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Fujita, Yuki
Kouda, Katsuyasu
Ohara, Kumiko
Nakamura, Harunobu
Iki, Masayuki

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Leptin mediates the relationship between fat mass and blood pressure

The Hamamatsu School-based health study

Yuki Fujita, PhD[a,*], Katsuyasu Kouda, MD, PhD[b], Kumiko Ohara, PhD[c], Harunobu Nakamura, MD, PhD[c], Masayuki Iki, MD, PhD[a]

Abstract

Animal studies have shown that leptin mediates the association between obesity and hypertension. However, only a few studies have assessed this relationship in population-based epidemiological studies. This study aimed to determine whether leptin mediates the relationship between body fat and blood pressure in school-aged children.

A cross-sectional survey was conducted among school-aged children in Hamamatsu, Japan. Body fat was measured using dual-energy X-ray absorptiometry. Height-normalized index of fat mass (fat mass index) was calculated by dividing fat mass by height squared. Serum leptin levels were measured by enzyme-linked immunosorbent assay. Multiple regression analysis was used to evaluate relationships between body fat, serum leptin levels, and blood pressure. The mediating effect of leptin on the association between body fat and blood pressure was assessed by causal mediation analysis and regression analysis.

Both fat mass index and leptin were significantly and positively associated with blood pressure. Fat mass index was also strongly associated with serum leptin levels. Body fat and blood pressure were no longer associated after adjusting for leptin. These findings suggest that the association between body fat and blood pressure is mediated by leptin. Of the total effect of fat mass index on blood pressure, the mediating effect of leptin accounted for 78.6% ($P = .03$) in boys and 42.2% ($P = .11$) in girls.

Our findings suggest that body fat is associated with blood pressure, and this association is mediated by leptin. Thus, leptin acts as a mediator that links body adiposity with blood pressure elevation in school-aged children.

Abbreviations: β = standardized regression coefficient, BIA = bioelectrical impedance analysis, BMI = body mass index, BP = blood pressure, DBP = diastolic blood pressure, DXA = dual-energy X-ray absorptiometry, r = correlation coefficient, SBP = systolic blood pressure, SNS = sympathetic nervous system, VIF = variance inflation factor.

Keywords: adipose tissue, blood pressure, child, densitometry, leptin

1. Introduction

Excessive body weight usually results from excessive fat weight. Obesity is defined as abnormal and excessive fat mass, and may impair health.[1] Obesity in childhood increases the risk of obesity in adulthood, and consequently, obese children are likely to become obese adults.[2] According to a previous study, 40% of obese children remain obese in adulthood.[3] In both childhood and adulthood, obesity is one of the most significant risk factors for hypertension.[4,5] Although studies are ongoing regarding the mechanisms by which obesity causes hypertension, one possible mechanism underlying obesity-induced hypertension involves leptin.[6,7] In experimental animal studies, leptin administration was shown to increase blood pressure (BP).[8,9] Previous studies also demonstrated that transgenic mice that overexpress leptin develop hypertension,[10] and that acute infusion of leptin in rabbits increases BP via stimulation of the sympathetic nervous system (SNS).[11] Thus, leptin plays an important role in BP elevation. Leptin is produced primarily by adipocytes in adipose tissue.[12,13] Therefore, leptin could potentially serve as a mediator in the association between adipose tissue and BP.[6,7,14]

No study to date has investigated the role of leptin as a mediator between body fat and BP in epidemiological studies targeting children and adolescents, although some have investigated the association between leptin and body weight. For instance, a positive relationship between serum leptin levels and body weight has been reported in epidemiological studies.[15,16] A study of obese children and adolescents found that leptin levels were positively associated with BP, when body weight or body mass index (BMI) was not controlled for.[17] In contrast, a cross-sectional study in school-aged healthy children reported that although serum leptin levels were significantly associated with BP, the association disappeared after adjusting for BMI.[18] Another cross-sectional study in school children found a positive association between leptin and BP, without adjustment for BMI,

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[a] Department of Public Health, Kindai University Faculty of Medicine, Osaka-sayama, [b] Department of Hygiene and Public Health, Kansai Medical University, Hirakata, [c] Department of Health Promotion and Education, Graduate School of Human Development and Environment, Kobe University, Kobe, Japan.

[*] Correspondence: Yuki Fujita, Department of Public Health, Kindai University Faculty of Medicine, 377-2 Oono-Higashi, Osaka-Sayama, Osaka 589-8511, Japan (e-mail: yfujita@med.kindai.ac.jp).

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and an inverse association after adjusting for BMI.[19] In addition, a population-based study of Danish and Norwegian children and adolescents reported that leptin mediated the association between BMI and BP, using empirical mediation analyses.[20]

Given that BMI consist not only of fat mass, but also of lean body mass, BMI is an unsuitable index for accurately assessing relationships between body fat and leptin. To accurately examine relationships between body fat, leptin, and BP, an accurate and precise technique for measuring body fat, as well as studies that involve the direct evaluation of body fat, are needed. Several techniques can be used to directly evaluate body fat. For example, bioelectrical impedance analysis (BIA) is commonly used to assess body fat in both clinical and research settings.[21] A previous epidemiological study found a positive relationship between leptin and body fat, as determined by BIA.[15] However, the accuracy and precision of BIA is limited for the following reasons: BIA generally underestimates body fat in obese subjects,[22] and moderate exercise or the ingestion of meals before BIA underestimates body fat percentage.[23,24] On the other hand, dual-energy X-ray absorptiometry (DXA) is a widely accepted method for evaluating fat mass, fat-free soft tissue mass, and bone mineral content,[25] and is a reliable and noninvasive modality.[26] DXA is increasingly being used as a criterion or reference for comparison with other body composition measurements.[21] The aim of the present epidemiological study was to investigate relationships between body fat and serum leptin levels, and between serum leptin levels and BP using DXA in school-aged healthy children, and to clarify the role of leptin as a mediator of the relationship between body fat and BP.

2. Methods

2.1. Study subjects

A cross-sectional survey was conducted in November or December 2010 and 2011 in Hamamatsu City, Japan. The source population was all 521 fifth grade children (268 boys and 253 girls) enrolled in Aritama Elementary School and Sekishi Elementary School. All parents and guardians were provided printed information about the study, and written consent was obtained prior to participation in the survey. Students were also allowed to decline participation on their own accord. This study was approved by the Ethics Committee of the Kindai University Faculty of Medicine.

2.2. Body fat measurements

Body fat was measured using a single DXA scanner (QDR-4500A, Hologic Inc, Bedford, MA) mounted on a mobile examination car at each school. An experienced radiological technologist performed all scans and scan analyses. Quality control of the DXA scanner was performed using the Step Phantom scan throughout the surveys. Subjects wore light clothing without metal objects while undergoing whole-body scanning. Fat mass (kg) was measured, and body fat percentage was calculated by dividing fat mass by body mass. Height-normalized index of fat mass (fat mass index, kg/m^2) was calculated by dividing fat mass in kilograms by the square of height in meters.

2.3. Anthropometric measurements

Body weight and height were measured in light clothing with no shoes. BMI (kg/m^2) was calculated by dividing body weight (in kilograms) by the square of height (in meters). To determine overweight and underweight status, international cut-offs corresponding to BMIs for adults of 25 kg/m^2 and 18.5 kg/m^2, respectively, were used. International cut-off points for overweight status have been defined as >20.55 kg/m^2 for boys and >20.74 kg/m^2 for girls,[27] and cut-off points for underweight status have been defined as <14.97 kg/m^2 for boys and <15.05 kg/m^2 for girls.[28]

2.4. BP measurements

Measurements of systolic BP (SBP) and diastolic BP (DBP) were conducted by an experienced physician using an automated device (BP-103i 2, OMRON COLIN, Tokyo, Japan). Arm circumference was measured midway between the olecranon and acromion process. Appropriate cuff size was selected based on arm circumference. BP was measured at least twice in the seated position with the left arm supported at the heart level after at least 5 minutes of rest. The mean value of 2 measurements was used for analysis.

2.5. Blood analysis

Blood samples were collected for measurement of leptin levels, and all serum samples were stored at − 80°C until analysis. Serum leptin levels were measured with a commercially available kit (Quantikine Human Leptin, R&D Systems, Inc, Minneapolis, MN). Intra- and interassay coefficients of variation were 2.0% and 5.2%, respectively.

2.6. Statistical analysis

Statistical analysis was performed with SAS software for Windows, ver. 9.4 (SAS Institute Japan Ltd, Tokyo, Japan). The level of significance was set at $P < .05$. Pearson correlation test and Spearman rank correlation test were used to assess associations between BP and covariates and associations between leptin levels and fat mass parameters. Multiple regression analysis was used to evaluate relationships between fat mass parameters (predictor) and BP (outcome). Multicollinearity was evaluated by pairwise correlation and the variance inflation factor (VIF), which was defined as the inverse of tolerance. Pairwise correlations greater than 0.70 to 0.80 between 2 independent variables or a VIF >4.0 are considered to signify a harmful multicollinearity between the 2 variables in a regression model.[29] Mediation analysis was used to examine the extent that leptin mediated the relationship between fat mass parameters and BP.

3. Results

Among the 521 children in the source population, we excluded 113 children who refused to participate or had missing data. Thus, the final study population included 400 children (197 boys and 203 girls) with a mean age of 11.2 ± 0.3 years. Table 1 summarizes general subject characteristics stratified by gender. Mean fat mass and SBP were 7.2 ± 3.7 kg and 105.5 ± 10.6 mm Hg, respectively, among boys, and 7.7 ± 3.2 kg ($P = .10$) and 106.7 ± 10.2 mm Hg ($P = .27$), respectively, among girls. Mean serum leptin levels were 2.5 ± 3.0 ng/mL among boys and 3.5 ± 3.0 ng/mL ($P < .01$) among girls.

Table 2 shows correlation coefficients between BP and covariates in boys and girls. SBP was significantly associated

Table 1

General subject characteristics by gender.

	Boys (N = 197)	Girls (N = 203)	*P*
Age, y	11.2 ± 0.3	11.2 ± 0.3	.56
Height, cm	141.4 ± 6.3	143.4 ± 6.9	<.01
Weight, kg	34.9 ± 7.3	35.3 ± 7.0	.62
BMI, kg/m^2	17.3 ± 2.6	17.0 ± 2.3	.23
Waist circumference, cm	63.2 ± 7.7	62.5 ± 6.4	.35
Fat mass, kg	7.2 ± 3.7	7.7 ± 3.2	.10
Fat mass index, kg/m^2	3.5 ± 1.7	3.7 ± 1.3	.23
Body fat percentage, %	19.2 ± 6.0	20.8 ± 4.6	<.01
SBP, mm Hg	105.5 ± 10.6	106.7 ± 10.2	.27
DBP, mm Hg	58.1 ± 7.4	59.6 ± 8.2	.06
Leptin, ng/mL	2.5 ± 3.0	3.5 ± 3.0	<.01
Overweight, N (%)	29 (14.7)	20 (9.9)	.17
Underweight, N (%)	16 (8.1)	17 (8.4)	1.00
Sedentary behavior (media use), N (%)			
<1 h/day	42 (21.3)	47 (23.2)	.17
≥1, <2 h/d	96 (48.7)	91 (44.8)	
≥2, <3 h/d	33 (16.8)	49 (24.1)	
≥3, <4 h/d	18 (9.1)	13 (6.4)	
≥4 h/day	8 (4.1)	3 (1.5)	
Pubic hair appearance, N (%)			
<5th Grade	0 (0.0)	13 (6.4)	<.01
5th Grade	10 (5.1)	55 (27.1)	
No appearance	187 (94.9)	135 (66.5)	

Values are presented as mean ± standard deviation, or N (percentage).
Fat mass index was calculated as fat mass divided by height squared.
BMI = body mass index, DBP = diastolic blood pressure, N = number, SBP = systolic blood pressure.

with fat mass parameters, leptin level, and height in both genders and with pubic hair appearance in boys. DBP was significantly associated with fat mass and leptin level in both genders and with fat mass index, body fat percentage, and height in boys.

Table 2

Correlation coefficients between blood pressure and covariates.

		Boys		Girls	
	Covariates	r	*P*	r	*P*
SBP	Fat mass	0.30	<.01*	0.33	<.01*
	Fat mass index	0.26	<.01*	0.31	<.01*
	Body fat percentage	0.21	<.01*	0.26	<.01*
	Leptin	0.30	<.01*	0.24	<.01*
	Height	0.26	<.01*	0.30	<.01*
	Sedentary behavior	0.12	.10†	0.14	.05†
	Pubic hair appearance	−0.16	.02†	−0.07	.29†
DBP	Fat mass	0.20	<.01*	0.16	.02*
	Fat mass index	0.20	<.01*	0.13	.07*
	Body fat percentage	0.17	.02*	0.08	.24*
	Leptin	0.16	.03*	0.22	<.01*
	Height	0.20	<.01*	0.13	.07*
	Sedentary behavior	0.03	.64†	−0.01	.89†
	Pubic hair appearance	−0.08	.29†	−0.02	.74†

* Pearson correlation coefficient.
† Spearman rank correlation coefficient. Fat mass index was calculated as fat mass divided by height squared.

Table 3 shows associations between body fat parameters and BP in boys and girls. All body fat parameters were significantly associated with SBP and DBP in boys (Model 1). In girls, all body fat parameters were significantly associated with SBP, and body fat percentage was significantly associated with DBP. However, after adjusting for leptin (Model 2), all body fat parameters were not significantly associated with BP in either gender, with VIF values of approximately 4.

Table 4 shows correlation coefficients between serum leptin levels and body fat parameters in boys and girls. Serum leptin levels were strongly correlated with every body fat parameter in both genders.

Table 3

Regression coefficient between fat mass and blood pressure.

			Boys			Girls		
Dependent variable		Independent variable	β	*P*	VIF	β	*P*	VIF
SBP	Model 1	Fat mass	0.21	<.01	1.21	0.27	<.01	1.28
		Height	0.21	<.01	1.21	0.11	.14	1.28
	Model 2	Fat mass	0.09	.53	4.92	0.18	.23	4.86
		Height	0.23	<.01	1.32	0.12	.11	1.35
		Leptin	0.13	.36	4.29	0.10	.44	4.18
	Model 1	Fat mass index	0.26	<.01		0.31	<.01	
	Model 2	Fat mass index	0.14	.34	4.16	0.22	.11	3.99
		Leptin	0.15	.30	4.16	0.11	.42	3.99
	Model 1	Body fat percentage	0.21	<.01		0.26	<.01	
	Model 2	Body fat percentage	−0.02	.87	2.97	0.08	.48	2.58
		Leptin	0.28	.02	2.97	0.24	.03	2.58
DBP	Model 1	Fat mass	0.17	.03	1.21	0.07	.36	1.28
		Height	0.09	.26	1.21	0.18	.02	1.28
	Model 2	Fat mass	0.01	.93	4.92	0.03	.83	4.86
		Height	0.11	.16	1.32	0.19	.02	1.35
		Leptin	0.17	.25	4.29	0.04	.76	4.18
	Model 1	Fat mass index	0.20	<.01		0.13	.07	
	Model 2	Fat mass index	0.08	.58	4.16	0.06	.68	3.99
		Leptin	0.14	.35	4.16	0.08	.58	3.99
	Model 1	Body fat percentage	0.17	.02		0.08	.24	
	Model 2	Body fat percentage	0.01	.95	2.97	−0.04	.71	2.58
		Leptin	0.20	.10	2.97	0.16	.15	2.58

Fat mass index was calculated as fat mass divided by height squared.
β = standardized regression coefficient, DBP = diastolic blood pressure, SBP = systolic blood pressure, VIF = variance inflation factor.

Table 4

Correlation coefficients between leptin levels and fat mass parameters.

	Boys		Girls	
	r	*P*	r	*P*
Fat mass	0.86	<.01	0.86	<.01
Fat mass index	0.87	<.01	0.87	<.01
Body fat percentage	0.81	<.01	0.78	<.01

r: Pearson's correlation coefficient. Fat mass index was calculated as fat mass divided by height squared.

Table 5 shows the mediating effects of leptin on the association between body fat parameters and BP by causal mediation analysis. In boys, of the total effect of fat mass index on SBP and DBP, 78.6% ($P=.01$) and 71.3% ($P=.25$) were mediated by leptin, respectively. In girls, of the total effect of fat mass index on SBP and DBP, 42.2% ($P=.11$) and 55.8% ($P=.38$), respectively, were mediated by leptin.

4. Discussion

The present study, which targeted school-aged children, found that leptin mediates the relationship between body fat and BP. A mediator refers to an intervening variable between a predictor variable and outcome variable, and lies in the causal pathway between the predictor of interest and outcome, thereby mediating the predictor's effects.[30] Regression analysis can be used to determine whether a variable mediates a relationship, based on the following analysis.[30] First, a relationship exists between a predictor variable and outcome variable. Second, a relationship also exists between the predictor variable and mediator variable. Third, the predictor variable should no longer be related to the outcome variable when adjusting for the mediator variable. In this regard, the present study found that body fat was significantly and positively associated with BP. Body fat was also strongly associated with serum leptin levels. However, the association between body fat and BP disappeared after adjusting for leptin. Based on this, we concluded that leptin mediates the relationship between body fat and BP. A cross-sectional study of children and adolescents from Denmark and Norway found that the association between BMI and BP was mediated by leptin, using empirical mediation analysis.[20] Our findings are consistent with that study. In the mediation analysis we performed, we observed a significant mediation effect of leptin on BP. This mediation effect showed a stronger tendency in boys than in girls. As shown in Table 1, there were no significant differences in body fat mass or BP between boys and girls, but serum leptin levels were lower in boys. This suggests that leptin may have a larger mediation effect on BP in boys.

Table 5

Proportion of mediating effect of leptin on the association between fat mass parameters and BP.

	Boys		Girls	
	%	*P*	%	*P*
Fat mass				
SBP	81.3	.08*	37.5	.18*
DBP	103.3	.08*	49.7	.60*
Fat mass index				
SBP	78.6	.03†	42.2	.11†
DBP	71.3	.25†	55.8	.38†
Body fat percentage				
SBP	129.4	<.01†	83.9	<.01†
DBP	101.2	.11†	125.6	.11†

Fat mass index was calculated as fat mass divided by height squared.
DBP = diastolic blood pressure, SBP = systolic blood pressure.
* Adjusted for height and sedentary behavior.
† Adjusted for sedentary behavior.

Multicollinearity may have been a factor in our multiple regression analysis, in which both leptin and body fat were used as independent variables in the same model to test for relationships with BP. The issue of multicollinearity arises when 2 or more independent variables in a regression model show high inter-correlations.[31] Multicollinearity increases the estimate of standard error of regression coefficients and results in wider confidence intervals, leading to a failure of rejecting the null hypothesis in significance tests.[31] Therefore, multicollinearity can lead to inaccurate conclusions regarding the relationship between outcome and predictor variables.[31,32] The present study found that the correlation coefficient between leptin and body fat is approximately 0.86, and that VIF values of fat mass parameters and leptin are higher than 4.0 when both leptin and body fat were used as independent variables in the same model to test relationships with BP. These findings are considered indicative of multicollinearity. Therefore, it may be inappropriate to adjust for leptin when evaluating the association between body fat and BP. It may also be inappropriate to adjust for body fat when evaluating the association between leptin and BP.

Previous studies have shown conflicting results regarding the association between leptin and BP,[17–19] which could be attributed at least in part to the mediating effect of leptin and/or multicollinearity described above. A follow-up study in Japanese male adolescents reported that although serum leptin levels were significantly associated with SBP, the 2 were no longer associated after adjusting for BMI.[18] A cross-sectional study in Brazil reported that leptin was positively correlated with SBP and DBP in obese children and adolescents, but no adjustments were made for body weight or BMI.[17] The Taipei Children's Heart Study reported a positive association between plasma leptin and BP, but an inverse association between these 2 after adjusting for BMI.[19] However, none of these studies tested whether leptin mediates the association between body weight and BP, or multicollinearity.

Although the mechanisms underlying the relationship between body fat and BP are not fully understood, 1 major mechanism may involve leptin. Serum leptin is a hormone that is secreted from adipose tissue and increases activation of the SNS in numerous organs such as the kidneys and blood vessels. Injection of leptin in rats increases activation of the SNS by increasing plasma norepinephrine and epinephrine levels.[33] Acute infusion of leptin in rabbits also increases BP via stimulation of the SNS.[11] These sympathoexcitatory effects of leptin have been shown to increase arterial pressure.[34,35]

The present study has several strengths. First, measurement of body fat was conducted using DXA, a technique that is more precise compared with BIA. Second, this was a single-center study, and thus there was no inter-center variation. Moreover, body composition was measured by a single radiological technologist. Third, the present population-based study reflects the health status of community-dwelling children in Japan. Anthropometric measurements of our study population were similar to those reported in the National Nutrition Survey in Japan (e.g., mean±SD of height in boys and girls, 144.1 ± 7.5 cm and 145.7 ± 7.5 cm, respectively; mean±SD of weight in boys and girls, 37.9 ± 8.0 kg and 38.5 ± 8.1 kg, respectively).[36]

The present study has also some limitations. First, this study was cross-sectional in design. Second, we did not obtain information regarding the tanner scale, physical activity, and nutrient intake, which are potential factors that can confound the association between leptin and BP in childhood.

In conclusion, we found that body fat is strongly associated with BP and serum leptin levels in school-aged healthy children, and that serum leptin levels are positively associated with BP. Our findings suggest that leptin mediates the relationship between body fat and BP, thereby highlighting leptin as a mediator that links body adiposity with BP elevation.

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Author contributions

Data curation: Yuki Fujita.
Formal analysis: Yuki Fujita.
Funding acquisition: Katsuyasu Kouda.
Investigation: Yuki Fujita, Katsuyasu Kouda, Kumiko Ohara, Harunobu Nakamura, Masayuki Iki.
Methodology: Yuki Fujita, Katsuyasu Kouda, Kumiko Ohara, Harunobu Nakamura, Masayuki Iki.
Project administration: Katsuyasu Kouda.
Writing – original draft: Yuki Fujita.
Writing – review & editing: Yuki Fujita, Katsuyasu Kouda, Kumiko Ohara, Harunobu Nakamura, Masayuki Iki.
Yuki Fujita orcid: 0000-0002-7490-258X.

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