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REGULAR ARTICLE

# Association of serum $NO_x$ level with clustering of metabolic syndrome components in middle-aged and elderly general populations in Japan

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**Abstract**
*Objectives* The aim of this study was to determine whether the serum nitrite plus nitrate ($NO_x$) level correlates with biomarkers that are known components of the metabolic syndrome (MetS).
*Methods* Serum $NO_x$ levels were measured using a commercial kit in 608 Japanese men and women between the ages of 39 and 85 years. Multivariate adjustments for age, smoking status, alcohol consumption and exercise were made in the analysis of covariance (ANCOVA). The components of the metabolic syndrome were defined based on the following criteria: body mass index (BMI) $\geq$25.0 kg/m$^2$, glycated hemoglobin (HbA1c) $\geq$5.6%, systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg, high-density lipoprotein-cholesterol (HDL-C) $\leq$1.03 mmol/l for men and $\leq$1.29 mmol/l for women and triglyceride $\geq$1.69 mmol/l.
*Results* The logarithmically transformed age-adjusted serum $NO_x$ ($\ln NO_x$) value was significantly higher in the low HDL-C group ($1.76 \pm 0.05$ μmol/l; $p < 0.05$) than MetS component groups ($1.65 \pm 0.01$ μmol/l) in men, but no difference was found in women. The means of serum $\ln NO_x$ after multivariate adjustment were 1.64, 1.65, 1.64, 1.66, and 1.81 μmol/l for 0, 1, 2, 3, and 4–5 MetS components for all subjects, respectively. The results of ANCOVA confirmed that the serum $\ln NO_x$ level was significantly correlated with the clustering of MetS components in both men and women ($p < 0.0001$ for trend).
*Conclusion* Our results suggest that an increase in the clustering of MetS components was associated with the increase in serum NO levels in our general population.

**Keywords** Analysis of covariance · Cyclic guanosine 3′5′-Monophosphate (cGMP) · Metabolic syndrome · Nitric oxide

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## Introduction

Metabolic syndrome (MetS), which has become increasingly prevalent in the developed countries of the world, including Japan [1, 2], is a common metabolic disorder most often defined as the presence of at least two of the following risk factors: obesity, hypertension, hyperglycemia (insulin resistance), decreased high-density lipoprotein cholesterol (HDL-C) and elevated triglycerides (TG) [National Cholesterol Education Program (NECP) Adult Treatment

Panel III (ATP-III) [3]; World Health Organization (WHO)]. People with MetS are known to be at increased risk of coronary heart disease and type 2 diabetes.

Nitric oxide (NO) is an inorganic free radical gas synthesized by the oxidation of L-arginine in a process catalyzed by nitric oxide synthase (NOS). Nitric oxide synthase is classified into three subtypes – neuronal NOS (nNOS), mainly found in the brain, endothelial NOS (eNOS), found in the vascular endothelium, and inducible NOS (iNOS) mainly expressed in activated macrophages during the inflammatory state [4]. NO is known to be the principal mediator of several functions, including vasodilation, anticoagulation, leukocyte adhesion, smooth muscle proliferation and the antioxidative capacity of endothelial cells [5–8].

In an experimental setting, Cook et al. [9] observed hypertension and insulin resistance in eNOS null mice, while Tsutsui et al. [10] observed a number of disorders, such as hypertension, arteriosclerosis, insulin resistance, visceral obesity and aging, in a recently developed line of genetically engineered mice that lacked all three NOS subtypes. In a clinical–epidemiological setting, Konukoglu et al. [11] suggested that the circulating NO level was lower in obese female subjects with hypertension than in the normal control subjects. Moreover, Kondo et al. [12] found that a reduction in NO bioactivity occurs with abdominal fat accumulation in women.

Based on these studies, we anticipated that the serum nitrite plus nitrate ($NO_x$) level might decline in patients with MetS due to reduced NO production or an increase in the NO consumption level. Although there have been very few epidemiological studies examining the correlation between circulating NO and MetS in general populations, Cui et al. [13] recently reported that the urinary extraction of cyclic guanosine 3′, 5′-monophosphate (cGMP), a second messenger of NO, was inversely correlated with the clustering of a number of MetS risk factors. However, to date, no data are available on the association between the circulating $NO_x$ level and any indicator of circulating NO level, and MetS risk factors in a general population. The purpose of this study was to explore whether the serum $NO_x$ level correlates with MetS risk factors using community-based epidemiological data.

## Materials and methods

### Study subjects

We studied a population of 608 Japanese (209 men and 399 women) aged between 39 and 85 years who were living in a rural area of Hokkaido, Japan in August 2005 who participated in an annual health checkup program, including a serological test and physical examination. The survey included a self-administered questionnaire, anthropometric measurements, including height and weight for the calculation of body mass index [BMI; weight in kg/(height in m)$^2$], and the collection of blood samples. The questionnaire addressed such lifestyle characteristics as physical activity, smoking status and alcohol consumption. Physical activity was divided into two categories ($\geq$1 h a week vs. $<$1 h). Smoking status was represented using the Brinkman Index calculated from data obtained from the questionnaires on the number of cigarettes smoked per day and length of the smoking habit (in years). Individuals who answered that they drink habitually were defined as current drinkers and the others as non-drinkers. All subjects gave their informed consent to answering the questionnaire and provided residual blood for analysis. The Ethics Committee of Nagoya University Graduate School of Medicine, Nagoya, Japan approved the study protocol.

### Biochemical analysis

A portion of each collected blood sample was immediately examined for glycated hemoglobin (HbA1c), total cholesterol (TC), HDL-C and TG; the remainder was stored at 4°C for a maximum length of 5 days until assayed for $NO_x$ concentrations. Since NO is unstable and quickly auto-oxidized to nitrate and nitrite after production, the concentrations of $NO_x$ in serum were measured using a commercial kit (Nitrate/Nitrite Colorimetric Assay kit, Cat No. 780001; Cayman Chemical, Ann Arbor, MI). Briefly, the subjects' serum samples were ultrafiltered (molecular cut-off of 10,000) at 6000 *g* for 60 min at 4°C. The ultrafiltrate was incubated for 3 h with nitrate reductase and its cofactor and then allowed to react with Griess reagents for 20 min. Absorbance was measured at 540 nm with a microplate reader (Molecular Devices, Crawley, UK). The within-day and between-day coefficients of variation for this assay were less than 5% at a concentration of 50 μmol/l [14]. Since serum $NO_x$ concentrations were found to be skewed in distribution, they were normalized with a logarithmic transformation in advance of all the analyses.

### Metabolic syndrome risk factors

Whereas the waistline was used to define obesity in the committee that evaluated the diagnostic standards for MetS [3, 15] have reported that the Asian criterion of obesity is BMI $\geq$ 25.0 kg/m$^2$, or a waist circumference of $\geq$80 cm for women and $\geq$90 cm for men. By referring to this report and the final report on the ATP guidelines of the NCEP, which defines the characteristics of the MetS components,

we adopted the following cutoff limits: (1) a high BMI, i.e., BMI $\geq$ 25.0 kg/m$^2$; (2) high blood pressure, i.e., systolic blood pressure $\geq$130 mmHg and/or a diastolic blood pressure $\geq$85 mmHg; (3) a high HbA1c, i.e, a percentage $\geq$5.6%; (4) a low HDL-C, i.e., a concentration of $\leq$1.03 mmol/l for men or $\leq$1.29 mmol/l for women; (5) a high TG, i.e., a concentration of $\geq$1.69 mmol/l. The subjects were classified based on the accumulated number of MetS risk factors (0, 1, 2, 3 and $\geq$4).

### Statistical analysis

All statistical analyses were conducted using the SPSS statistical package for Windows ver. 11.0 (SPSS, Chicago, IL), and two-sided $p$ values of $<0.05$ were considered to be statistically significant. Age-adjusted means of the basic characteristics of subjects were first examined in a general linear model (GLM) for any differences between men and women. In the second GLM analysis, differences in the least-square means of the logarithmically transformed age-adjusted serum $NO_x$ levels ($lnNO_x$) were examined for each MetS component followed by further adjustments for such covariates as physical activity, smoking status and alcohol drinking habits. In the third GLM analysis, the serum $NO_x$ level was treated as a dependent variable, which was predicted by the main predictor, the accumulated number of MetS components and other covariates. This GLM modeling was initially conducted for all subjects in the main analysis and then for men and women separately in the sub-analysis.

To test the trend in $NO_x$ levels, we used a linear contrast on the assumption that the number of MetS components was equally spaced from none to $\geq$4. A comparison of $NO_x$ levels was also conducted between those with $\leq$3 MetS components (component 0, 1, 2 and 3 groups) and those with $\geq$4 in the GLM analysis.

## Results

The sex-specific characteristics of 608 subjects are shown in Table 1. While there were significant differences between men and women in age, TC, HDL-C and HbA1c, no such differences were observed in serum $lnNO_x$ values or systolic and diastolic blood pressure. Men and women with high BMI accounted for 33.0 and 30.8% of the study population, with high blood pressure, for 64.6 and 62.4%, with high TG, for 22.5 and 14.8%, with low HDL-C, for 7.7 and 2.3% and with high HbA1c, for 17.7 and 9.5%. Age- and multivariate-adjusted $lnNO_x$ values for each MetS component are summarized in Table 2. Significant differences in age-adjusted $lnNO_x$ can be seen between HDL-C $\leq$1.03 mmol/l and HDL-C $>$1.03 mmol/l for men

**Table 1** Age-adjusted, sex-specific mean values of basic characteristics and the presence of metabolic risk factors

| | Men | Women | $p$ for difference[a] |
|---|---|---|---|
| Number | 209 | 399 | |
| Mean ± SEM | | | |
| Age (year) | 63.4 ± 0.7 | 60.5 ± 0.5 | 0.001 |
| Serum $NO_x$ (μmol/l)[b] | 1.65 ± 0.01 | 1.65 ± 0.01 | 0.975 |
| BMI (kg/m$^2$) | 23.8 ± 0.2 | 23.7 ± 0.2 | 0.655 |
| BMI (kg/m$^2$) $\geq$25 | 33.0% | 30.8% | |
| Systolic blood pressure (mmHg) | 133.5 ± 1.3 | 135.3 ± 0.9 | 0.234 |
| Systolic blood pressure (mmHg) $\geq$130 | 60.8% | 59.9% | |
| Diastolic blood pressure (mmHg) | 81.8 ± 0.7 | 81.0 ± 0.5 | 0.381 |
| Diastolic blood pressure (mmHg) $\geq$85 | 40.2% | 36.8% | |
| High blood pressure (%) | 64.6 | 62.4 | |
| Total cholesterol (mmol/l) | 5.27 ± 0.06 | 5.59 ± 0.04 | $<$0.0001 |
| HDL cholesterol (mmol/l) | 1.40 ± 0.02 | 1.58 ± 0.02 | $<$0.0001 |
| HDL cholesterol $\leq$1.03 for men, $\leq$1.29 for women (%) | 7.7 | 2.3 | |
| Triglyceride (mmol/l) | 1.28 ± 0.04 | 1.15 ± 0.03 | 0.009 |
| Triglyceride (mmol/l) $\geq$150 | 22.5% | 14.8% | |
| HbA1C (%) | 5.20 ± 0.04 | 5.03 ± 0.03 | $<$0.0001 |
| HbA1C (%) $\geq$5.6 | 17.7 | 9.5 | |
| People who exercise (yes) (%) | 37.3 | 36.8 | |
| Brinkman Index | 548.1 ± 24.0 | 55.3 ± 17.3 | $<$0.0001 |
| Drinking (%) | 60.8 | 20.6 | |

BMI, Body mass index; SEM, standard error of the mean; HDL, high-density lipoprotein; HbA1C, glycated hemoglobin

[a] General linear model analysis

[b] Logarithmically transformed serum $NO_x$

(1.76 ± 0.05 vs. 1.65 ± 0.02 μmol/l). This difference remained significant after additional adjustments for physical activity, smoking status and alcohol drinking habit. The serum $NO_x$ level showed no significant associations with other MetS components.

The relationship between serum $NO_x$ levels and the accumulated number of MetS components is shown in Table 3. The means of serum $lnNO_x$ after multivariate adjustments were 1.64, 1.65, 1.64, 1.66, and 1.81 μmol/l for 0, 1, 2, 3, and ≥4 MetS components, respectively, for all subjects. Significant associations between the number of MetS components and multivariate-adjusted $lnNO_x$ were shown for all subjects, along with a significant trend toward an increase in $NO_x$ levels according to the accumulation of MetS components (Fig. 1). The sex-specific subanalysis also showed a significant association and linear trend (Table 3; Fig. 1).

## Discussion

Metabolic syndrome has attracted increasing attention due to its growing prevalence among people in developed countries. Our primary concern in this study was to investigate the relationship between the varying levels of circulating NO and MetS, as the latter places individuals at an elevated risk for coronary heart disease and other diseases related to plaque buildups in the vascular wall. There have been some reports on the relationship between circulating or exhaled NO and such MetS-induced diseases as arteriosclerosis, coronary heart disease and type 2 diabetes [16–18]. To date, however, the underlying kinetics of the circulating NO levels in individuals with MetS has not yet been resolved. In this context, Cui et al. [13] examined the relationship between MetS and urinary excretion of cGMP and found that a clustering of MetS risk factors was inversely associated with urinary cGMP excretion.

In our study, we assumed that the production or availability of NO declines in proportion to the prevalence of clustering MetS risk factors in a healthy general population. Unexpectedly, however, our results showed that serum $NO_x$ levels tended to increase with the accumulation of MetS components, and this occurred in both the main analysis and the sex-specific sub-analysis, even though there were no correlations of serum $NO_x$ levels with high BMI, high blood pressure, high TG or high HbA1c. Notably, serum $NO_x$ levels were significantly higher in those individuals having ≥4 MetS components than in the others, whereas there were no significant differences in serum $NO_x$ levels among the component 0, 1, 2 and 3

**Table 2** Age- and multivariable-adjusted $lnNO_x$ (μmol/l) for each metabolic syndrome (MetS) component

| | Men | | | Women | | |
|---|---|---|---|---|---|---|
| | No | Yes | *p* for difference[b] | No | Yes | *p* for difference[a] |
| High BMI | | | | | | |
| Number | 140 | 69 | | 276 | 123 | |
| Age-adjusted NO | 1.65 ± 0.02 | 1.67 ± 0.03 | 0.727 | 1.65 ± 0.01 | 1.66 ± 0.02 | 0.694 |
| Multivariable-adjusted NO[b] | 1.65 ± 0.02 | 1.66 ± 0.03 | 0.753 | 1.65 ± 0.01 | 1.67 ± 0.02 | 0.514 |
| Hypertension | | | | | | |
| Number | 74 | 135 | | 150 | 249 | |
| Age-adjusted NO | 1.65 ± 0.02 | 1.66 ± 0.02 | 0.578 | 1.65 ± 0.02 | 1.65 ± 0.01 | 0.973 |
| Multivariable-adjusted NO[b] | 1.64 ± 0.03 | 1.66 ± 0.02 | 0.627 | 1.66 ± 0.02 | 1.65 ± 0.02 | 0.936 |
| High triglyceride | | | | | | |
| Number | 162 | 47 | | 340 | 59 | |
| Age-adjusted NO | 1.66 ± 0.02 | 1.67 ± 0.03 | 0.714 | 1.65 ± 0.01 | 1.67 ± 0.03 | 0.464 |
| Multivariable-adjusted NO[b] | 1.65 ± 0.02 | 1.67 ± 0.03 | 0.673 | 1.65 ± 0.01 | 1.66 ± 0.03 | 0.777 |
| Low HDL | | | | | | |
| Number | 193 | 16 | | 390 | 9 | |
| Age-adjusted NO | 1.65 ± 0.01 | 1.76 ± 0.05 | 0.043 | 1.65 ± 0.01 | 1.68 ± 0.07 | 0.671 |
| Multivariable-adjusted NO[b] | 1.64 ± 0.02 | 1.77 ± 0.05 | 0.025 | 1.65 ± 0.01 | 1.67 ± 0.07 | 0.762 |
| High HbA1c | | | | | | |
| Number | 172 | 37 | | 361 | 38 | |
| Age-adjusted NO | 1.65 ± 0.02 | 1.7 ± 0.03 | 0.170 | 1.65 ± 0.01 | 1.67 ± 0.03 | 0.465 |
| Multivariable-adjusted NO[b] | 1.65 ± 0.02 | 1.7 ± 0.03 | 0.186 | 1.65 ± 0.01 | 1.67 ± 0.03 | 0.698 |

[a] General linear model analysis

[b] Adjusted for age, Brinkman Index, drinking (yes) and exercise (yes)

**Table 3** Age- and multivariable-adjusted $lnNO_x$ (μmol/l) according to number of metabolic syndrome components

| | Number of metabolic syndrome components | | | | | *p* for difference[a] | *p* for trend[b] |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | ≥4 | | |
| Total | | | | | | | |
| Number | 151 | 230 | 146 | 67 | 14 | | |
| Age-adjusted NO | 1.64 ± 0.02 | 1.653 ± 0.01 | 1.64 ± 0.02 | 1.66 ± 0.03 | 1.81 ± 0.05 | 0.064 | <0.0001 |
| Multivariable-adjusted NO[c] | 1.65 ± 0.02 | 1.656 ± 0.01 | 1.65 ± 0.02 | 1.66 ± 0.03 | 1.81 ± 0.05 | 0.081 | <0.0001 |
| Men | | | | | | | |
| Number | 46 | 70 | 55 | 29 | 9 | | |
| Age-adjusted NO | 1.62 ± 0.03 | 1.67 ± 0.01 | 1.65 ± 0.03 | 1.64 ± 0.04 | 1.82 ± 0.07 | 0.099 | <0.0001 |
| Multivariable-adjusted NO[c] | 1.62 ± 0.03 | 1.67 ± 0.01 | 1.65 ± 0.03 | 1.64 ± 0.04 | 1.82 ± 0.07 | 0.109 | <0.0001 |
| Women | | | | | | | |
| Number | 105 | 160 | 91 | 38 | 5 | | |
| Age-adjusted NO | 1.65 ± 0.02 | 1.65 ± 0.02 | 1.64 ± 0.02 | 1.67 ± 0.03 | 1.78 ± 0.09 | 0.557 | <0.0001 |
| Multivariable-adjusted NO[c] | 1.66 ± 0.02 | 1.65 ± 0.02 | 1.65 ± 0.02 | 1.67 ± 0.03 | 1.77 ± 0.09 | 0.681 | <0.0001 |

$lnNO_x$, Logarithmically transformed age-adjusted serum $NO_x$levels

[a] General linear model analysis

[b] Test for trend was performed using a linear contrast assuming equal spacing from 0 to ≥4

[c] Adjusted for age, Brinkman index, drinking (yes) and exercise (yes)

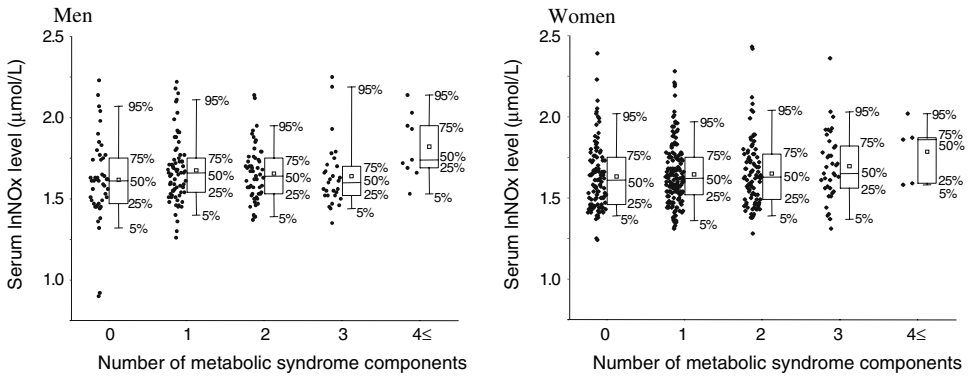


**Fig. 1** Individual values and box plots of serum logarithmically transformed age-adjusted serum $NO_x$levels ($lnNO_x$; μmol/l) and metabolic syndrome components. *Open squares* in box plot show averages

groups (data not shown). This result explicitly indicates that a surge in $NO_x$ levels observed in the ≥4 MetS components group contributed largely to the significant overall linear trend toward an increase in $NO_x$ levels associated with the clustering of MetS components.

Although a clear explanation for why our results contradicted our initial expectation remains to be explored, evidence supporting this relationship has been reported earlier. Rydén et al. [19] found that eNOS expression in the omental tissue of obese patients was significantly elevated. Maejima et al. [17] demonstrated that hypertensive diabetic subjects had a significantly higher serum $NO_3^-$ level than normotensive diabetic ones and ascribed those higher levels to advanced diabetic microvascular complications, indicating a compensatory mechanism for reducing the vascular response to various intrinsic vasoactive agents. In line with these previous findings, our results raise the possibility of an up-regulation in which the circulating NO levels are affected by a positive feedback regulation of NO-related homeostasis in those at a high risk for MetS. More recently, Osawa et al. [20] reported that the survival rate declined in proportion to $NO_x$ levels in 127 subjects aged 65–101 years. This finding can be interpreted as providing additional evidence for the positive association of high $NO_x$ levels with a high risk for adverse health outcomes, although no causal explanation was reported by these authors. To confirm the role of $NO_x$ in individuals with a high clustering of MetS components, such as seen in this study, we are currently continuing our investigation of these subjects; the results from repeated $NO_x$ measurements and follow-up observations should facilitate an interpretation of the results reported here.

High-density lipoprotein cholesterol has been reported to induce activation of the PI3-kinase–Akt kinase pathway and MAP kinase to cause an increase in eNOS protein [21]. However, our results are not consistent with this previous

report; we found a significant difference in age-matched $NO_x$ levels in men with HDL-C $\leq$1.03 mmol/l versus those with HDL-C >1.03 mmol/l, suggesting that low HDL-C was associated with higher $NO_x$ levels. We assume that the discrepancy between our results and those of the previous investigation can be attributable to the study designs, i.e., in vitro experiment versus systematic body reactions. Furthermore, it is possible that the reason for the lack of a similar difference in $NO_x$ levels associated with HDL-C in women may be related to menopause, which can induce oxidative stress following the depletion of estrogens [22], resulting in the attenuation of the relationship between the serum $NO_x$ and HDL-C levels.

Our results also contradict those of Cui et al. [13], who reported an inverse correlation between the urinary excretion of cGMP and the clustering of metabolic risk factors. However, any evaluation of urinary cGMP as a substitute for NO bioavailability requires some caution. Since urinary cGMP excretion is likely to depend on the renal function [23, 24], the renal dysfunction of cGMP and $NO_x$ excretion in urine may result in the increase of circulating $NO_x$ levels. Moreover, it has been reported that a creatinine adjustment of urinary biological indicators assayed in spot urine is not a reliable tool for conversion into 24-h urinary excretion [25].

There were some limitations to this study. Because of the relatively small sample size of the subpopulation with >4 MetS components ($n = 9$ for men and $n = 5$ for women), the possibility remains that a few subjects with an extremely high $NO_x$ level brought about the observed strong trend. While the box plots (Fig. 1) indicate that no extreme outliers in the >4 MetS components group resulted in an overestimation of the association by severely affecting the $NO_x$ distribution, a definitive conclusion in this respect may not be drawn until we have confirmed the reproducibility of the results among our subjects in the follow-up investigation now underway or in other general populations. Another concern is the possibility that NO metabolites degenerated in the serum samples stored at 4°C for the maximum length of 5 days after collection. By assaying $NO_x$ in paired serum samples from five healthy adults, one immediately after the collection and the other stored at 4°C for 5 days until ultrafiltrated, we confirmed that there was no significant within-individual variation in $NO_x$ ($37.4 \pm 14.1$ versus $37.8 \pm 13.9$ μmol/l, $p = 0.96$) in that time interval.

The subjects included in this study participated in annual health check-ups and represented approximately 6% of the residents in the relevant age range. Moreover, most of these had undergone repeated check-ups in previous years, resulting in the possible interventional effect of confounding the relationship between $NO_x$ levels and the clustering of MetS components. The impact of these limitations will be evaluated in a future investigation for which we plan a follow-up and the recruitment of new subjects.

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## References

1. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes Care. 2005;28:2745–9.
2. Miyatake N, Kawasaki Y, Nishikawa H, Takenami S, Numata T. Prevalence of metabolic syndrome in Okayama prefecture, Japan. Intern Med. 2006;45:107–8.
3. National Cholesterol Educaton Program (NCEP) (2002) 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, or ATP III (updated 2004). Circlation 106:3143–221.
4. Stuehr DJ. Enzymes of the L-arginine to nitric oxide pathway. J Nutr. 2004;134:2748S–51S.
5. Moncada S, Palmer RM, Higgs EA. The discovery of nitric oxide as the endogenous nitrovasodilator. Hypertension. 1988;12:365–72.
6. Binion DG, Fu S, Ramanujam KS, Chai YC, Dweik RA, Drazba JA, et al. iNOS expression in human intestinal microvascular endothelial cells inhibits leukocyte adhesion. Am J Physiol. 1998;275:G592–603.
7. Zuckerbraun BS, Stoyanovsky DA, Sengupta R, Shapiro RA, Ozanich BA, Rao J, et al. Nitric oxide-induced inhibition of smooth muscle cell proliferation involves S-nitrosation and inactivation of RhoA. Am J Physiol Cell Physiol. 2007;292:C824–31.
8. Thippeswamy T, McKay JS, Quinn JP, Morris R. Nitric oxide, a biological double-faced janus – is this good or bad? Histol Histopathol. 2006;21:445–58.
9. Cook S, Hugli O, Egli M, Vollenweider P, Burcelin R, Nicod P, et al. Clustering of cardiovascular risk factors mimicking the human metabolic syndrome X in eNOS null mice. Swiss Med Wkly. 2003;133:360–3.
10. Tsutsui M, Shimokawa H, Morishita T, Nakashima Y, Yanagihara N. Development of genetically engineered mice lacking all three nitric oxide synthases. J Pharmacol Sci. 2006;102:147–54.
11. Konukoglu D, Serin O, Turhan MS. Plasma leptin and its relationship with lipid peroxidation and nitric oxide in obese female patients with or without hypertension. Arch Med Res. 2006;37:602–6.
12. Kondo T, Ueyama J, Imai R, Suzuki K, Ito Y. Association of abdominal circumference with serum nitric oxide concentration in healthy population. Environ Health Prev Med. 2006;11:321–5.
13. Cui R, Iso H, Pi J, Kumagai Y, Yamagishi K, Tanigawa T, et al. Metabolic syndrome and urinary cGMP excretion in general population. Atherosclerosis. 2007;190:423–8.
14. Kitaichi K, Wang L, Takagi K, Iwase M, Shibata E, Nadai M, et al. Decreased antipyrine clearance following endotoxin administration: in vivo evidence of the role of nitric oxide. Antimicrob Agents Chemother. 1999;43:2697–701.
15. Ko GT, Cockram CS, Chow CC, Yeung V, Chan WB, So WY, et al. High prevalence of metabolic syndrome in Hong Kong Chinese–comparison of three diagnostic criteria. Diabetes Res Clin Pract. 2005;69:160–8.

16. Clini E, Volterrani M, Pagani M, Bianchi L, Porta R, Gile' LS, et al. Endogenous nitric oxide in patients with chronic heart failure (CHF): relation to functional impairment and nitrate-containing therapies. Int J Cardiol. 2000;73:123–30.
17. Maejima K, Nakano S, Himeno M, Tsuda S, Makiishi H, Ito T, et al. Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complications. 2001;15:135–43.
18. Savino A, Pelliccia P, Schiavone C, Primavera A, Tumini S, Mohn A, et al. Serum and urinary nitrites and nitrates and Doppler sonography in children with diabetes. Diabetes Care. 2006;29:2676–81.
19. Rydén 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;25:811–5.
20. Osawa M, Hayashi T, Nomura H, Funami J, Miyazaki A, Ignarro LJ, et al. Nitric oxide (NO) is a new clinical biomarker of survival in the elderly patients and its efficacy might be nearly equal to albumin. Nitric Oxide. 2007;16:157–63.
21. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res. 2006;98:1352–64.
22. Arteaga E, Villaseca P, Rojas A, Arteaga A, Bianchi M. Comparison of the antioxidant effect of estriol and estradiol on low density lipoproteins in post-menopausal women. Rev Med Chil. 1998;126:481–7.
23. Zeballos GA, Bernstein RD, Thompson CI, Forfia PR, Seyedi N, Shen W, et al. Pharmacodynamics of plasma nitrate/nitrite as an indication of nitric oxide formation in conscious dogs. Circulation. 1995;91:2982–8.
24. Wever KE, Masereeuw R, Miller DS, Hang XM, Flik G. Endothelin and calciotropic hormones share regulatory pathways in multidrug resistance protein 2 (Mrp2-) mediated transport. Am J Physiol Renal Physiol. 2007;292:F38–46.
25. Alessio L, Berlin A, Dell'Orto A, Toffoletto F, Ghezzi I. Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. Int Arch Occup Environ Health. 1985;5:99–106.