Original Article

A Positive Association between Leptin and Blood Pressure of Normal Range in Japanese Men

Keiko WADA[1)], Hiroshi YATSUYA[1)], Koji TAMAKOSHI[1)], Rei OTSUKA[1)], Chie FUJII[1)], Kunihiro MATSUSHITA[1),2)], Kaichiro SUGIURA[1),2)], and Hideaki TOYOSHIMA[1)]

The results of previous studies on the relationship between leptin and blood pressure are discordant. We investigated to what extent the serum leptin level was related to blood pressure independent of the degree of insulin resistance. The subjects were 1,916 men aged 34–69 years whose mean body mass index (BMI) was 23.0 kg/m^2. Blood pressure was regressed by leptin concentrations with adjustments for age, BMI, insulin resistance, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol, physical activity, drinking habits and smoking status. Leptin was associated with diastolic blood pressure (DBP) (standardized β: 0.092, p=0.003), but not with systolic blood pressure (SBP) (standardized β: 0.035, p=0.25), although insulin resistance was positively associated with both SBP and DBP (standardized β: 0.175 for SBP, p<0.001 and 0.114 for DBP, p<0.001) among all subjects. After subjects were divided into those with normal blood pressure (SBP<130 mmHg and DBP<85 mmHg) and those with higher blood pressure, leptin was positively and significantly associated with DBP (standardized β: 0.106, p=0.012) independent of the degree of insulin resistance, but not with SBP (standardized β: 0.064, p=0.13) among subjects in the normal blood pressure range. Among the subjects with higher blood pressure, however, neither the association of leptin with SBP nor that of leptin with DBP was statistically significant. These findings suggest that leptin may maintain and increase arterial tone, resulting in the elevation of DBP only within normal blood pressure range. It is also likely that leptin is a physiological mediator—or at least a marker—of some degree of DBP elevation in obesity. (*Hypertens Res* 2006; 29: 485–492)

***Key Words*: leptin, blood pressure, insulin resistance, body mass index, epidemiology**

Introduction

Although increased sympathetic nerve activity has been suggested to contribute to the development of obesity-related hypertension (*1–5*), the pathophysiological mechanism responsible for this effect remains poorly understood.

An association between leptin and blood pressure has been reported in both experimental (*6*, *7*) and epidemiologic studies (*8–17*). It was shown that leptin administration raised blood pressure in animals (*6*, *7*), and that transgenic skinny mice that over-expressed leptin were hypertensive (*18*). Positive associations between leptin levels and blood pressure have been reported in case-control (*8*, *9*), cross-sectional (*10–*

From the [1)]Department of Public Health/Health Information Dynamics and [2)]Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan.

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Address for Reprints: Hideaki Toyoshima, M.D., Ph.D., Department of Public Health/Health Information Dynamics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466–8550, Japan. E-mail: toyosima@med.nagoya-u.ac.jp

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13) and longitudinal studies (*14*). A graded positive relationship between plasma leptin levels and blood pressure was observed among 457 untreated employees (*11*). A statistically significant positive correlation was observed between the changes in mean blood pressure and the changes in leptin levels after a 3 month weight reduction in women (*14*). However, several other reports did not find a significant association between leptin and blood pressure (*15–17*), and the reason for this disagreement remains to be determined.

There are several factors that might explain the discrepancy among these studies. First, insulin resistance and insulin concentration are also associated with blood pressure (*19*). Insulin and leptin are reported to cooperate in the modulation of vascular tone (*20–22*), and insulin has been shown to stimulate leptin production (*23*). Second, although the primary effect of leptin on blood pressure may be the central stimulation of sympathetic nerve activity (*24*), other studies have suggested that leptin may have another, contradictory effect on blood pressure—*i.e.*, a vasodilative action that may or may not be related to a release of nitric oxide from the vascular endothelium (*25–27*), and the stimulatory effect of leptin on nitric oxide was possibly modified by the degree of obesity (*28*).

In the present study, therefore, we investigated the extent to which the serum leptin level affects blood pressure while controlling for the degree of insulin resistance and obesity in a large cohort of Japanese adult men.

Methods

Subjects and Design

This analysis was performed as part of a cohort study on cardiovascular disease whose participants included public servants and employees of a manufacturing company in Aichi Prefecture, Japan (*29*, *30*). In 1997, they were requested to answer a self-administered questionnaire about their medical history and lifestyle, including physical activity, drinking habits and smoking status. They underwent an annual physical examination including height, weight and blood pressure measurement, and provided fasting blood samples. Blood samples were collected after 12 h of overnight fasting, and serum was isolated immediately. After measurement of fasting glucose (FBS) and other routine parameters, the sera were centrifuged and kept frozen in a deep freezer at −80°C until the determination of serum leptin and insulin concentrations in the year 2000. Of the 7,683 male workers at enrollment, there were 7,426 men who responded to questions about lifestyle and whose blood pressure was measured. Among those, there were 2,155 subjects for whom blood data on serum leptin and insulin concentrations were available. After excluding individuals with FBS≥140 mg/dl (n=82) and those who had been prescribed antihypertensive drugs or hypoglycemic medication (n=157), 1,916 eligible men aged 34–69 years remained for the analysis. The study protocol and informed consent procedure were approved by the Ethics Committee of Nagoya University Graduate School of Medicine, Nagoya, Japan.

Lifestyle Characteristics

Physical activity was assessed by 2 questionnaire items: one on the frequency (seldom, 1–3 times per month, 1–2 times per week, or ≥3 times per week) and one on the intensity (vigorous, moderate, light) of activity. Vigorous activity was defined in the questionnaire as a level of activity that left participants out of breath. Similarly, moderate activity was defined as a level that left participants breathing rather hard. Those who engaged in vigorous activity 1–3 times or more per month, moderate activity 1–2 times or more per week, or light activity 3 times or more per week were classified as "active." All others on these questions were classified as "not active." Drinking habits were first assessed by the number of drinking days per week (0, 1–3, 4–6, and daily). If present (greater than 0), they were further categorized into two levels (light or heavy) by weekly consumption; that is, daily alcohol consumption multiplied by days of drinking per week. Light and heavy drinking corresponded to less than 23 g and ≥23 g of ethanol consumption per day, respectively. Smoking status was classified into 3 levels (never, past, or current smoker).

Measurements

Height and weight were measured when subjects were dressed in light indoor clothing and without footwear. Body mass index (BMI) was calculated as (weight in kg)/(height in m)^2. Serum insulin concentration (FIRI) was measured by solid phase radio-immunoassay (RIABEAD II; Dainabot Co., Ltd., Chiba, Japan), and homeostasis model assessment–insulin resistance (HOMA-R), which was calculated as FBS (mg/dl) × FIRI (μU/ml)/405, was used as an index of insulin resistance (*31*). Serum leptin concentration was determined with a radio-immunoassay (HUMAN LEPTIN RIA KIT; Linco Research, Inc., St. Charles, USA) in a commercial laboratory. The detection limit of the leptin assay was 0.5 ng/ml, the specificity of the human leptin was 100%, and the inter-assay coefficients of variation were 1.79% and 1.75% for low and high concentration controls, respectively.

Blood pressure was measured by auscultation using a mercury sphygmomanometer or by an automated sphygmomanometer (UK-15; Parama-Tech Co., Tokyo, Japan). For both measurement methods, Korotkoff phases 1 and 5 were taken as the systolic (SBP) and diastolic blood pressure (DBP), respectively. As a rule, measurements were taken from the right arm with the subjects in a sitting position after a minimum 5 min of rest. If the first measurement values were outside the range of SBP<140 mmHg and DBP<90 mmHg, the measurements were repeated after the subjects sat quietly for several more minutes. Since only the lower values among the measurements were recorded on the health check-up card,

Table 1. Characteristics of Studied Subjects

	All	Normal	Higher BP	*p**
n	1,916	1,088	828	
Age (years)[a]	49.0±6.0	48.1±5.7	50.2±6.2	<0.001
BMI (kg/m^2)[a]	23.0±2.7	22.4±2.5	23.7±2.7	<0.001
FIRI (μU/ml)[b]	4.78 (2.76–8.27)	4.30 (2.54–7.29)	5.49 (3.18–9.46)	<0.001
HOMA-R[b]	1.14 (0.63–2.07)	1.00 (0.57–1.76)	1.35 (0.75–2.43)	<0.001
Leptin (ng/ml)[b]	2.89 (1.78–4.71)	2.59 (1.63–4.12)	3.33 (2.06–5.40)	<0.001
TG[a]	137.9±95.4	124.1±85.3	156.1±104.4	<0.001
HDL-C[a]	55.4±14.9	55.9±15.1	54.6±14.5	0.052
LDL-C[a]	129.5±31.4	128.5±31.2	131.0±31.7	0.084
SBP (mmHg)[a]	126.4±16.9	114.9±9.1	141.6±12.0	<0.001
DBP (mmHg)[a]	78.4±11.1	71.4±7.2	87.7±8.2	<0.001
Physical activity[c]				
Not active	1,547 (80.7%)	884 (81.3%)	663 (80.1%)	0.52
Active	369 (19.3%)	204 (18.7%)	165 (19.9%)	
Drinking habit[c]				
None	516 (26.9%)	320 (29.4%)	196 (23.7%)	
Light	454 (23.7%)	276 (25.4%)	178 (21.5%)	<0.001
Heavy	946 (49.4%)	492 (45.2%)	454 (54.8%)	
Smoking status[c]				
Never	642 (33.5%)	346 (31.8%)	296 (35.7%)	
Past	342 (17.8%)	171 (15.7%)	171 (20.7%)	<0.001
Current	932 (48.6%)	571 (52.5%)	361 (43.6%)	

BMI, body mass index; FIRI, fasting immunoreactive insulin; HOMA-R, homeostasis model assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. **p* value by Student' *t*-test or χ^2-test for the difference by the blood pressure category. [a]Mean±SD; [b]geometrical mean and range of ±1 SD, [c]number of each category.

these values were used in this study.

Statistical Analysis

The levels of insulin, HOMA-R and leptin were skewed and hence normalized by logarithmic transformation in all analyses. The geometric mean and mean±SD were computed using the log-transformed values and converted back to the original scale of measurement.

We divided the subjects into those with normal blood pressure (n=1,088) and those with higher blood pressure (n=828) to elucidate whether the effect of leptin on blood pressure differed by blood pressure level. In the present analysis, normal blood pressure was defined as SBP<130 mmHg and DBP<85 mmHg according to the guidelines of the European Society of Hypertension/European Society of Cardiology (ESH-ESC) (*32*) and the Japanese Society of Hypertension (JSH) (*33*). The characteristics of subjects in each blood pressure level were assessed, and those showing differences between the two blood pressure groups were examined by Student's *t*-test for continuous variables or χ^2-test for discrete variables. Pearson's correlation analysis was used to detect the association among age, BMI, HOMA-R, leptin level, and blood pressure. The association between leptin concentrations and blood pressure was assessed by multiple linear regression analysis. Blood pressure was used as the outcome variable and leptin concentrations as the explanatory variable. Covariates were potential confounders including age, BMI, HOMA-R, triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), physical activity, drinking habits and smoking status. In this model, drinking habits were re-categorized into 2 levels (none or light, and heavy). Smoking status was also re-categorized into 2 levels (never or past, and current).

All analyses were conducted using the SPSS statistical package for Windows Version 11.5 (SPSS Inc., Chicago, USA). All p values were calculated by a two-sided test. A p value of less than 0.05 was considered statistically significant in all analyses.

Results

The characteristics of study subjects are shown in Table 1. The mean age was 49.0 years (SD: 6.0 years) and the mean BMI was 23.0 kg/m^2. The mean SBP and DBP were 126.4 mmHg and 78.4 mmHg, respectively. The geometric means of FIRI, HOMA-R and leptin were 4.78 μU/ml, 1.14, and 2.89 ng/ml, respectively. Subjects with higher blood pressure

Table 2. Pearson's Correlation Coefficients between Age, BMI, HOMA-R, Leptin and Blood Pressure

Variables	log [HOMA-R]		log [leptin]		SBP		DBP	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
All (n=1,916)								
Age	0.02	0.37	0.08	<0.001	0.18	<0.001	0.17	<0.001
BMI	0.54	<0.001	0.67	<0.001	0.28	<0.001	0.28	<0.001
log [HOMA-R]	—	—	0.59	<0.001	0.29	<0.001	0.27	<0.001
log [leptin]	—	—	—	—	0.28	<0.001	0.30	<0.001
Normal (n=1,088)								
Age	−0.03	0.34	0.05	0.079	0.001	0.98	0.08	0.008
BMI	0.52	<0.001	0.64	<0.001	0.23	<0.001	0.20	<0.001
log [HOMA-R]	—	—	0.58	<0.001	0.20	<0.001	0.14	<0.001
log [leptin]	—	—	—	—	0.23	<0.001	0.23	<0.001
Higher BP (n=828)								
Age	−0.02	0.92	0.02	0.56	0.15	<0.001	0.06	0.076
BMI	0.51	<0.001	0.65	<0.001	0.06	0.079	0.09	0.010
log [HOMA-R]	—	—	0.54	<0.001	0.11	<0.001	0.10	0.004
log [leptin]	—	—	—	—	0.05	0.17	0.12	<0.001

BMI, body mass index; HOMA-R, homeostasis model assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure.

were older than subjects in the normal blood pressure range (p<0.001). BMI, FIRI, HOMA-R, TG, leptin, SBP and DBP were all higher among subjects with higher blood pressure than those with normal blood pressure (p<0.001). Physical activity was not associated with the blood pressure level. The proportion of heavy drinkers was higher among subjects with higher blood pressure (p<0.001). The proportion of current smokers was higher among the subjects with normal blood pressure (p<0.001).

Age was significantly correlated with leptin, SBP and DBP (p<0.001), but not with HOMA-R among all subjects (Table 2). BMI was significantly associated with HOMA-R, leptin, SBP and DBP (p<0.001). BMI was more strongly correlated with HOMA-R and leptin (Pearson's r: 0.54 and 0.67, respectively) than with SBP and DBP (Pearson's r: 0.28 and 0.28, respectively). HOMA-R was significantly correlated with SBP and DBP (Pearson's r: 0.29 and 0.27, respectively; both p<0.001). Leptin was also significantly correlated with SBP and DBP (Pearson's r: 0.28 and 0.30, respectively; both p<0.001). Pearson's correlation coefficient between HOMA-R and leptin was 0.59 (p<0.001).

Stratified analysis by blood pressure levels showed that the associations of BMI with HOMA-R and leptin remained strong in both blood pressure level groups. The BMI of subjects in the normal blood pressure range was more strongly correlated with SBP and DBP (Pearson's r: 0.23 and 0.20, respectively) than that of those with higher blood pressure (Pearson's r: 0.06 and 0.09, respectively). HOMA-R was significantly correlated with SBP and DBP at both blood pressure levels. Leptin was significantly correlated with SBP and DBP (Pearson's r: 0.23 and 0.23, respectively; both p<0.001) among subjects with normal blood pressure. Among subjects with higher blood pressure, leptin was associated with DBP (Pearson's r: 0.12, p<0.001), but not with SBP (Pearson's r: 0.05, p=0.17). Pearson's r between HOMA-R and leptin was 0.58 and 0.54 in subjects with normal blood pressure and in those with higher blood pressure, respectively (both p<0.001).

In multiple linear regression analysis, leptin was associated with DBP (standardized β: 0.092, p=0.003), but not with SBP (standardized β: 0.035, p=0.25) among all subjects (Table 3). Each of age, BMI, HOMA-R, TG and HDL-C was positively and significantly associated with SBP and DBP (standardized β for SBP and DBP: 0.172 and 0.160 for age, 0.167 and 0.149 for BMI, 0.175 and 0.114 for HOMA-R, 0.098 and 0.094 for TG, and 0.082 and 0.069 for HDL-C, respectively). Stratified analysis by blood pressure level revealed that leptin was positively and significantly associated with DBP (standardized β: 0.106, p=0.012), but not with SBP (standardized β: 0.064, p=0.13) among subjects in the normal blood pressure range. Among the subjects with higher blood pressure, however, neither the association of leptin with SBP nor that of leptin with DBP was statistically significant (standardized β for SBP and DBP: −0.055, p=0.26 and 0.065, p=0.19, respectively). HOMA-R was associated with SBP, but not with DBP among subjects with either blood pressure level.

Additionally, we examined the association of leptin with DBP in two age groups, a group of subjects below 50 years of age (n=1,114) and a group 50 years old or over (n=802). The results showed that leptin was associated with DBP in the younger group (standardized β: 0.104, p=0.013), but not in the older group (standardized β: 0.062, p=0.19).

We also performed the multiple linear regression analysis when HOMA-R was replaced with the serum insulin level. Leptin was associated with DBP (standardized β: 0.107,

Table 3. Multiple Regression Analysis for Blood Pressure

Independent variables	All (n=1,916)		Normal (n=1,088)		Higher BP (n=828)	
	β[a]	p	β[a]	p	β[a]	p
SBP						
Age	0.172	<0.001	0.014	0.63	0.153	<0.001
BMI	0.167	<0.001	0.165	<0.001	0.057	0.24
log [HOMA-R]	0.175	<0.001	0.082	0.032	0.136	0.002
log [leptin]	0.035	0.25	0.064	0.13	−0.055	0.26
TG	0.098	<0.001	0.065	0.046	0.040	0.29
HDL-C	0.082	0.001	0.077	0.026	0.070	0.090
LDL-C	−0.014	0.54	0.000	0.99	−0.018	0.62
Physical activity	0.009	0.67	0.007	0.81	0.029	0.41
Drinking habit	0.127	<0.001	0.116	<0.001	0.019	0.61
Smoking status	−0.080	<0.001	−0.106	<0.001	0.008	0.83
	Total R^2=17.7%		Total R^2=10.2%		Total R^2=4.3%	
DBP						
Age	0.160	<0.001	0.088	0.003	0.062	0.079
BMI	0.149	<0.001	0.130	0.001	0.036	0.47
log [HOMA-R]	0.114	<0.001	0.005	0.89	0.052	0.24
log [leptin]	0.092	0.003	0.106	0.012	0.065	0.19
TG	0.094	<0.001	0.058	0.074	0.046	0.23
HDL-C	0.069	0.006	0.040	0.25	0.071	0.085
LDL-C	0.003	0.90	0.018	0.55	−0.002	0.96
Physical activity	−0.006	0.77	−0.026	0.39	0.017	0.62
Drinking habit	0.130	<0.001	0.126	<0.001	0.020	0.60
Smoking status	−0.115	<0.001	−0.139	<0.001	−0.062	0.10
	Total R^2=17.2%		Total R^2=9.5%		Total R^2=3.0%	

BMI, body mass index; HOMA-R, homeostasis model assessment; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. Normal, subjects with SBP<130 mmHg and DBP<85 mmHg. Higher BP, subjects with SBP≥130 mmHg and/or DBP≥85 mmHg. [a]Standardized coefficient β.

p=0.001), but not with SBP (standardized β: 0.054, p=0.085) among all subjects. Insulin was positively associated with SBP and DBP (standardized β for SBP and DBP: 0.131, p<0.001 and 0.076 p=0.007, respectively). In stratified analysis by blood pressure level, the relation of leptin with DBP (standardized β: 0.120, p=0.005) was stronger than that of leptin with SBP (standardized β: 0.086, p=0.042) among subjects in the normal blood pressure range. Among the subjects with higher blood pressure, neither the association of leptin with SBP nor that of leptin with DBP was statistically significant (standardized β for SBP and DBP: −0.055, p=0.26 and 0.064, p=0.19, respectively).

Furthermore, we did the same analyses when normal blood pressure was re-defined as SBP<140 mmHg and DBP<90 mmHg to examine whether this subtle change in definition would influence the association. The association between leptin and blood pressure did not change; leptin was positively related with DBP among the subjects in the normal blood pressure range (n=1,431) (standardized β: 0.131 p<0.001). The association of HOMA-R with blood pressure also did not substantially change, although β of HOMA-R for SBP was attenuated among subjects with higher blood pressure level (0.096, p=0.10).

Discussion

Our study showed that leptin was positively related with DBP independent of insulin resistance among the subjects in the normal blood pressure range, but this relationship was not found among the subjects having higher blood pressure. The positive relationship between leptin and insulin resistance, which was in agreement with the results of several other studies (*34–36*), was not altered by stratifying the subjects into a normal blood pressure and higher blood pressure group. The relationship between leptin and DBP was not influenced by changing the cut-off value of blood pressure for defining normotension. These results suggest that the hypertensive state itself might change the association between leptin and blood pressure. Although the association between leptin and DBP was independent of BMI, BMI adjustment had attenuated the association remarkably, suggesting that leptin is a physiological mediator—or at least a marker—of some degree of DBP elevation in obesity.

The results of two experimental studies (*6*, *7*) indicated that

leptin exerts a pressor effect. In addition, it has been reported that blood pressure in leptin deficient *ob*/*ob* mice is low (*37*), and that transgenic skinny mice over-expressing leptin have high blood pressure (*18*). Leptin has been thought to stimulate sympathetic nerve activity based on the observations that intravenous administration of leptin increased sympathetic nerve activity in rats (*24*). Leptin has also been reported to have effects that activate the renin-angiotensin system (RAS) (*38*). However, leptin has also been reported to have depressor effects; that is, leptin was shown to function as a diuretic/natriuretic factor (*39*) and to induce a release of nitric oxide that opposes the pressor action (*25*).

Although many epidemiologic researchers had studied the association of leptin with blood pressure and the sympathetic nervous system, these results were not consistent (*8–17*, *40–45*). Our results may partly explain this diversity of findings. We showed that leptin has a pressor action only in the normal blood pressure range. This may be due to a particular characteristic of leptin—that is, leptin exists in a free form as well as a receptor protein-bound form within a living body. The protein-bound rather than the free leptin levels were reported to be correlated with basal sympathetic outflow in normotensive men (*46*). Because the circulating soluble leptin receptor concentration and the bound fraction of leptin were reported to decrease in the obese (*47*, *48*), leptin's excitation of sympathetic nervous activity may have been decreased among the subjects with higher blood pressure, whose mean BMI was higher than that of normotensives (Table 1).

Another possible explanation is that leptin's depressor action may be elevated in hypertensive people. It was reported that arterial dilation mediated by leptin-induced nitric oxide occurred only *in vitro* at concentrations well above those typically observed in morbidly-obese humans (*49*). Physiological concentrations of leptin may exert a pressor-dominant effect through the sympathetic system, and higher leptin concentrations may exert a depressor-dominant effect through direct interaction with vascular endothelial dysfunction.

Furthermore, the present results suggested that the association of leptin with DBP was stronger than that of leptin with SBP. However, insulin resistance was more strongly associated with SBP than DBP. It is known that pressure waves reflected off the peripheral resistance constitute diastolic pressure. In the elderly whose large arteries are stiffened, reflected waves from the upper and especially from the lower body travel faster and return to the central aorta in early to mid-systole to increase SBP. In the younger individuals whose large arteries are not stiffened, the reflected wave returns at the end of systole or early diastole to amplify DBP (*50*). Thus, the inter-individual variation in DBP observed in the present population, who had a relatively narrow younger age range (34–69 years), may have been the result of inter-individual variation in peripheral resistance. It may be possible that leptin is associated more with peripheral resistance through its sympathetic system activation than with other mechanism. In contrast, variation in SBP may be more attributable to other mechanisms, such as RAS activation or established arterial sclerosis, which may be associated more with insulin resistance (*51*, *52*). Although elucidating the underlying mechanisms is beyond the scope of the present study, these speculations derived from our results should be confirmed physiologically or by observation in larger cohorts, including women and other ethnic populations.

In this study, HOMA-R was used as an index of insulin resistance. Because HOMA-R is strongly correlated with the hyperinsulinemic glucose clamp method, which has been regarded as the reference method for an accurate assessment of insulin sensitivity, it can be reliably used in epidemiological studies (*53*). One limitation of our study is that blood pressure was not always measured twice, and that the index of sympathetic nerve activity such as heart rate was not measured. These measurements are needed in further studies. Another limitation was that BMI was used for the adjustment of the degree of obesity. It is possible that BMI did not necessarily mirror the quantity of adiposity and the body fat distribution. However, the association of leptin concentrations with BMI was similar to that with the other surrogates for obesity, including waist circumference and hip circumference (*54*). Thus, it seems unlikely that these were solely responsible for the associations observed in our data. Finally, the generalizability of our study is limited by the fact that our subjects were ethnically homogeneous Japanese men.

In conclusion, our study showed that leptin was positively related with DBP among men in the normal blood pressure range, but this relationship was not found among men with higher blood pressure. The association between leptin and blood pressure was independent of insulin resistance. These findings suggest that leptin may maintain and increase arterial tone, resulting in the elevation of DBP only within normal blood pressure range. It is also likely that leptin is a physiological mediator—or at least a marker—of some degree of DBP elevation in obesity.

References

1. Montani JP, Antic V, Yang Z, Dulloo A: Pathways from obesity to hypertension: from the perspective of a vicious triangle. *Int J Obes Relat Metab Disord* 2002; **26**: S28–S38.
2. Aihara A, Imai Y, Sekino M, *et al*: Discrepancy between screening blood pressure and ambulatory blood pressure: a community-based study in Ohasama. *Hypertens Res* 1998; **21**: 127–136.
3. Masuo K, Mikami H, Ogihara T, Tuck ML: Weight gain–induced blood pressure elevation. *Hypertension* 2000; **35**: 1135–1140.
4. Flaa A, Mundal HH, Eide I, Kjeldsen S, Rostrup M: Sympathetic activity and cardiovascular risk factors in young men in the low, normal, and high blood pressure ranges. *Hypertension* 2006; **47**: 396–402.
5. Kaushik RM, Mahajan SK, Rajesh V, Kaushik R: Stress profile in essential hypertension. *Hypertens Res* 2004; **27**:

619–624.
6. Shek EW, Brands MW, Hall JE: Chronic leptin infusion increases arterial pressure. *Hypertension* 1998; **31**: 409–414.
7. Casto RM, VanNess JM, Overton JM: Effects of central leptin administration on blood pressure in normotensive rats. *Neurosci Lett* 1998; **246**: 29–32.
8. Agata J, Masuda A, Takada M, *et al*: High plasma immunoreactive leptin level in essential hypertension. *Am J Hypertens* 1997; **10**: 1171–1174.
9. Sheu WHH, Lee WJ, Chen YT: High plasma leptin concentrations in hypertensive men but not in hypertensive women. *J Hypertens* 1999; **17**: 1289–1295.
10. de Courten M, Zimmet P, Hodge A, *et al*: Hyperleptinaemia: the missing link in the metabolic syndrome? *Diabet Med* 1997; **14**: 200–208.
11. Barba G, Russo O, Siani A, *et al*: Plasma leptin and blood pressure in men: graded association independent of body mass and fat pattern. *Obes Res* 2003; **11**: 160–166.
12. Hirose H, Saito I, Tsujioka M, Mori M, Kawabe H, Saruta T: The obese gene product, leptin: possible role in obesity-related hypertension in adolescents. *J Hypertens* 1998; **16**: 2007–2012.
13. Takizawa H, Ura N, Saitoh S, *et al*: Gender difference in the relationships among hyperleptinemia, hyperinsulinemia, and hypertension. *Clin Exp Hypertens* 2001; **23**: 357–368.
14. Itoh K, Imai K, Masuda T, *et al*: Relationship between changes in serum leptin levels and blood pressure after weight loss. *Hypertens Res* 2002; **25**: 881–886.
15. Hu FB, Chen C, Wang B, Stampfer MJ, Xu X: Leptin concentrations in relation to overall adiposity, fat distribution, and blood pressure in a rural Chinese population. *Int J Obes Relat Metab Disord* 2001; **25**: 121–125.
16. El-Gharbawy AH, Kotchen JM, Grim CE, *et al*: Gender-specific correlates of leptin with hypertension-related phenotypes in African Americans. *Am J Hypertens* 2002; **15**: 989–993.
17. Mallamaci F, Cuzzola F, Tripepi G, *et al*: Gender-dependent differences in plasma leptin in essential hypertension. *Am J Hypertens* 2000; **13**: 914–920.
18. Aizawa-Abe M, Ogawa Y, Masuzaki H, *et al*: Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* 2000; **105**: 1243–1252.
19. Sung KC, Kim BJ, Kim BS, *et al*: In normoglycemic Koreans, insulin resistance and adipocity are independently correlated with high blood pressure. *Circ J* 2004; **68**: 898–902.
20. Verma S, Bhanot S, McNeill JH: Sympathectomy prevents fructose-induced hyperinsulinemia and hypertension. *Eur J Pharmacol* 1999; **373**: R1–R4.
21. Galipeau D, Arikawa E, Sekirov I, McNeill JH: Chronic thromboxane synthase inhibition prevents fructose-induced hypertension. *Hypertension* 2001; **38**: 872–876.
22. Vecchione C, Aretini A, Maffei A, *et al*: Cooperation between insulin and leptin in the modulation of vascular tone. *Hypertension* 2003; **42**: 166–170.
23. Kolaczynski JW, Nyce MR, Considine RV, *et al*: Acute and chronic effects of insulin on leptin production in humans: studies *in vivo* and *in vitro*. *Diabetes* 1996; **45**: 699–701.
24. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI: Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 1997; **100**: 270–278.
25. Fruhbeck G: Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes* 1999; **48**: 903–908.
26. Matsuda K, Teragawa H, Fukuda Y, Nakagawa K, Higashi Y, Chayama K: Leptin causes nitric-oxide independent coronary artery vasodilation in humans. *Hypertens Res* 2003; **26**: 147–152.
27. Zanetti M, Barazzoni R, Vadori M, Stebel M, Biolo G, Guarnieri G: Lack of direct effect of moderate hyperleptinemia to improve endothelial function in lean rat aorta: role of calorie restriction. *Atherosclerosis* 2004; **175**: 253–259.
28. Beltowski J, Wojcicka G, Jamroz A: Stimulatory effect of leptin on nitric oxide production is impaired in dietary-induced obesity. *Obes Res* 2003; **11**: 1571–1580.
29. Yatsuya H, Tamakoshi K, Hattori H, *et al*: Serum phospholipid transfer protein mass as a possible protective factor for coronary heart diseases. *Circ J* 2004; **68**: 11–16.
30. Mabuchi T, Yatsuya H, Tamakoshi K, *et al*: Association between serum leptin concentration and white blood cell count in middle-aged Japanese men and women. *Diabetes Metab Res Rev* 2005; **21**: 441–447.
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
32. European Society of Hypertension–European Society of Cardiology Guidelines Committee: 2003 European Society of Hypertension–European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003; **21**: 1011–1053.
33. Japanese Society of Hypertension Guidelines Subcommittee for the Management of Hypertension: Guidelines for the management of hypertension for general practitioners. *Hypertens Res* 2001; **24**: 613–634.
34. Kim-Motoyama H, Yamaguchi T, Katakura T, *et al*: Serum leptin levels are associated with hyperinsulinemia independent of body mass index but not with visceral obesity. *Biochem Biophys Res Commun* 1997; **239**: 340–344.
35. Zimmet PZ, Collins VR, de Courten MP, *et al*, Mauritius NCD Study Group: Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. *Int J Obes Relat Metab Disord* 1998; **22**: 171–177.
36. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M: Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 1996; **45**: 695–698.
37. Mark AL, Shaffer RA, Correia ML, Morgan DA, Sigmund CD, Haynes WG: Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens* 1999; **17**: 1949–1953.
38. Bornstein SR, Torpy DJ: Leptin and the renin-angiotensin-aldosterone system. *Hypertension* 1998; **32**: 376–377.
39. Jackson EK, Li P: Human leptin has natriuretic activity in the rat. *Am J Physiol* 1997; **272**: F333–F338.
40. Suter PM, Locher R, Hasler E, Vetter W: Is there a role for the ob gene product leptin in essential hypertension? *Am J Hypertens* 1998; **11**: 1305–1311.
41. Corica F, Corsonello A, Ientile R, *et al*: Leptin and norepi-

nephrine plasma concentrations during glucose loading in normotensive and hypertensive obese women. *Am J Hypertens* 2001; **14**: 619–626.
42. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M: Interactions between leptin and the human sympathetic nervous system. *Hypertension* 2003; **41**: 1072–1079.
43. Narkiewicz K, Kato M, Phillips BG, *et al*: Leptin interacts with heart rate but not sympathetic nerve traffic in healthy male subjects. *J Hypertens* 2001; **19**: 1089–1094.
44. Fogari R, Derosa G, Zoppi A, *et al*: Comparison of the effects of valsartan and felodipine on plasma leptin and insulin sensitivity in hypertensive obese patients. *Hypertens Res* 2005; **28**: 209–214.
45. Nishina M, Kikuchi T, Yamazaki H, Kameda K, Hiura M, Uchiyama M: Relationship among systolic blood pressure, serum insulin and leptin, and visceral fat accumulation in obese children. *Hypertens Res* 2003; **26**: 281–288.
46. Tank J, Jordan J, Diedrich A, *et al*: Bound leptin and sympathetic outflow in nonobese men. *J Clin Endocrinol Metab* 2003; **88**: 4955–4959.
47. Sinha MK, Opentanova I, Ohannesian JP, *et al*: Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *J Clin Invest* 1996; **98**: 1277–1282.
48. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR: Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. *Obes Res* 2003; **11**: 760–768.
49. Knudson JD, Dincer UD, Zhang C, *et al*: Leptin receptors are expressed in coronary arteries and hyperleptinemia causes significant coronary endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 2005; **289**: H48–H56.
50. Smulyan H, Safar ME: The diastolic blood pressure in systolic hypertension. *Ann Intern Med* 2000; **132**: 233–237.
51. Kirpichnikov D, Sowers JR: Role of ACE inhibitors in treating hypertensive diabetic patients. *Curr Diab Rep* 2002; **2**: 251–257.
52. Imazu M: Hypertension and insulin disorders. *Curr Hypertens Rep* 2002; **4**: 477–482.
53. Bonora E, Targher G, Alberiche M, *et al*: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; **23**: 57–63.
54. Haffner SM, Gingerich RL, Miettinen H, Stern MP: Leptin concentrations in relation to overall adiposity and regional body fat distribution in Mexican Americans. *Int J Obes Relat Metab Disord* 1996; **20**: 904–908.