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Plasma resistin levels are associated with homocysteine, endothelial activation, and nitrosative stress in obese youths

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ABSTRACT

Objective: To evaluate whether serum resistin levels are related to cardiovascular risk in obese children.

Design and methods: Cross-sectional study of 110 children (40 normal weight and 70 severely obese). Clinical and biochemical parameters, including lipid profile, fasting glucose and insulin, and homocysteine, were determined. The levels of adipokines (adiponectin, leptin, and resistin), markers of inflammation (high-sensitivity C-reactive protein (hs-CRP)), endothelial activation (serum concentrations of soluble intercellular and vascular cellular adhesion molecule-1 (sICAM-1, sVCAM-1)), and oxidative/nitrosative stress (malondialdehyde and urinary nitrate/nitrite) were measured.

Results: A partial correlation adjusted by gender, Tanner stage, and body mass index in obese children showed that resistin was significantly related to central obesity ($p < 0.002$), insulin resistance ($p < 0.005$), and homocysteine ($p < 0.001$). No association was found with other metabolic risk factors or hs-CRP levels. Malondialdehyde ($p < 0.043$) and sVCAM-1 ($p < 0.002$) were positively correlated whereas urinary nitrate/nitrite was negatively correlated ($p < 0.007$). In multiple regression analysis homocysteine, sVCAM-1, and urinary nitrate/nitrite remained independent determinants of resistin levels (R^2 adjusted = 0.347, $p = 0.000$).

Conclusions: Resistin could be considered as a promising marker for future cardiovascular disease in obese children.

Introduction

Obesity confers an increased risk of comorbidities and premature death, mainly due to the accumulation of visceral fat. Thus, adipose tissue is now considered to be an endocrine organ that produces several bioactive molecules, most of which negatively affect the development of cardiometabolic risk. Regarding the background of this complex disorder, a role for oxidative and nitrosative stress is suggested [1].

Resistin was originally described as an adipokine that was found to induce insulin resistance or impaired liver sensitivity to insulin [2]. Thereafter, widespread research on the relationship between resistin, obesity, and associated cardiovascular risk has been conducted, mainly in experimental fields [3,4]. Homocysteine (Hcys), a known independent risk factor for cardiovascular disease, induces resistin expression and secretion from adipocytes [5]. Resistin is also associated with the activation of inflammatory processes [6]. Thus, it has been subsequently proposed that resistin can act as an effector molecule that leads to an atherosclerotic state, possibly through several mechanisms. It has been shown that resistin has direct effects on endothelial cell activation by inducing the expression of cell adhesion molecules, thereby enhancing leukocyte adhesion [7,8]. Previous "in vitro" experimental studies on endothelial cells and atherosclerotic plaque progression also showed that resistin can impair endothelium-dependent relaxation, promote angiogenesis [9], and induce vascular inflammation [10]. An increase in resistin concentration significantly decreases endothelial nitric oxide synthase expression and nitric oxide (NO) production through oxidative stress in cultured human coronary artery endothelial cells [11], suggesting that the effects of resistin can be mediated by oxidative stress. However, the precise role of resistin in the clinical scenario remains to be fully elucidated.

Given that cardiometabolic risk factors continue from early life to adulthood, resistin could be considered as a link between obesity in youth and adult cardiovascular disease. This further issue is still debated. Very few studies have been performed on children in this setting, and recent studies have failed to find an association between resistin levels and obesity in children, as determined by an increase in body mass index (BMI) [12–14]. Moreover, the levels appear to be related to pubertal

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development [15]. To our knowledge, the association between the circulating levels of resistin and the degree of oxidative/nitrosative stress has not been evaluated in youths. Because children present the early stages of the development of complications linked to obesity, we postulated that a dysregulation of this molecule would reflect an association with comorbidities and could have predictive value. We tested the hypothesis that resistin can be a marker for the identification of individuals who are "at risk" for premature atherosclerosis. To achieve this goal, we investigated the relationship of resistin levels with circulating biomarkers of vascular endothelial function and parameters of oxidative and nitrosative stress in a cohort of obese children.

Methods

Subjects

The participants in this cross-sectional study were 110 Spanish children (63 boys) aged between 7 and 14 years. We enrolled 70 severely obese children who were referred to our unit from October 2012 to March 2013 to participate in an outpatient weight-loss program. The definition of BMI-based obesity employed was as proposed by Cole and colleagues for childhood [16]. BMI variability with age and sex was adjusted using the standard deviation score for BMI (SDS-BMI), based on WHO charts [17]. In total, 40 age-matched healthy children from a school health program and with a normal BMI (<85th percentile for age and gender according to reference standards) were selected as a control group for the purpose of comparison of serum resistin levels. Pubertal development (Tanner stage) was clinically assessed. All of the children were instructed to follow a nitrate-restrictive diet (leafy vegetables, cured meat, and sausages) during the 3 days before blood and urine sampling. Children were excluded if they had known genetic abnormalities or underlying systemic diseases or if they were within 1 month of any acute infectious process. The study followed the Helsinki guidelines and all participants and their parents gave their written, informed consent to participate. The protocol was approved by the Ethics Committee of Dr. Peset University Hospital.

Anthropometric and clinical measurements

Weight and height were measured according to a standardized protocol. Waist circumference was determined with non-elastic tape and was measured at the smallest circumference between the costal margin and the iliac crest. Waist-to-height ratio was then calculated. Resting blood pressure was measured in the dominant arm with an electronic sphygmomanometer (Dinamp 200; GE Medical Systems Information Technologies, Inc., Milwaukee, WI, USA), and the average of three measurements was recorded.

Bioelectrical impedance was assessed using a Tanita BC-418MA instrument with eight-contact electrodes (Tanita Europe BV, Hoofddorp, The Netherlands). The fat mass was normalized for height^2 and used as fat mass index.

Blood sample and urine analysis

Blood and urine samples for analysis were collected simultaneously with routine clinical investigations. Fasting blood samples were collected and centrifuged, and an aliquot of plasma and serum was deep-frozen until experimental determinations. Routine biochemical parameters were measured by automated methods, and insulin and Hcys were measured by an automated electrochemiluminescence immunoassay (Aeroset System® and Architect c8000®; Abbott Clinical Chemistry, Wiesbaden, Germany). Insulin resistance was assessed using a widely validated mathematical formula for the homeostasis model assessment index (HOMA-IR = insulin (μU/mL) × glucose (mmol/L) / 22.5). A value of 3.16 was considered as the normal limit [1]. Urine was collected in sterile containers for a 2-hour period after voiding the bladder in the morning.

Assays

High-sensitivity C-reactive protein (hs-CRP) levels were determined by kinetic nephelometry (Immage Nephelometer®; Beckman Coulter Inc., Brea, CA, USA). Adiponectin, leptin, and resistin were measured in the plasma using the MILLIPLEX MAP Human Adipocyte Magnetic Bead Panel and the HADCYMAG-61K (Millipore, Billerica, MA, USA) in the Luminex 100 IS Analyser System (Luminex Corp, Austin, TX, USA). The coefficients of variation for the three analytes ranged from 6.5% to 10%. The serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) were measured with commercial ELISA kits (Bender MedSystems, Vienna, Austria). Assays were performed according to the manufacturer's instructions, with an interassay coefficient of variation of 8.5% for sVCAM-1 and 9.7% for sICAM-1. The concentration of malondialdehyde–thiobarbituric acid adduct in the plasma was measured based on absorbance at 532 nm in a Waters HPLC System (Milford, MA, USA) following isocratic separation in a reverse-phase C-18 column of 5 μm, 4.6 mm × 150 mm; eluted with 50 mmol/L potassium phosphate buffer (pH 6.8) and methanol (58:42, v/v) [18].

Because NO is a very labile molecule, its direct measurement in biological samples is difficult. In aqueous solution, NO reacts with molecular oxygen, accumulates in the plasma, and is excreted in the urine as the stable oxidation end-products nitrite (NO_2^-) and nitrate (NO_3^-) ions, also known as NOx, which can be readily measured in biological fluids and have been used in vitro and in vivo as indicators of NO production. The levels of NO end-products were measured using a modified Griess assay. Briefly, the assay involves the enzymatic conversion of nitrate to nitrite by nitrate reductase (*Aspergillus* species), followed by the measurement of nitrite through the formation of a magenta-colored azo dye as a product of the Griess reaction with Griess reagent (1 g/L sulfanilamide, 0.1 g/L N-1-naphthylethylenediamine, and 25 g/L phosphoric acid). Absorbance was read at 540 nm using a Multiskan EX microplate reader (Thermo Lab Systems, Helsinki, Finland). The conditions were essentially those described by Moshage et al. [19]. All samples were assayed in duplicate. Values of nitrate/nitrite in the urine were expressed relative to levels of creatinine (μmol/mmol) [20].

Statistical analysis

Comparisons of dichotomic values between groups were done by Chi-squared test. Quantitative data are described as the median and interquartile range (25th to 75th percentile). The conformity of data with a normal distribution was controlled with the Kolmogorov–Smirnov test, and skewed variables were log-transformed prior to using parametric tests. Comparisons of continuous values between groups were done by analysis of covariance adjusted by gender and Tanner stage or by Student's t test in the case of age. The significance of the differences between resistin levels in the control and obese groups with or without insulin resistance was tested by analysis of covariance, with gender, Tanner stage, and BMI as covariates, and a post-hoc Bonferroni analysis. A partial correlation coefficient adjusted for gender, Tanner stage, and BMI was determined to identify variables with a statistically significant association with serum resistin levels. Predictors identified in the bivariate analysis with a significance of $p < 0.01$ were included in a multiple linear regression analysis. Collinear variables were not included in the model simultaneously. Backward stepwise elimination was applied to the initial multiple regression model to determine the contribution of factors influencing resistin levels after adjusting for potential confounding variables. A value of $p < 0.05$ was considered to be statistically significant. The data were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows, version 17, SPSS Inc., Chicago, IL, USA).