

A Longitudinal Assessment of Hormonal and Physical Alterations during Normal Puberty in Boys. V. Rising Leptin Levels May Signal the Onset of Puberty*

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ABSTRACT

Leptin, the product of the *ob* gene, is an adipocyte-derived hormone that signals the amount of adipose tissue energy stores to the brain and exerts major effects on energy homeostasis and neuroendocrine function. Leptin has recently been shown to affect reproductive function in leptin-deficient and normal rodents. As puberty, the process of sexual maturation and acquisition of reproductive competence, has been proposed to be triggered by the attainment of a critical amount and/or distribution of fat, we examined whether changes in circulating leptin levels could represent the hormonal signal responsible for triggering the onset of puberty in humans. Eight prepubertal boys (Tanner genital stage 1 or early stage 2 at the initiation of the study) were evaluated longitudinally for 2.5–5.1 yr depending on when Tanner stage 5 of genital development was achieved. Sera for the determination of leptin, testosterone, and dehydroepiandrosterone sulfate

were obtained every 4 months during the period of the study. Compared to baseline prepubertal levels (8 months before the onset of puberty, as defined by the initial rise in testosterone above the detection limit of the assay), leptin levels rose by approximately 50% just before the onset of puberty and decreased to approximately baseline values after the initiation of puberty ($P < 0.01$, by ANOVA), remaining stable for more than 2 yr. These changes occurred despite constantly increasing body mass index ($P < 0.05$, by ANOVA). No significant association between leptin and dehydroepiandrosterone sulfate concentrations was detected. In conclusion, the levels of circulating leptin are consistent with the hypothesis that this molecule is an important signal responsible for triggering the onset of puberty. The stimulus for a surge in leptin levels just before the onset of puberty is currently unknown. (*J Clin Endocrinol Metab* 82: 1066–1070, 1997)

PUBERTY is the process of sexual maturation by which reproductive competence is attained. The changes in physical appearance and behavior that characterize puberty are the direct result of rising concentrations of gonadal steroids, testosterone in boys and estradiol in girls; their production is driven by increased levels of pituitary gonadotropins, which are, in turn, regulated by the release from hypothalamic neurons of GnRH (1). The onset of puberty requires increased functional activity of the GnRH neuron. Before the onset of puberty, GnRH functional activity is under feedback control by low levels of plasma sex steroids produced by the prepubertal gonads and is also restrained by higher central nervous system control mechanisms (1). Although a number of factors influence GnRH neuronal function by eliminating this restraint, the molecular identity

of factors that herald the onset of puberty at the level of the GnRH neuron has remained elusive (1). An influence of adequate nutrition on the timing of puberty has long been recognized, and one prominent theory to account for the onset of puberty has invoked adipose tissue mass and the concept of a critical body weight (2–4). According to this theory, menarche is triggered by the attainment of a critical percentage of fat, and the maintenance of menstrual cycles requires the persistence of a minimal level of body fat (2, 3). This theory has been criticized on the basis of a number of methodological and empirical shortcomings (5). Recent evidence suggests that body fat distribution, rather than total body fat mass, is more closely related to estradiol and/or testosterone levels at the onset of puberty (6). However, in the absence of a molecular mechanism to link adipose mass or function to the activation of the hypothalamic-pituitary-gonadal axis and given the imperfect correlation between body fat mass and the onset of puberty, the validity of the critical weight theory has remained a subject of debate.

Leptin, the product of the *ob* gene (7), is an adipocyte-derived hormone that signals the state of energy stores to the brain (8–10). Genetic alterations that prevent either the production of this molecule (*ob/ob*) or the response to it (*db/db*) produce severe obesity, with both decreased energy expenditure and hyperphagia (11). Treatment of *ob/ob* mice with leptin reverses these defects, suggesting that leptin functions physiologically to limit obesity (12–15). More recently, a role

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* This work was supported by NIH Grant DK-28082 (to J.S.F.), Beth Israel Hospital General Clinical Research Center Grant M01-RR-01032, University of Virginia Health Sciences Center General Clinical Research Center Grant RR-00847, and a grant from Genentech (for partial support to A.D.R. during the data-gathering phases of this project).

† Supported by the Division of Endocrinology, Beth Israel Deaconess Medical Center, and the Clinical Investigator Training Program, Beth Israel Deaconess Medical Center, Harvard-Massachusetts Institute of Technology, Health Sciences and Technology, in collaboration with Pfizer.

for leptin in the regulation of neuroendocrine function has been defined (16). First, in addition to reversing their obesity, chronic leptin treatment of ob/ob mice reverses the reproductive failure (17, 18) that characterizes these mice. Second, and possibly more relevant to the physiological function of leptin, the changes in neuroendocrine and reproductive function that accompany a 48-h fast in normal mice are blunted or reversed by the administration of leptin (16). Finally, leptin treatment of normal weanling female mice was shown to accelerate the onset of puberty, without producing a measurable effect on body weight (19, 20). Thus, it is reasonable to speculate that leptin may signal the onset of puberty in humans.

Subjects and Methods

Subjects

Eight healthy Caucasian boys participated in this longitudinal study. At entry, the height and weight of each subject were between the 5th and 95th percentiles for age (according to standards from the National Center for Health Statistics). All were in late Tanner stage 1 or early stage 2 genital development at the initiation of the study and had reached Tanner stage 5 of genital development by the end of the study. Ages ranged from 9.75–11.9 yr at entry into the study, with a study duration of 2.5–5.1 yr depending on the timing and tempo of pubertal development of each boy. All subjects progressed normally through puberty during the observation period. A consent form was signed by a parent or guardian, and assent was received from each subject. The study and consent forms were approved by the human investigation committee at the University of Virginia.

Study design

The study design called for an in-patient evaluation every 4 months. Although the vast majority of studies occurred at 4-month intervals, occasionally unforeseeable personal circumstances prevented subjects from undergoing testing at the prescribed time. On these occasions, testing was carried out at a 5-month interval. At each evaluation, the subjects were admitted to the Clinical Research Center at the University of Virginia, where a physical exam was conducted (including pubertal staging), and height and weight were recorded. Body mass index (BMI) was calculated as weight (kilograms) divided by height squared (meters). Early morning samples were used for the hormonal measurements described in this report. Serum leptin was measured by RIA as described previously (21). Serum testosterone was performed using commercially available RIAs (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). Serum dehydroepiandrosterone sulfate (DHEAS) was measured by Endocrine Sciences. The sensitivity for the testosterone assay used is 25 ng/dL. Inter- and intraassay coefficients of variation are less than 8%.

Statistical analyses

To quantitate the effect of leptin on puberty, we averaged the leptin levels of study subjects at the four time points specified in relation to the onset of puberty, taking the point in time when testosterone concentrations became detectable for the first time (testosterone, ≥ 25 ng/dL) as the onset of puberty. Leptin levels 8 months before the onset of puberty were considered the prepubertal levels. Leptin levels at the time when testosterone reached a maximum were averaged as reflecting postpubertal levels, whereas levels at the midpoint between initiation of puberty and postpuberty were averaged and accepted as the midpubertal levels. Comparisons among means and trends across puberty were sought using repeated measures ANOVA. Statistical significance was assumed at the conventional level of $P \leq 0.05$.

Results

Baseline characteristics

The mean (\pm SD) age of the study participants at initiation of the study was 10.07 (0.98) yr. Their mean height (\pm SD) was 140.04 (6.54) cm, and their mean weight (\pm SD) was 33.89 (6.4) kg at presentation and increased significantly during the study.

Leptin and testosterone concentrations

Each of the boys displayed normal progression of puberty as determined by the progressive rise in testosterone from below the levels of detection to mature levels, as well as appropriate development of secondary sexual characteristics. The duration of prepubertal period varied among the subjects (Fig. 1). Despite this variability, it is apparent from inspection of Figure 1 that the peak level of leptin is found in the specimen immediately before or at the time of the initial rise in testosterone in every subject. In those subjects with the longest period of puberty, it is striking that leptin levels rise a mean of approximately 100% in the months just before the onset of puberty. In all subjects, leptin levels fell during the period of the initial rapid rise in testosterone, crossing the rising testosterone curve in every case, either to return to the prepubertal level or decrease even further.

To quantitate this phenomenon, we averaged the leptin levels of all study subjects at four distinct time points. Compared to baseline prepubertal levels, leptin concentrations increased by approximately 100% at puberty (Fig. 2a). Moreover, leptin concentrations decreased to approximately baseline values after the onset of puberty and remained at approximately the same level for more than 2 yr (Fig. 2a). These changes, expressed as a percentage of the levels at the onset of puberty, were highly statistically significant by repeated measures ANOVA ($P < 0.01$; Fig. 2b). The change in mean leptin levels at the same four time points evaluated by repeated measures ANOVA was also statistically significant ($P < 0.05$).

Leptin and DHEAS concentrations

No significant association between leptin concentrations and adrenarche, as indicated by rising DHEAS levels, could be detected (data not shown).

Leptin and BMI

Both the increase in leptin concentrations before and at the onset of puberty and the subsequent decrease in leptin's concentrations to baseline in the period after puberty occurred despite constantly increasing BMI ($P < 0.05$; Fig. 3).

Discussion

The onset of puberty requires changes in hypothalamic GnRH secretion that entrain alterations in the secretion of pituitary gonadotropins and thereby augmented secretion of gonadal steroids (1, 22). The identity of factors that act on the GnRH neuron to initiate puberty remains unknown (22). The critical weight theory of puberty, although attractive on several counts, has not itself provided a molecular link between weight or adiposity and the central control of reproduction

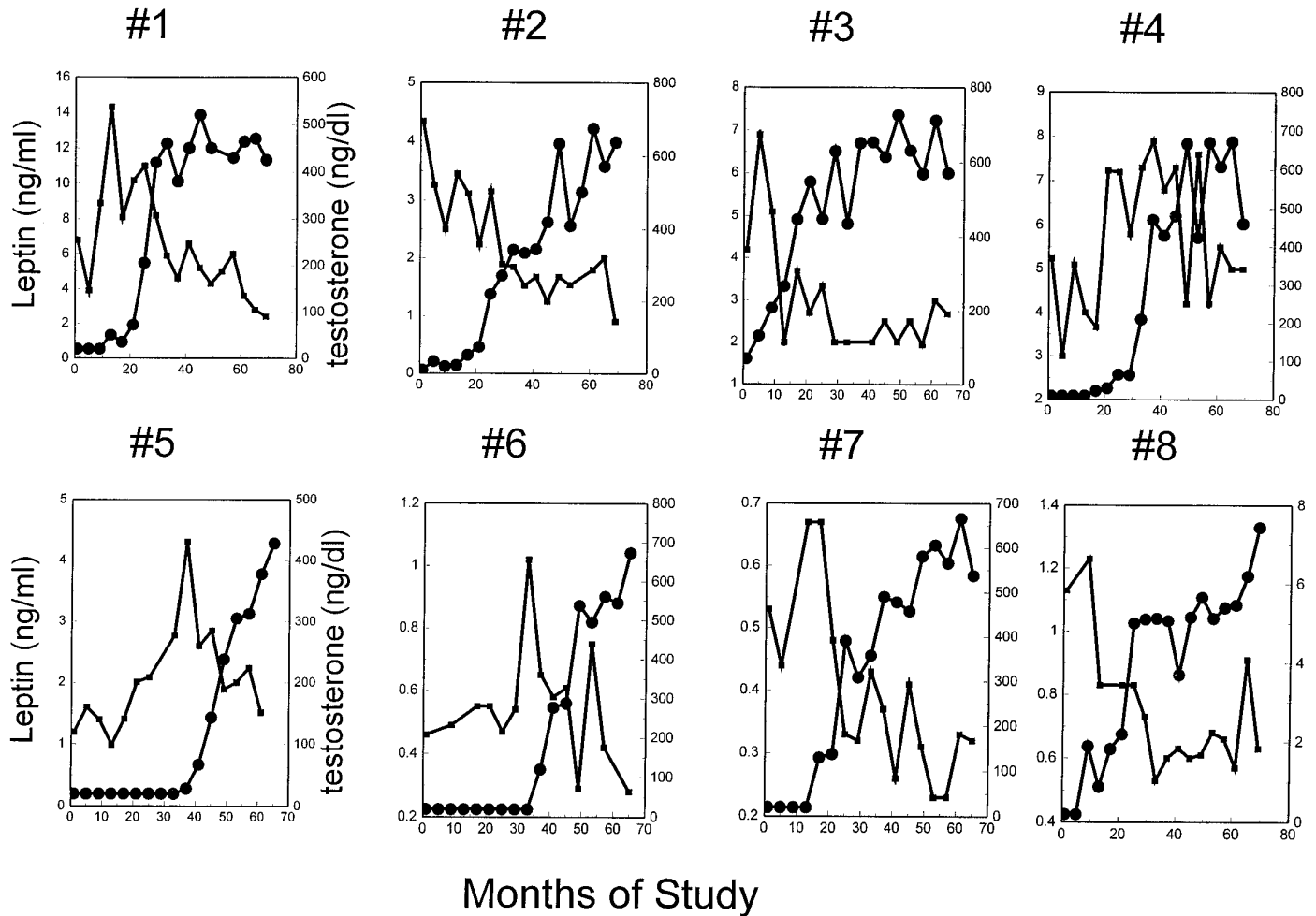


FIG. 1. Leptin and testosterone concentration changes over the study period. Numbers 1–8 refer to individual study subjects.

(2–4). Based on several lines of evidence, leptin is a plausible candidate for such a link. First, leptin-deficient *ob/ob* mice have hypothalamic hypogonadism, and this is reversed after chronic leptin treatment (17, 18). Second, in normal mice the starvation-induced fall in leptin, a key factor in the neuroendocrine adaptation to starvation, is clearly associated with diminution of the activity of the hypothalamic-pituitary-gonadal axis. The activity of this axis is maintained by exogenous leptin administration (16). Finally, daily leptin administration accelerates the onset of puberty in normal mice (19, 20). To address the possible role of leptin in the onset of human puberty, we measured leptin during a comprehensive longitudinal study of puberty in normal boys. The results are entirely consistent with a role for leptin in the onset of puberty. The pituitary hormonal changes associated with the onset of puberty are sleep-associated surges in LH secretion. The fact that leptin levels display circadian variation, with higher levels at night (11), suggests that leptin may be responsible for the nocturnal surges in LH secretion that characterize the initial stages of puberty.

In seven of eight subjects, testosterone levels were initially undetectable and then rose sharply to mature levels over the next 24 months. Initial leptin levels varied widely among the subjects before puberty, ranging from 0.4–6 ng/mL. Inde-

pendent of the absolute concentrations, leptin levels were at or near their peak at the onset of puberty, as judged by the initial rise in testosterone and the increase in testicular volume. In addition, leptin levels declined from this early pubertal peak in every subject, so that the curves for leptin and testosterone concentrations crossed near the middle of the rising testosterone curve. This overall pattern is particularly well seen in subjects 1, 3, 5, 6, and 7, in whom both the sharp rise in the early or prepubertal period and the sharp decline in the midpubertal period are seen, but the outline of a similar pattern is evident in all subjects. The similarity of these patterns is all the more remarkable given that they are derived from single early morning specimens obtained 4 months apart.

Several questions emerge from these findings. First, the factor(s) responsible for the changes in leptin levels over time in these subjects is not clear. Leptin levels are influenced by a number of factors, among which a positive correlation with adipose mass is the best defined (9–11). Although increases in adiposity occur before pubertal onset (3), the magnitude of the serial rise in leptin during this period (2-fold increase compared to the prepubertal period) seems out of proportion to what might be occurring within the adipose mass during this period. Direct measures of adipose mass were not ob-

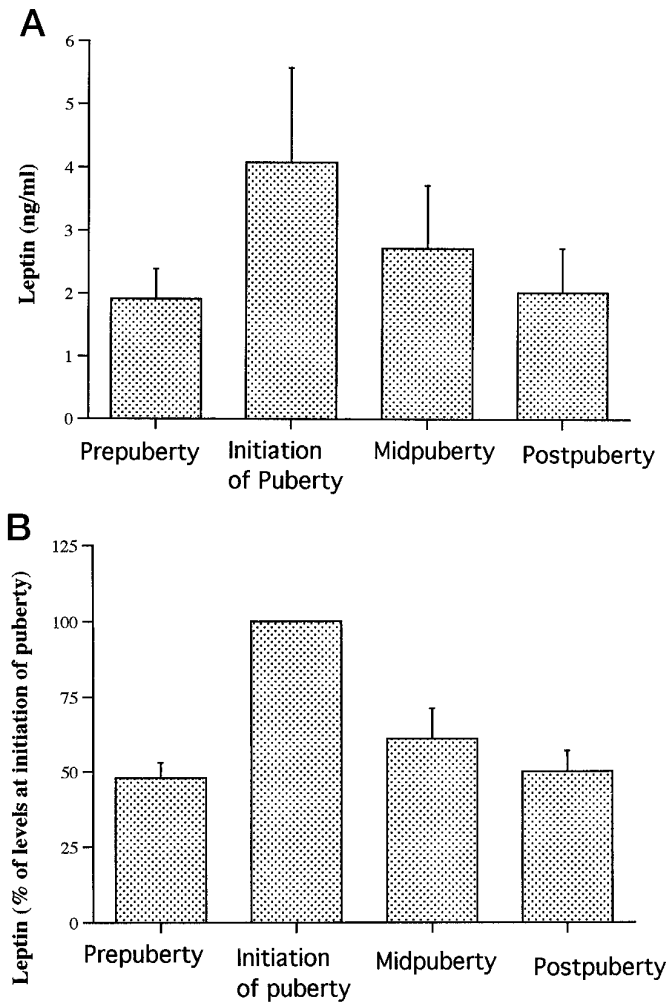


FIG. 2. a, Mean (\pm SD) leptin concentrations during the prepubertal period, the onset of puberty, and the midpubertal and postpubertal periods. b, Leptin concentrations (expressed as a percentage of the leptin concentrations at the onset of puberty) during the prepubertal period, the onset of puberty, and the midpubertal and postpubertal periods.

tained in this study, so we must rely on the indirect assessment of adiposity provided by the BMI, which may not provide as adequate an assessment of adiposity during late pubertal stages as it does in sexually mature adults (23, 24). However, most studies reveal good correlations between BMI and percent body fat in early puberty (25, 26). Indeed, BMI rose through puberty in these boys as their leptin levels fell, in direct contrast to the finding of a strong positive relationship between leptin and BMI in adults. Future studies in which adipose mass as well as the regional distribution of body fat are determined will be necessary to clarify this issue.

Factors other than fat mass can influence leptin levels in experimental animals and man (11). Positive factors include insulin (11, 27–29) and glucocorticoids (11, 30–32); negative factors include adrenergic agonists (33–36) and, possibly, other lipolytic factors (11). It will be interesting to determine whether these or other factors contribute, along with changing fat mass, to the peripubertal rise in leptin concentrations and their subsequent fall as puberty progresses.

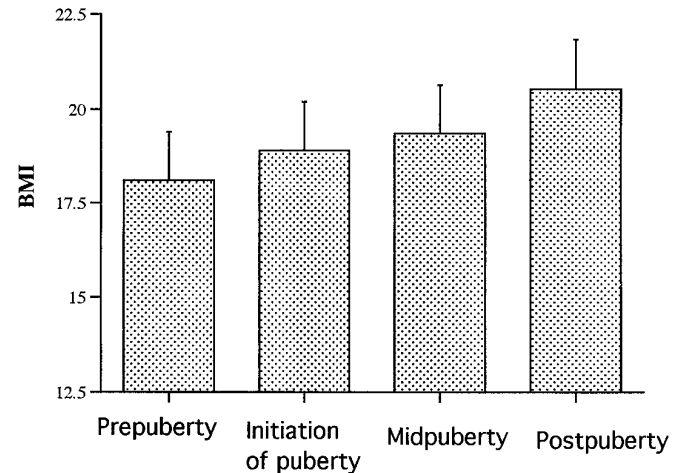


FIG. 3. Mean (\pm SD) BMI during the prepubertal period, the onset of puberty, and the midpubertal and postpubertal periods.

In humans, the increase in adrenal androgen production, adrenarche (22), represents the first recognizable hormonal change of puberty and is characterized by rising plasma levels of adrenal androgens, particularly DHEAS, followed by dehydroepiandrosterone and androstendione (22). They usually precede the onset of any other hormonal evidence of pubertal maturation. Although this process generally occurs independently of sexual maturation (gonadarche), we investigated a potential relationship between DHEAS, on the one hand, and leptin or testosterone, on the other, in our subjects. No relationship between DHEAS and either leptin or testosterone levels was identified in the group of boys studied. Thus, it appears that activation of the hypothalamic-pituitary-adrenal axis is not an important factor responsible for the increase in leptin concentrations before puberty. Additionally, we can conclude that leptin does not play a role in initiating adrenarche. The factor(s) that modulates the changes in adrenal androgen secretion during adrenarche remain elusive and controversial. Finally, we and others have shown that GH levels are elevated in late puberty (37, 38), and it is widely accepted that this increase is due to increasing gonadal steroid concentrations (39, 40). It remains to be determined whether rising leptin levels are the physiological trigger initiating the gonadal steroid-induced increase in GH secretion.

The precise mechanism through which increased leptin levels may affect the onset of puberty is as yet uncertain. In rodents it is clear that leptin acts on hypothalamic centers involved in the regulation of energy balance, and that the arcuate nucleus is one important target (11, 41–43). It is currently accepted that the intermittent secretion of GnRH is regulated by a neural oscillator (22). It remains unknown, however, the extent to which the neural oscillator function responsible for intermittent GnRH secretion is innate to the GnRH-producing cells (22, 44) as opposed to being induced by other neuropeptides or neurotransmitters in the central nervous system. Neuropeptide Y, a target of leptin action in the arcuate nucleus (41, 42), can positively regulate GnRH secretion *in vitro* and *in vivo* (45). Future studies will determine whether the action of leptin to influence the hypothalamic

lamic-pituitary-gonadal axis is through direct actions on the GnRH neuron and/or indirect through the intermediation of another factor(s). It will also be of great interest to determine the extent to which defects in the leptin pathway may be responsible for one or more disorders of puberty, especially those in which obesity is part of the syndrome, for example the Prader-Willi or Kallmann syndrome.

Acknowledgments

We express our gratitude to Ms. Catherine Kern and Ms. Ginger Bauler for technical assistance in the Core Laboratory of the General Clinical Research Center, Ms. Sandra Jackson and her nursing staff of the University of Virginia General Clinical Research Center, Ms. Melanie Bishop for coordination and data management, the pediatric fellows for patient care, and Dr. Mark Stene at Endocrine Sciences for DHEAS measurements. We acknowledge our long term collaborator, Dr. Robert M. Blizzard, for helpful discussions in setting up and executing the original protocol.

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