0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 10
Printed in U.S.A.

Leptin Is Inversely Related to Age at Menarche in Human Females*

VELIMIR MATKOVIC, JASMINKA Z. ILICH, MARIO SKUGOR, NANCY E. BADENHOP, PREM GOEL, ALBERT CLAIRMONT, DINO KLISOVIC, RAMZI W. NAHHAS, AND JOHN D. LANDOLL

Bone and Mineral Metabolism Laboratory, Departments of Physical Medicine, Medicine, and Nutrition, Davis Medical Research Center (V.M., J.Z.I., M.S., N.E.B., A.C., D.K., J.D.L.); and the Department of Statistics (P.G., R.W.N.), Ohio State University, Columbus, Ohio 43210

ABSTRACT

Over the last century there has been a trend toward an earlier onset of menarche attributed to better nutrition and body fatness. With the discovery of the obesity gene and its product, leptin, we reexamined this hypothesis from a new perspective. As delayed menarche and leanness are considered risk factors for osteoporosis, we also evaluated the relation between leptin and bone mass. Body composition and serum leptin levels were measured, and the timing of menarche was recorded in 343 pubertal females over 4 yr. Body composition was measured by dual x-ray absorptiometry, and leptin by a new RIA. All participants were premenarcheal at baseline (aged 8.3–13.1 yr). Leptin was strongly associated with body fat ($r = 0.81$; $P < 0.0001$) and change in body fat ($r = 0.58$; $P < 0.0001$). The rise in serum leptin concentration up to the level of 12.2 ng/mL (95% confidence interval, 7.2–16.7) was associated with the decline in age at menarche. An increase of 1 ng/mL in serum leptin lowered the age at menarche by 1 month. A serum leptin level of 12.2 ng/mL corresponded to a relative percent body fat of 29.7%, a body mass index of 22.3, and body fat of 16.0 kg. A gain in body fat of 1 kg lowered the timing of menarche by 13 days. Leptin was positively related to bone area ($r = 0.307$; $P < 0.0001$) and change in bone area ($r = 0.274$; $P < 0.0001$).

A critical blood leptin level is necessary to trigger reproductive ability in women, suggesting a threshold effect. Leptin is a mediator between adipose tissue and the gonads. Leptin may also mediate the effect of obesity on bone mass by influencing the periosteal envelope. This may have implications for the development of osteoporosis and osteoarthritis. (*J Clin Endocrinol Metab* **82:** 3239–3245, 1997)

THE AGE AT which women develop menarche is an important factor in determining population size, breast cancer, and osteoporosis, all of which are of enormous present-day concern (1–3). Therefore, understanding all potential factors responsible for early menarche is of considerable interest. Over the last century there has been a trend toward earlier onset of puberty and menarche in affluent societies, attributed primarily to the improvement in nutritional status and general health of younger generations of women (4, 5). The onset of menarche was closely related with the achievement of a certain body weight (6) or percent body fat (7, 8). The only explanation given for this association was the influence of an unknown mediator on the hypothalamic-pituitary-gonadal axis. Kennedy and Mitra suggested that a metabolic signal related to fat stores is a signal for estrus in the rat (9, 10).

With the recent discovery of the obesity gene (*ob*) and its product, leptin (11), it is possible to reexamine the relationship between body fatness and the timing of menarche from a new perspective. In support of this are recent discoveries in *ob/ob* mice treated with leptin showing signs of early onset of ovarian maturation and reproductive function (12–14). Similar data for humans do not exist.

We, therefore, measured body composition and serum leptin levels every 6–12 months and recorded menarche in a cohort of young females entering puberty over a 4-yr period of follow-up. We also examined the relationship between leptin and bone mass of pubertal girls, knowing the role body weight has in predicting bone mass and in osteoporosis prevention.

Subjects and Methods

Subjects

Three hundred and forty-three healthy Caucasian girls were recruited from the school districts in central Ohio to participate in a 4-yr longitudinal study of skeletal development during adolescence. All subjects were premenarcheal at baseline, aged 8.3–13.1 yr, and in pubertal stage 2, based on either breast or pubic hair development [mean of the two expressed as sexual maturity index (SMI)]. They were all ambulatory, free of any acute or chronic disease, and did not take medications known to affect body weight. Baseline metabolic and skeletal characteristics of the entire population were previously presented (15, 16). All participants and their parents gave informed consent according to guidelines of the human subjects committee at Ohio State University. Menstrual history, anthropometry, nutritional status, energy expenditure, body composition, and blood samples were obtained every 6–12 months over a 4-yr period.

Anthropometry, nutritional status, and energy expenditure

The subject's weight was measured to the nearest 0.1 kg in normal indoor clothing without shoes. Standing height was recorded without shoes on a wall-mounted stadiometer to the nearest 0.1 cm with man-

Received May 15, 1997. Revision received June 27, 1997. Accepted July 2, 1997.

Address all correspondence and requests for reprints to: V. Matkovic, M.D., Ph.D., Bone and Mineral Metabolism Laboratory, Davis Medical Research Center, Ohio State University, 480 West 9th Avenue, Columbus, Ohio 43210.

* This work was supported in part by grants from the NIH (RO1-AR-40736–01A1, CRC-NIH M01-RR-00034, and NRICGP/USDA-37200–7586), Procter & Gamble Co., Ross Abott Laboratories, and National Dairy Management.

dible plane parallel to the floor. Pubertal stage based on breast development and pubic hair distribution was self-assessed by marking corresponding figures of sexual development (15–17). The estimates of energy intake were obtained from the 3-day dietary food records taken over 2 weekdays and 1 weekend. Energy intake from the food record was analyzed by Nutritionist III software package, version 8.5 (The Hearst Corp., San Bruno, CA). Energy expenditure was estimated every 6 months by using a 2-day (1 week day and 1 weekend day) activity record (18). The process requires participants to record their dominant activity in 15-min periods throughout 24 h. Recording was simplified by having subjects use an activity list. Each activity record was analyzed separately, and the mean activity, measured as the total daily energy expenditure, was calculated.

Assessment of body composition

Body composition was measured by a dual x-ray absorptiometry technique with a pencil beam Lunar DPX-L machine with 1.3q software (Lunar, Madison, WI). All measurements were performed using the medium speed scan mode with 8 cm/s detector speed and sample size 4.8 over 9.6 mm, with the subject in the supine position along the middle axis of the scanning table and within the field of view of the detector. The data for total body bone mineral content (TBBMC), body fat, and lean body mass (LBM) were recorded. The precision errors [coefficients of variation (CVs)] for the measurements of TBBMC, body fat, and LBM in our laboratory were 0.9%, 2.6%, and 1.1%, respectively (15, 16).

Serum leptin, gonadotropins, and estradiol

After obtaining a blood sample, serum was stored at −80° C. Serum leptin was measured using the new sensitive RIA described by Ma *et al.* (19) at Linco Research (St. Charles, MO). The percent CVs within and between runs for leptin ranged from 3.4–8.3% and from 3.6–6.2%, respectively. FSH was measured by a standard sandwich immunoassay procedures; the within-run CV ranged from 1.6–2.4%, and the between-run CV ranged from 2.8–3.2% (Technicon, Immuno 1 System, Miles, Tarrytown, NY). LH and estradiol were measured by Coat-A-Count RIA kits (Diagnostic Products Corp., Los Angeles, CA). Within-run and between-run CVs for LH were 1.0–1.6% and 2.2–7.1%, respectively; and within-run and between-run CVs for E_2 were 4.3–7.0% and 4.2–8.1%, respectively.

Statistical analysis

Basic descriptive statistics were used to describe each variable at three time points (cross-sectional models: early premenarche, before menarche, and at menarche; Table 1). The data for menarche and leptin before menarche (highest r^2) were initially smoothed by using a LOWESS smoother (making no *a priori* assumptions about the model) to assess the overall shape of the plot. The plot indicated that the variables of interest could be modeled as a segmented linear and plateau regression. SAS Proc NONLINR (SAS, Cary, NC) and S-Plus (StatSci, MathSoft, Seattle, WA) were used to fit the model running on a HP6000/615 work station. For this model, we also determined the 95% confidence interval (CI) at the change point (serum leptin level) between the regression lines. The association between leptin and menarche was also evaluated according to the different timing of menarche used as a categorical variable (menarche groups: group 1 = still premenarcheal after 4 yr of follow-up, to group 5 = developed menarche during the first year of follow-up; results presented as box plots).

To evaluate the determinants of menarche and bone variables, standard multiple regression and forward stepwise regression analyses were used. All predictor variables and first order interactions were included initially in the regression model, then, one by one, the most significant predictor variables (smallest *P* value) were added to the model. To avoid growth-related artifacts in the identification of menarche, height, body composition variables, and index of pubertal development (SMI) were included in each model. The goal of the regression analysis was to obtain a predictor model that contained relatively few significant determinant variables with the highest coefficient of determination (r^2). Due to the skewness of serum leptin and body fat data, the log-transformed variables were used as well. All calculations were performed using Data Desk Professional version 4.1 (Data Description, Ithaca, NY) and Statistica/Mac version 4.1 (StatSoft, Tulsa, OK). $P < 0.05$ was considered significant throughout.

Results

The relation between serum leptin and absolute and relative body fat in adolescent females before menarche is shown in Fig. 1, A and B. The association between serum leptin and body fat is best represented by the linear regression model; r = 0.81, $P < 0.0001$, equation: leptin (ng/mL) = $-0.27 + 0.00078 \times$ body fat (g). The scatter plot of serum leptin and percent body fat was best described by an exponential growth model; variance explained = 68%, equation: leptin (ng/mL) = $0.22 + \exp(0.61 + 0.057 \times$ percent body fat), $P < 0.0001$. Leptin also strongly correlated with BMI, with r = 0.75, $P < 0.0001$, equation: leptin (ng/mL) = $-21.09 + 1.27 \times$ BMI. The relation between the change (Δ) in serum leptin level and body fat over time in young females is presented in Fig. 1C; r = 0.58, $P < 0.0001$, linear equation: leptin (ng/mL/3 yr) = $-0.15 + 0.001 \times \Delta$ body fat (g/3 yr).

The relation between the serum leptin concentration and menarche in young females is presented in Fig. 2A. Body composition and serum leptin measurements obtained before menarche were used for this analysis. The scatterplot shown with linear-plateau least squares fit model with equations: menarche (yr) = 13.43–0.082 × leptin (ng/mL) for

TABLE 1. Descriptive statistics of anthropometry and body composition, energy balance, and serum leptin in young females at early premenarche (~23 months before the onset of the first menstrual period), before menarche (~6 months before the onset of the first menstrual period), and at menarche (±2 months)

Variable	Early premenarche	Before menarche	Menarche
Age (yr)	10.9 ± 0.8	12.2 ± 1.0	12.8 ± 0.9
Sexual maturity index	1.9 ± 0.4	3.0 ± 0.8	3.5 ± 0.7
Ht (cm)	145.8 ± 7.1	155.2 ± 6.8	158.5 ± 6.3
Wt (kg)	40.4 ± 8.2	49.2 ± 10.4	52.9 ± 10.8
Body mass index	18.9 ± 3.1	20.4 ± 3.6	21.0 ± 3.7
Body fat (g)	9,688 ± 5,563	12,252 ± 7,540	13,519 ± 7,965
% Body fat	24.3 ± 9.1	25.0 ± 9.5	25.7 ± 9.4
Lean body mass (g)	27,989 ± 3,957	33,786 ± 4,309	36,117 ± 4,189
Total body BMC (g)	1,335 ± 235	1,703 ± 312	1,886 ± 325
Energy intake (kJ/day)	8,289 ± 1,736	8,109 ± 1,724	8,017 ± 1,820
Energy expenditure (kJ/day)	7,590 ± 1,812	9,280 ± 2,272	10,100 ± 2,397
Serum leptin (ng/mL)	8.6 ± 6.4	9.2 ± 7.2	9.9 ± 7.8

Results presented as the mean ± SD. Serum FSH (5.9 ± 1.9 mIU/mL), LH (2.4 ± 2.5 mIU/mL), and E_2 (24.2 ± 16.1 pg/mL) were measured in blood samples before menarche.

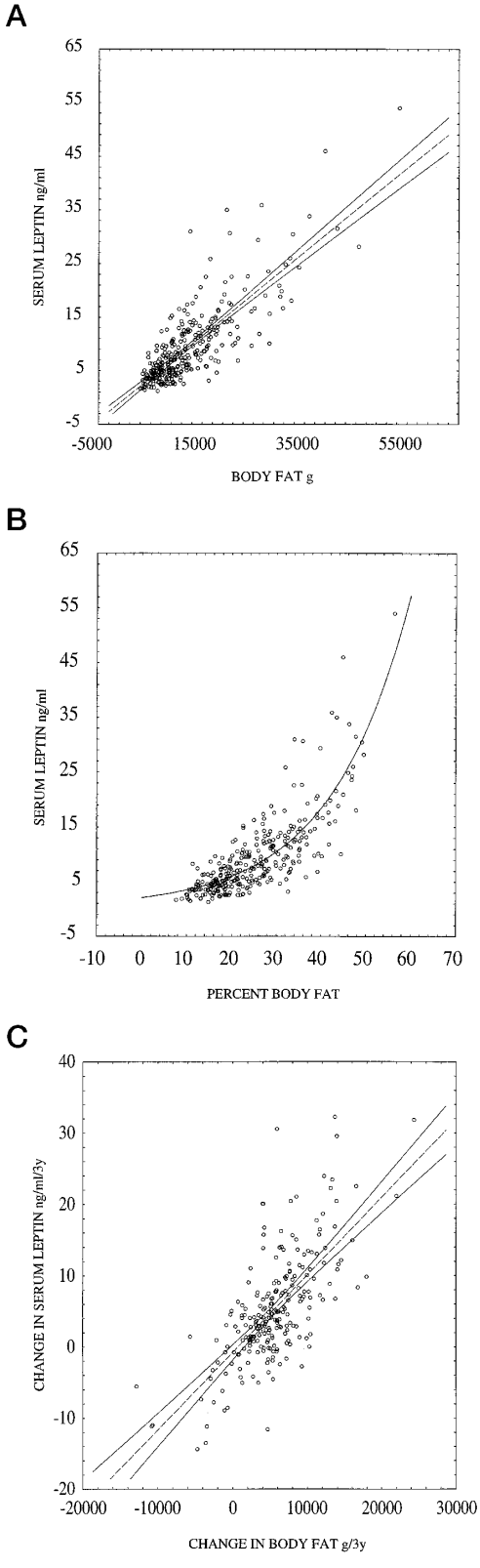

FIG. 1. A, Relation between serum leptin and body fat in 314 young females before menarche; B, relation between serum leptin and percent body fat in young females before menarche; C, relation between the change (Δ) in serum leptin level and body fat over time in young females. The scatterplots are shown with the 95% confidence curves and regression lines.

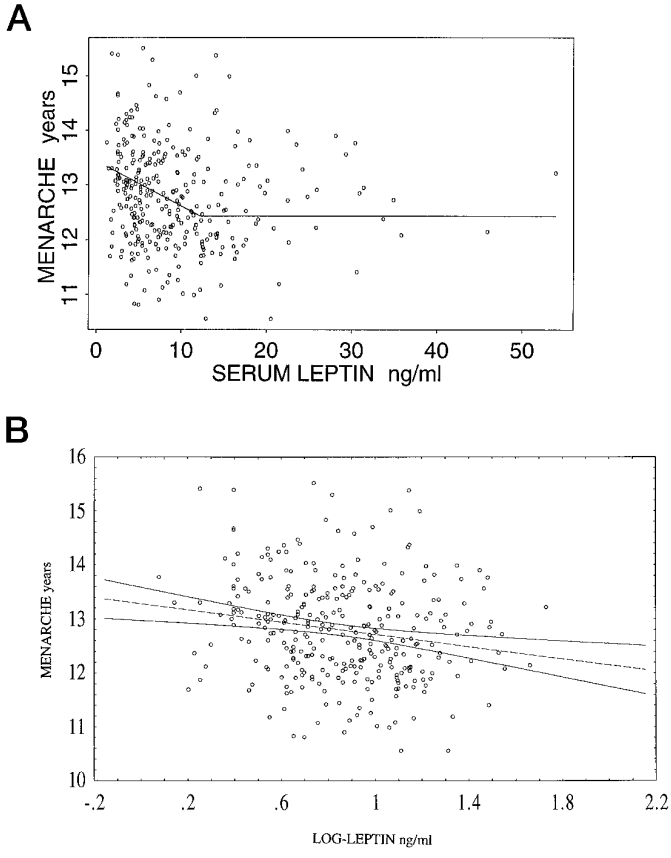

FIG. 2. A, Relation between serum leptin concentration before menarche and age at menarche in 311 young females. The scatter plot is shown with the linear-plateau least squares fit model. The 95% CI for the slope in the lower region, showing the inverse relation between menarche and leptin, is −0.12 to −0.05 (yr). The 95% CI for the change point (serum leptin; $x \times 0$) is 7.6–16.7 ng/mL. B, Relation between log-transformed serum leptin and menarche. The scatter plot is shown with the 95% confidence curves and the regression line.

serum leptin less than 12.2 ng/mL as menarche (yr) = 12.2 + 0 × leptin (ng/mL) otherwise. The 95% CI for the slope in the lower region, showing the inverse relation between menarche and leptin, is −0.12 to −0.05 (yr). The 95% CI for the change point (serum leptin; $x \times 0$) is 7.6–16.7 ng/mL. The negative association between menarche and leptin is even better depicted with Log-transformed leptin data (Fig. 2B); equation: menarche (yr) = 13.1–0.4 × log-leptin, r = −0.184, $P < 0.001$. The timing of menarche (years) and the change (Δ) in body fat over time were negatively related; r = −0.18, $P < 0.0017$, linear equation: menarche (yr) = 13.01–0.000035 × Δ body fat (g/3 yr). Table 2 shows the summary of the forward stepwise regression procedures with regression weights for menarche as the dependent variable in the models with different obesity, anthropometry, and energy balance parameters as predictor variables measured before menarche (−6 months). Independent variables with corresponding model r^2 were as follows: model A: log-leptin, height, SMI, LBM, TBBMC, energy intake, energy expenditure, $r^2 = 0.26$; model B: log-percent body fat, height, SMI, LBM, TBBMC, energy intake, energy expenditure, $r^2 = 0.24$; model C: log-body fat, height, SMI, LBM, TBBMC, energy intake, energy expendi-

TABLE 2. Summary of the forward stepwise regression procedures with regression weights for menarche as the dependent variable in the models with different obesity, anthropometry, and energy balance parameters as predictor variables measured before menarche (−6 months) (n = 291)

Menarche as dependent variable	Step	Selected variable	Partial r^2	B	SE of B	*t*	*P* level
Model A	1	SMI	0.185	0.380	0.067	5.7	0.0000
	2	Ht	0.047	0.033	0.008	4.2	0.0000
	3	Log leptin	0.023	−0.459	0.155	−3.0	0.0034
Model B	1	SMI	0.185	0.390	0.067	5.8	0.0000
	2	Ht	0.047	0.033	0.008	4.2	0.0000
	3	Log percent body fat	0.015	−0.677	0.280	−2.4	0.0163
Model C	1	SMI	0.185	0.392	0.067	5.8	0.0000
	2	Ht	0.047	0.036	0.008	4.5	0.0000
	3	Log body fat	0.013	−0.453	0.202	−2.2	0.0258

The selected independent variables with corresponding model r^2 in models A, B, and C are shown.

ture, $r^2 = 0.24$ (n of cases = 291). The corresponding intercepts were 7.1, 7.7, and 8.4, respectively. F to enter 3.0. Sexual maturity index, height, and log-leptin, log-percent body fat, and log-body fat were selected as determinants of menarche in the models. Logarithmic transformations of serum leptin concentration and absolute and relative body fat were always negatively associated with menarche in the respective models, each controlled for sexual maturation and body size. This negative association between menarche and absolute and relative body fat remained significant with corresponding correlation coefficients ($r = -0.133$ and $r = -0.136$, and *P* values of 0.013 and 0.014, respectively). Individuals with early-onset menarche were shorter in stature than subjects with late-onset menarche.

Serum leptin remained low in young females who did not develop menarche after 4 yr of follow-up (Fig. 3). Group 1 (n = 11; 3.2%) did not have a menstrual period by the end of the fourth year of follow-up, group 2 (n = 39; 11.4%) had a first menstrual period during the fourth year, group 3 (n = 93; 27.1%) had a first menstrual period during the third year, group 4 (n = 132; 38.5%) had a first menstrual period during the second year, and group 5 (n = 68; 19.8%) started menstruating during the first year of the study. Box plots of serum leptin according to menarche groups at yr 1 of the study are presented in Fig. 3A. The group effect is significant by ANOVA at $P < 0.05$. Serum leptin by yr 4 of the study in different menarche groups is presented in Fig. 3B. The difference in serum leptin between group 1 and pooled groups 2–5 is significant by *t* test at $P < 0.008$.

Figure 4 shows the three-dimensional plane surface model ($z = a + bx + cy$) developed by the least squares criterion of the relationship among the timing of menarche (z), energy intake (y), and serum leptin level (x) in young females. Data for energy intake and leptin were obtained before menarche. Subjects with lower energy intake and lower serum leptin levels had late-onset menarche in this model and *vice versa*.

A scatterplot with the regression lines and 95% confidence curves of the relation between total body bone area and log-transformed serum leptin data at menarche (n = 311; r = 0.307; $P < 0.0001$) is presented in Fig. 5A, and the change in total body bone area and serum leptin over time (n = 270; r = 0.274; $P < 0.0001$) is presented in Fig. 5B. Table 3 shows multiple regression analysis with total body bone area (square centimeters) as the dependent variable, and age, SMI, height, LBM, TBBMC, and log-leptin as independent variables in the model (n = 311; adjusted $r^2 = 0.95$). Height, LBM, TBBMC, and log-leptin partially predict total body bone area

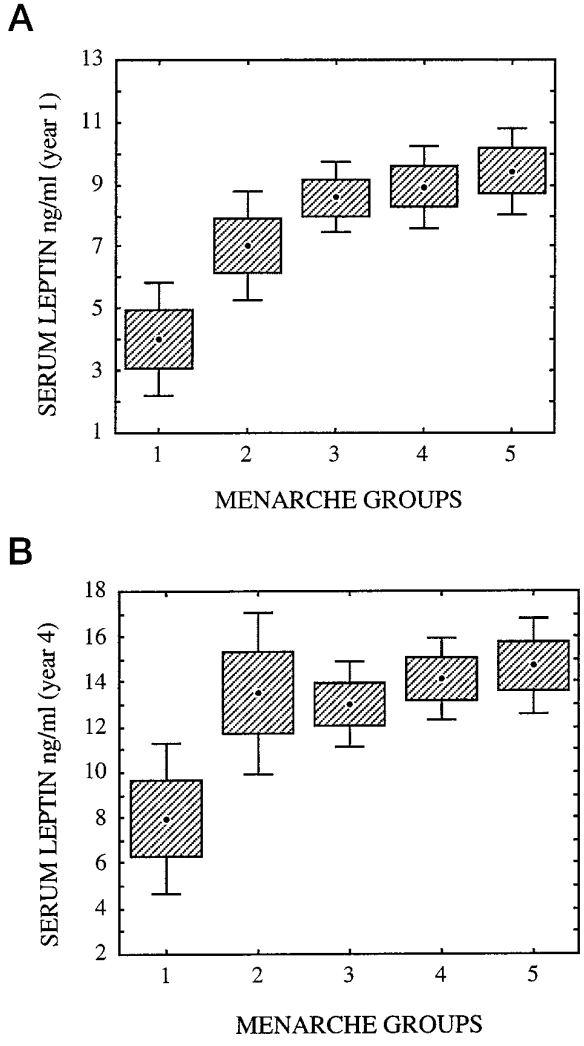

FIG. 3. A, Box plots of serum leptin in participating females according to menarche groups at yr 1 of the study. B, Box plots of serum leptin of the same subjects according to menarche groups at yr 4 of the study. Data are presented as the mean ± SE ± 1.96 SE.

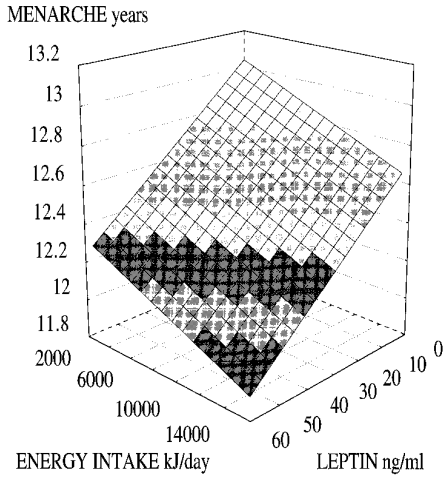

FIG. 4. Three-dimensional plane surface model of the relationship among the timing of menarche (z), energy intake (y), and serum leptin level (x) in young females. Data for energy intake and leptin were measured before the onset of menarche.

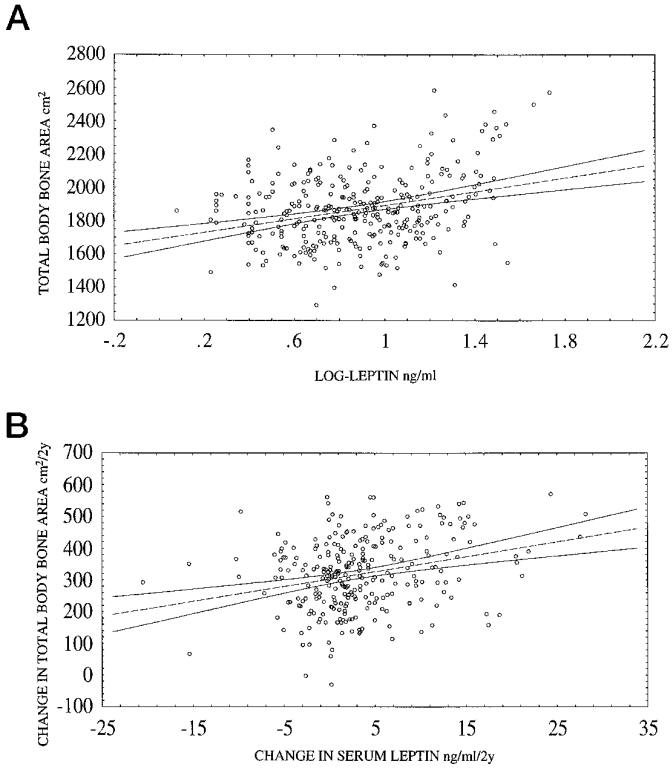

FIG. 5. A, Scatterplot with the regression lines and 95% confidence curves of the relation between total body bone area and log-transformed serum leptin data at menarche (n = 311). B, Scatterplot with the regression lines and 95% confidence curves of the relation between the change in total body bone area and serum leptin over time (n = 270).

after being adjusted by other variables. Table 4 shows multiple regression analysis, with the change (Δ) in total body bone area (cm^2/2 y) as the dependent variable and the changes in SMI, height, LBM, TBBMC, and serum leptin level over 2-yr period as independent variables in the model (n = 270; adjusted r^2 = 0.93). Changes in height, LBM, TBBMC, and serum leptin partially predict the change in total body bone area over time after being adjusted by other variables.

TABLE 3. Multiple regression analysis with total body bone area (square centimeters) as the dependent variable and age, SMI, height, LBM, TBBMC, and log leptin as independent variables in the model

Variable	b	Partial correlation	R^2	t	P
Age	−0.01	−0.05	0.20	−0.88	0.3825
SMI	−0.00	−0.01	0.21	−0.12	0.9063
Ht	0.22	0.54	0.60	11.27	0.0000
LBM	0.27	0.50	0.77	10.14	0.0000
TBBMC	0.55	0.77	0.77	20.91	0.0000
Log leptin	0.09	0.35	0.24	6.45	0.0000

All parameters were measured at menarche. Adjusted r^2 = 0.95; n = 311. The table shows standardized regression coefficients, and partial and semipartial correlations with corresponding r^2, t, and P values for each determinant variable in the model.

TABLE 4. Multiple regression analysis with the change (Δ) in total body bone area (square centimeters per 2 yr) as the dependent variable and the changes in SMI, height, LBM, TBBMC, and serum leptin level over a 2-yr period as the independent variables in the model

Variable	b	Partial correlation	r^2	t	P
Δ SMI	0.00	0.01	0.27	0.10	0.9208
Δ Ht	0.39	0.64	0.69	13.70	0.0000
Δ LBM	0.12	0.22	0.75	3.69	0.0003
Δ TBBMC	0.56	0.83	0.54	23.80	0.0000
Δ Leptin	0.05	0.19	0.15	3.13	0.0019

Adjusted r^2 = 0.93; n = 270. The table shows standardized regression coefficient, and partial and semipartial correlations with corresponding r^2, t and P values for each determinant variable in the model.

Discussion

According to our knowledge this is the first study to examine longitudinally the influence of body fatness and serum leptin on the timing of menarche in human females. In addition, this is the first study to evaluate, either cross-sectionally or longitudinally, the relationship between leptin and body fat in children. Serum leptin is strongly associated with body fat and indexes of body fatness (percent body fat and BMI) as well as with the change in body fat over time. As leptin is encoded by the *ob* gene and produced only in the fat cells, its serum concentration indirectly reflects body fat stores (11, 20). The above data are in agreement with the results obtained in a small group of teenage females who participated in a diurnal variation study of leptin (21) and also with the data obtained in adults (22). In addition, using a segmented linear plateau model we found an inverse relation between menarche and serum leptin up to 12.2 ng/mL (95% CI, 7.2–16.7 ng/mL). As indicated by the estimated slope of the regression line in the lower region, an increase of 1 ng/mL in serum leptin level lowers the timing of menarche by 1 month, on the average. A partial independent effect of leptin on menarche was confirmed when the simultaneous effects of growth and pubertal development were controlled for in the multiple regression model. The results of this research indicate that a certain degree of blood leptin

level is necessary to trigger the reproductive ability of young women. Therefore, it is very likely that fat cells exert their regulatory effect on menarche through synthesis of leptin.

Serum FSH, LH, and estradiol did not provide any additional predictive capability for the timing of menarche in the multivariate statistics. Only serum leptin was selected by the forward stepwise regression procedure as a significant predictor variable in the model (n = 201; partial $r^2 = 0.036$; $t = -2.7$; $P < 0.007$; F to enter 3.0). The only relationship between leptin and gonadotropins was present when the serum FSH concentration before menarche was regressed on the change in serum leptin level during puberty (nanograms per mL/3 yr; n = 193; r = 0.176; $P < 0.014$). It is most likely that leptin acts as a hormone and provides a direct stimulus to gonads by triggering a reproductive cycle in human females. The exact understanding of the mechanism involved will require a clinical trial with leptin in premenarcheal girls of low body fatness. A persistent defect of the hypothalamic-pituitary-gonadal axis was described in *ob/ob* mice (23); these abnormalities were corrected by leptin administration (12, 13), although the exact sequence of the events in the restoration of the axis still remains unknown. Absolute and relative body fat were also negatively related to menarche as well as to the change in body fat over time. Young females who did not develop menarche by the fourth year of follow-up in this study have lower body fat than their peers who started menstruating earlier. A serum leptin level of 12.2 ng/mL corresponds to a relative body fat of 29.7%, a BMI of 22.3, and body fat of 16.0 kg among our participants. A gain in body fat of 1 kg lowers the timing of menarche by approximately 13 days.

It is anticipated, therefore, that leptin deficiency is a primary reason for delayed puberty and menarche in individuals and in populations accustomed to absolute or relative dietary energy deficiency. In menstruating women, a negative energy balance caused by either fasting and/or exercise could cause secondary amenorrhea (24–26), presumably due to low levels of circulating leptin. Low serum leptin levels were found in young amenorrheic athletes (27) and in women suffering from anorexia nervosa (28). A decrease in the serum leptin concentration was documented in older women in response to exercise (29).

As late onset of menarche and leanness are considered risk factors for osteoporosis (3, 30–32), we evaluated the association between leptin and bone mass of young females during peak growth. The association between body weight and bone mass has previously been attributed to either mechanical forces exerted on the skeleton and/or to a more favorable estrogen status associated with obesity (33). This study showed a positive relation between serum leptin and whole body bone mineral areal density (r = 0.204; $P < 0.0002$); however, in a multiple regression model, leptin did not show any influence on bone mineral content when bone area was among the predictor variables. Instead, serum leptin was related to bone area, suggesting an influence on the periosteal envelope. We propose that obesity may have an additional protective effect on bone mass through its influence on cortical bone and its periosteal envelope, and that this could be mediated by leptin. Periosteal bone apposition increases bone volume, with a corresponding change in peak bone mass (3). It is the bone size that ultimately determines the risk of fracture, as documented for the spine (34). The definitive data in this regard, however, should come from intervention studies with leptin in an animal model by looking at the skeleton as the target organ. The above finding could help understanding the pathophysiology of osteoporosis and osteoarthritis. Most of the patients with generalized osteoarthritis are obese and have higher peak bone mass and a hyperactive periosteal envelope (35). Their serum leptin levels are higher than those in patients with osteoporosis (36).

In summary, this observational study conducted in young women showed an inverse relation between menarche and serum leptin up to 12.2 ng/mL, suggesting a threshold effect of serum leptin with regard to reproductive ability. Leptin is a hormone acting as a mediator between adipose tissue and the gonadal-hypothalamic axis in human females. Leptin may also mediate the effect of obesity on bone mass by influencing periosteal envelope expansion. This may have implications for the development of osteoporosis and osteoarthritis.

References

1. **Zacharias L, Rand WM, Wurtman RJ.** 1976 A prospective study of sexual development and growth in American girls: the statistics of menarche. Obstet Gynecol Surv. 31:S325–S337.
2. **MacMahon B, Cole P, Brown J.** 1973 Etiology of human breast cancer: a review. J. Natl. Cancer Inst. 50:21–42.
3. **Matkovic V, Jelic T, Wardlaw GM, et al.** 1994 Timing of peak bone mass in caucasian females and its implicaton for the prevention of osteoporosis. Inference from a cross-sectional study. J Clin Invest. 93:799–808.
4. **Zacharias L, Wurtman RJ.** 1969 Age at menarche. N Engl J Med. 280:868–875.
5. **Wyshak G, Frisch RE.** 1982 Evidence for a secular trend in age of menarche. N Engl J Med. 306:1033–1035.
6. **Frisch RE, Revelle R.** 1970 Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science. 169:397–399.
7. **Frisch RE, McArthur JW.** 1974 Menstrual cycles: fatness as determinant of minimum weight for height necessary for their maintenance or onset. Science. 185:949–951.
8. **Maclure M, Travis LB, Willett W, MacMahon B.** 1991 A prospective cohort study of nutrient intake and age at menarche. Am J Clin Nutr. 54:649–656.
9. **Kennedy GC, Mitra J.** 1963 Hypothalamic control of energy balance and the reproductive cycle in the rat. J Physiol. 166:395–407.
10. **Kennedy GC, Mitra J.** 1963 Body weight and food intake as initiating factors for puberty in the rat. J Physiol. 166:408–418.
11. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425–432.
12. **Barash IA, Cheung CC, Weigle DS, et al.** 1996 Leptin is a metabolic signal to the reproductive system. Endocrinology. 137:3144–3147.
13. **Chehab FF, Mounzih K, Lu R, Lim ME.** 1997 Early onset of reproductive function in normal female mice treated with leptin. Science. 275:88–90.
14. **Ahima RS, Prabakaran D, Mantzoros C, et al.** 1996 Role of leptin in the neuroendocrine response to fasting. Nature. 382:250–252.
15. **Matkovic V, Ilich JZ, Andon MB, et al.** 1995 Urinary calcium, sodium, and bone mass of young females. Am J Clin Nutr. 62:417–425.
16. **Ilich JZ, Hangartner TA, Skugor M, Roche AF, Goel P, Matkovic V.** 1996 Skeletal age as a determinant of bone mass in young females. Skel Radiol. 25:431–439.
17. **Duke PM, Litt IF, Gross RT.** 1980 Adolescent's self-assessment of sexual maturation. Pediatrics. 66:918–920.
18. **Bouchard C, Tremblay A, Leblanc C, et al.** 1983 A method to assess energy expenditure in children and adults. Am J Clin Nutr. 37:461–465.
19. **Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M.** 1996 Radioimmunoassay of leptin in human plasma. Clin Chem. 42:942–946.
20. **Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV.** 1996 Leptin: the tale of an obesity gene. Diabetes. 45:1455–1462.
21. **Matkovic V, Ilich JZ, Badenhop NE, et al.** 1997 Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. J Clin Endocrinol Metab. 82:1368–1372.
22. **Considine RV, Sinha MK, Heiman ML, et al.** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Eng J Med. 334:292–295.

23. **Swerdloff RS, Batt RA, Bray GA.** 1976 Reproductive hormonal function in the genetically obese (*ob/ob*) mouse. Endocrinology. 98:1359–1364.
24. **De Souza MJ, Metzger DA.** 1991 Reproductive dysfunction in amenorrheic athletes and anorexic patients: a review. Med Sci Sports Exerc. 23:995–1007.
25. **Van Der Spuy ZM.** 1985 Nutrition and reproduction. Clin Obstet Gynecol. 12:579–604.
26. **Vigersky RA, Andersen AE, Thompson RH, Loriaux DL.** 1977 Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. N Engl J Med. 297:1141–1145.
27. **Laughlin GA, Yen SSC.** 1997 Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 82:318–321.
28. **Grinspoon S, Gulick T, Askari H, et al.** 1996 Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab. 81:3861–3863.
29. **Kohort WE, Landt M, Birge SJ.** 1996 Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. J Clin Endocrinol Metab. 81:3980–3985.
30. **Matkovic V, Ilich JZ, Skugor M, Saracoglu M.** 1995 Primary prevention of osteoporosis. Physical Med Rehab Clin North Am. 6:595–627.
31. **Heaney RP.** 1996 Bone mass, nutrition, and other lifestyle factors. Nutr Rev. 54:S3–S10.
32. **Rosenthal DL, Mayo-Smith W, Hayes CW, et al.** 1989 Age and bone mass in premenopausal women. J Bone Miner Res. 4:533–5538.
33. **Siiteri PK.** 1987 Adipose tissue as a source of hormones. Am J Clin Nutr. 45:277–282.
34. **Gilsanz V, Loro ML, Roe TF, Sayre J, Gilsanz R, Schulz EE.** 1995 Vertebral size in elderly women with osteoporosis. Mechanical implications and relationship to fractures. J Clin Invest. 95:2332–2337.
35. **Matkovic V.** 1996 Nutrition, genetics, and skeletal development. J Am Coll Nutr. 15:556–569.
36. **Matkovic V, Skugor M, Ilich JZ, et al.** 1997 Serum leptin and β3-adrenergic-receptor gene (βarg) polymorphism in osteoarthritic (OA) and osteoporotic (OP) patients. J Bone Miner Res. 12:S257.