

Original Article

## Hyperleptinemia Is Associated with Hypertension in Japanese Males

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Leptin is a hormone which is predominantly secreted by adipose tissue. Recent studies have shown that leptin increases arterial blood pressure. Although data from available animal studies clearly indicate an association between leptin and hypertension, results of human studies have been less definitive. We conducted a case-control study to examine the association between serum leptin levels and hypertension in 111 hypertensive subjects and 222 male controls, using conditional logistic regression analyses. Mean serum leptin levels were found to be marginally higher in the case subjects than in the control subjects (3.3 ng/ml versus 3.0 ng/ml), however, conditional logistic regression analysis revealed that subjects in the highest quartile had a significantly increased risk of hypertension compared with those in the lowest quartile, even after adjusting for drinking status and diabetes mellitus (adjusted OR, 2.11; 95% CI, 1.01-4.39). Our findings suggest that leptin plays an important role in the development of hypertension.

**Key words:** epidemiological study, hyperleptinemia, hypertension, leptin

**O**besity increases the risk of cardiovascular disease through multiple mechanisms including hypertension, diabetes, dyslipidemia and atherosclerosis. Adipose tissue has long been considered passive tissue that specializes in energy storage, but recent research has demonstrated that adipose tissue produces and secretes various bioactive substances, which are known as adipocytokines [1, 2]. Leptin is an adipocyte-derived hormone that acts in the hypothalamus to regulate appetite and energy expenditure. Recent research has found that leptin is a pleiotropic

hormone with multiple actions that are potentially relevant not only to the control of appetite but also to cardiovascular function, insulin secretion, angiogenesis, immune response and haematopoiesis [3]. Landsberg *et al.* report that hyperinsulinemia and insulin resistance are associated with obesity-related hypertension and may play a role in the pathogenesis of hypertension through their effects on the sympathetic nervous system and kidneys [4]. However, in experimental and clinical studies, insulin infusion has not been shown to cause a hypertensive shift in blood pressure [5, 6]. The association between obesity and hypertension suggests that the adipose mass may be an important tissue in the regulation of blood pressure [7]. Considine *et al.* report that obese subjects have

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higher serum leptin levels than normal-weight subjects [8, 9]. Additionally, Aizawa-Abe *et al.* found that obesity-induced hyperleptinemia may increase sympathetic nerve activity, contributing to a significant elevation in blood pressure [10]. Therefore, it is reasonable to examine leptin as a potential link between obesity and high blood pressure. Data from animal studies clearly indicate an association between leptin and hypertension [7, 10–13] but the results of human studies are less definitive [14–17]. Therefore, we performed a matched case-control study to evaluate whether or not serum leptin levels are associated with hypertension.

## Materials and Methods

**Subjects.** The subjects of the present study were 111 male employees who were participants in health insurance society A in Fukuoka Prefecture, Japan and who received an annual health check-up in 2000. All subjects had hypertension, which was defined as having a systolic blood pressure (SBP) of 140 + mmHg and/or a diastolic blood pressure (DBP) of 90 + mmHg, and/or being under antihypertensive treatment. The creatinine levels of all study subjects were within reference range, and no subject suffered secondary hypertension due to endocrine disease. For each case subject, 2 control subjects were randomly selected and matched with the case subject for age (within  $\pm 1$  year) and body mass index (BMI; within  $\pm 1$ ). The control subjects were normotensive subjects with no history of hypertension. Informed consent was obtained from each subject. This study was approved by the Ethics Committee of Fukuoka University.

**Measurements.** A fasting blood sample was drawn from a peripheral vein into a siliconized disposable plastic tube. Serum samples were kept at  $-80^{\circ}\text{C}$  for subsequent assays. The level of serum leptin was evaluated by enzyme immunoassay (Human Leptin RIA Kit; Linco Research, St. Charles, MO, USA). Serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically using commercial enzyme kits (Wako, Osaka, Japan and Daiichi Kagaku, Tokyo, Japan respectively). Hemoglobin A1c (HbA1c) was measured by latex agglutination using a commercially available kit (Fuji Rebio, Tokyo, Japan). Uric acid was measured

by the uricase F-DAOS method using a commercially available kit (Wako). SBP and DBP were measured in the daytime (09:00–15:00) using a standard mercury sphygmomanometer with the cuff on the right arm and the subjects in a sitting position. When the SBP or DBP was  $\geq 140$  mmHg or  $\geq 90$  mmHg, respectively, it was measured again twice after a rest period. The first BP measurement was used as the BP value for the purposes of this study. We defined hypercholesterolemia as a TC value of  $\geq 240$  mg/dl and/or the use lipid-lowering agents, and diabetes mellitus as a HbA1c value of  $\geq 6.5\%$  and/or treatment for diabetes mellitus with insulin or oral agents. BMI, weight (in kilograms) divided by the square of height (in meters), was used as an index of relative weight. Each subject completed a questionnaire covering his clinical history, smoking status and alcohol consumption. The subjects were assigned to 1 of 3 smoking status groups: current smokers, ex-smokers and never-smokers. Alcohol intake was likewise classified into 3 categories: regular drinkers, ex-drinkers and never-drinkers.

**Statistical analysis.** All analyses were conducted using the Statistical Analysis System (SAS, Version 9.1; SAS, Cary, NC, USA). For continuous variables, results are presented as mean  $\pm$  standard deviation (SD) or median (interquartile range), and differences between the case and control groups were evaluated using an unpaired *t* test. Categorical variables are presented by frequency counts, and intergroup comparisons were tested for statistical significance using a  $\chi^2$  test. Because the distributions of serum leptin and triglyceride levels were skewed, log transformation was used, which yielded more normally distributed data. Associations between hypertension and serum leptin levels were analyzed by conditional logistic regression analysis.

## Results

The clinical characteristics of the study subjects are presented in Table 1. Drinking status and diabetes mellitus were found to differ significantly between case subjects and control subjects (non-drinkers, 8.2% versus 22.2%; current drinkers, 91.8% versus 76.0%; ex-drinkers, 0% versus 1.8%; diabetes mellitus, 10.8% versus 2.3%). No differences were found in smoking status or hypercholesterolemia

**Table 1** Basic characteristics of the study population

Characteristics	Case subjects			Control subjects			P value
	n			n			
Age (years)	111	49.3	(5.1)	222	48.9	(5.1)	0.59
Smoking status (%)							
	Non-smokers	59	52.7	133	59.7		0.26
	Current smokers	41	37.3	63	28.5		
	Ex-smokers	11	10.0	26	11.8		
Drinking status (%)							
	Non-drinkers	9	8.2	49	22.2		<.001
	Current drinkers	102	91.8	169	76.0		
	Ex-drinkers	0	0.0	4	1.8		
Body mass index, mean (Kg/m <sup>2</sup> )	111	24.2	(2.8)	222	24.0	(2.6)	0.45
Systolic blood pressure (mmHg)	111	145.8	(12.3)	222	121.5	(10.6)	<.0001
Diastolic blood pressure (mmHg)	111	92.4	(7.3)	222	75.4	(8.9)	<.0001
Diabetes mellitus (%)	12	10.8		5	2.3		<.001
Hypercholesterolemia (%)	21	18.9		33	14.9		0.34

SD: standard deviation

Data are expressed as mean (SD) or as frequency counts (%) as appropriate.

P-value refers to overall differences across groups as derived from an unpaired *t*-test (for continuous variables) or from a  $\chi^2$  test (for categorical variables).

**Table 2** Biochemical characteristics of the study population

Characteristics	Case subjects			Control subjects			P value
Leptin level (ng/ml)							
	mean (SD)	3.3	(1.5)	3.0	(1.6)		
	median (IQR)	2.9	(2.3-4.3)	2.7	(2.0-3.7)		
Log-leptin level, mean (SD)		0.5	(0.2)	0.4	(0.2)		0.079
Triglyceride (mg/dl), mean (SD)		191.8	(173.4)	185.0	(184.9)		
Log-triglyceride, mean (SD)		2.2	(0.3)	2.2	(0.3)		0.88
Cholesterol level (mg/dL), mean (SD)							
	TC	206.4	(34.1)	201.1	(34.0)		0.18
	LDL-C	108.8	(28.8)	110.6	(29.8)		0.61
	HDL-C	63.0	(15.0)	57.0	(12.7)		<0.001
Creatinine (mg/dl), mean (SD)		1.1	(0.1)	1.1	(0.1)		0.49
Uric acid (mg/dl), mean (SD)		5.9	(1.6)	5.9	(1.3)		0.81
HbA1c (%), mean (SD)		5.1	(1.1)	4.9	(0.7)		0.07

TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; HbA1c, hemoglobin A1c; SD, standard deviation; IQR, interquartile range.

P-value refers to overall differences across groups as derived from an unpaired *t*-test (for continuous variables) or from a  $\chi^2$  test (for categorical variables).

between the 2 groups. The metabolic characteristics of the study subjects are shown in Table 2. Log-transformed serum leptin levels and HbA1c were marginally higher in the case subjects than in the control subjects (log-leptin, 0.5 ng/ml versus 0.4 ng/

ml; HbA1c, 5.1% versus 4.9%), and HDL-C levels in the case subjects were significantly higher than in the control subjects (63.0 mg/dl versus 57.0 mg/dl). There were no significant differences between case subjects and control subjects in the levels of log-

transformed triglyceride, TC, LDL-C, creatinine or uric acid.

Next, we analyzed the association between serum leptin levels and hypertension. The results of conditional logistic regression analysis are shown in Table 3. The case subjects and control subjects were grouped into quartiles for serum leptin levels with interquartile cut-off points of 2.1, 2.7 and 3.7 ng/mL. Subjects in the highest quartile of serum leptin levels had a significantly increased risk of hypertension, compared with those in the lowest quartile (odds ratio, OR, 2.35; 95% confidence interval, 95% CI, 1.15–4.81). Moreover, high serum leptin levels were significantly associated with hypertension even after adjustment for drinking status and diabetes mellitus (adjusted OR, 2.11; 95% CI, 1.01–4.39). These data suggest that hyperleptinemia is an independent variable for hypertension.

### Discussion

The present study has demonstrated that serum leptin levels are associated with hypertension. As shown in Table 2, serum leptin levels were similar in normotensive and hypertensive subjects. However, when we performed conditional logistic regression analysis to analyze the association between serum leptin levels and hypertension, we found that subjects in the highest quartile of serum leptin levels had a

significantly increased risk of hypertension, compared with those in the lowest quartile. These results suggest that leptin plays an important role in the development of hypertension. Our data are only partly consistent with those recently published by Barba *et al.* [18], who report that men with higher leptin levels have a significantly greater probability of being hypertensive, adjusting for differences in age, total adiposity and fat distribution. In the present study, no dose-response relationship was detected.

The results of earlier studies on the role of leptin in human hypertension are not consistent. Some epidemiological studies report significantly higher leptin levels in essential hypertensive patients than in controls [19, 20], or a significant correlation between leptin levels and blood pressure [16]. Hirose *et al.* note that the correlation of mean blood pressure with leptin remains in obese adolescents even after adjustment for age and BMI [17]. However, these results were not confirmed by Kokot *et al.* [15] or Uckaya *et al.* [14]. No association was found between 24-h DBP and BMI-adjusted leptin by Narkiewicz *et al.* [21], while Suter *et al.* report a significant relationship between SBP and plasma leptin levels in hypertensive women but not in hypertensive men [22].

However, recent studies report that fasting plasma leptin levels appear to be related to various cardiovascular diseases including stroke [23] and myocardial infarction [24]. Leptin thus seems to play an impor-

Table 3 Crude and adjusted odds ratios for hypertension

	Case subjects	Control subjects	Crude	Adjusted <sup>1</sup>
	n (%)	n (%)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
Serum leptin level (ng/ml)				
0–2.1	23 (20.7)	63 (28.4)	1 (reference)	1 (reference)
2.2–2.7	27 (24.3)	53 (23.9)	1.38 (0.72–2.66)	1.43 (0.73–2.81)
2.8–3.7	26 (23.4)	59 (26.6)	1.22 (0.64–2.35)	1.05 (0.52–2.10)
3.8+	35 (31.5)	47 (21.2)	2.35 (1.15–4.81)	2.11 (1.01–4.39)
			p for trend <b>0.14</b>	p for trend <b>0.15</b>
Drinking status				
Non-drinkers	9 ( 8.2)	49 (22.2)	1 (reference)	1 (reference)
Drinkers	102 (91.8)	173 (77.8)	3.14 (1.49–6.63)	2.78 (1.28–6.04)
Diabetes mellitus	12 (10.8)	5 ( 2.3)	5.65 (1.81–17.62)	5.06 (1.58–16.22)

Data are expressed as odds ratios, 95% confidence interval, 95% CI.

The case subjects were matched with controls for age and BMI.

<sup>1</sup>Odds ratios were adjusted for drinking status and diabetes mellitus.

tant role in the development of cardiovascular diseases. Recently, the concept of selective leptin resistance was proposed, allowing the hypothesis that the sympathoexcitatory actions of leptin are preserved despite resistance to its satiety and weight-reducing action [25]. If leptin resistance is selective in human obesity, then leptin could contribute to increased sympathetic activity and elevated arterial pressure. The pathogenesis of essential hypertension remains to be clarified.

The present study has several limitations. First, unmeasured variables related to blood pressure and/or leptin may confound the association between leptin and hypertension. For example, we were unable to measure fasting insulin levels in our subjects. Therefore, no statement regarding the interaction between leptin and fasting insulin can be made without further study. Second, sampling was performed at different times of day; a factor which may affect the measurement of leptin level since circulating leptin shows a circadian rhythm. Nevertheless, there is no reason to believe that the spread of sampling times differed between the case subjects and control subjects.

To summarize, serum leptin levels were marginally higher in case subjects than in control subjects in the present study. After adjusting for confounding factors, conditional logistic regression analysis showed that serum leptin levels were significantly associated with hypertension in Japanese men. Thus, leptin may be an independent predictor of hypertension.

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