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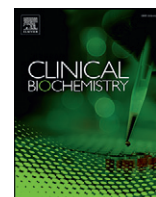

Enhanced reduction in oxidative stress and altered glutathione and thioredoxin system response to unsaturated fatty acid load in familial hypercholesterolemia

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ABSTRACT

Objectives: Familial hypercholesterolemia (FH) is characterized by increased oxidative stress (OS) levels. In the postprandial state, lipids and lipoproteins modulate OS status through their impact on pro-oxidant and antioxidant mechanisms. The objective of this study was to evaluate in patients with FH the response to an unsaturated oral fat load test (OFLT) by analyzing the mRNA levels of genes involved in the glutathione and thioredoxin antioxidant systems.

Design and Methods: We analyzed 14 FH patients and 20 normolipidemic and normoglycemic controls. In both groups, mRNA values of antioxidant enzyme genes (glutathione and thioredoxin systems) were determined at baseline and at 2, 4, 6, and 8 h after OFLT by real time PCR.

Results: In the fasting state the mRNA levels of antioxidant enzymes GPX4 and the GSR, GSS, and GCLC enzymes (involved in glutathione regeneration and synthesis) and thioredoxin (TXN), were significantly increased in the FH group compared to the healthy controls. Some genes (GPX1 and GPX4) were increased at 4 h in both groups, but values for the rest of the antioxidant enzyme mRNAs were decreased in FH patients after 4 h from unsaturated OFLT and were increased in controls.

Conclusions: We concluded that an OFLT with predominantly unsaturated fat has a different effect on postprandial antioxidant enzyme mRNA levels in controls than in FH patients. Increased antioxidant enzyme mRNA is not the main way to reduce postprandial oxidative stress in FH. This difference could determine the influence of dietary patterns in these patients.

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Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations which reduce the activity of the low density lipoprotein receptor (LDL-R) gene. FH is characterized by high cholesterol levels and specifically, elevation of LDL-cholesterol (LDL-C), tendon xanthoma, and early onset of coronary heart disease (CHD) and atherosclerosis [1,2]. CHD is influenced by different cardiovascular risk factors such as, age, gender, weight, blood pressure, LDL-C and high-density lipoprotein cholesterol (HDL-C) plasma values, mutations in LDL-R, and diet [2,3]. Moreover, it is thought that oxidative stress (OS) plays a particularly important role in the development of cardiovascular diseases [4]. Increased OS caused by the production of reactive oxygen

Abbreviations: cDNA, complementary DNA; CHD, coronary heart disease; FH, familial hypercholesterolemia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCLC, gamma-glutamylcysteine synthetase; GPX, glutathione peroxidase; GSH, reduced glutathione; GSR, glutathione reductase; GSS, glutathione synthetase; GSSG, oxidized glutathione; HDL-C, high lipoprotein density cholesterol; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; mRNA, RNA messenger; OFLT, oral fat load test; OS, oxidative stress; ROS, reactive oxygen species; TXN, thioredoxin; TXNRD, thioredoxin reductases.

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species (ROS) and/or reduced antioxidant mechanisms present in arterial walls and circulating cells are the main causes of atherosclerosis and endothelial dysfunction [5]. Previous studies have also shown increased OS levels in other diseases with a high risk of developing atherosclerosis [6]. We have recently shown altered antioxidant enzymatic activity in hypertensive patients [7] and increased oxidative stress in FH patients, but normal antioxidant activity [8]. Furthermore, we also reported in hypertensive subjects that the response of the main cytoplasmic antioxidant systems to chronic OS is inadequate [9].

In many situations increases in pro-oxidant activity exceed the capacity of antioxidant enzymes, leading to the increase of OS. ROS are usually inactivated by antioxidant enzymes: catalases, superoxide dismutases, and members of the glutathione and thioredoxin systems. The glutathione system includes oxidized and reduced glutathione (GSSG and GSH respectively), and different enzymes that control their levels, such as glutathione peroxidases (GPX), glutathione reductase (GSR), glutathione synthetase (GSS), and gamma-glutamyl cysteine synthetase (GCLC). The thioredoxin system mainly involves genes codifying plasmatic and mitochondrial thioredoxins (TXN and TXN2) and thioredoxin reductases (TXNRD1, TXNRD2, and TXNRD3). Both systems have important implications in human health [10,11].

Dietary fat has been proposed to be critical in OS regulation due to the important role that different lipids and lipoproteins have in modulating OS status [12]. Several epidemiological and experimental studies evaluating the impact of fat-challenges in healthy people have shown that the quality and quantity of fat change OS status [13]. We have recently reported that an oral fat load test (OFLT) with predominantly unsaturated fat improves fasting OS and decreases postprandial OS status in patients with FH, as shown by GSH, GSSG, and malondialdehyde (MDA) measurements. The mechanisms underlying these alterations are not well understood, but we hypothesized that the postprandial unsaturated fat processing reaction might affect the expression of antioxidant enzyme genes. Therefore, the objective of this study was to evaluate the OFLT response to unsaturated fat in FH patients and healthy subjects by determining the mRNA levels of genes involved in the glutathione and thioredoxin systems in lymphomonocytes.

Methods

Subjects and study protocol

We have studied 14 individuals with FH selected from our outpatient clinic according to the following criteria: plasma levels of total LDL and cholesterol above the 95th percentile when corrected for age and sex; the presence of tendon xanthoma, coronary heart disease (CHD) in a first degree relative, and bimodal distribution of total LDL and cholesterol levels in the family, indicating an autosomal dominant IIa phenotype. All FH patients were carriers of LDL receptor gene mutations who were previously screened and described by our group [14]. Twenty healthy volunteers were randomly recruited as control group and met the following criteria: plasma total cholesterol less than 5.2 mmol/L, triglycerides less than 1.7 mmol/L, apolipoprotein B less than 1.2 g/L, fasting glucose less than 5.6 mmol/L, and absence of personal or family history of dyslipidemia, cardiovascular disease, or diabetes. All participants gave informed consent and the study protocol was conducted in accordance with the guidelines for human and animal research outlined in the Declaration of Helsinki and was approved by the ethics committees of our institution. Exclusion criteria were: individuals with CHD (presence of angina pectoris, signs of ischemia in the stress electrocardiogram, history of myocardial infarction, acute coronary syndrome, or coronary revascularization procedures), diabetes, hypertension, consumption of more than 30 g of alcohol per day, smokers, body weight fluctuation of more than 10% in the previous three months, secondary hyperlipidemia, renal or hepatic insufficiency and hypothyroidism, infection, or inflammatory disease within six weeks before the study.

A complete medical history, anthropometric examination and clinical chemistry analysis were performed in all participants by standard procedures. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared and abdominal circumference in cm (point between the low costal rim and the iliac crest) was measured. Genotyping for apolipoprotein E was done; only subjects with a E3/E3 genotype participated in the OFLT. In addition, medication known to modify the lipid profile or OS was withdrawn at least six weeks before the study.

Subjects with FH followed a cholesterol-lowering National Cholesterol Education Program-1 diet during the six weeks preceding the study, whereas the control group maintained their usual dietary habits. A cannula was placed for venous blood sampling after overnight fasting, and a urine sample was collected before administering the OFLT. The participants remained in a sitting or supine position for the next 8 h, and peripheral venous specimens were collected into sodium ethylenediaminetetraacetic acid (EDTA) tubes (for Ficoll-Hypaque isolation of lymphomonocytes) before (0 h) and every 2 h for a period of 8 h after the OFLT.

Oral fat load test

Subjects ingested a high-fat meal with a commercial liquid preparation of long chain triglycerides (Supracal; SHS International Ltd, Liverpool, UK). Each 100 mL contains 50 g of fat (450 kcal), of which 9.6 g is saturated, 28.2 g is mono unsaturated, and 10 g is polyunsaturated. The ratio u6/u3 is 20/1. The dose ingested was 50 g of fat per meter squared of body surface. Complete information on the Supracal product can be obtained at Nutricia.com.

Biochemical profile

Blood samples were drawn from the antecubital vein. Glucose, total cholesterol, and triglyceride plasma concentrations were measured using automatic standard enzymatic methods with clinical chemistry Olympus AU5400 analyzers. Plasma apolipoprotein B was determined by immunoturbidimetry using goat antibodies Apo-B100 (Beckman Coulter) [15]. High density lipoprotein cholesterol (HDL-C) values were calculated after precipitation of apolipoprotein B-containing lipoproteins with polyanions and very-low-density lipoprotein (VLDL) cholesterol after separation of VLDL ($d < 1006$ g/mL) by ultracentrifugation [16,17]. The LDL-C was calculated by subtraction of VLDL and high-density lipoprotein cholesterol from total cholesterol. The coefficient of variation for both lipids and lipoproteins was less than five percent.

Oxidative stress assays

Blood plasma was obtained by centrifugation and mononuclear cells were isolated from whole blood obtained in Vacutainer tubes containing EDTA using Ficoll-Hypaque isolation. Reduced glutathione (GSH) and GSSG contents were measured in mononuclear cells by high-performance liquid columns (HPLC) as previously described [18]. Samples were prepared with 0.3 perchloric acid to 50 μL of mononuclear cells. Then, samples were taken to pH 10 with KOH, centrifuged and samples were mixed with 300 μL of 1% 1-fluoro-2,4-dinitrobenzene. After this, derivatization was completed and samples were analyzed in duplicate by HPLC and ultraviolet detection. After this, derivatization was completed and samples were analyzed in duplicated by HPLC and ultraviolet detection. The coefficient of variation was less than seven percent. MDA was measured in plasma by HPLC [19]. The protein content was determined using the Bradford method.

mRNA-level analysis by real-time PCR

Total RNA from mononuclear cells was extracted with TRIzol reagent using a chloroform extraction protocol as previously described [20].

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