[Environmental Health and Preventive Medicine 9, 41–46, March 2004]

Original Article

Serum Leptin Levels in Healthy Adolescents: Effects of Gender and Growth

Tiankui WANG[1], Ikuharu MORIOKA[2], Yoshiaki GOWA[3], Yuko IGARASHI[4], Nobuyuki MIYAI[1], Hiroichi YAMAMOTO[1], Mikio ARITA[2], Shintaro TAKEDA[2] and Kazuhisa MIYASHITA[1]

[1]*Department of Hygiene, Wakayama Medical University, Wakayama, Japan*
[2]*Nursing College, Wakayama Medical University, Wakayama, Japan*
[3]*Faculty of Human Science, Osaka International University, Osaka, Japan*
[4]*Akashi Junior High School affiliated with the Faculty of Human Development, Kobe University, Akashi, Japan*

Abstract

Objectives: The purposes of this paper were to evaluate the serum leptin levels in healthy adolescents and to establish standard age variation curves.

Methods: Nine hundred six (414 boys and 492 girls) healthy adolescents were investigated. The maximum increment age in height (MIA) was identified in 124 boys and 130 girls. The menarcheal age (MA) was obtained for 130 girls. Fasting leptin levels were measured by enzyme immunoassay. The MIA was calculated by proportional allotment of yearly height increments.

Results: Serum leptin levels did not change in boys and girls from the ages of 9 to 11. They decreased after the age of 11 in boys, while they increased in girls. Stepwise multiple regression analysis revealed that serum leptin levels were closely related to pubertal stage. The levels decreased remarkably after MIA in boys and increased remarkably after MA in girls. We drew standard age variation curves of serum leptin levels by calculating the 25th, 50th and 75th percentiles for each age in both boys and girls. The percentile curves for boys were divided into pre-MIA and post-MIA curves. Those for girls were divided into pre-MA and post-MA curves.

Conclusion: We have devised a potentially useful method for evaluating serum leptin levels in adolescents considering the effects of gender and growth.

Key words: leptin, standard curves, maximum increment age (MIA), menarcheal age (MA), adolescent growth

Introduction

Leptin, a product of the ob gene, is produced by the adiposities and plays a role in suppressing food intake and stimulating energy expenditure. The serum leptin level is closely correlated with fat mass and body mass index (BMI) (1) and thus is a potentially useful index in evaluating obesity. Serum leptin is associated with some diseases related to obesity (2, 3). In healthy adolescents, BMI and percentage of body fat are related to adolescent growth and vary greatly (4-6). Serum leptin levels show great variability in healthy adolescents even at the same age and the same gender. This variability does not allow the evaluation of serum leptin levels by a simple standard value. Thus, few studies have reported standard values of serum leptin levels in healthy adolescents (7, 8).

The purposes of our study were to clarify the serum leptin levels in healthy adolescents and to create standard age variation curves of serum leptin levels considering the effects of gender and adolescent growth.

Materials and Methods

Study population

The subjects were children in 5 elementary schools, 5 junior high schools and 6 high schools in Wakayama and Hyogo Prefectures, Japan. Medical checkups for lifestyle-related diseases were carried out for 906 (414 boys and 492 girls) adolescents aged 9 to 17 years from 1998 to 2001. The subjects underwent standardized medical examinations consisting

Received May. 29 2003/Accepted Dec. 12 2003
Reprint requests to: Tiankui WANG
Department of Hygiene, Wakayama Medical University, 811-1 Kimiidera, Wakayama, 641-8509, Japan
TEL: +81(73)441-0646, FAX: +81(73)441-0646
E-mail: wtiankui@hotmail.com

Table 1 Physical developments of subjects

Age (Years)	Boys				Girls			
	n	Height (cm)	Weight (kg)	BMI (kg/m^2)	n	Height (cm)	Weight (kg)	BMI (kg/m^2)
9	9	133.4±3.1	30.9±4.4	17.3±1.9	8	138.6±8.4	31.4±5.3	16.2±1.4
10	31	138.6±5.2	34.1±6.7	17.7±2.9	29	140.8±7.8	32.4±7.5	16.2±2.5
11	18	142.5±6.1	38.6±10.6	18.8±3.9	28	145.3±7.2	36.9±8.8	17.3±2.9
12	69	152.5±7.2	44.6±8.8	19.1±3.2	98	153.7±6.2	42.7±7.6	18.0±2.6
13	89	160.2±7.6	49.9±10.8	19.2±3.3	94	156.1±4.9	45.9±5.2	18.8±1.7
14	38	165.9±6.1	52.3±7.3	18.9±1.8	46	156.4±5.2	48.0±7.4	19.6±2.8
15	54	169.6±5.5	57.8±11.4	20.0±3.6	72	158.3±4.5	50.3±7.6	20.1±3.2
16	72	169.3±7.2	59.9±11.4	20.9±3.6	70	158.7±4.7	50.9±8.5	20.2±2.9
17	34	171.1±7.0	60.6±8.0	20.4±2.2	47	159.1±6.2	51.2±9.6	20.1±3.0
Total	414	160.2±12.7	50.8±12.9	19.5±3.3	492	154.8±7.8	45.9±9.4	19.0±2.9

of anthropometrical and physiological measurements. The subjects also completed a questionnaire about their personal and parental medical histories, medications used and habits of daily living. Written informed consent, as well as clearance for the examination data to be used, was obtained from the subjects and their guardians after they had received an explanation of the study aims and procedures. Table 1 shows the physical developments of the subjects. All the children were healthy according to assessment of personal medical history, physiological examination and hematological and biochemical tests. There were no clinically diagnosed obese or thin cases.

Medical examinations

Body height was measured to the nearest 0.1 cm with a portable steelyard. Body weight was determined using a calibrated electronic scale with a precision of 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m). Blood pressure was measured twice by a digital automatic sphygmomanometer employing a Korotkoff device (Omron, HEM-907) after 10 min of rest in a supine position. The average of the two readings was used as the representative value.

Blood samples were collected during fasting for hematological and biochemical analysis. Serum leptin levels were measured by enzyme-linked immunosorbant assay (ELISA). Luteinizing hormone (LH) was measured by immunoradiometric assay (IRMA).

To measure serum leptin levels, a Maxisorp 96-well microtiter plate (NUNC) was incubated overnight with monoclonal antibodies to human leptin (Otsuka). The wells were then rinsed with PBS with 1% BSA (Sigma). Next, serum samples were incubated in the wells overnight. After washing, the incubation was done again the following night with polyclonal antibodies to human leptin (Otsuka) as the primary antibody. The wells were washed after incubation and were exposed to a horseradish peroxidase–conjugated secondary antibody (Biosource). The peroxidase color reaction was developed by using orthophenylenediamine dihydrochloride (Sigma). The plate was then read on a microplate reader (Corona Electric, MTP-450) with an end-point program at an OD of 492 nm. The serum leptin concentration and the incubation times were optimized to ensure testing in the linear range.

Maximum increment age and menarcheal age

The maximum increment age in height (MIA) was calculated by proportional allotment of yearly height increments of adolescents, as previously reported (9, 10). The formula used in this method is as follows:

$$\mathrm{MIA}=A_{max}+\frac{I_{max}-I_{-1}}{(I_{max}-I_{-1})+(I_{max}-I_{+1})}-\frac{1}{2}$$

A_{max}=M–B+{(m+b)/12}
I_{max}: maximal annual increment in height
I_{-1}: annual increment in height in the year preceding that of I_{max}
$I_{+1:}$ annual increment in height in the year following that of I_{max}
A_{max}: median of I_{max} age class
M: measurement year of I_{max}
m: measurement month
B: birth year
b: birth month.

Menarcheal age (MA) was computed according to the menarcheal date shown in the health records of the subjects in junior high and high schools (9).

In terms of MIA, the pubertal stages were classified as MIA1 to MIA5. MIA1 represents the stage the year before MIA; MIA2, the year of MIA; MIA3, the first year after MIA2; MIA4, the second year after MIA2; and MIA5, more than two years after MIA2. The pubertal stages of the girls were classified as MA1 to MA5 in the same manner as MIA.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package v9.0 for Windows (SPSS Inc.). The distribution of serum leptin levels was lognormal; therefore, the values of serum leptin levels were transformed to the logarithms. Correlations between serum leptin level and age were tested by Pearson's correlation coefficient. To analyze factors relevant to serum leptin, multiple regression analysis (stepwise) was used. The following factors were included in the model as independent variables: age, pubertal stage, BMI, systolic and diastolic blood pressures, serum concentration of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG) and LH (girls) from the results of biochemical analysis. A

comparison of the differences in serum leptin levels at different pubertal stages was performed by one-way ANOVA. Any p-values less than 0.05 were considered statistically significant.

Results

1. Factors relevant to serum leptin levels in healthy adolescents

The median and interquartile ranges of serum leptin levels were computed at each age. The results are shown in Table 2 and Fig. 1. The serum leptin levels showed no significant change in the boys and girls from the ages of 9 to 11. However, they decreased remarkably in the boys and increased in the girls after the age of 11. The serum leptin levels were correlated positively with age in the girls ($r=0.32$, $p<0.001$), but negatively with age in the boys ($r=-0.35$, $p<0.001$).

In order to examine factors relevant to serum leptin levels, multivariate regression analysis was carried out. Among all subjects, MIA was obtained from 124 boys and 130 girls, and MA was obtained from 130 girls. The significant variables selected for the models are shown in Table 3. For boys, serum leptin levels were predicted by BMI, pubertal stage according to MIA, and TC. For girls, pubertal stage according to MIA, HDL-C, LH and TC were major factors relevant to serum leptin levels when we used the pubertal stages according to MIA. Even when the pubertal stages according to MA were used, pubertal stage according to MA, HDL-C and LH were major factors.

The serum leptin levels in the boys and girls according to the pubertal stages are shown in Table 4. The serum leptin levels in the boys decreased significantly from MIA2. In contrast, those in the girls increased continuously from MIA1 to MIA5 and from MA1 to MA5. Those in the girls were significantly higher in MA3 to MA5 than those in MA1.

2. Standard age variation curves of serum leptin levels according to pubertal stages

First, using 906 subjects' data, we calculated the 25th, 50th and 75th percentiles for each age in boys and girls and drew the dashed lines in Fig. 2 as the percentile curves. The curves were smoothed by eye, but very little smoothing was necessary at the 50th percentile curves. In Fig. 2, the dashed lines are seen only after the age of 11 for boys or from the ages of 11 to 12 for girls because the solid lines overlap the dashed lines before the age of 11.

Second, to establish the standard age variation curves of serum leptin levels considering the effects of adolescent growth, we classified 237 subjects, whose MIA for boys or MA for girls were identified, into two groups. For the boys, one group was pre-MIA, and the other was post-MIA. For the girls, one group was pre-MA, and the other was post-MA. We then calculated the 25th, 50th and 75th percentiles for each age in the groups and drew the percentile curves (solid line in Fig.2). For the boys, the left solid lines of pre-MIA represent the serum leptin levels before MIA, and the right solid lines of post-MIA represent those after MIA. The 50th percentile curve of pre-MIA in the boys does not significantly change until the age of 11 and slowly decreases after that age. After MIA, the serum leptin

Table 2 Serum leptin levels (ng/ml) by 25th, 50th and 75th percentiles in boys and girls according to age from 9 to 17

Age (Years)	Boys, percentile				Girls, percentile			
	n	25th	50th	75th	n	25th	50th	75th
9	9	1.7	5.2	10.3	8	2.7	3.6	4.5
10	31	1.6	4.7	13.0	29	1.7	3.4	6.6
11	18	2.1	6.0	14.8	28	2.7	4.6	5.9
12	69	0.8	2.2	7.6	98	2.5	3.9	8.9
13	89	0.8	1.6	3.7	94	4.2	5.8	8.9
14	38	0.9	1.4	3.4	46	4.6	7.4	9.0
15	54	0.6	1.1	2.5	72	4.9	6.9	9.5
16	72	0.5	1.1	2.4	70	5.0	8.0	10.9
17	34	0.6	1.1	1.8	47	5.3	7.6	11.0
Total	414	0.7	1.5	4.1	492	3.6	5.9	9.2

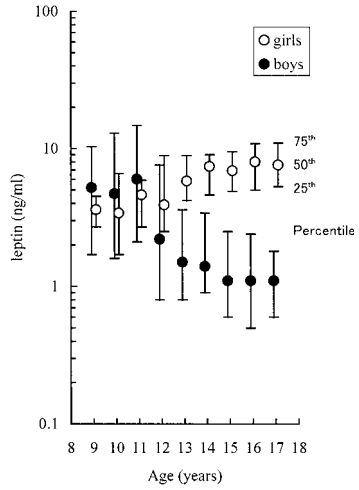

Fig. 1 Serum leptin level according to age

Table 3 Variables relevant to serum leptin levels by multivariate regression analysis (stepwise)

a) MIA was used as an index of pubertal stages in boys and girls

	Variables	β	R^2	P
Boys				
	BMI	0.659	0.496	<0.001
	Stage (MIA)	–0.398		<0.001
	TC	0.195		0.007
Girls				
	Stage (MIA)	0.282	0.177	0.001
	HDL-C	–0.252		0.003
	LH	0.211		0.012
	TC	0.172		0.044

b) MA was used as an index of pubertal stages in girls

	Variables	β	R^2	P
Girls				
	Stage (MA)	0.269	0.142	0.002
	HDL-C	–0.224		0.009
	LH	0.192		0.025

Stage (MIA) shows pubertal stages according to MIA.
Stage (MA) shows pubertal stages according to MA.
β: Standardized regression coefficient.
R^2: Adjusted R square.
Leptin and LH data were used after logarithmic transformation.

Table 4 Serum leptin levels (ng/ml) by 25th, 50th and 75th percentiles in boys and girls according to pubertal stage

Stage (MIA)	n	Percentile			Stage (MA)	n	Percentile		
		25th	50th	75th			25th	50th	75th
Boys									
1	26	1.4	2.8	5.9					
2	19	0.6	0.8a	2.0					
3	16	0.7	0.9a	1.8					
4	20	0.5	1.1a	2.1					
5	43	0.4	0.8b	2.1					
Girls					Girls				
1	12	2.7	4.5	4.9	1	21	2.5	3.9	6.4
2	14	3.3	6.2	7.9	2	29	3.4	5.6	8.8
3	30	3.2	5.6	8.1	3	35	4.6	7.8a	9.8
4	28	5.5	7.8	9.7	4	20	6.3	8.1b	10.3
5	46	5.8	8.0	11.5	5	25	6.3	7.9b	11.2

Stage (MIA) shows pubertal stages according to MIA.
Stage (MA) shows pubertal stages according to MA.
The significance of differences was determined by ANOVA after logarithmic transformation.
a: Significant difference compared with MIA1 or MA1 ($p<0.05$).
b: Significant difference compared with MIA1 or MA1 ($p<0.01$).

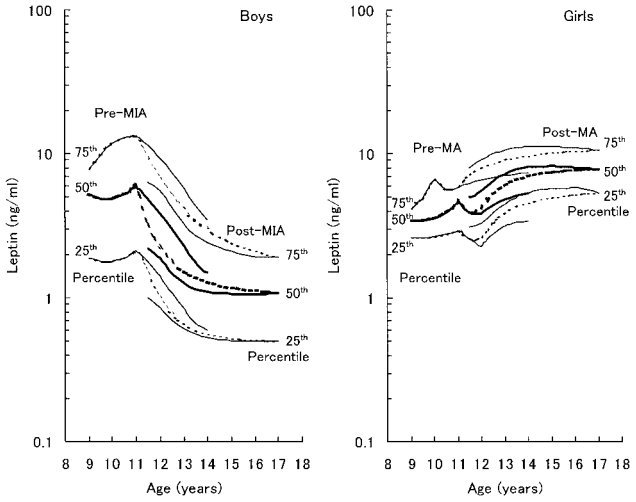

Fig. 2 Standard age variation curves of serum leptin levels in boys and girls

levels remarkably decrease to the left solid line of post-MIA, which continues to decrease slowly. For the girls, the left solid lines of pre-MA represent the serum leptin levels before MA, and the right solid lines of post-MA represent those after MA. Before MA, the 50th percentile curve of pre-MA increases from the ages of 9 to 11 and transitionally decreases from the ages of 11 to 12 because of the remarkable body growth around the MIA period. After MA, the serum leptin levels increase remarkably to the left solid line of post-MA, which continues to increase slowly.

Discussion

Several studies on children have demonstrated a large variation in serum leptin levels according to age and gender (11, 12). In our present study, the serum leptin levels in the girls increased progressively in an age-related way. In contrast, the serum leptin levels in the boys were similar to those in the girls at the ages of 9 to 11, but a striking decrease occurred after the age of 11. The different pattern between boys and girls has also been reported in previous cross-sectional studies (7, 13) and in a longitudinal study of boys (14). For adolescent children, we should assess serum leptin levels according to not only chronological age variations but also pubertal stage variations because children who are in the same age group are probably at different pubertal stages.

Adolescent growth induces large individual differences in height and other variables. Our results of multivariate regression analysis revealed that pubertal stage strongly predicted serum leptin levels in both sexes. This finding shows that serum leptin levels in healthy adolescents may mainly change in relation to changes in pubertal stage in both sexes. Thus, pubertal stages should be considered when drawing standard age variation curves.

Outside Japan, many studies on serum leptin levels at different pubertal stages have been carried out according to the Tanner stages (11, 12, 15). In our study, we used MIA and MA to classify the pubertal stages. MIA shows the maximum growth speed in height. It is an objective and easily obtainable index to evaluate the growth of adolescent children (10) because height is regularly (at least once a year) measured in Japanese elementary, junior high and high schools. MA is an objective and accurate index for the grade of sexual maturity in girls (16, 17). Using MA, we can easily understand the stages of adolescent growth in girls. MA is closely related to MIA and comes 1.5 years after MIA (9, 10). In our study, the serum leptin levels in the boys decreased significantly during the MIA2 pubertal stage and decreased gradually from MIA3 to MIA5. However, the levels in the girls increased slowly from the MIA1 to MIA5 pubertal stages. The serum leptin levels in the girls increased more remarkably through the pubertal stages according to MA than through the pubertal stages according to MIA. These results were not contradictory to those of the research using pubertal stages classified by the Tanner stages (7, 11, 15).

In our study to establish standard age variation curves, we

set chronological age as the basis. As markers of the pubertal stages, we adopted MIA for the boys and MA for the girls. Because MA is more obvious than MIA and because serum leptin levels significantly increased after MA, MA was adopted for the girls instead of MIA. MIA or MA were represented by two lines for the percentile curves: pre-MIA or MA and post-MIA or MA. The tendencies of the two lines after the age of 11 were the same, but the post-MIA lines were located below the pre-MIA lines, and the post-MA lines were located above the pre-MA lines.

We selected 237 subjects, whose MIA for boys or MA for girls were identified, to establish the standard age variation curves of serum leptin levels. The 25th, 50th and 75th percentiles at each age showed larger variation than those using all subjects. However, the trends of the smoothed lines corresponded to those using all subjects. Thus, rectifying the lines was not necessary.

The subjects were schoolchildren in 16 schools in Wakayama and Hyogo Prefectures. We asked for the cooperation of the schools which have grasped well the information on growth and the habits of daily living of the schoolchildren. However, the mean heights and weights of the subjects corresponded to the physical developments in the Statistical Report of the School Health Survey (18). The subjects lived normal lives and showed normal eating styles, judging from the results of the questionnaires on habits of daily living. No regional difference was obtained. Thus, our students seemed to be normal schoolchildren in Japan.

Standard age variation curves were established for healthy adolescents. The standards for serum leptin levels for healthy adolescents are shown in the Appendix. In order to verify these standard age variation curves, we selected one boy (11 years old at the first year) and one girl (13 years old at the first year) whose serum leptin levels had been measured continuously for four years. The serum leptin level of the boy was slightly higher than that of the 50th percentile of pre-MIA boys in the first year. In the second year it gradually decreased and corresponded to the 50th percentile curve of pre-MIA boys. After his MIA, it dropped remarkably. However, it was still on the 50th percentile curve of post-MIA boys. The serum leptin of the girl was slightly lower than that of the 50th percentile of pre-MA girls. After her MA, it increased remarkably and then was on the 50th percentile curve of post-MA girls at the third and fourth years. During these four years we could not find significant changes in BMI in either gender (boy; 15.8–17.6, girl; 17.1–18.7). By using these curves, we can accurately distinguish boys and girls with high serum leptin levels in a healthy adolescent population, excluding the effects of gender and growth.

Industrialized societies around the world are experiencing an epidemic of childhood obesity. It is important to apply preventive strategies earlier. These standard age variation curves can be applied to health education to prevent obesity (19). Further study is needed in order to apply the standard age variation curves to the evaluation of serum leptin levels in overweight or obese adolescents.

Conclusion

We established standard age variation curves of serum leptin levels in healthy adolescents by calculating the 25th, 50th and 75th percentiles for each age in both boys and girls. The percentile curves in boys were divided into pre-MIA and post-MIA curves. Those in girls were divided into pre-MA and post-MA curves. These curves are a potentially useful method for evaluating serum leptin levels in adolescents considering the effects of gender and growth.

References

(1) Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425–432.

(2) 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.

(3) Tershakovec AM, Kuppler KM, Zemel BS, Katz L, Weinzimer S, Harty MP, Stallings VA. Body composition and metabolic factors in obese children and adolescents. Int J Obes Relat Metab Disord 2003; 27: 19–24.

(4) Arslanian S, Suprasongsin C, Kalhan SC, Drash AL, Brna R, Janosky JE. Plasma leptin in children: relationship to puberty, gender, body composition, insulin sensitivity, and energy expenditure. Metabolism 1998; 47: 309–312.

(5) Roemmich JN, Clark PA, Berr SS, Mai V, Mantzoros CS, Flier JS, Weltman A, Rogol AD. Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. Am J Physiol 1998; 275: E543–551.

(6) Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI. Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol Metab 1997; 82: 2148–2152.

(7) Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol Metab 1997; 82: 2849–2855.

(8) Ahmed ML, Ong KK, Morrell DJ, Cox L, Drayer N, Perry L, Preece MA, Dunger DB. Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. J Clin Endocrinol Metab 1999; 84: 899–905.

(9) Shiraishi T, Morioka I, Yoshida Y, Konishi H, Mino T, Nagal N, Matshmoto K, Takeda S. The Temporai Relationship between Maximum Increment Age in height and Menarcheal Age in Japanese girls. Wakayama Med Rep 1988; 30: 147–151.

(10) Matsumoto K, Miyata H, Mino T, Takeda S. A calculation method of the maximum growth age in height. Wakayama Med Rep 1978; 21: 79–86.

(11) Ellis KJ, Nicolson M. Leptin levels and body fatness in children: effects of gender, ethnicity, and sexual development. Pediatr Res 1997; 42: 484–488.
(12) Horlick MB, Rosenbaum M, Nicolson M, Levine LS, Fedun B, Wang J, Pierson RN Jr, Leibel RL. Effect of puberty on the relationship between circulating leptin and body composition. J Clin Endocrinol Metab 2000; 85: 2509–2518.
(13) Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab 1997; 82: 2904–2910.
(14) Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab 1997; 82: 1066–1070.
(15) Hassink SG, Sheslow DV, de Lancey E, Opentanova I, Considine RV, Caro JF. Serum leptin in children with obesity: relationship to gender and development. Pediatrics 1996; 98: 201–203.
(16) Morioka I, Miyashita K, Gowa Y, Ishii N, Kuroda M, Takeda S, Hirase E, Matsumoto K. Physical characteristics at menarche in high school girls. Adolescentology 1996; 14: 116–120.
(17) Gowa Y, Shiraishi T, Morioka I, Miyashita K, Takeda S, Hirase E, Matsumoto K. Temporal relationship between menarche and physical growth in high school girls. Adolescentology 1995; 13: 249–254.
(18) Ministry of Education, Culture, Sports, Science and Technology. Means and standard deviations of height, weight and sitting height by age. In: Statistical Report of the School Health Survey 2000. Tokyo; Ministry of Education, Culture, Sports, Science and Technology. 2000; 3
(19) Kondo T, Yoshida A, Okada R, Kanda T, Kobayashi I, Murakami M. Circulating leptin: a marker of health in female students. J Int Med Res 2002; 30: 109–115.

Appendix

Table Serumleptin levels (ng/ml) by 25th, 50th and 75th percentiles in boys and girls according to age and pubertal stage. For source of data and method construction see text

Age (Years)	Boys, percentile						Girls, percentile					
	25th		50th		75th		25th		50th		75th	
	Pre-MIA	Post-MIA	Pre-MIA	Post-MIA	Pre-MIA	Post-MIA	Pre-MA	Post-MA	Pre-MA	Post-MA	Pre-MA	Post-MA
9	1.9		5.2		7.7		2.6		3.4		4.1	
10	1.8		4.9		11.6		2.7		3.6		6.6	
11	2.1		5.9		13.2		2.9		4.6		5.8	
12	1.4	0.9	3.8	1.9	9.4	5.5	2.3	3.5	3.8	5.5	6.6	9.0
13	0.8	0.6	2.3	1.3	5.7	3.2	3.1	4.5	4.8	7.2	7.1	10.5
14	0.6	0.5	1.5	1.1	3.5	2.4	3.4	5.4	5.3	8.1	7.4	11.2
15		0.5		1.1		2.1		5.7		8.2		11.2
16		0.5		1.1		1.9		5.8		8.0		10.9
17		0.5		1.1		1.9		5.3		7.8		10.6