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Serum nitric oxide metabolites are associated with the risk of hypertriglyceridemic-waist phenotype in women: Tehran Lipid and Glucose Study

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ABSTRACT

Background and aim: There are some controversial issues regarding the association of nitric oxide and obesity-related states. This study was conducted to investigate whether serum nitric oxide metabolites (NOx) could predict the occurrence of visceral lipid accumulation, defined as hypertriglyceridemic-waist (HTW) phenotype.

Methods: We used a prospective approach for this study conducted on participants of the Tehran Lipid and Glucose Study, 2243 adult men and women were followed for a median of 6.3 years. Serum NOx concentrations were measured at baseline (2006–2008), and demographics, anthropometrics and biochemical variables were evaluated at baseline and again after a 3-year (2009–2011) and a 6-year follow-up (2012–2014). The occurrence of HTW phenotype, defined as waist circumference ≥90 cm in men and ≥85 cm in women, along with serum triglyceride levels ≥177 mg/dL, were assessed across serum NOx tertiles.

Results: Mean age of participants was 41.5 ± 14.5 years at baseline and 39.4% were male. The cumulative incidence of HTW phenotype was 37.6% (33.2% in men, 40.5% in women). There was no significant association between serum NOx and the occurrence of HTW phenotype in men. After adjustment of confounding variables, risk of HTW phenotype in women, in the highest compared to the lowest tertile of serum NOx (≥30.9 *vs.* <19.9 μmol/L), increased by 39% (OR = 1.39, 95% CI = 1.05–1.93, *P* for trend = 0.053).

Conclusion: Serum NOx level was an independent predictor of HTW phenotype in women.

1. Introduction

There is growing interest regarding the potential effects of nitric oxide (NO) and its metabolites, nitrate and nitrite (NOx), in physiological pathways and pathophysiologic conditions [1–3]. Recent studies show that the impaired nitrate-nitrite-NO pathway, either reduced or overproduction of NO may be a risk factor and/or prognosis for development of cardiometabolic disorders especially vascular dysfunction, cardiovascular disease, chronic kidney disease, endocrine disorders, insulin resistance, and type 2 diabetes [4–7].

Major sources of nitrate in the body are endogenous production and the diet [2,8]. Main endogenous sources of plasma nitrate is L-arginine-NO pathway while nitrite is produced by oxidation of NO or reduction of nitrate [2]; In humans and rodents, most plasma nitrate and nitrite are derived from nitric oxide synthase (NOS) activity [9,10], and vascular endothelium is considered as the main source of total NO synthesis [11].

It have been shown that adipose tissue is also a potential source of NO production, which occurs by endothelial NOS (eNOS) and inducible NOS (iNOS), in both white and brown adipose tissue

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[12,13]; these observations raise the hypothesis that nitrate-nitrite-NO pathway may be important in regulation of energy homeostasis and adipose tissue metabolism.

Inconsistent data are available regarding the association of NO and its metabolites with obesity; in some studies, increased serum levels of NOx, overexpression of eNOS as well as overproduction of NO have been observed in obese human [14,15]. In contrast, lower eNOS expression in both adipose tissue and skeletal muscle of obese humans and rodents, reduced eNOS activity as well as decreased NO bioavailability have been reported in some investigations [16–18]. A similar controversy was also observed regarding the association between NO metabolites and various obesity-related measures in some previous cross-sectional studies; Fujita et al. studying 80 Japanese adults, reported a higher level of serum NOx in obese subjects and a great correlation between NO metabolites and visceral fat area [19]. Increased serum NOx levels along with increase in body mass index (BMI), waist circumference (WC), and waist to hip ratio were observed in women, but not men [20]. A negative correlation between NOx concentration and abdominal adiposity was also reported only in women in a cross-sectional investigation [21].

To our knowledge, this controversial issue has not yet been investigated in the framework of a prospective longitudinal examination; such a setting could probably help to better justify and provide causality regarding the association between NOx and obesity. The main focus in this study, therefore, was to assess whether serum NOx concentration, an indicator of systemic NO synthesis, could predict the occurrence of hypertriglyceridemic-waist (HTW) phenotype, as a dichotomous surrogate marker of visceral adiposity.

2. Methods

2.1. Study population

This study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS), an ongoing community-based prospective study being conducted to investigate and prevent non-communicable diseases, in a representative sample in the district 13 of Tehran, the capital city of Iran [22]. In the current study, 3505 adult men and women (≥20 years) participants of the third (2006–2008) TLGS examination, with measurements of serum NOx, were enrolled. We excluded pregnant women, subjects who had chronic or frequent diarrhea, those with prevalent coronary artery disease or type 2 diabetes, participants with renal dysfunction (serum creatinine > 123.8 μmol/L), subjects with incomplete data on their WC or TG levels, or those who had HTW^+ at baseline. The remaining participants ($n = 2243$) were followed up to the fourth (2009–2011) and fifth (2012–2014) TLGS examinations. Participants who had left the study before follow-up examinations without diagnosed HTW^+ ($n = 309$), were also excluded and final analyses were conducted on 1934 adults (762 men, 1172 women).

Written informed consents were obtained from all participants and the study protocol was approved by the ethics research council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

2.2. Data collection

2.2.1. Demographic and anthropometric measures

Demographics, anthropometrics, and biochemical measures were evaluated during a median of 6.3 years follow-up, in the three intervals. Baseline measurements were conducted in 2006–2008; second and third examinations were also carried out in 2009–2011, and 2012–2014, respectively. Trained interviewers collected information using pretested questionnaires. Smoking status was obtained using face-to-face interviews. Weight was measured to the nearest 100 g using digital scales, while the subjects were minimally clothed, without shoes. Height was measured to the nearest 0.5 cm, in a standing position without shoes, using a tape meter. Body mass index was calculated as weight (kg) divided by square of the height (m^2). Waist circumference was measured to the nearest 0.1 cm, midway between the lower border of the ribs and the iliac crest at the widest portion, over light clothing, using a soft measuring tape, without any pressure to the body.

2.2.2. Biochemical measures

Fasting blood samples were taken after 12–14 h, from all study participants at baseline and follow-up phases. Serum triglyceride (TG) levels were measured by enzymatic colorimetric analysis with glycerol phosphate oxidase (Pars Azmun Company, Tehran, Iran). Both inter- and Intra-assay coefficients of variations of the assays were <5%. Serum NOx concentration was measured by a rapid, simple spectrophotometric method which has been developed by Miranda et al. for simultaneous detection of nitrate and nitrite [23]. This method has been validated in our laboratory and a review paper regarding serum NOx measurement has been published by our group [24,25]. Inter- and Intra-assay coefficients of variations of the assays were 5.2% and 4.4%, respectively; the sensitivity of the assay was 2.0 μmol/L and its recovery was 93 ± 1.5% [20].

2.2.3. Definition of terms

Hypertriglyceridemic waist, a simple and accurate marker of central adiposity, has been previously developed based on the combination of WC and TG levels [26,27]. Hypertriglyceridemic waist was defined as WC ≥ 90 cm in men, and ≥85 cm in women, along with serum TG levels ≥177 mg/dL [28].

Diabetes was defined as fasting serum glucose ≥126, 2 h serum glucose ≥200 or anti-diabetic medications [29]. Current smoker was defined as a person who smoked cigarettes daily or occasionally. Cardiovascular disease (CVD) was defined as any coronary heart disease (CHD) or stroke. Coronary heart disease was defined as myocardial infarction (MI), probable MI, unstable angina pectoris and angiographic proven CHD [30]. According to the World Health Organization classification, menopause was defined as the absence of spontaneous menstrual bleeding for more than 12 months, for which no other pathologic or physiologic cause could be determined [31].

2.3. Statistical methods

Log-transformed of the variables with non-normal distribution (serum NOx and TG) were used in the analyses. The mean (±SD) values and the proportions of baseline characteristics of the participants with and without the HTW phenotype, in each sex, were compared using the independent sample t test or Chi square test, respectively.

Dietary intakes of the study participants were compared across serum NOx tertiles using analysis of covariance with adjustment for total energy intake. Serum TG levels and WC at baseline and follow-up examinations were compared across serum NOx tertiles using analysis of variance and Bonferroni pairwise comparison test.

A univariate analysis was performed for each potential confounder including age, using of medications, smoking, menopause status, systolic and diastolic blood pressures, and body mass index; variables with $P_E < 0.2$ in the univariate analysis were selected for the multivariable models; P_E (P value for entry) determines which variables should be included in the final multivariable model. To determine the incidence of HTW^+ across tertiles