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Brief Communication

Elevated concentration of asymmetric dimethylarginine (ADMA) in individuals with metabolic syndrome

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ABSTRACT

The metabolic syndrome (MS) is a cluster of pathophysiological alterations that includes the presence of hypertension, insulin resistance, dyslipidemia, and abdominal obesity. MS is associated with increased risk of developing diabetes and cardiovascular diseases. Endothelial dysfunction with impaired nitric oxide (NO) bioavailability has been implicated in insulin resistance and hypertension. NO is synthesized by nitric oxide synthase (NOS) using L-arginine as substrate. Asymmetric dimethyl arginine (ADMA) is a major and potent endogenous NOS inhibitor, associated with cardiovascular and renal diseases. We tested the hypothesis that plasmatic ADMA levels are increased in patients with MS.

We studied 85 adult individuals from Talca, Chile, separated in two groups, 48 individuals with MS (according to modified ATP III criteria), and 37 individuals without MS as controls. ADMA levels were significantly increased in the MS group (mean ± standard deviation 0.71 ± 0.38 vs. 0.48 ± 0.28 μmol/L, $p = 0.0009$). Furthermore, the levels of ADMA were modestly but significantly correlated with waist circumference ($p = 0.01$) but not with the other components of MS (blood pressure, glycemia, triglycerides and high density lipoprotein cholesterol HDL-c). These results suggest a possible link between increased ADMA levels and the MS.

Introduction

The metabolic syndrome (MS) is associated with abdominal obesity, blood lipid disorders, inflammation, insulin resistance, and increased risk of developing cardiovascular diseases and diabetes [1]. There is growing evidence that endothelial dysfunction is also a component of the MS [2]. Endothelium-derived nitric oxide (NO), synthesized by nitric oxide synthase (NOS), plays a critical role in the endothelial function [3]. There is abundant experimental data that endothelial dysfunction is caused by reduced bioavailability of NO in the vascular wall [4]. Evidence indicates that endogenous NOS inhibitors may be causally related to this process [5,6]. Among these inhibitors, asymmetric dimethylarginine (ADMA) has emerged as an important predictor element associated with cardiovascular risk factors, endothelial dysfunction, hypertension, atherosclerosis and cardiovascular mortality [7].

ADMA is a naturally occurring amino acid that competitively inhibits NOS activity. It is produced by methylation of arginine residues of intracellular proteins via protein arginine *N*-methyltransferases (PRMTs). When these methylated proteins are hydrolyzed, ADMA is released. ADMA is excreted in the urine, but its primary route of clearance is the enzymatic degradation by dimethylarginine dimethylaminohydrolases (DDAH). As a potent NOS inhibitor, ADMA has cardiovascular effects: induces vasoconstriction, increasing blood pressure [8,9] and increases monocytes adhesiveness to endothelial cells [10,11]. Epidemiologically, ADMA levels have been associated with several causes of mortality [12]. ADMA has been positively associated to coronary events [13,14] and a recent population study of women revealed that those in the highest ADMA quintile (>0.62 μmol/L) had a 30% increased risk of myocardial infarction and stroke over 24 years of follow-up [14,15].

We have recently characterized a high incidence of MS in the population of Talca, Chile [16]. Furthermore we have found in this population increased levels of soluble factors related to endothelial dysfunction, namely the soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble CD40L [17]. The goal of this study was to evaluate the levels of ADMA in individuals with and without MS

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and its association with the components of the syndrome and other soluble factors involved in endothelial dysfunction.

Materials and methods

Subjects

The study subjects were randomly selected within the individuals of the "Cardiovascular profile of adults of Talca, 2005" project [18]. We selected 85 nonsmoker individuals, 45–64 years old from two groups: a group conformed by subjects with MS ($n = 48$) and a group without MS ($n = 37$) as control. All subjects were Hispanic.

We used the ATPIII (Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults) criteria for diagnosis of MS [5]. It consists of the presence of three or more of the following factors: waist circumference (>102 cm men and >88 cm women), high blood pressure ($\geqslant$130/85 mm Hg or arterial hypertension in treatment), hyperglycemia ($\geqslant$100 mg/dL or diabetes in treatment (modified according to ADA 2005) [19], hypertriglyceridemia ($\geqslant$150 mg/dL) and low high density lipoprotein cholesterol (HDL-c, <40 men and <50 mg/dL women).

The anthropometric and arterial pressure measurements, as well as blood extractions, were performed at the Health Sciences School, Universidad de Talca. All the participants provided written informed consent. The Institutional Review Board of the Universidad de Talca and the Health Service of Maule, Chile, according to the Declaration of Helsinki, approved the protocol. Weight, size, and waist perimeter were measured according to the Technical General Rule, ESPA, Ministry of Health, Chile, 2006. For these measurements, a calibrated scale and an anthropometer were used. The Body Mass Index (BMI) was determined by the formula: weight (kg)/height (m^2). Blood pressure was measured using an inflated automatic monitor (OMRON HEM-741C, Columbia, USA).

Laboratory assays

Blood samples were obtained from each person after 12-h fasting. A portion of the sample was collected without anti-clotting to obtain serum. Another portion was treated with sodium citrate (3.2%) in a ratio 9:1 (blood: anticoagulant). Serum and plasma were separated, aliquoted and stored at −70 °C until use. Glycemia and lipid profile were measured using enzymatic methods (Roche Diagnostics, Mannheim, Germany) and an automatic spectrophotometer (Hitachi 717, Japan). The low density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [20]. Plasma levels of ADMA were determined by ELISA (Alexis Biochemical, San Diego, California). Plasminogen activator inhibitor-1 (PAI-1) was quantified using ELISA (Zymutest, Hyphen Biomed, Mason, Ohio). Soluble intercellular adhesion molecule 1 (sVCAM-1), sE-Selectin and sCD40 ligand (sCD40L) were determined separately by solid-phase ELISA (R&D Systems, Minneapolis, Minnesota). For different ELISAs the optical densities (OD) were read at 450 nm using a StatFax-2600 microplate reader (Awareness Technology Inc, Palm City, Florida). Plasmatic levels of fibrinogen were determined using the Clauss method (DG-FIB kit, Diagnostics Grifols) with a Clot-1 coagulometer (RAL, Barcelona, Spain). Uric acid was measured using the activity of urate oxidase to convert uric acid to allantoin and H_2O_2, coupled to peroxidase (Valtek Diagnostics, Santiago, Chile). LDL oxidized was measured by ELISA (Mercodia AB, Sweden).

Measurement of thiobarbituric acid reactive species (TBARS) in plasma

Plasma concentrations of malondialdehyde (MDA) are an index of lipid peroxidation and oxidative stress. Thiobarbituric acid reacts with MDA in the presence of acidic medium at high temperature to generate the TBARS that were measured spectrophotometrically at 530 nm [21].

HOMA-IR

In each subject, the degree of insulin resistance was estimated at the baseline by HOMA according to the method described by Matthews et al. [22]. In particular, an insulin resistance score (HOMA-IR) was computed with the formula: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5. Insulin was measured using the Elecsys Insulin assay, which employs two monoclonal antibodies which together are specific for human insulin (Roche-Mannheim, Germany). Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity (insulin resistance).

Statistical methods

Data are expressed as means ± standard deviation. Comparison between two groups was assessed by Student unpaired t test. In order to identify which MS diagnostic criteria individually affect serum levels of ADMA, a multivariate regression analysis was performed with adjustments for age and gender. SAS 9.1.3 and SPSS 14.0 softwares (SPSS, Inc, Chicago, Illinois) were used for statistical analysis. A value of $p < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the population studied are summarized in Table 1. Both groups were similar in age. Glycemia, HDL-c, triglycerides, waist circumference and blood pressure were all significantly elevated in the MS group, for both genders (all $p < 0.05$). The percentages of subjects in the MS and non-MS groups meeting MS criteria according to the ATP III definition are presented in Table 2.

In Table 3 are shown the levels of biomarkers of oxidative stress and insulin resistance. MS group showed increased level of TBARS ($p = 0.012$ for men, $p = 0.01$ for women), LDL oxidized ($p < 0.001$ for women) and uric acid ($p = 0.013$ for men and $p = 0.008$ for women). Additionally, insulin concentration ($p = 0.017$ for men and $p < 0.001$ for women) and HOMA-IR index ($p = 0.04$ for men and $p < 0.001$ for women) were both elevated in the MS group. These data indicate the presence of oxidative stress and insulin resistance in the MS patients.

Plasma concentrations of ADMA were significantly higher in subjects with MS (mean ± standard deviation 0.71 ± 0.38 μmol/L) in comparison with the non-MS subjects (0.48 ± 28 μmol/L, $p = 0.0009$, Fig. 1). We did not find significant differences in plasma concentrations of ADMA grouped by sex ($p = 0.929$) or age ($p = 0.356$). We performed a multiple-regression analysis to study the independent relationship between ADMA levels (log transformed) and the MS components (Table 4). ADMA levels were significantly and independently associated with waist circumference ($r = 0.488$, $p < 0.01$). This association showed significant differences by gender ($p = 0.009$, Fig. 2), being significant in men ($r = 0.698$, $p < 0.001$) but not in women ($r = 0222$, $p = 0085$). ADMA did not show correlation with the other components of the MS: glycemia, triglycerides, HDL-c, systolic and diastolic pressure. We also analyzed whether the levels of ADMA correlated with other molecules involved in endothelial dysfunction, elevated in this population, such as the soluble factors fibrinogen, PAI-1, sCD40L, sVCAM-1 and sE-Selectin. ADMA levels did not correlate significantly with any of these factors (Table 4).