy optimally targeting the pathogenesis? Ther. Adv. Cardiovasc. Dis. **1**, 7–26

Skeaff C. M., Hodson L., McKenzie J. E. (2006): Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. J. Nutr. **136,** 565–569

Siscovick D. S, Raghunathan T. E, King I., Weinmann S., Wicklund K. G. (1995). Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA **27,** 1363–1367

Tavella M., Corder C. N., McConathy W. (1993): Effect of gemfibrozil on fatty acids in lipid fractions of plasma from patients with hypertriglyceridemia. J. Clin. Pharmacol. **33**, 35–39

USDA (2005): US Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 18. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp

WHO Technical Report Series 916 (2003): Diet, nutrition and the prevention of chronic disease. Report of the Joint WHO, FAO Expert Consultation, Geneva, Switzerland

Wood D., De Backer G., Faergeman O., Graham I., Mancia G., Pyorala K. (1998): Prevention of coronary heart disease in clinical practice: recommendations of the Second joint task force of European and other societies on coronary prevention. Atherosclerosis **140**, 199–270

Yu-Poth S., Zhao G., Etherton T., Naglak M., Jonnalagadda S., Kris-Etherton P. M. (1999): Effects of the National Cholesterol Education Program s Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am. J. Clin. Nutr. **69**, 632–646

Warensjö E., Sundström J., Lind L., Vessby B. (2006): Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. Am. J. Clin. Nutr. **84,** 442–448

Warensjö E., Sundström J., Vessby B., Cederholm T., Risérus U. (2008): Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. Am. J. Clin. Nutr. **88**, 203–209