Original Article

Elevated Nitric Oxide Metabolites are Associated with Obesity in Women

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Abstract

Background: Nitric oxide (NO) plays a role in almost every biologic system including regulation of energy balance and food intake. This study aimed at determining association between serum nitric oxide metabolite (NO_x) levels and obesity in a population-based study.

Methods: In a cross-sectional study, NO_x levels were measured in 3505 adult participants. Pregnant women and those with diabetes, renal dysfunction, chronic diarrhea, and hospitalization within the past three months, subjects using antihypertensive medications or aspirin, and those with missing data were excluded. Finally, 2445 subjects (1004 men and 1441 women) were included.

Results: Women with body mass index (BMI) > 30 kg/m^2 compared to those with BMI < 25 kg/m^2, had significantly higher serum NO_x concentrations after multivariable adjustment (B = 5.24, P = 0.002). In addition, there was a significant trend of increasing serum NO_x concentrations in categories of BMI in women. Women with a waist circumference (WC) ≥ 90 cm had significantly higher serum NO_x concentration even after multivariate adjustment. Women with waist-to-hip ratio (WHR) ≥ 0.90 had significantly higher serum NO_x concentration; however, the association was not significant after multivariable adjustment. None of the parameters were significantly associated with NO_x in men.

Conclusion: A positive association between BMI and WC and serum NO_x concentration was found in women which might be a reflection of increased NO production.

Keywords: Nitric oxide metabolites, obesity, population

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Introduction

Nitric oxide (NO), a colorless inorganic free radical gas, is produced in all tissues[1,2] and plays a role in almost every biologic system.[3] NO is produced from oxidation of N atoms of *L*-arginine[4] by three NO synthase (NOS, EC 1.14.13.39) isozymes including endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) enzymes.[5] The short half-life and low concentration of NO in vivo make it difficult to measure directly; therefore, its stable end products (nitrate + nitrite = NO_x) are measured.[6] NO_x measurement is the most suitable method for assessment of NO synthesis in vivo[4] and a high correlation between endogenous NO production and serum NO_x levels has been reported.[6] Although beneficial effects of NO in physiologic ranges have been established,[7] high NO_x levels are positively associated with risk for adverse health outcomes[8] and increased serum NO_x levels have been reported in a group of diseases including cardiovascular diseases (CVD),[1] hyperlipidemia,[1] diabetes,[1,9] and metabolic syndrome.[9] Excessive NO production by iNOS is a mediator of nonspecific tissue damage and may be involved in the pathogenesis of metabolic disorders, including obesity-linked type 2 diabetes.[10]

NO plays an important role in regulation of energy balance and food intake in mice.[11] Both eNOS and iNOS are present in human adipose tissue, which may be a source of NO production.[12,13] NO has an inhibitory effect on both basal and catecholamine-stimulated lipolysis and plays a physiologic role in the regulation of lipolysis in human.[14] iNOS is markedly induced in the fat of obese rats and iNOS-mediated NO production may cause obesity-linked insulin resistance.[15]

Based on our knowledge, there is no documented study on the relation between serum NO_x and obesity indices in an epidemiologic setting; therefore, this study aimed at determining the association between serum NO_x levels and obesity in a population-based study.

Subjects and Methods

Study subjects

The Tehran Lipid and Glucose Study (TLGS) is a prospective study being conducted with the aim of determining the prevalence of noncommunicable disease risk factors.[16] In the TLGS, 10368 people, aged 20 years and over, living in district 13 of Tehran, were selected by a multistage cluster random-sampling method. In the third phase of TLGS, 2006 through 2007, NO_x measurements were done for 3505 participants, aged over 20 years. Excluded were pregnant women, participants with renal dysfunction (creatinine > 123.8 μmol/L), chronic diarrhea, and hospitalization during the past three months, as were subjects who had diabetes, those using antihypertensive medication (including nitrites, diuretics, calcium channel blockers, angiotensin- converting enzyme inhibitors, and beta- blockers), or aspirin and any subject with missing data. After application of exclusion criteria, 2445 subjects (1004 men and 1441 women) were enrolled in the study.

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Table 1. Characteristics of the study participants[a]

Parameters	Men (n = 1004)	Women (n = 1441)	P-value[b]
Age (years)	42.1 ± 15.4	39.8 ± 13.5	< 0.001
Body mass index (kg/m^2)	26.4 ± 4.2	27.1 ± 4.9	< 0.001
Waist circumference (cm)	93.9 ± 10.9	85.5 ± 13.0	< 0.001
Waist-to-hip ratio	0.95 ± 0.06	0.83 ± 0.08	< 0.001
Systolic blood pressure (mm Hg)	117 ± 15	108 ± 16	< 0.001
Diastolic blood pressure (mm Hg)	73.6 ± 9.7	70.0 ± 10.1	< 0.001
Fasting serum glucose (mmol/L)	4.99 ± 0.48	4.86 ± 0.47	0.001
Total cholesterol (mmol/L)	4.86 ± 1.03	4.84 ± 0.98	0.715
Triglycerides (mmol/L)	1.59 (1.54–1.65)	1.24 (1.22–1.28)	< 0.001
HDL-C (mmol/L)	0.99 ± 0.22	1.18 ± 0.26	< 0.001
LDL-C (mmol/L)	3.00 ± 0.88	3.00 ± 0.83	0.904
Creatinine (μmol/L)	103 ± 11	86 ± 11	< 0.001
Current smoker (%)	19.5	1.5	< 0.001
History of CVD (%)	2.0	0.5	< 0.001

[a]: Data are mean ± SD and percent for continuous and categorical variables respectively except for triglycerides for which geometric mean (95 % confidence interval) is presented; [b]: By independent t-test and chi-square test for continuous and categorical variables respectively; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CVD: cardiovascular disease.

Table 2. Association between serum NO_x concentration (as dependent variable) and BMI, WC, and, WHR (as independent variables)

Parameters	Men			Women		
	B	SE	P-value	B	SE	P-value
Unadjusted						
BMI: 25–30	-0.05	1.35	0.973	3.23	1.28	0.012
BMI > 30	-0.20	1.82	0.914	7.31	1.46	< 0.001
WC ≥ 90	-1.04	1.31	0.428	5.19	1.14	< 0.001
WC ≥ 95	-1.28	1.22	0.297	4.81	1.28	< 0.001
WHR ≥ 0.95 [men]; ≥ 0.90 [women]	0.423	1.23	0.731	4.59	1.32	< 0.001
Multivariable adjusted*						
BMI: 25–30	-0.47	1.36	0.728	2.14	1.38	0.122
BMI > 30	-0.90	1.86	0.627	5.4	1.67	0.001
WC ≥ 90	-1.61	1.36	0.235	2.82	1.36	0.038
WC ≥ 95	-1.68	1.24	0.176	1.69	1.47	0.250
WHR ≥ 0.95 [men] and ≥ 0.90 [women]	-0.26	1.33	0.844	0.735	1.54	0.406

*Adjustments were done for age, serum total cholesterol, serum triglycerides, systolic blood pressure, fasting serum glucose, and menopausal status in women; and for age, fasting serum glucose, smoking, history of cardiovascular disease, and using medications for thyroid disorders and dyslipidemia in men; BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; B: regression coefficient; SE: standard error.

The Ethics Committee of Shahid Beheshti University of Medical Sciences approved the proposal of this study, and a written informed consent was obtained from all subjects.

Anthropometric, clinical, and laboratory assessments

Details of data collection in the TLGS have been published previously;[17] in brief, weight and height were measured according to standard protocols. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2). Two measurements of systolic and diastolic blood pressures were performed after a 15- minute rest in a sitting position; the mean of the two measurements was considered the subject's blood pressure.

To avoid the influence of drugs, smoking, and nitro-compounds from food, blood samples were taken after 12 – 14 hours overnight fasting, and centrifuged within 30 – 45 minutes of collection; all blood analyses were done at the TLGS research laboratory on the day of blood collection. Serum NO_x concentration was measured by the Griess reaction,[6] which has been validated in our laboratory.[18] In brief, serum samples were deproteinized by zinc sulfate (15 mg/mL), and centrifuged at 10000 g for 10 minutes; a 100 μL of the supernatant was transferred to a microplate well, and 100 μL vanadium (III) chloride (Aldrich®) (8 mg/mL) was added to each well to reduce nitrate to nitrite, as the Griess reaction detects only nitrite. Griess reagents [50 μL sulfanilamide (2 %) and 50 μL N-(1-Naphthyl) ethylendiamine dihydrochloride (Sigma®) (0.1 %)] were then added and samples were incubated for 30 minutes at 37 ºC; absorbance was read at 540 nm using the enzyme-linked immunosorbent assay (ELISA) reader (Sunrise, Tecan, Austria). Serum NO_x concentration was determined from the linear standard curve established by 0 – 100 μM sodium nitrate. Inter- and intra-assay coefficients of variation (CV) were 5.2 % and 4.4 %, respectively. The sensitivity of the assay was 2.0 μmol/L and its recovery was 93 ± 1.5 %.

Serum glucose was measured using the enzymatic colorimetric method with glucose oxidase. For the oral glucose tolerance test, 75 grams of glucose solution was administered orally to subjects and a blood sample was taken two hours later. Total cholesterol (TC) was assayed using the enzymatic colorimetric assay with cholesterol esterase and cholesterol oxidase. Serum triglycerides (TG) were assayed, using enzymatic colorimetric method with glycerol phosphate oxidase. Measurement of high-density lipoprotein cholesterol (HDL-C) was done after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid. The analyses were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands); low-density lipoprotein cho-

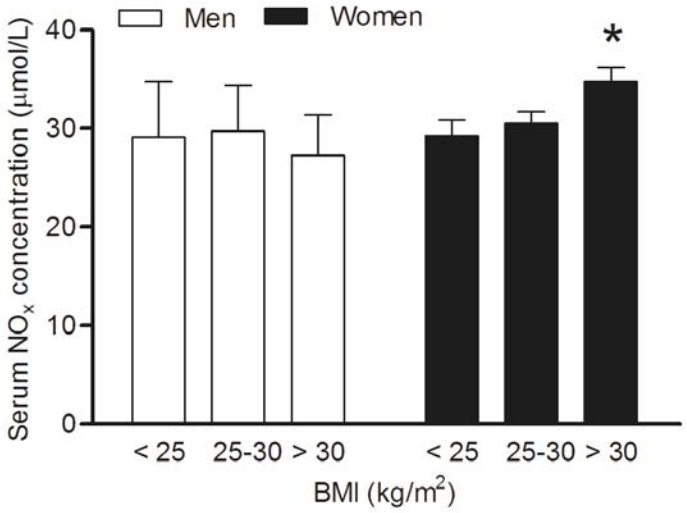

Figure 1. Multivariable- adjusted mean values of serum NO_x concentrations according to BMI groups and sex. *: P for trend was 0.001 in women.

lesterol (LDL-C) concentrations in samples were calculated with the Friedewald equation: LDL-C = TC-HDL-C–TG/5.[19] Intra-and interassay CVs were both 2.2 % for glucose. For both TC and HDL-C, intra- and interassay CVs were 0.5 % and 2.0 %, respectively. Intra- and interassay CVs for TG were 0.6 % and 1.6 %, respectively.

Definition of terms

We used the American Diabetes Association definition of diabetes, i.e., fasting serum glucose ≥ 7.0 mmol/L or two-hour serum glucose ≥ 11.1 mmol/L and/or pharmacologic treatment.[20] Cut-off points of waist circumference (WC) were selected according to the first report of the Iranian National Committee of Obesity, which proposed WC ≥ 90 cm as at risk and WC ≥ 95 cm as high risk for CVD events.[21] Cut-off points for waist-to-hip ratio (WHR) were selected according to Hadaegh, et al. to be ≥ 0.95 in men and ≥ 0.90 in women for predicting cardiovascular outcomes.[22] Family history of CVD reflected prior diagnosis of CVD in first-degree female relatives, under 65 years of age or first-degree male relatives, aged less than 55 years.[23] Smoking was defined as using ≥ one cigarette per day or using waterpipe.

Statistical analysis

Data were analyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 17). Independent sample *t*-test and chi-square test were used for comparing continuous and categorical variables, respectively between men and women. Multiple regression analysis was used to determine association between serum NO_x concentrations and BMI; adjustments were done for those variables that had a p_E less than 0.2 in univariate analyses (age, fasting serum glucose, history of CVD, smoking, and using thyroid or antithyroid drugs in men; age, TC, TG, systolic blood pressure, fasting serum glucose, and menopausal status in women); P_E or P- value for entry determines how many variables are eventually included in the model.[24] Two-sided P- values less than 0.05 were considered statistically significant.

Results

In this cross-sectional study, 2445 subjects (1004 men and 1441 women), aged 20 – 87 years, were investigated. Characteristics of the study participant according to gender are shown in Table 1. Men were older and, compared to women, had higher values for systolic and diastolic blood pressures, TG, WC, WHR, and creatinine and lower values of BMI and HDL-C.

In women, there was a significant positive correlation between BMI and serum NO_x concentration ($r = 0.153$, $P < 0.001$, $n = 1441$) and correlation was higher for BMI ≥ 40 kg/m² ($r = 0.441$, $P = 0.04$, $n = 22$). No significant correlation between BMI and serum NO_x concentration was found in men ($r = 0.013$, $P = 0.676$, $n = 1004$).

Associations between serum NO_x concentrations and obesity indices (BMI, WC, and WHR) according to regression analysis are shown in Table 2. None of the parameters were significantly associated with NO_x in men. After multivariable adjustment, women with BMI > 30 kg/m² compared to those with BMI < 25 kg/m², had significantly higher serum NO_x concentrations ($B = 5.24$, $P = 0.002$). In addition, there was a significant trend of increasing serum NO_x concentrations in categories of BMI in women (Figure 1). Women with WC ≥ 90 cm had significantly higher serum NO_x concentrations, a significance that remained after multivariate adjustment; women with WHR ≥ 0.90 however also had significantly higher serum NO_x concentration but the association did not remain significant after multivariable adjustment.

Discussion

The results of this study showed a positive association between BMI and WC and serum NO_x concentrations in women selected from a population-based study. Increased serum NO_x levels in overweight and obese women might be a reflection of increased NO production.

In this study, NO_x was associated with BMI and WC in women, but not in men; in line with our results, Olszanecka-Glinianowicz, et al. studying 154 women, reported higher serum NO concentrations in overweight and obese women, compared to controls;[12] again, similar to our findings, they also found significant positive correlations between serum NO and BMI. Fujita, et al. reported higher serum NO_x concentrations in obese subjects due to NO production by visceral fat.[25] In agreement with our results,

Kondo, et al. reported no significant correlation between abdominal obesity and NO_x concentration in men[26] and Higashi, et al. have found no difference between plasma NO_x concentrations in normal weight and obese men, although their results were limited by a low number of subjects.[27] Even in healthy subjects, there is controversy over the difference between serum NO_x concentrations in men and women.[28] Although from the results of our study we could not explain the reason underlying the difference in NO_x levels between obese men and women, but it has been speculated that "men might not be as susceptible as women in terms of endothelial responsiveness to the circulating adipocytokines produced by visceral fat tissue".[26]

Increased NO metabolites in overweight and obese adolescents, aged 14 – 19 years, have previously been reported and it has been proposed that obesity leads to increase in NO production in humans.[29] We also previously reported that the upper limit of reference values for serum NO_x concentrations were higher in otherwise healthy overweight and obese girls, aged four to 19 years.[30] However, contradictory to our results, Gruber, et al. reported a decrease in serum NO_x in obese juveniles, due to increased oxidative stress.[31] Recently, Higashino, et al. reported increased serum NO_x levels in a group of diseases and suggested that it may be due to increase in NO production through cytokine-induced iNOS induction;[1] a similar mechanism may occur in overweight and obesity, findings in support of which, Lin, et al. have reported marked decrease in serum NO_x concentration in obese subjects after weight reduction surgery.[32] In addition, it has been shown that obese subjects have a capacity for increased NO production.[33]

Nitrite and nitrate have previously been considered as inert oxidation products of NO and as surrogates for endothelial function; however, recently it has been shown that nitrite/nitrate are physiologically relevant storage reservoirs of NO.[34] It has been shown that NO synthesized within a specified location could be transported in blood as nitrite to distant organs and provide protection against ischemic injury;[35] therefore, it could be speculated that increased levels of NO_x in overweight and obese subjects may be a compensatory mechanism against obesity-related changes.

As limitations of the study, we did not record the diet of the participants, which is a source for NO_x in body fluids in addition to endogenous NO synthesis;[36] however, we measured samples after 10 – 12 hours fasting, when plasma NO_x is mainly reflective of endogenous NO production.[36,37] In addition, due to the cross-sectional design of our study we could not draw a causal relationship.

In conclusion, the results of this study showed a positive association between BMI and WC and serum NO_x concentrations in women. Increased serum NO_x levels in overweight and obese women might be a reflection of increased NO production.

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References

1. Higashino H, Tabuchi M, Yamagata S, Kurita T, Miya H, Mukai H, et al. Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects. *Journal of Medical Sciences.* 2010; **10:** 1 – 11.
2. Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J.* 1994; **298 (Pt 2):** 249 – 258.
3. Yoon S, Moon J, Shin C, Kim E, Jo SA, Jo I. Smoking status-dependent association of the 27-bp repeat polymorphism in intron 4 of endothelial nitric oxide synthase gene with plasma nitric oxide concentrations. *Clinica Chimica Acta.* 2002; **324:** 113 – 120.
4. Tsikas D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007; **851:** 51 – 70.
5. Tsikas D. Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. *Free Radic Res.* 2005; **39:** 797 – 815.
6. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide.* 2001; **5:** 62 – 71.
7. Colasanti M, Suzuki H. The dual personality of NO. *Trends Pharmacol Sci.* 2000; **21:** 249 – 252.
8. Ueyama J, Kondo T, Imai R, Kimata A, Yamamoto K, Suzuli K, et al. Association of serum NO_x level with clustering of metabolic syndrome components in middle-aged and elderly general populations in Japan. *Environ Health Prev Med.* 2008; **13:** 36 – 42.
9. Zahedi Asl S, Ghasemi A, Azizi F. Serum nitric oxide metabolites in subjects with metabolic syndrome. *Clin Biochem.* 2008; **41:** 1342 – 1347.
10. Perreault M, Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat Med.* 2001; **7:** 1138 – 1143.
11. Joost HG, Tschop MH. NO to obesity: does nitric oxide regulate fat oxidation and insulin sensitivity? *Endocrinology.* 2007; **148:** 4545 – 4547.
12. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A. Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-alpha, and TNF soluble receptors in women with overweight and obesity. *Metabolism.* 2004; **53:** 1268 – 1273.
13. Choi JW. Enhanced nitric oxide production is closely associated with serum lipid concentrations in adolescents. *Clinica Chimica Acta.* 2004; **347:** 151 – 156.
14. Ryden M, Elizalde M, van Harmelen V, Ohlund A, Hoffstedt J, Bringman S, et al. Increased expression of eNOS protein in omental versus subcutaneous adipose tissue in obese human subjects. *Int J Obes Relat Metab Disord.* 2001; **25:** 811 – 815.
15. Dallaire P, Marette A. Obesity-linked insulin resistance: is nitric oxide the missing link? *Can J Diabetes.* 2004; **28:** 59 – 66.
16. Azizi F, Rahmani M, Emami H, Mirmiran P, Hajipour R, Madjid M, et al. Cardiovascular risk factors in an Iranian urban population: Tehran Lipid and Glucose Study (phase 1). *Soz Praventivmed.* 2002; **47:** 408 – 426.
17. Azizi F, Ghanbarian A, Momenan AA, Hadaegh F, Mirmiran P, Hedayati M, et al. Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. *Trials.* 2009; **10:** 5.
18. Ghasemi A, Hedayati M, Biabani H. Protein precipitation methods evaluated for determination of serum nitric oxide end products by the Griess assay. *JMSR.* 2007; **2:** 43 – 46.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; **18:** 499 – 502.
20. American Diabetes Association. Standards of medical care in diabetes--2011. *Diabetes Care.* 2011; **34(Suppl 1):** S11 – S61.
21. Azizi F, Khalili D, Aghajani H, Esteghamati A, Hosseinpanah F, Delavari A, et al. Appropriate waist circumference cut-off points among Iranian adults: the first report of the Iranian National Committee of Obesity. *Arch Iran Med.* 2010; **13:** 243 – 244.
22. Hadaegh F, Zabetian A, Sarbakhsh P, Khalili D, James WP, Azizi F. Appropriate cutoff values of anthropometric variables to predict cardiovascular outcomes: 7.6 years follow-up in an Iranian population. *Int J Obes (Lond).* 2009; **33:** 1437 – 1445.
23. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 2001; **285:** 2486 – 2497.
24. Hosmer DW LS. *Applied Logistic Regression*. Second ed. New York: John Wiley & Sons; 2000.
25. Fujita K, Wada K, Nozaki Y, Yoneda M, Endo H, Takahashi H, et al. Se-

rum nitric oxide metabolite as a biomarker of visceral fat accumulation: clinical significance of measurement for nitrate/nitrite. *Med Sci Monit.* 2011; **17:** CR123 – 131.
26. Kondo T, Ueyama J, Imai R, Suzuki K, Ito Y. Association of abdominal circumference with serum nitric oxide concentration in healthy population. *Environmental Health and Preventive Medicine.* 2006; **11:** 321 – 325.
27. Higashi Y, Sasaki S, Nakagawa K, Kimura M, Noma K, Hara K, et al. Low body mass index is a risk factor for impaired endothelium-dependent vasodilation in humans: role of nitric oxide and oxidative stress. *J Am Coll Cardiol.* 2003; **42:** 256 – 263.
28. Ghasemi A, Zahedi Asl S, Mehrabi Y, Saadat N, Azizi F. Serum nitric oxide metabolite levels in a general healthy population: relation to sex and age. *Life Sci.* 2008; **83:** 326 – 331.
29. Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. *Clin Chem.* 2001; **47:** 1106 – 1109.
30. Ghasemi A, Zahediasl S, Azizi F. Reference values for serum nitric oxide metabolites in pediatrics. *Nitric Oxide.* 2010; **23:** 264 – 268.
31. Gruber HJ, Mayer C, Mangge H, Fauler G, Grandits N, Wilders-Truschnig M. Obesity reduces the bioavailability of nitric oxide in juveniles. *Int J Obes (Lond).* 2008; **32:** 826 – 831.
32. Lin LY, Lee WJ, Shen HN, Yang WS, Pai NH, Su TC, et al. Nitric oxide production is paradoxically decreased after weight reduction surgery in morbid obesity patients. *Atherosclerosis.* 2007; **190:** 436 – 442.
33. Mather KJ, Lteif A, Steinberg HO, Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. *Diabetes.* 2004; **53:** 2060 – 2066.
34. Calvert JW. Cardioprotective effects of nitrite during exercise. *Cardiovasc Res.* 2011; **89:** 499 – 506.
35. Elrod JW, Calvert JW, Gundewar S, Bryan NS, Lefer DJ. Nitric oxide promotes distant organ protection: evidence for an endocrine role of nitric oxide. *Proc Natl Acad Sci U S A.* 2008; **105:** 11430 – 11435.
36. Baylis C, Vallance P. Measurement of nitrite and nitrate levels in plasma and urine--what does this measure tell us about the activity of the endogenous nitric oxide system? *Curr Opin Nephrol Hypertens.* 1998; **7:** 59 – 62.
37. Grau M, Hendgen-Cotta UB, Brouzos P, Drexhage C, Rassaf T, Lauer T, et al. Recent methodological advances in the analysis of nitrite in the human circulation: nitrite as a biochemical parameter of the L-arginine/NO pathway. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007; **851:** 106 – 123.