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Arginase I and the very low-density lipoprotein receptor are associated with phenotypic biomarkers for obesity

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ABSTRACT

Objective: Obesity is a serious health problem implicated in many metabolic disorders (i.e., hypertension, dyslipidemia, and cardiovascular disease). We examined whether the mRNA of tested genes were linked to blood lipid concentrations and vascular endothelial function as features of obesity.

Methods: In healthy subjects (30–69 y old; normal weight, $n = 22$, body mass index 18.5–23 kg/m^2; overweight, $n = 25$, body mass index $\geq$23 kg/m^2) the following parameters were measured in the blood circulation: total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triacylglycerol, apolipoprotein AI, apolipoprotein B, adiponectin, high-sensitivity C-reactive protein, and soluble intercellular adhesion molecule-1. The mRNA levels of genes (arginase I [*ARG1*], very low-density lipoprotein receptor [*VLDLR*], adiponectin receptor-1 [*ADIPOR1*], *ADIPOR2*, and nitric oxide synthase-3 [*NOS3*]) were tested in the subjects' peripheral blood mononuclear cells.

Results: The expression levels of all tested genes were investigated for their associations with the blood concentrations of each parameter. In the expression study, only *ARG1* (4.5-fold) and *VLDLR* (4-fold) expressions were significantly upregulated in the overweight group compared with the normal-weight group. The *ARG1* mRNA levels were positively associated with blood concentrations of total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, and soluble intercellular adhesion molecule-1. The *VLDLR* mRNA levels showed a positive relation with triacylglycerol and glucose concentrations and a negative relation with adiponectin levels.

Conclusion: Significant upregulations of *ARG1* and *VLDLR* were observed in the overweight condition and their expression levels are likely to be closely linked to the phenotypic biomarkers for obesity (disturbed lipid profiles and endothelial dysfunction).

Introduction

The prevalence of obesity has been increasing around the world, and nearly 31% of adults older than 20 y are considered overweight in Korea [1]. A person with a body mass index (BMI) greater than 23 kg/m^2 is considered overweight or obese based on the guidelines from the Korean Society for the Study of Obesity. Because of its close link to several metabolic disturbances such as hypertension, dyslipidemia, and insulin resistance, the understanding of obesity has been an interest of many researchers. Each metabolic condition, known collectively as *metabolic syndrome*, serves as an independent risk factor for atherosclerosis and contributes to the development of type 2 diabetes mellitus and coronary heart disease [2,3].

One of the comprehensive ways to investigate the cause of obesity or the characteristics associated with obesity is to analyze the transcriptional profiles in obesity [4–6]. In our preliminary microarray experiment, we observed that the mRNA abundance of very low-density lipoprotein receptor (*VLDLR*) and arginase I (*ARG1*) was remarkably higher in the peripheral blood

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mononuclear cells (PBMCs) of obese subjects compared with their normal-weight counterparts (unpublished), raising the question of the roles of these genes in the pathogenesis of obesity and obesity-related complications.

Arginase is an enzyme in the urea cycle that catalyzes the hydrolysis of L-arginine to urea and L-ornithine [7]. Emerging evidence has implicated this enzyme in the regulation of nitric oxide (NO) synthesis through several mechanisms, for example, potentially inhibiting the production of NO by competition with NO synthase (NOS) for the substrate L-arginine and the uncoupling of NOS, leading to the production of the NO scavenger [8]. There is also evidence that the administration of L-arginine restores NO synthesis and vascular function, suggesting that an impaired L-arginine availability underlies these vascular pathologies [9,10]. Thus, it is suggested to be a crucial mediator of endothelial dysfunction in several pathologic states including hypertension, diabetes, and vascular disease [11–13]. *VLDLR*, a member of the *LDLR* family, is involved in the uptake of remnant lipoprotein particles into peripheral tissues in cooperation with lipoprotein lipase. The roles of *VLDLR* in the development of atherosclerosis and insulin resistance have been investigated in previous studies and the *VLDLR* has been suggested as a target to ameliorate disease states [14,15]. For instance, knocking out the *VLDLR* hindered insulin resistance induced by a high-fat and high-calorie diet and limited weight gain in *ob*/*ob* mice, which are genetically prone to be obese [16].

To further clarify the relation of *ARG1* and *VLDLR* to the clinical features of obesity including endothelial dysfunction and lipid abnormalities, we examined whether there were differences in the mRNA abundance of *ARG1* and *VLDLR* in addition to two adiponectin receptors (*ADIPOR1* and *ADIPOR2*) and *NOS3* in PBMCs between overweight/obese and normal-weight individuals. We then investigated whether there were associations between the mRNA levels of the genes tested and changes in blood lipid profiles as features of obesity and plasma levels of proteins for vascular endothelial cell functions.

Materials and methods

Study population

The study subjects were 30 to 69 y old and subdivided into two group based on the level of their BMI (normal-weight group [healthy control], BMI 18.5–23 kg/m^2; overweight/obese group, BMI $\geq$23 kg/m^2) based on the guidelines from the Korean Society for the Study of Obesity. Subjects were excluded from the study if they had any history of 1) clinical or electrocardiographic evidence of coronary artery disease, stroke, myocardial infarction, or peripheral arterial occlusive disease; 2) diabetes mellitus (fasting glucose levels $\geq$126 mg/dL or 2-h serum glucose level $\geq$200 mg/dL after a 75-g oral glucose tolerance test); 3) abnormal liver or renal function; 4) thyroid or pituitary disease; 5) acute or chronic inflammatory disease; 6) orthopedic limitations; 7) body weight loss/gain in the previous 1 y; and 8) regular use of any medications that could affect cardiovascular function and/or metabolism. We included 47 genetically unrelated Koreans in this study (25 healthy controls and 22 overweight/obese subjects). The purpose of the study was carefully explained to all participants and their informed consent was obtained before their participation. The study protocol was approved by the institutional review board of the Yonsei University College of Medicine and carried out in accordance with the Declaration of Helsinki.

Anthropometric parameters and blood collection

Body weight and height were measured for unclothed subjects and the BMI was calculated as body weight (kilograms) divided by the square of the height (meters). The waist circumference was measured at the umbilical level with the subjects standing after normal expiration. After a 12-h fasting period, venous blood specimens were collected in tubes containing ethylenediaminetetraacetic acid or plain tubes, centrifuged into plasma or serum, and then stored at −70°C until analysis.

Serum lipid profiles, apolipoproteins A1 and B

Serum cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were measured with commercially available kits (Choongwae, Seoul, Korea) using enzymatic methods. Serum triacylglycerols were analyzed using a total glycerol test kit (Roche, Basel, Switzerland). All measurements were performed on a Hitachi 747 autoanalyzer (Hitachi, Ltd., Tokyo, Japan). Each sample was measured in duplicate, and the average value was used. If there was a large variation between the two values, the samples were remeasured. Serum apolipoprotein (apo) A1 and B levels were determined by turbidimetry at 340 nm using a specific antiserum (Roche).

Measurement of fasting glucose and plasma adiponectin

Fasting glucose was measured by a glucose oxidase method using a Glucose Analyzer (Beckman Instruments, Irvine, CA, USA). Plasma adiponectin concentration was measured using an enzyme immunoassay (human adiponectin enzyme-linked immunosorbent assay kit; B-Bridge International, Inc., Cupertino, CA, USA). The assay was read using a Victor^2 reader (Perkin-Elmer Life Sciences, Turku, Finland) at 450 nm and the wavelength correction was set to 540 nm.

Measurement of high-sensitivity C-reactive protein and soluble intercellular adhesion molecule-1

Serum concentrations of high-sensitivity C-reactive protein (hs-CRP) were measured with an ADVIA 1650 (Bayer, Tarrytown, NY, USA) using a commercially available kit (high-sensitivity CRP-Latex[II] X2 kit, Seiken Laboratories, Ltd., Tokyo, Japan) that allowed the detection of CRP in the range of 0.001 to 31 mg/dL. Plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) were measured using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA.). The resultant color reaction was read at 450 nm using a Victor^2 reader.

RNA isolation and real-time polymerase chain reaction

Total RNA was isolated from the PBMCs using a QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The primers for real-time polymerase chain reaction (PCR) analysis were designed using the FastPCR 6.0 (Primer Digital Ltd., Helsinki, Finland) program. The sequences of the designed primers were as follows: *ARG1* (sense, 5′-CCT TTC TCA AAG GGA CAG CCA-3′; antisense, 5′-GAT GGG TCC AGT CCG TCA ACA-3′), *ADIPOR1* (sense, 5′-GCT CCT GCC AGT AAC AGG GAA; antisense, 5′-CCA GCT CCA GTG ATG TAC ATC-3′), *ADIPOR2* (sense, 5′-CTT TGT GGC CCC TCT GCA AGA; antisense, 5′-CAG TGC ATC CTC TTC ACT GCA-3′), *VLDLR* (sense, 5′-GGA TGA AGA CTG TGT TGA CGG-3′; antisense, 5′- TTA TGG ATG CAC TCG CCA GAG-3′), and *NOS3* (sense, 5′-TAC TCC CAC CAG CGC CAG AAC-3′; antisense, 5′-GCC CCC AAT TTC CAG CAG CAT-3′). β-Actin (sense, 5′-CCA ACT GGG ACG ACA TGG AGA-3′; antisense, 5′-CGA TCC ACA CGG AGT ACT TGC-3′) was measured for each sample as a reference. Real-time PCR reactions were then carried out in a 20-μL reaction mixture (2 μL of cDNA and 20 μL of Superscript II Reverse Transcriptase; Invitrogen, Carlsbad, CA, USA). The PCR program was initiated at 95°C for 5 min before 40 thermal cycles, each for 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, were conducted. The data that were obtained were analyzed using the comparative-cycle threshold method and were normalized by the β-actin expression value. Melting curves were generated for each PCR reaction to ensure the purity of the amplification product. The delta-delta-cycle threshold ($\Delta\Delta C_T$) method was used to measure relative quantification. Values were expressed as fold changes over the control and expressed as mean ± standard deviation.

Statistical analysis

Statistical analyses were performed with SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Differences in continuous variables between the two subject groups were tested by an independent *t* test (Student's *t* test), and non-continuous variables were tested by chi-square tests. Pearson correlation coefficients were used to examine the relations between variables. The Kolmogorov-Smirnov test was used to test the normality of the distribution, and skewed variables were logarithmically transformed for statistical analysis. For descriptive purposes, mean values are presented using untransformed values. The results are expressed as mean ± standard deviation or percentage. Two-tailed $P < 0.05$ was considered statistically significant.

Results

Clinical and biochemical characteristics of study subjects

Table 1 compares the general and chemical characteristics of the healthy controls (normal weight, BMI 18.5–23 kg/m^2) and the