Association of Abdominal Circumference with Serum Nitric Oxide Concentration in Healthy Population

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Abstract

Objectives: We determined the relationship between abdominal circumference and the concentration of nitric oxide (NO), an endothelial cell product known to play an important role in the regulation of vascular tone and thrombocyte activations.

Methods: Subjects were 177 men and 339 women aged 40 or over who were free from a history of diabetes or malignancy. Analysis of covariance was applied to examine the gender-specific and smoking-status-specific associations of abdominal fat volume measured as waist circumference, waist-hip ratio, and waist-to-stature ratio, with serum NO level represented by the concentration of NO metabolites (NOx; nitrate plus nitrite).

Results: Although men showed no statistical association between abdominal fat accumulation and NOx concentration, abdominal adiposity seemed to inversely affect the serum NOx concentration of never- and current-smoking women.

Conclusion: Our results suggest that a reduction in NO bioactivity occurs with abdominal fat accumulation in women. The underlying biological mechanism might involve adipocytokines secreted from visceral fat, but is yet to be elucidated.

Key words: metabolic syndrome, nitric oxide, adipocytokine, endothelial dysfunction, analysis of covariance

Introduction

Nitric oxide (NO) plays important physiological roles in the endothelium (1), where free radical gas NO is synthesized from the amino acid L-arginine by endothelial constitutive NO synthase (eNOS, NOS3) and released (2). Endothelium-derived NO is a potent vasodilator as well as an inhibitor of platelet aggregation and adhesion (3, 4), and is associated with the development of cardiovascular disease (CVD) in the presence of endothelial dysfunction (ED), which plays an early and prominent role in atherosclerotic plaque formation (5, 6). Moreover, ED occurs in several cardiovascular settings, such as dyslipidemia, diabetes, and essential hypertension (7, 8), which are the currently established components of metabolic syndrome (MS) (9).

Numerous studies have confirmed the increased risks of CVD, hypertension, metabolic complications, or mortality due to centrally located body fat (10–14). In this respect, waist circumference (WC), the use of which is a convenient means of evaluating abdominal fat accumulation, has thus far been related to cardiovascular risk factors (15, 16) and found to be associated more with CVD risk factors than body mass index (BMI) (17). Furthermore, previous cohort studies showed that waist-to-hip ratio (WHR) is a better predictor of CVD than other indices of obesity, independent of BMI (18). Because WC or WHR serves as a useful marker of intra-abdominal fat and strongly correlates with visceral adipose tissue, the relationship between abdominal fat accumulation and metabolic risk factors can be argued in terms of the biological properties of visceral adipose tissue. Growing evidence has thus far demonstrated that visceral adipose tissue is characterized by a high frequency of genes encoding secretory proteins, classified as adipocytokines, which, once released, flow easily into systemic circulation and might eventually affect the entire body (9, 19).

We raised the question as to whether the adipocytokines secreted from the visceral fat in the accumulated abdominal fat contribute to the development of CVD by affecting endothelial

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NO concentration even under normal or preclinical conditions. Currently, there are no reports yet dealing with the relationship between abdominal adiposity and endothelial NO concentration, and experimental results regarding the mechanism of adipocytokines for regulating endothelial function are limited and hypothetical (20). The purpose of this study is to examine whether abdominal fat accumulation is associated with serum NO level independently of the effect of BMI in healthy persons.

Subjects and Methods

A total of 607 residents (210 men and 397 women) of a rural area of Hokkaido attended a health checkup program conducted in August 2005 and gave written informed consent before answering a lifestyle questionnaire and providing residual blood for analysis. Defining eligible subjects as those who were 40 years of age or over, not taking medication for diabetes, and had no history of malignancy, we recruited 177 men and 339 women, accounting for 4.0% and 8.0% of the residents over age 40, respectively. Fasting serum samples were collected from these subjects, stored at 4°C, and ultrafiltrated within three days of the collection for NO assay. Since NO is unstable and rapidly auto-oxidizes to nitrate and nitrite after its production, the concentrations of stable metabolites of NO (NOx; nitrite plus nitrate) were determined using the method described in a previous study (21). Nitrate concentration was measured as nitrite after enzymatic conversion by nitrate reductase, and nitrite concentration was measured after deproteinization using the Griess color reaction, which was read at a wavelength of 540 nm. The values obtained by this procedure represent the sum of the amounts of nitrite and nitrate derived from NO. Serum NOx concentration was found to be skewed in distribution and normalized by logarithmic transformation before all the analyses. For the assessment of abdominal fat accumulation, we measured girth circumference at the level of the waist and hip using a flexible, plastic tape measure with the lightly attired subjects breathing normally in a standing position, and obtained three abdominal adiposity indices: WC, WHR, and waist-to-stature ratio (WSR).

For statistical analyses, we first examined the association of each of the MS components with log-transformed NOx concentration. The characteristics of the MS components used as predictors were defined by the following cutoff limits detailed in the *Definition and the Diagnostic Standard for Metabolic Syndrome* (22): 1) a high blood pressure, that is, a systolic blood pressure >130 mmHg or a diastolic blood pressure >85 mmHg; 2) a high triglyceride concentration, that is, a concentration >150 mg/dl; 3) a low high-density lipoprotein cholesterol (HDL-C) concentration, that is, a concentration <40 mg/dl; and 4) a high glucose concentration, that is, a fasting blood glucose concentration >110 mg/dl. For women, menopausal status (postmenopausal or not) was also used as a main predictor variable. In all of these statistical tests for difference, analysis of covariance (ANCOVA) was adopted to adjust for the effects of age and smoking status.

Next, we conducted ANCOVA to examine the association of log-transformed serum NOx concentration treated as a dependent variable with WC, WHR, and WSR as predictor variables. By including age, smoking status, and, for women, menopausal status as confounding variables, the multivariate-adjusted correlation was calculated (model-1). Furthermore, BMI was also added as a confounder to confirm the BMI-independent effects of the abdominal adiposity indices (model-2). Because smoking most likely affects abdominal circumference (23), subanalyses were accompanied with statistical models built separately on the basis of three smoking categories (never, former, and current). All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) for Windows version 11.0, and 2-sided p values less than 0.05 were considered statistically significant.

The Ethics Committee of Nagoya University School of Health Sciences, Nagoya, Japan, approved the study protocol.

Results

Gender-specific subject characteristics were summarized in Table 1. The men were significantly higher in average age and WC than the women, whereas WSR was significantly lower

Table 1 Basic characteristics of study subjects

Characteristics	Men (n=177)		Women (n=339)	
	Mean	(Range)	Mean	(Range)
Age (year)	63.2	(42–85)	60.6	(40–84)
Body mass index (kg/m ²)	23.9	(17.3–33.3)	23.6	(16.4–35.7)
Waist circumference (cm)	84.5	(66.4–106)	80.6	(56.4–107)
Waist-to-hip ratio	0.91	(0.73–1.04)	0.90	(0.67–1.12)
Waist-to-stature ratio	0.52	(0.52–0.65)	0.53	(0.37–0.69)
NOx concentration (μmol/l)	50.9	(8.0–179.1)	49.8	(17.2–271.8)
	Frequency		Frequency	
Smoking status				
Never	44		282	
Former	84		29	
Current	49		28	
Menopausal status				
Premenopausal	—		88	
Postmenopausal	—		251	

Table 2 NOx concentrations adjusted for age and smoking status on the basis of metabolic risk factors and menopausal status

Metabolic risk factors and menopausal status	Men (n=117)				Women (n=339)			
	No.	Yes	No	p value for difference ^a	No.	Yes	No	p value for difference ^a
Abdominal circumference ≥85 cm (men), 90 cm (women)	86	55.3	49.6	0.06	61	51.6	53.4	0.91
Triglyceride ≥150 mg/dl	39	52.4	52.3	0.69	50	55.3	52.2	0.39
HDL cholesterol <40 mg/dl	14	72.9	50.7	<0.05	6	59.9	52.8	0.34
Systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg	115	52.7	51.6	0.60	213	53.0	53.0	0.80
Fasting blood glucose >110 mg/dl	24	58.8	51.3	0.73	28	62.9	52.2	0.25
Postmenopausal	—				251	53.7	51.1	0.35

^a Log-transformed NOx concentration was used for the statistical test.

Table 3 Association of abdominal adiposity indices with NOx concentration^a by smoking status

Smoking status	All subjects		Never		Former		Current	
	Standardized coefficient (×10 ⁻³)		Standardized coefficient (×10 ⁻³)		Standardized coefficient (×10 ⁻³)		Standardized coefficient (×10 ⁻³)	
Variables	model-1 ^b	model-2 ^c	model-1 ^d	model-2 ^e	model-1 ^d	model-2 ^e	model-1 ^d	model-2 ^e
Men	n=177		n=44		n=84		n=49	
Waist circumference	5.0	0.9	-0.46	-22.4	3.6	6.3	3.6	6.3
Waist-to-hip ratio	2.4	-5.8	-1.1	-26.2	-0.79	-5.0	-0.79	5.0
Waist-to-stature ratio	8.8	3.5	3.3	-9.0	3.7	4.5	3.7	4.5
Women	n=339		n=282		n=29		n=28	
Waist circumference	-1.4	-8.9*	-0.82	-9.2*	-0.56	-0.32	-14.4	-12.9
Waist-to-hip ratio	-5.8	-9.7**	-5.1	-9.3*	-0.20	-0.80	-30.3*	-32.9*
Waist-to-stature ratio	-1.8	-13.4	-0.60	-13.2	-0.54	-3.6	-29.5	-42.2

^a NOx concentration was log-transformed.

^b Adjusted for age, menopausal status (women only), and smoking status.

^c Adjusted for age, menopausal status (women only), smoking status, and body mass index.

^d Adjusted for age, and menopausal status (women only).

^e Adjusted for age, menopausal status (women only), and body mass index.

* p<0.05, ** p<0.01.

in the men than in the women. No significant differences were observed between the men and the women in BMI, WHR and NOx concentration. Never-smoking subjects accounted for 25% and 83% of the men and women, respectively. Nearly three quarters of the women self-reported themselves as postmenopausal.

In Table 2, a tendency was observed for the multivariate-adjusted means of NOx concentration being higher in the presence of MS components, although the difference in the means was significant only in the relationship with HDL-C for the men.

In the ANCOVA model for the men, WC, WHR, and WSR showed no significant relationship with NOx concentration across the three smoking categories in both model-1 and model-2 (Table 3). In the women, WC and WHR showed a significant inverse correlation with NOx concentration in model-2, and this relationship was found to persist among never-smoking subjects. Current smoking women showed a significant relationship between WHR and NOx concentration in model-2, whereas formerly smoking women did not.

Discussion

Although our study focussed on the relationship between abdominal adiposity and serum NOx concentration in a healthy

population, we considered that the effects of certain metabolic risk factors, which might be potential confounders of the relationship of interest, must be discussed. Although studies indicating that a reduced endothelium-derived NOx concentration reflects the development of atherosclerotic disorders are numerous (1, 7, 24, 25), we found no significant differences in adjusted means in relation to all the MS components but a low HDL-C concentration in men, which is consistent with a previous result obtained using the biomarker cyclic guanosine 3',5'-monophosphate (cGMP), a second messenger of NO, excreted in urine collected from a large population-based sample (26). Consequently, we did not treat these MS components as covariates in the ANCOVA analyses. Furthermore, a circadian variation in serum NOx concentration must be considered. A previous paper demonstrating a borderline trend for a circadian variation in NOx concentration in healthy male subjects performing normal daily activities including dining indicated only a small degree of daytime NOx fluctuation (27).

This study showed that the abdominal adiposity expressed by WC and WHR is inversely associated with the serum NOx concentration in never-smoking women, and this relationship was independent of the effects of BMI and menopausal status. An age-adjusted relationship between BMI and serum NOx concentration was tested in our preliminary analysis; however, it showed no statistical significance in either men or women

across the three smoking categories (data not shown), which is in agreement with that of a previous report (26). Our results imply that, as visceral fat accumulates in the abdomen, never-smoking women become more likely to develop ED as represented by a decrease in NOx bioactivity. In addition, current smoking women showed a similar significant relationship between abdominal adiposity (WHR) and NOx concentration, whereas formerly smoking women did not. A previous longitudinal observation demonstrated a paradoxical increase in WHR due to smoking despite weight loss and suggested the harmful effects of smoking on the pattern of body fat distribution (23). Moreover, smoking no doubt causes ED, and this pathological change seems to be due to the proapoptotic effects of cigarette smoking (28). One could explain the steep negative coefficient observed in the relationship between the abdominal adiposity and NOx in currently smoking women as being partly attributable to the combination of these underlying anthropometric and

molecular mechanisms.

No significant relationship between abdominal adiposity and NOx concentration was observed in men, regardless of smoking status. In our speculation, men might not be as susceptible as women in terms of the endothelial responsiveness to the circulating adipocytokines produced by visceral fat tissue. Although no studies have yet been carried out to elucidate the underlying mechanism in this respect, we have launched a new epidemiological study to investigate the role of adipocytokines affecting the endothelial function in the presence of abdominal fat accumulation.

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