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Serum nitric oxide metabolite as a biomarker of visceral fat accumulation: Clinical significance of measurement for nitrate/nitrite

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: A visceral fat area of more than 100 cm^2 as measured by computed tomography (CT) at the umbilical level has been included as a criterion for obesity in all the proposed criteria for metabolic syndrome. However, CT cannot be used frequently because of radiation exposure. We evaluated the usefulness of measurement of the serum levels of nitric oxide (NO), instead of CT and the waist circumference, as a marker of abdominal visceral fat accumulation.

Material/Methods: The study was carried out in 80 subjects. The serum levels of NO metabolites (nitrate/nitrite) were measured using the Griess reagent.

Results: Simple and multiple regression analysis revealed that the serum levels of NO metabolites showed the greatest degree of correlation with the visceral fat area ($r=0.743$, $p<0.0001$), and corresponded to a visceral fat area of 100 cm^2, as determined using the ROC curve, was 21.0 µmol/ml (sensitivity 88%, specificity 82%); this method was more sensitive than the waist circumference for evaluation of the visceral fat accumulation.

Conclusions: Measurement of the serum levels of NO metabolites may be a simple, safe, convenient and reliable method for the evaluation of visceral fat accumulation in clinical diagnostic screening.

key words: **visceral fat • obesity • NO metabolite • waist scale • metabolic syndrome**

Abbreviations: **BMI** – body mass index; **MetS** – metabolic syndrome; **WHO** – World Health Organization; **AHA** – American Heart Association; **NHLBI** – National Heart, Lung, and Blood Institute; **IDF** – international diabetes federation; **NO** – nitric oxide; **eNOS** – endothelial nitric oxide synthase; **iNOS** – inducible nitric oxide synthase; **NO metabolite** – Nitrate+Nitrite; **CT** – computed tomography; **hsCRP** – high-sensitive C-reactive protein; **HOMA-IR** – homeostasis model assessment of insulin resistance; **L/S ratio** – CT attenuation value of the liver to that of the spleen; **FBS** – fasting blood sugar; **WC** – waist circumference; **SFA** – subcutaneous fat area; **VFA** – visceral fat area; **TFA** – total fat area; **AST** – alanine aminotransferase; **TG** – triglyceride

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Background

Recently, rapid socioeconomic growth has led to a more sedentary lifestyle and change in diet over the past several decades in developed nations. As a result of economic prosperity, obesity has become a serious health problem in several Western countries. Although the prevalence of obesity in Asian populations is lower than that in Westerners, it has been reported that the health risks associated with obesity in Asians are observed at lower body mass index (BMI) [1] as compared to Westerners.

Many studies have demonstrated a close relationship between body fat accumulation and occurrence of metabolic syndrome [2,3]. Specifically, excessive accumulation of abdominal adipose tissue, especially intra-abdominal visceral fat, leads to obesity-related complications [4,5]. The metabolic syndrome, a cluster of glucose intolerance, hypertension, and dyslipidemia with visceral fat accumulation, is a common health problem in developed nations [6–8].

A simple obesity index is needed to monitor the development of obesity because of the worldwide increase in obesity [9,10]. In the mid-1990s, waist circumference was used as an independent risk indicator to identify individuals who needed weight management [11–13]. According to the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria, waist circumference of 40 inches or more for men and 35 inches or more for women is a risk factor for development of metabolic syndrome. At the annual meeting of the Japanese Society of Internal Medicine in April 2005, one of the diagnostic criteria for metabolic syndrome is abdominal circumference of 85 cm (33.46 inches) for men and 90 cm (35.43 inches) for women, measured at the umbilical level [14]. However, subcutaneous and visceral fat cannot be differentially evaluated by measuring only abdominal circumference. Therefore, a criterion was set for measuring the area of visceral fat (more than 100 cm^2) by computed tomography (CT) at the umbilical level [15]. However, CT cannot be used frequently because of the risk from radiation exposure.

We therefore considered serum nitric oxide (NO) level for use in the evaluation of abdominal visceral fat accumulation, instead of CT and waist circumference. NO is a vasodilator with a short half-life and acts within a small distance, and is thus ideally suited to act as a tissue hormone [16,17]. It is thought that NO is involved in adipose tissue biology by influencing adipogenesis, insulin-stimulated glucose uptake, and lipolysis [16]. The enzymes for NO generation in adipose cells are endothelial NO synthase (eNOS) and inducible NO synthase (iNOS) [18–20]. It has been reported that NO inhibits the proliferation, but stimulates the expression, of 2 adipogenic marker genes – peroxisome proliferator-activated receptor γ and uncoupling protein 1, *in vitro* in rat brown preadipocytes [21]. Lipid accumulation and lipogenic enzymes are also induced by NO in rat white preadipocytes [22]. In addition, it is reported that insulin-stimulated glucose uptake in rat white adipose tissue is dependent on NO synthesis *in vivo* [23]. Basal as well as catecholamine-stimulated lipolysis is inhibited by NO in human and rat subcutaneous adipose tissue depots [24–27].

Cytokine-dependent regulation of iNOS has also been reported in fat cells [28]. Based on these findings, NO appears to be an important mediator for adipocyte physiology. Thus, we investigated the relationship between abdominal visceral fat accumulation and serum NO level, and showed the clinical significance of measurement of NO metabolites in serum for the evaluation of fat accumulation.

Material and Methods

Subjects

We evaluated the clinical indices in patients who were admitted to the Yokohama City University Hospital from 2006 to 2009. The protocol was reviewed and approved by the institutional ethics review committee. Informed consent was obtained from all subjects before examination. The study was carried out in 80 subjects admitted to our hospital, and was restricted to men and postmenopausal women to eliminate the influence of pregnancy and female hormone replacement therapy. The study was also restricted to persons over 20 years of age to reduce the possible confounding effect of growth and development. A total of 80 Japanese subjects (38 men and 42 postmenopausal women, 58.0±7.8 years, BMI 24.6±1.1 kg/m^2) were evaluated in this study (Table 1). The proportions of subjects with history of diabetes mellitus, hyperlipidemia, and hypertension were 25.6%, 24.3%, and 22.7%, respectively.

Measurement for various indices

Venous blood samples were obtained after the patients had fasted overnight (12 hours), for measurement of the serum ALT, glucose, insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, Fe, Ferritin, high-sensitive C-reactive protein (hsCRP), type IV collagen 7s domain, and hyaluronic acid. The serum insulin levels were measured by radioimmunoassay, while the other laboratory biochemical parameters were measured in a conventional automated analyzer.

As NO itself is unstable in *in vivo* physiological condition, we therefore measured serum NO metabolites (Nitrate/Nitrite) as indicators of NO level in blood [29]. Plasma samples (50 µl) were deproteinized by incubation with 140 µl of deionized H_2O and 10 µl of 30% $ZnSO_4$ at room temperature for 15 min, and the samples were centrifuged at 2000 g for 10 min. Nitrate was converted to nitrite using cadmium beads, and total nitrite was measured spectrophotometrically using a Nitrate/Nitrite Colorimetric assay kit (Cayman Chemical, Ann Arbor, MI).

Insulin resistance was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: [fasting serum insulin (µU/ml) × fasting plasma glucose (mg/dl)/405]. However, the HOMA-IR was performed in only 72 subjects for whom the fasting plasma glucose was under 170 mg/dl, because HOMA-IR has been reported to be a suitable method for evaluating the presence of insulin resistance in patients only when the fasting glucose levels are under 170 mg/dl [30].

Circadian rhythm of NO metabolite levels in human

To examine circadian rhythm of NO metabolite levels in human, we measured NO metabolite levels 10 times a day (6:00 am, 8:00 am, 9:00 am, 12:00 pm, 2:00 pm, 3:00 pm,

Table 1. Clinical characteristics of the study subjects.

n	80	hsCRP (mg/dl)	0.149±0.034
Age (years)	58±7.8	Total cholesterol	199.0±4.0
Sex (male/female)	38/42	HDL-cholesterol (mg/dl)	59.0±2.0
Body Weight (kg)	62.3±1.8	LDL-cholesterol (mg/dl)	112.0±4.0
BMI (kg/m^2)	24.6±1.1	Triglyceride (mg/dl)	139.0±10.0
WC (cm)	87.4±1.6	Fe (μg/dl)	128.0±10.0
L/S ratio	1.096±0.033	Ferritin (ng/ml)	245.0±38.0
Visceral fat (cm^2)	100.3±6.0	Hyaluronic acid (ng/ml)	55.0±10.0
Subcutaneous fat (cm^2)	155.0±15.5	Type IV collagen 7S (ng/ml)	4.8±0.3
FBS (mg/dl)	99.0±2.0	Nitrate+Nitrite (μmol/ml)	25.84±3.55
Insulin (μU/ml)	7.3±0.8	Hypertension (%)	22.7
HOMA-IR	1.740±0.203	Hyperlipidemia (%)	24.3
ALT (IU/l)	45.0±5.0	Diabetes mellitus (%)	25.6

Data are means ±SD.

6:00 pm, 8:00 pm, 9:00 pm, 6:00 am) for inpatients on whom liver biopsy was performed. Since certain foods are known to be rich sources of NO metabolites, all patients ate meals at the same times (7:00 am, 1:00 pm, 7:00 pm), and venous blood samples were obtained 1 hour before, 1 hour after, and 2 hours after eating a meal.

Detection of NO generation and iNOS expression in human visceral adipose cells

Human adipose cells were purchased from Cell Garage Co., Ltd. (Ishikari, Hokkaido, Japan) and cultured in human primary mesenterium visceral adipose cells in a 37°C incubator room. Cells were stimulated with obesity-associated hormones such as insulin, leptin, and angiotensin II, and supernatant for measurement of NO metabolite, and samples for Western blot analysis were prepared. NO metabolite in supernatant was measured by Nitrate/Nitrite Colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). The samples for Western blot analysis (25 μg of protein/lane) were separated by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to a nitrocellulose membrane using an electroblotting transfer apparatus. The nitrocellulose membranes were incubated in blocking buffer (10 mM Tris, 100 mM NaCl, 0.1% Tween 20, and 5% nonfat milk) overnight at 4°C. Thereafter, the membranes were incubated with rabbit polyclonal anti-iNOS antibody (Cayman Chemical, Ann Arbor, MI) for 90 min at room temperature. The membranes were washed 3 times for 10 min each in washing buffer, and incubated with the secondary antibody diluted in blocking buffer (anti-rabbit peroxidase-conjugated antibody) for 60 min at room temperature. The signals were then detected by ECL-plus Western blotting starter kit (GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, England) as described by the manufacture.

Anthropometry and abdominal fat distribution

Anthropometric measurements (height, weight and waist circumference [WC]) were performed in a standing position. The weight and height of the patients were measured with a calibrated scale after the patients removed their shoes and any heavy items of clothing. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). WC at the umbilical level was measured with a non-stretchable tape in the late exhalation phase while standing [31]. Abdominal fat distribution was determined using CT (computed tomography) scanning while the subjects were the supine position, in accordance with a previously described procedure [32]. Ordinary CT parameters were used, specifically, 120 kV and 200 mA, as well as a slice thickness of 5 mm, a scanning time of 2 seconds, and a field of view of 400 mm. The subcutaneous fat area (SFA) and intra-abdominal visceral fat area (VFA) were measured at the level of the umbilicus and determined by a standardized method with CT numbers. Briefly, a region of interest of the subcutaneous fat layer was defined by tracing its contour on each scan, and the attenuation range of CT numbers (in Hounsfield units) for fat tissue was calculated. A histogram for fat tissue was computed on the basis of mean attenuation ±2SD. Total and intraperitoneal tissue with attenuation within the mean ±2SD were considered to be the total fat area (TFA) and VFA, and the SFA was defined by subtracting the VFA from the TFA.

Definition of metabolic syndrome

Metabolic syndrome was defined according to the 2005 guidelines of the Japanese Society of Internal Medicine or the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria [33,34], and World Health Organization (WHO). In the Japanese guideline, which is similar to the IDF criteria [35,36], subjects with metabolic syndrome must have: abdominal obesity (defined as WC more than 85 cm in men or more than 90 cm in women [37]), plus any 2 of the following 3 factors: (1) dyslipidemia – hypertriglyceridemia (serum triglyceride concentration more than 150 mg/dl {1.69 mmol/L}) and/or low HDL-cholesterol (serum concentration less than 40 mg/dl

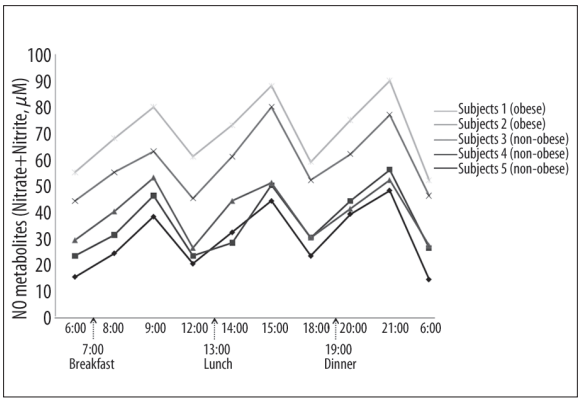


Figure 1. The influence of a meal on the levels of NO metabolites in serum. Samples were collected 10 times a day before 1 hour, after 1 hour, after 2 hour taking a meal. Serum NO metabolite levels were increased and the influence of a meal was certainly recognized after 2 hours having a meal in all 5 patients, but fasting serum NO metabolite levels were quite stable.

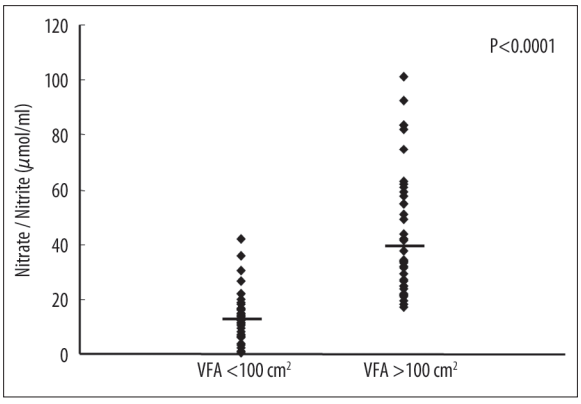


Figure 2. Comparison of serum NO metabolite level between visceral fat area (VFA) under 100 cm^2 and over 100 cm^2. Each plot represents the individual serum NO metabolite level, respectively. Each bar represents mean, respectively. Significant elevation of serum NO metabolites in obese subjects were observed in comparison to that in non-obese subjects.

{1.04 mmol/L}); (2) hypertension – systolic blood pressure more than 130 mmHg and/or diastolic blood pressure more than 85 mmHg; (3) high fasting glucose – serum glucose concentration more than 110 mg/dl {6.1 mmol/L}). In the present study, abdominal obesity was defined as WC more than 85 cm in men or more than 90 cm in women as reported for the Japanese population [37].

Assessment of hepatic fat content

The degree of liver steatosis was measured by CT. Previous studies have shown a strong correlation between the CT attenuation values of the liver and the extent of fatty infiltration as measured by biopsy [38,39]. The ratio of the CT attenuation value of the liver to that of the spleen (L/S ratio) was used for quantitative estimation of the hepatic fat content, with an L/S ratio of<1 being considered to represent fatty liver [37].

Statistical analysis

All the data were expressed as means ±SD. The relationship between any 2 variables was analyzed by standard correlation analysis conducted using the StatView version 5.0 software (SAS, Cary, NC). ANOVA was applied for group comparisons, followed by Student's *t* test for confirmation of the statistical associations. The relationship between the L/S ratio, VFA, or SFA and other relevant covariates was examined by multiple regression analysis and determination of the standardized correlation coefficients. Statistical significance was assumed when the *P* value was <0.05.

Results

Characteristic of subjects

Characteristics of study subjects by sex are presented in Table 1. Of the 80 total subjects, 38 were men and 42 were women. The mean BMI was 24.2±1.0 kg/m^2 in men, and 25.0±1.1 kg/m^2 in women. There was no significant difference between sexes for BMI, L/S ratio, all serum markers, existence of obesity-related disorder and total abdominal fat area. However, the mean subcutaneous fat area and waist circumference were higher in women (163.2±23.1 cm^2, 84.5±0.8 cm, respectively) than in men (145.8±10.4 cm^2, 88.2±3.6 cm), although the mean visceral fat area in men was higher (104.3±3.1 cm^2) than in women (96.2±15.4 cm^2) ($p<0.05$).

Stability of NO metabolites

As NO is an unstable vasodilator with a short half-life in physiological condition, we therefore measured serum NO metabolites (Nitrate/Nitrite) as NO level in blood [27]. To confirm the stability of NO metabolites after sample collection, we examined the stability of NO metabolites under the condition of –80°C for 7 days, and room temperature for 24 hours. As shown in Supplemental Figures 1 and 2, NO metabolites were stable under the storage condition of –80°C for 7 days and room temperature for 24 hours.

Circadian rhythm of NO metabolite levels in humans

Since certain foods are known to be rich sources of NO metabolites, we measured NO metabolite levels 10 times a day to examine circadian rhythm and the influence of eating a meal. Nevertheless, serum NO metabolite levels were increased and the influence of a meal was clearly recognized 2 hours after eating a meal in all 5 patients (Figure 1); serum NO metabolite levels were also waning 2 hours after eating a meal, and fasting serum NO metabolite levels were quite stable (Figure 1).

Comparison of serum NO metabolite levels under 100 cm^2 and over 100 cm^2 of visceral fat area

We compared serum NO metabolite levels of the group with visceral fat area under 100 cm^2 to the group that had visceral fat area over 100 cm^2. Significant elevation of serum NO metabolites (nitrate/nitrite) in obese subjects (visceral fat area over 100 cm^2) were observed in comparison to that in non-obese subjects (visceral fat area under 100 cm^2) (Figure 2).

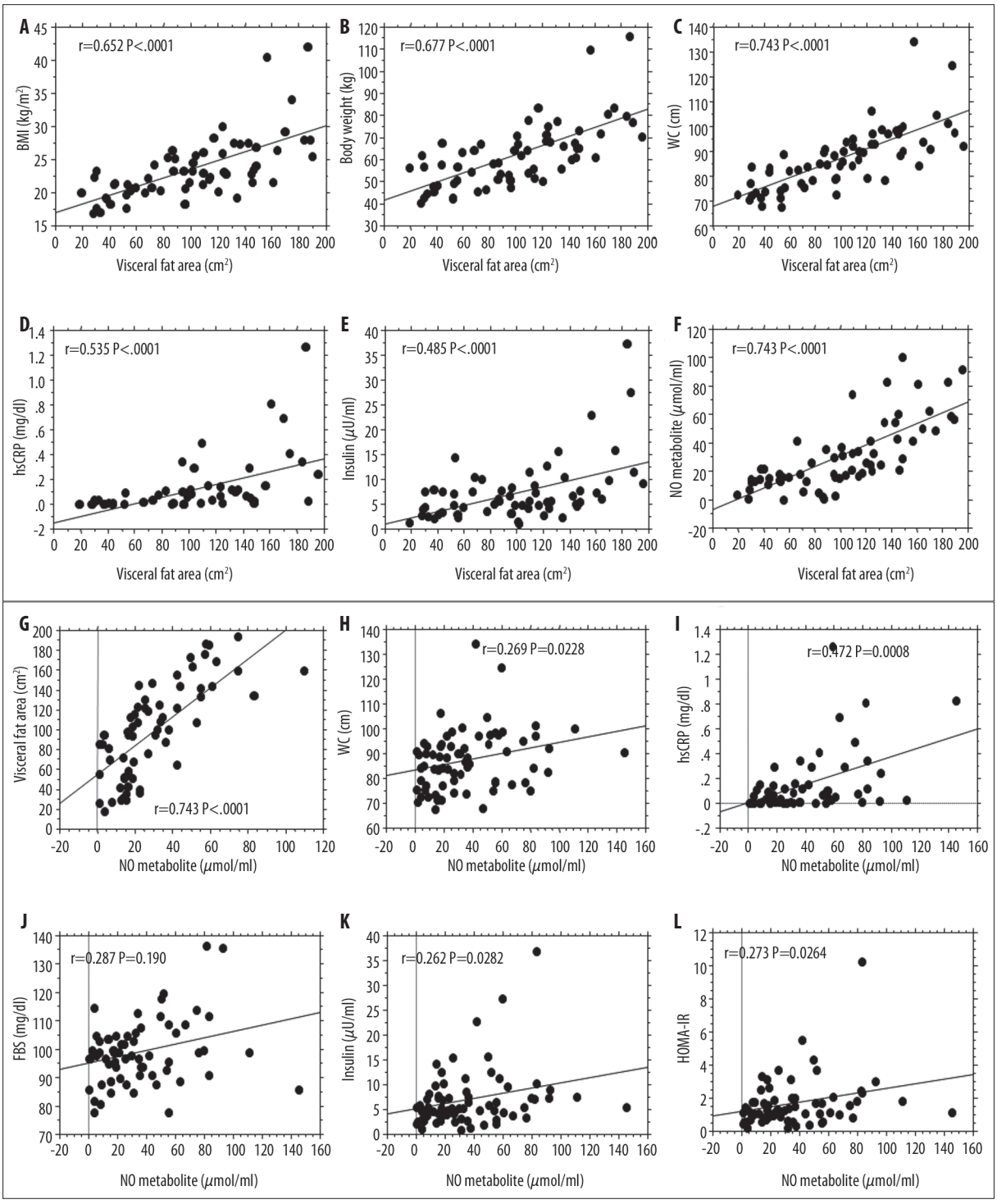


Figure 3. Simple regression analysis of the relationship between visceral fat area and other factors, and the relationship between NO metabolites and other factors. The visceral fat area was correlated with the BMI (r=0.652, p<0.0001), body weight (r=0.677, p<0.0001), WC (r=0.743, p<0.0001), hsCRP (r=0.535, p<0.0001), serum insulin concentration (r=0.485, p<0.0001), and NO metabolite (r=0.743, p<0.0001). The NO metabolites was correlated with the visceral fat area (r=0.743, p<0.0001), WC (r=0.269, p=0.0228), hsCRP (r=0.472, p=0.0002), FBS (r=0.287, p=0.0190), serum insulin concentration (r=0.262, p=0.0282), and HOMA-IR (r=0.273, p=0.0264).

Relationship between visceral fat accumulation and clinical parameters

The relationship between visceral fat area and the other factors is shown in Figure 3. The visceral fat area was correlated with: BMI (r=0.652, p<0.0001; Figure 3A); body weight (r=0.677, p<0.0001; Figure 3B); WC (r=0.743, p<0.0001; Figure 3C); hsCRP (r=0.535, p<0.0001; Figure 3D); serum insulin concentration (r=0.485, p<0.0001; Figure 3E); and NO metabolite (r=0.743, p<0.0001; Figure 3F). WC and NO

Table 2. Multiple regression analysis of the relationship between VFA, NO metabolite and other associated variables for the entire group.

Risk factors	Regression coefficient	SE	Standardized regression coefficient	P-Value
Age	.320	.330	.082	.3385
Sex	20.704	12.238	.210	.0983
Body weight	–.210	.973	–.065	.8299
BMI	1.179	2.814	.122	.6775
WC	1.756	.702	.469	.0164**
NO metabolite	.752	.196	.390	.0055**
Insulin	.207	.803	.028	.7983
hsCRP	22.543	19.870	.108	.2632

The dependent variable is the VFA. The independent variables are age, sex, body weight, BMI, WC, NO metabolite, serum insulin, and serum hsCRP.

Risk factors	Regression coefficient	SE	Standardized regression coefficient	P-Value
Age	–.276	.338	–.113	.4180
Sex	–8.898	11.463	–.150	.4409
WC	16.558	16.383	.146	.1165
Visceral fat	.222	.107	.387	.0319**
HOMA-IR	–.051	.073	–.182	.5898
Insulin	.072	.115	.083	.5311
FBS	–.022	.054	–.058	.6867
hsCRP	5.010	3.288	.252	.1332

The dependent variable is the NO metabolite. The independent variables are age, sex, WC, visceral fat, HOMA-IR, serum insulin, serum fasting blood sugar, and serum hsCRP. ** $P<0.05$ R2 for entire model =0.766. NO metabolite = Nitrate+Nitrite.

metabolites were closely correlated with visceral fat area. Multiple regression analysis was performed to quantify the impact of the measured indices (age, sex, and 6 correlative factors [BMI, body weight, WC, hsCRP, serum insulin concentration, NO metabolites]) on the visceral fat area. The results shown in Table 2 indicate that the WC and NO metabolites, but not other factors, were significantly related to the visceral fat area.

Relationship between NO metabolites and clinical parameters

Next, we examined the relationship between NO metabolites in serum and other factors. As shown in Figure 3, the level of serum NO metabolites was correlated with the visceral fat area ($r=0.743$, $p<0.0001$; Figure 3G), WC ($r=0.269$, $p=0.0228$; Figure 3H), hsCRP ($r=0.472$, $p=0.0002$; Figure 3I), FBS ($r=0.287$, $p=0.0190$; Figure 3J), serum insulin concentration ($r=0.262$, $p=0.0282$; Figure 3K), and HOMA-IR ($r=0.273$, $p=0.0264$; Figure 3L). In particular, the visceral fat area was closely correlated with the NO metabolites. Multiple regression analysis was also performed to quantify the impact of the measured indices (age, sex, and 6 correlative factors [FA, WC, hsCRP, FBS, serum insulin concentration, HOMA-IR]) on the NO metabolites. The results shown in Table 2 indicate that only the visceral fat area, but not other factors, were significantly related to the NO metabolites.

Comparison of NO metabolites and waist circumference in the association of visceral fat accumulation

As our results indicated that visceral fat area was strongly correlated with WC and NO metabolites, we compared the NO metabolites and waist circumference for the association with visceral fat area. As shown in Table 3, any of 3 criteria of metabolic syndrome respectively indicated another waist circumference. We compared NO metabolite with each of the 3 criteria of WC. The cut-off value for NO metabolite, which corresponds to VFA 100 cm^2 using the ROC curve, was 21.0 μmol/ml (sensitivity 88%, specificity 82%). Using the same method, the cut-off value for WC of 2005 guidelines of the Japanese Society was 85 cm for men and 90 cm for women (sensitivity 75%, specificity 90%), WHO was 37 inches for men and women (sensitivity 56%, specificity 95%), and AHA was 40 inches for men and 35 inches for women (sensitivity 50%, specificity 98%) (Table 3).

Detection of NO generation and iNOS expression in human visceral adipose cells

We confirmed that visceral adipose cells express NO synthase and can generate NO by stimulation with obesity-associated hormones such as insulin, leptin, and angiotensin II. As shown in Figure 4A, expression of iNOS protein was observed in cultured human visceral adipose cells by the stimulation of obesity-associated hormones. In addition, increase

Table 3. Comparison the NO metabolite with waist circumference that correspond to VFA 100 cm^2 in 80 subjects.

	Sensitivity (Se)			Specificity (Sp)		
	Se(all)	Se(m)	Se(f)	Sp(all)	Sp(m)	Sp(f)
WC (Japanese criteria)	75%	78%	71%	90%	87%	95%
WC (WHO criteria)	56%	50%	64%	95%	100%	91%
WC (AHA criteria)	50%	25%	75%	98%	100%	95%
Nitrate + Nitrite	88%	90%	85%	82%	85%	77%

WC – Waist Circumference; Se – Sensitivity; Sp – Specificity; m – male;f – female.

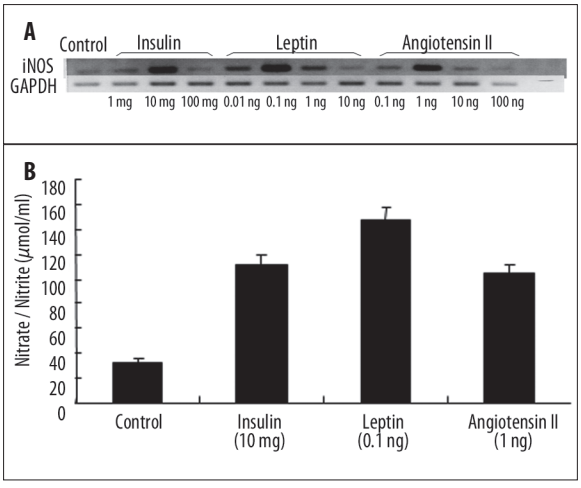


Figure 4. Detection of NO generation and iNOS expression in human visceral adipose cells. (**A**) Expression of iNOS protein in cultured human visceral adipose cells stimulated with obese-associated hormones, such as insulin, leptin, angiotensin II, was detected by western blot analysis. (**B**) Release of NO metabolite (Nitrate/Nitrite) from cultured human visceral adipose cells stimulated with obese-associated hormones. Each column represents mean.

in NO generation from cultured human visceral adipose cells by the stimulation of obesity-associated hormones was also observed (Figure 4B). These results indicate that the majority of elevated serum NO was generated from visceral fat.

Discussion

In the present study, we demonstrated marked elevation of serum NO metabolites (nitrate/nitrite) in obese subjects in comparison to that in non-obese subjects, even after adjustment for age, sex, waist circumference, hsCRP, BMI, subcutaneous fat area, and insulin resistance. We clearly showed that NO metabolites, but not other factors, were significantly related to the visceral fat area. This is the first report to demonstrate a strong association between visceral fat area and serum levels of NO metabolites.

WC is currently widely used for estimation of intra-abdominal visceral fat area. The increase in WC has been demonstrated to be a good index of cardiovascular disease [40] and an important diagnostic marker for metabolic syndrome. Although CT scanning and magnetic resonance imaging are used for precise measurements of visceral fat [41,42], simple anthropometric measurements for estimating the amount of visceral fat are essential for simple screening of the general population. On the basis of prior studies, WC has been shown to directly reflect abdominal fat mass [43]. WHO initially proposed a definition for metabolic syndrome in 1998 [44], and AHA proposed useful criteria for the diagnosis of the metabolic syndrome in 2001 [45]. However, the measurements offered for cut-off criteria for abdominal circumference of more than 94 cm in men and women by WHO, and more than 102 cm in men and more than 88 cm in women by AHA for abdominal obesity, are based on Westerners' criteria. Therefore, based on these criteria, the prevalence of metabolic syndrome in the Asian population is very low [46], because Asians have higher risk of diabetes and/or metabolic syndrome even though they have lower fat measurement. Asian populations have been shown to have a higher body fat deposition at a lower BMI than Westerners [47–49]. Abdominal obesity promotes insulin resistance and leads to metabolic syndrome [50–52]. Therefore, Asians have higher glucose intolerance and cardiovascular risk factors compared to Westerners at a relatively lower BMI [53]. In 2002, a study of 1193 Japanese subjects revealed that WC measurement that equated with a visceral fat area of 100 cm^2 was a useful cut-off value for the prediction of patients with more than 1 clinical diagnosis (dyslipidemia, hyperglycemia and hypertension). This 100 cm^2 value is observed in more than 70% of those Japanese patients identified with coronary heart disease. Therefore, Japanese guidelines have established a cutoff point for waist circumference to be 85 cm in men and 90 cm in women [54].

Based on such reports, it is suggested that WC that corresponds to VFA 100 cm^2 is extremely different among races and between the sexes. Moreover, visceral and subcutaneous fat obesity cannot be clearly distinguished, and the intraobserver and interobserver variability for waist circumference were higher than those for body mass index [55]. Abdominal visceral fat area can be evaluated by CT; however, CT cannot be used frequently because of the risk of radiation exposure. Thus, a simple, safe, convenient and reliable marker for the evaluation of visceral fat accumulation is required in clinical diagnostic screening. We therefore focused on serum NO level for the evaluation of abdominal visceral fat accumulation, instead of CT and waist circumference.

Conclusions

NO is a vasodilator with a short half-life and acts within a short distance, and is thus ideally suited to act as a tissue

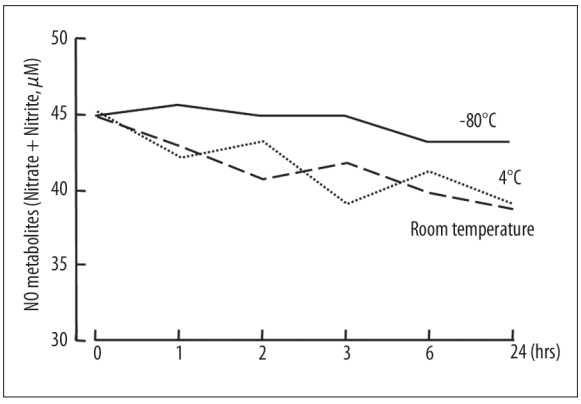
hormone [16,17]. In our preliminary experiments, production of NO metabolites was increased in visceral adipose tissue in rats [56]. In the present study we clearly show that obesity-associated hormones such as insulin, leptin, and angiotensin II regulate the production of NO in human cultured visceral adipose cells. These results indicate that visceral fat might be an important source of NO in humans. We suggest that measurement of serum NO metabolites may be a good biomarker for the evaluation of visceral fat accumulation. In the present study, we clearly demonstrated the marked elevation of serum NO metabolites in obese subjects in comparison to non-obese subjects. Our data also indicate that the marked elevation in serum NO metabolites were closely correlated with visceral fat area. Therefore, the monitoring of serum NO metabolite levels may be a more useful, safer, and more cost-effective biomarker for the diagnosis of visceral fat accumulation than CT and WC.

Since certain foods, especially processed meats, are known to be rich sources of NO metabolites [57], a long-term diet rich in NO metabolites could affect the serum NO metabolite level, and care must be taken to avoid those foods before measuring serum NO metabolite level.

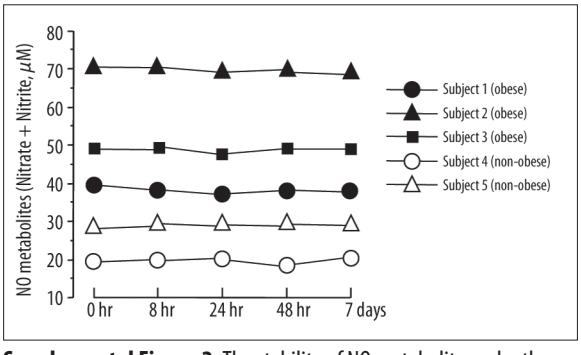
In conclusion, serum NO metabolites are significantly related to visceral fat accumulation. The monitoring of serum NO metabolite levels may be a simple, safe, convenient and reliable biomarker for the evaluation of visceral fat accumulation in clinical diagnostic screening.

Conflict of interest

This manuscript does not contain any conflicts of interest.



Supplemental Figure 1. The stability of NO metabolites under the condition of –80°C, 4°C or room temperature for 24 hours.



Supplemental Figure 2. The stability of NO metabolite under the condition of –80°C for 8 hours to 7 days.

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