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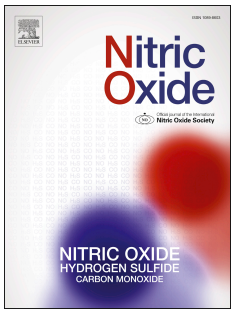

Serum nitric oxide metabolites are associated with the risk of hypertriglyceridemic-waist phenotype in women: Tehran Lipid and Glucose Study

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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.

Key words: Nitric oxide, nitric oxide metabolites, population, hypertriglyceridemic-waist phenotype

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 [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 was 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 206-2008; 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 of serum NOx, logistic regression models with adjustment for potential confounding variables were used. To assess the overall trends of odds ratios across tertiles of serum NOx, the median of each tertile was used as a continuous variable in logistic regression models.

All statistical analysis were conducted using SPSS (Version 16.0; Chicago, IL), and *P* values < 0.05 were considered significant.

3- 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) after a median

follow-up of 6.3. Baseline characteristics of study population are presented in Table 1. Women identified as HTW^+ were more likely to be older; both HTW^+ men and women had higher BMI, WC, and TG levels at baseline. Mean serum NOx was significantly higher in women with HTW^+ (28.7 *vs.* 24.5 μmol/L, *P*= 0.001).

Dietary intakes of the study participants across serum NOx tertile are shown in Table 2. There was no significant difference in dietary intakes, including energy intakes, macronutrients, dietary fiber, total vegetables, high- and medium-nitrate vegetables as well as grains and processed meats across serum NOx tertiles.

Serum TG levels and WC at baseline and follow-up examination across serum NOx tertiles are shown in Table 3. A higher serum TG levels was observed in the highest compared to the lowest tertile of serum NOx in both sexes at baseline; a similar significant difference was also observed in follow-up examination only in women. Women in the third tertiles of serum NOx had also higher WC both at baseline and follow-up examinations.

Table 4 shows the occurrence of the HTW phenotype across tertiles of serum NOx. There was no significant association between serum NOx and the occurrence of HTW^+ in men. The risk of HTW in women, in the highest compared to the lowest tertile of serum NOx, increased by 46% (OR= 1.46, 95% CI= 1.07-2.01, *P* for trend=0.016), in age-adjusted model. A similar association was also observed after additional adjustment for using medications, and systolic and diastolic blood pressures. In the fully adjusted model, after entering BMI, the association remained significant (OR= 1.39, 95% CI= 1.05-1.93, *P* for trend= 0.053).

4- Discussion

In this population-based prospective study of adult men and women, followed for a median of 6.3 years, we demonstrated that serum NOx was an independent predictor of the HTW phenotype only in women; among men, after controlling potential confounders, serum NOx had no predictive effect on the incidence of HTW^+. Increased serum NOx ≥ 30.9 μmol/L in women was accompanied with a 39% increased risk of HTW^+; moreover, a significant increasing trend for HTW^+ was observed across increasing serum NOx levels in women.

Hypertriglyceridemic-waist phenotype, defined as an elevated WC along with elevated TG levels, is a simple and accurate marker of central adiposity and is associated with a higher deposition of visceral fat [26-28; 32]. It is also relevant to mention that HTW phenotype, beyond a visceral fat accumulation index, has also been introduced as a simple and accurate predictor of cardiovascular events and metabolic disorders [33; 34].

In our study, serum NOx levels of the participants could be considered independent of the dietary intakes; in this study, there were no significant differences in dietary intakes of total vegetables, high- and medium-nitrate vegetables as well as grains and processed meats as main dietary sources of nitrate and nitrite, across tertiles of serum NOx levels. Moreover, to exclude short-term effects of dietary intakes of nitrate and nitrite on serum NOx, overnight fasting blood samples were used; this approach is considered in epidemiological studies to prevent the confounding effect of diet on serum NOx measurements [35; 36]. It has been shown that in fasted subjects ~ 90% of circulating nitrite derived from the L-arginine-NO pathway [37].

An overview of the previous cross-sectional studies indicates controversial findings as well as a gender difference regarding the association of NO metabolites with obesity; both increased and decreased serum NOx have been reported in overweight and obese subjects and the associations mainly were significant in women. There is insufficient data clarifying gender differences of

NOx and obesity-related parameters however it has been proposed that NO metabolism in women is more sensitive in response to adipocyte inflammatory cytokines [21].

Similar to our findings, Choi et al studying 363 adolescents, reported a positive correlation between NOx concentration and BMI as well as body fat; in this study, serum NOx concentrations were 4.1 and 4.2 fold higher in overweight males and females, compared to underweight subjects [14]. Fujita et al. in a cross-sectional study demonstrated that serum NOx had a great degree of correlation with visceral fat areas and introduced NOx as a simple and reliable method for the evaluation of visceral fat accumulation; moreover it has been clearly shown that obesity associated hormones such as insulin, leptin, and angiotensin II regulate NO production in human visceral adipocytes [19]. A previous cross-sectional study of our population [20] also showed a significant positive correlation between serum NOx and BMI only in women; higher levels of NOx were observed in women who had abdominal obesity and high waist to hip ratios.

Nitric oxide overproduction, as a compensatory response against obesity-related disorders [20], induction of iNOS in response to increased pro-inflammatory cytokines, and insulin concentrations [38], decreased eNOS activity and NO bioavailability in obese subjects [16] are putative underlying mechanisms that have been discussed to justify cross-sectional associations of NOx concentration and obesity. Although current literature is rather confusing, it can be speculated that overproduction of NO in obesity is mainly due to increased iNOS activity [39; 40].

Emerging evidence shows that NO has a central role in adipocyte physiology, regulation of energy metabolism and body composition [17; 39]; physiological levels of NO affect adipose tissue metabolism through activation of the peroxisome proliferator-activated receptor γ,

uncoupling protein-1, stimulation of mitochondrial biogenesis [3], as well as regulation of adipogenesis, lypolysis and insulin-stimulated glucose uptake [41; 42]. Overall, it seems that higher levels of NO have lipogenic properties due to stimulation of lipogenesis, reduction of catecholamine-stimulated lypolysis, reduction of energy expenditure in white adipose tissue, and also promotion of preadipocytes differentiation to mature adipocytes [17; 39]. Recent studies have also suggested that NO has hyperphagic effects and increases food intake; this could be due to the counterbalancing effect of the leptin and serotonergic system [17; 43; 44]. Our study had not potential to indicate underlying mechanisms regarding the association of serum NOx and HTW phenotype, however we speculated that higher incidence of HTW phenotype, observed among women with higher levels of serum NOx could be attributed to these adipogenic effects of NO metabolites.

Although HTW phenotype is an accurate index of abdominal adiposity, use of this index rather than a gold standard method for measurement of visceral fat accumulation may be considered as a limitation of this study. Dual-energy X-ray absorptiometry (DEXA) scan is an accurate and a gold standard method of body fat measurement, including both abdominal and subcutaneous fats, however this method mainly has clinical applications especially in pediatrics; due to some limitations, this method is less used in population-based epidemiological studies; practical considerations such as cost and feasibility must influence the choice of measure in many studies of large populations and use of simple measurements and indices such as waist circumference, skinfold thickness, and body mass index for measure of obesity is more practical in epidemiological studies [45; 46].

5- Conclusion

To our knowledge this is the first attempt to clarify the potential ability of NO to predict central adiposity, using a prospective population-based approach. In conclusion, our findings imply that increased serum NOx, most probably due to endogenous overproduction of NO, may be considered as an independent factor contributing to the development of visceral fat accumulation in women. With respect to the potential of the HTW phenotype in prediction of cardiometabolic disorders, it may be concluded that higher serum NOx is accompanied with future risk of cardiovascular disease and type 2 diabetes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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Table 1. Baseline characteristics of the participants

	Men (n=762)			*Women (n=1172)*		
	HTW $^{-}$	HTW $^{+}$	*P* value	HTW $^{-}$	HTW $^{+}$	*P* value
Age at baseline *(y)*	43.7±16.5	41.0±14.2	0.066	36.7±12.9	46.3±12.9	0.001
Smoking *(%)*	21.4	31.8	0.051	none	none	
Serum NOx[¶] *(μmol/L)*	26.3 (26.0-27.4)	26.0 (25.3-28.7)	0.52	24.5 (23.6-25.5)	28.7 (27.9-31.2)	0.001
Body mass index *(kg/m^2)*	25.4±4.2	27.3±3.8	0.001	25.6±4.5	27.6±4.1	0.001
Systolic blood pressure *(mm Hg)*	117±16.9	116±14.8	0.59	105±13.5	111±18.4	0.001
Diastolic blood pressure *(mm Hg)*	73.1±10.0	74.5±10.5	0.15	68.0±9.4	71.7±10.2	0.001
Waist circumference *(cm)*	90.9±10.5	95.8±9.9	0.001	81.0±12.0	87.0±9.8	0.001
Serum triglycerides [¶] *(mg/dL)*	107 (102-110)	192 (181-206)	0.001	84.7 (82.2-87.3)	150 (144-156)	0.001

Data are mean ± SD

[¶] Data are geometric mean (95% confidence interval)

Table 2. Dietary intakes of the participants across tertiles of serum NOx

	Serum NOx							
	Men (n=762)				*Women (n=1172)*			
	Tertile1	*Tertile2*	*Tertile3*	*P*	*Tertile1*	*Tertile2*	*Tertile3*	*P*
Serum NOx *(μmol/L)*								
Range	<20.9	20.9-29.9	≥29.9		<19.9	19.9-30.9	≥30.9	
Median	16.9	24.9	40.9		15.9	25.0	42.9	
Energy intake *(kcal/d)*	2447±67	2504±64	2486±66	0.81	2491±57	3535±53	2423±55	0.34
Carbohydrate *(% energy)*	57.0±0.5	57.2±0.5	57.4±0.5	0.88	57.4±0.4	57.5±0.4	56.7±0.4	0.28
Protein *(% energy)*	13.2±0.1	13.6±0.1	13.8±0.1	0.23	13.5±0.1	13.4±0.1	13.6±0.1	0.46
Total fats *(% energy)*	31.8±0.5	31.9±0.5	31.7±0.5	0.94	31.6±0.4	31.6±0.4	32.3±0.4	0.37
Total fiber *(g/d)*	41.5±1.2	42.0±1.2	41.0±1.2	0.40	39.8±1.1	40.9±1.1	39.9±1.1	0.71
Total vegetables *(g/d)*	333±13.0	312±12.5	291±12.9	0.07	309±10.0	302±9.5	300±10.9	0.81
High-nitrate vegetables *(g/d)*	48.0±3.4	41.0±3.2	41.8±3.4	0.28	41.8±2.5	41.1±2.4	42.1±2.5	0.96
Medium-nitrate vegetables *(g/d)*	34.7±2.5	37.4±2.4	32.2±2.4	0.31	35.9±1.9	36.1±1.8	35.4±1.9	0.96
Grains *(g/d)*	22.8±1.7	22.3±1.6	22.3±1.7	0.79	20.0±1.4	23.6±1.3	24.0±1.4	0.09
Processed meats	8.2±1.1	9.8±1.1	8.4±1.1	0.60	9.2±0.9	9.3±0.8	8.8±0.8	0.89

Data are mean ± SE

Analysis of covariance with adjustment for energy intakes was used.

Table 3. Serum triglyceride levels and waist circumference at baseline and follow-up examinations across tertiles of serum NOx

	Serum NOx					
	Men (n=762)			*Women (n=1172)*		
	Tertile1	*Tertile2*	*Tertile3*	*Tertile1*	*Tertile2*	*Tertile3*
Serum NOx *(μmol/L)*						
Range	<20.9	20.9-29.9	≥29.9	<19.9	19.9-30.9	≥30.9
Median	16.9	24.9	40.9	15.9	25.0	42.9
Serum triglycerides *(mg/dL)*						
At baseline	121 (114-129)	131 (124-140)	135 (126-145)[a]	101 (95-106)	105 (100-111)	114 (109-120)[a,b]
After 3 years	126 (118-134)	135 (128-145)	138 (129-148)	105 (96-108)	112 (108-119)[a]	124 (117-130)[a,b]
After 6 years	134 (126-143)	137 (129-145)	144 (134-154)	112 (104-115)	115 (111-123)	127 (120-133)[a,b]
Waist circumference *(cm)*						
At baseline	92.2±10.8	93.7±10.6	93.3±10.1	82.5±12.3	85.5±13.5	87.7±13.3[a,b]
After 3 years	94.7±10.2	96.1±10.7	95.5±10.2	89.1±11.9	91.6±12.3	93.9±13.3[a,b]
After 6 years	95.3±10.5	96.5±10.6	95.6±10.0	89.6±11.5	91.3±12.5	93.3±13.2[a,b]

Data are mean ± SD (geometric mean , 95% confidence interval)
Analysis of variance was used.
[a] Significantly different (P<0.05) from tertile 1
[b] Significantly different (P<0.05) from tertile 2

Table 4. The occurrence of HTW phenotype across tertiles of serum NOx

	Serum NOx							
	Men (n=762)				*Women (n=1172)*			
	Tertile1	*Tertile2*	*Tertile3*	*P for trend*	*Tertile1*	*Tertile2*	*Tertile3*	*P for trend*
Serum NOx *(μmol/L)*								
Range	<20.9	20.9-29.9	≥29.9		<19.9	19.9-30.9	≥30.9	
Median	16.9	24.9	40.9		15.9	25.0	42.9	
HTW Phenotype								
Model 1	Ref.	1.46 [¶] (1.00-2.13)	1.23 (0.83-1.23)	0.36	1	1.17 (0.86-1.60)	1.46 (1.07-2.01)	0.016
Model 2	Ref.	1.42 (0.97-2.09)	1.19 (0.81-1.77)	0.44	1	1.18 (0.86-1.62)	1.47 (1.07-2.03)	0.017
Model 3	Ref.	1.41 (0.95-2.07)	1.16 (0.78-1.72)	0.55	1	1.19 (0.86-1.64)	1.39 (1.05-1.93)	0.053

[¶] Data are odds ratio (95% confidence interval).

Model 1: age-adjusted; Model 2: additional adjustment for smoking (only for men), drugs (only for women) and systolic and diastolic blood pressures (only for women); Model 3: additional adjustment for body mass index (categorical; <25, 25-29.9, ≥30).