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Original Article

The effect of the plasma n-3/n-6 polyunsaturated fatty acid ratio on the dietary LDL phenotype transformation – Insights from the LIPGENE study☆

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SUMMARY

Background & aims: LDL phenotype B is associated with obesity, insulin resistance, hypertriglyceridemia and oxidative stress. The effect of plasma n-3/n-6 PUFA ratio on LDL phenotype transformation was investigated.

Methods: Patients with metabolic syndrome ($n = 99$) received one of four isocaloric diets: (A) High-fat (38% energy) SFA-rich diet (HSFA); (B) High-fat (38% energy), MUFA-rich diet (HMUFA); (C), low-fat (LF) (28% energy), high-complex carbohydrate diet with 1.24 g/d oleic sunflower oil (LFHCC) and (D): low-fat (28% energy), high-complex carbohydrate diet, with 1.24 g/d LC n-3 PUFA (LFHCC n-3) for 12 weeks. Analysis of plasma lipid profile and LDL phenotype was done pre- and post-interventions.

Results: Post-dietary change of LDL density was a main effect observed in all groups. LFHCC n-3 and HFMUFA diets resulted in favorable alteration of LDL phenotype from B to A and decreased LDL density. In contrast, increased LDL density was observed in HSFA and LFHCC groups. The plasma pre-n3/n6 PUFA, Apo E change and pre-Apo CIII/CII ratios explained in 65% the post-dietary change of LDL density in diet LFHCC n-3 consumers.

Conclusions: Study demonstrates efficacy of dietary n-3 PUFA to modify pro-atherogenic to less atherogenic LDL phenotype in patients with metabolic syndrome.
Study identifier at ClinicalTrials.gov was NCT00429195.

1. Introduction

The small, dense low-density lipoprotein (sdLDL) phenotype B is associated with obesity, insulin resistance, hypertriglyceridemia and oxidative stress, indicating the importance of these particles in cardiovascular complications of metabolic syndrome (MetS).[1] LDL particle size is inversely related with carotid intima-media thickness, a surrogate marker of early atherosclerosis.[2] LDL size is an important predictor of cardiovascular events and progression of coronary heart disease.[3,4] LDL size was also demonstrated to influence etiology and clinical presentation of ischemic stroke.[5] Identification of the most appropriate dietary intervention for the treatment of the atherogenic dyslipidemia is not yet defined. Regular consumption of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) of marine origin can improve serum lipid profile and reduce cardiovascular risk.[6] It has been demonstrated that eicosapentaenoic (EPA) in comparison to docosahexaenoic (DHA) acids exerts different effect on serum lipids and lipoproteins. The LDL particle size is increased after DHA but not after EPA supplementation.[7] DHA supplementation lowered concentrations of triacylglycerol (TG) and small, dense LDL particles.[8]

Non-standard abbreviations: CHO, carbohydrate; HSFA, high-SFA; HMUFA, high-MUFA; LF, low-fat; LFHCC, low-fat high carbohydrate; LFHCC n-3, low-fat, high carbohydrate + DHA + EPA.

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The aim of the study was to determine the influence of four isocaloric diets which differed in the amount and composition of fatty acids[9] on LDL phenotype in subjects with the MetS.

2. Research design and methods

2.1. Study design

The current work was conducted as the part of the EU Framework 6 Integrated Project LIPGENE "Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis".[9] Study was carried out in the Lipid and Atherosclerosis Research Unit at the Reina Sofia University Hospital and in the Department of Clinical Biochemistry, Jagiellonian University School of Medicine. The experimental protocol was approved by the local ethic committee at each of the intervention centers, according to the Helsinki Declaration of 1975 as revised in 1983. Volunteers were recruited using various methods including use of general practitioner databases, and poster and newspaper advertisements. Initially volunteers were screened over the telephone using a volunteer suitability questionnaire which assessed dairy food consumption, fish consumption and willingness to consume sandwiches during the study, to ensure potential alteration of diets. This was followed up in those fulfilling the inclusion criteria by completion of a health and lifestyle questionnaire, and anthropometric and biochemical tests. Considered in the present analysis were volunteers from two centers who completed the 12-week intervention trial and provided satisfactorily completed food records. In total, 99 volunteers with the metabolic syndrome (65 females and 34 males) with an average age of 54.5 years (range 35–70 years) started the study across the two centers. Each volunteer was randomly stratified (according to gender, age and fasting plasma glucose concentration) to one of four dietary interventions for 12 weeks. A minimisation procedure was used centrally to randomize volunteers to one of four study diets as previously described.[9]

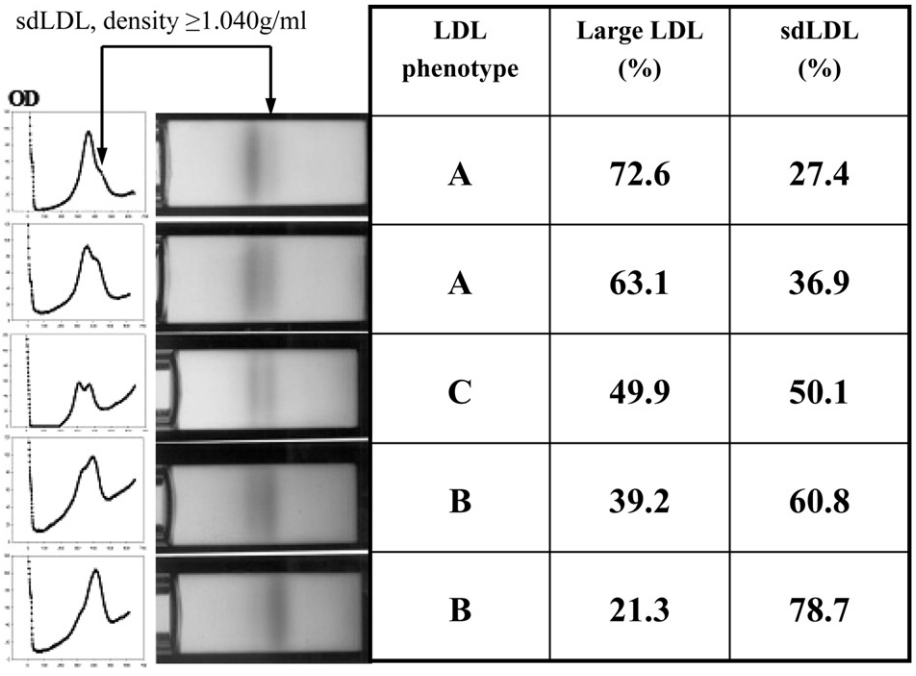
Anthropometric and fasting blood biochemical measurements were determined pre-intervention (week 0) and at post-intervention (week 12). Then serum or plasma was separated and supplemented with 10 mM phenylmethylsulfonylfluoride and 5 μl per ml plasma of Trasylol (10,000 KIE/ml). Plasma and serum samples were frozen at −80 °C for further biochemical analysis.

2.2. Dietary intervention

The diets differed in fat quantity and quality whilst remaining isoenergetic. Patients received one of the four dietary regime: (A) High-fat (38% energy) SFA-rich diet (HSFA); (B) High-fat (38% energy), MUFA-rich diet (HMUFA); (C) Isocaloric low-fat (LF) (28% energy), high-complex carbohydrate diet supplemented with 1.24 g/d high oleic sunflower oil (LFHCC) and (D) Isocaloric low-fat (28% energy), high-complex carbohydrate diet, supplemented with 1.24 g/d LC n-3 PUFA (LFHCC n-3) for 12 weeks. Intervention foods were provided by Unilever Best Foods (Vlaardingen, The Netherlands). The LC n-3 PUFA supplement (Marinol™ C-38) and the placebo high-oleic acid sunflower seed oil (HOSO) supplement were provided by Loders Croklaan (Wormerveer, The Netherlands).

2.3. Biochemical measurements

The TG-rich lipoprotein (TRL) fraction was isolated from 4 ml of plasma obtained from EDTA tubes. Plasma was put into the bottom of a 10 ml open top polycarbonate ultracentrifuge tube and overlayed with 6 ml of a preservative solution with NaCl (0.15 mol/l), sodium azide (0.05 g/l), chloramphenicol (0.05 g/l), gentamicin sulfate (40 mg/l) and EDTA (1 mmol/l), (pH 7.4, $d < 1.006$ kg/l). Ultracentrifugation was performed in a type 70Ti rotor at 183 000 × g and 4 °C during 24 h. TRL were collected by aspiration, placed directly into individual vials and stored at −80 °C until assay. Plasma cholesterol and TG were assayed by standard enzymatic tests. HDL and LDL cholesterol concentrations were determined using homogenous, direct assays. The LDL sub-fraction density profile (LDL phenotype) was determined after KBr density gradient ultracentrifugation (DGUG) of plasma.[10] The density of the main LDL sub-fraction was determined. Phenotype B was defined by the predominance of small, dense particle in LDL pool with the density cut-off point 1.040 g/ml (Fig. 1). Accuracy of the single band density measurement, controlled by refractometry, is limited to 0.0001 g/



LDL phenotype	Large LDL (%)	sdLDL (%)
A	72.6	27.4
A	63.1	36.9
C	49.9	50.1
B	39.2	60.8
B	21.3	78.7

Fig. 1. The DGUG method used for LDL phenotyping, description (Swinkels et al.[10]).