0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 2
Printed in U.S.A.

Circulating Concentrations of Nocturnal Leptin, Growth Hormone, and Insulin-Like Growth Factor-I Increase before the Onset of Puberty in Agonadal Male Monkeys: Potential Signals for the Initiation of Puberty*

K. J. SUTER, C. R. POHL, AND M. E. WILSON

Department of Anatomy and Neurobiology and The Animal Reproduction and Biotechnology Laboratory (K.J.S.), Colorado State University, Fort Collins, Colorado 80523; School of Health Sciences (C.R.P.), Duquesne University, Pittsburgh, Pennsylvania 15282; and Yerkes Primate Research Center (M.E.W.), Emory University, Lawrenceville Georgia 30043

ABSTRACT

The factor(s) responsible for initiating the developmental increase in nocturnal gonadotropin-releasing hormone secretion, defining the onset of puberty, are not known. Although signals regulating prepubertal growth seem to be obvious candidates to control such a process, it is unclear whether prepubertal alterations occur in these growth-related factors such that they might provide the brain information on changing body size. Using samples analyzed previously describing the initiation of nocturnal pulsatile LH secretion in agonadal male monkeys (*Endocrinology* 139: 2774–2783, 1998), developmental changes in plasma concentrations of leptin, GH, and insulin-like growth factor I (IGF-I) were determined to test the hypothesis that an increase in circulating levels of one or all of these growth-derived signals precedes the onset of puberty. Hormone concentrations were determined in five juvenile males at 10-day intervals from approximately 60 days before and 50 days after the initiation of pulsatile nocturnal LH secretion. Leptin concentrations were determined in samples obtained at 1000 and 2200 h, 36 and 48 h before the nocturnal assessment of pulsatile LH. Mean nocturnal GH concentrations were determined from the sequential samples collected at night. IGF-I was determined in the 1000- or 2200-h presequential samples. Although daytime leptin concentrations did not increase developmentally, nocturnal leptin levels increased significantly during the 30 days before the onset of puberty. Furthermore, both nocturnal GH and IGF-I concentrations showed a significant sustained increase from the early prepubertal period to the 30 days preceding the onset of puberty. These data are the first to demonstrate an increase in nocturnal leptin and GH-induced IGF-I secretion prior to the onset of puberty in the agonadal male monkey and that these developmental changes occur independent of the gonadal influences. These findings provide justification for empirical investigation of the role of leptin and the GH axis, in particular IGF-I, in regulating developmental increases in pulsatile nocturnal gonadotropin-releasing hormone secretion initiating puberty in primates. (*J Clin Endocrinol Metab* **85:** 808–814, 2000)

THE RATE-LIMITING step for the onset of puberty in monkeys is the initiation of an hourly release of nocturnal gonadotropin-releasing hormone (GnRH) from the hypothalamus (1). This process seems to be induced by changes in stimulatory and/or inhibitory input to GnRH neurons by neurons immunoreactive for glutamate (2–6), gamma aminobutyric acid (7–9), and neuropeptide-Y (NPY; Refs. 10 and 11), as well as the influence of glial-derived transforming growth factor-α (12, 13) within the mediobasal hypothalamus (MBH). Despite our understanding of these putative neurobiological mechanisms, the factor(s) responsible for the developmental change in the nature of neurochemical inputs controlling GnRH release *in situ* are not known (14).

Factors that regulate and/or emanate from the rapid increments in skeletal and ponderal growth at the transition to puberty may be important in regulating the onset of puberty (15–17). These findings have lead to the so-called "somatometer" hypothesis, suggesting that a central growth-tracking device that is sensitive to circulating peripheral growth-related cues might underlie the increase in GnRH release at puberty (16). Two such growth-derived signals, leptin and insulin-like growth factor I (IGF-I), are leading candidates to regulate the developmental increase in GnRH secretion (17). Importantly, an increase in these growth-derived signals must be shown to be a prepubertal event independent of gonadal influence, temporally preceding the developmental increase in gonadotropin secretion, and the direct manipulation of these signals must alter the timing of the onset of puberty.

With respect to leptin, the prevailing hypothesis is that a developmental increase in leptin secretion initiates puberty by signaling the brain that energy stores are sufficient to support reproduction (18–23). Indeed, maturational stage is a better predictor of serum leptin levels than chronological age (24). However, data obtained thus far on the role of leptin-initiating mammalian puberty are equivocal. Puberty is accelerated by leptin treatment to fed-mice in some studies (25, 26) and is normalized in diet-restricted rats (27). Moreover, studies of juvenile monkeys have failed to define a temporal relationship between daytime leptin levels and LH (28) or testosterone (29).

Received June 30, 1999. Revision received September 22, 1999. Accepted October 20, 1999.

Address correspondence and requests for reprints to: Mark E. Wilson, Yerkes Primate Research Center of Emory University, Field Station, 2409 Taylor Lane, Lawrenceville, Georgia 30043. E-mail: Markw@rmy.emory.edu.

* With the exception of three profiles of GH, all the leptin, IGF-I, and the remaining GH determinations were performed in the Assay Services Laboratory at Yerkes, supported by Grants RR-00165 and HD-16305.

The GH axis has also been implicated in regulating puberty (15, 16). GH deficiency delays puberty (30), whereas GH therapy normalizes the progression of puberty (31, 32). In addition, GH-induced hepatic expression of IGF-I in rats increases before puberty, resulting in an increase in IGF-I messenger RNA (mRNA) in the median eminence (33). Moreover, bone age better predicts the timing of menarche than does chronological age (34), suggesting a link between bone development and sexual maturation. Although some studies report that IGF-I infusion does not affect puberty onset in female rats (35), intracerebroventricular administration of IGF-I increases serum LH via GnRH and initiates puberty in juvenile rats (33). However, it is believed that the endogenous developmental increases in GH-induced IGF-I secretion are caused, at least in part, by the maturational rise in gonadal secretion (36, 37), implying that any elevation in the GH axis occurs after the onset of puberty. In contrast, data from agonadal female monkeys has demonstrated that although estradiol augments the developmental increase in GH and IGF-I release, serum concentrations do increase developmentally in the absence of estradiol (38, 39). Accordingly, the precise relationship between increases in GH, IGF-I, and onset of puberty remains unclear.

To better understand the potential relationship between these growth-derived signals and the onset of puberty, the present analysis examined the hypothesis that the developmental increase in circulating leptin and/or GH and IGF-I levels occurs before the onset of puberty, defined as the increase in nocturnal pulsatile LH secretion. This hypothesis was tested by examining samples for leptin, GH, and IGF-I that were collected as a part of another study that clearly defined the onset of puberty in juvenile male rhesus monkeys (40). The same mechanism that results in the onset of the pubertal rise in LH secretion observed in the present study in agonadal animals likely is responsible for the reactivation of the hypothalamic-pituitary-testicular axis in intact animals.

Materials and Methods

Animals

Samples from five agonadal male rhesus monkeys (*Macaca mulatta*) in which the time of the pubertal increase in pulsatile LH release had been previously determined (40) were analyzed. For the study, animals were maintained in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Surgical procedures, daily care, and collection of blood samples from these chronically catheterized animals has been described in detail (40). To define the age of the pubertal increase in GnRH release, the animals received an intermittent infusion of GnRH to sensitize the pituitary to endogenous stimulation. In four subjects, samples were taken at 10-day intervals, whereas in one subject samples were collected every 20 days. At each sampling, samples were collected every 12 min from 1900–0200 h after the GnRH infusion had been temporarily interrupted for 96 h. Because the same mechanism that results in the activation of the pituitary-gonadal axis in intact animals likely results in the increase in LH secretion observed in agonadal animals, for purposes of analysis and discussion such changes are referred to as "pubertal." Accordingly, samples were analyzed for LH, and an algorithm (based on developmental changes in mean levels of LH) was used to define the onset of puberty or day 0 (40). With the exception of one male, morning samples (1000 and 1030 h) and evening samples (2200 and 2230 h) were also obtained 36 and 48 h, respectively, after the interruption of GnRH infusion.

Plasma concentrations of leptin were determined in the morning and evening samples obtained before the collection of the sequential samples. If the evening samples were not available, the 2200-h sample from the sequential bleeds was used. Leptin was measured using a primate-specific RIA (Linco Research, Inc., St. Charles, MO). The assay has a sensitivity of 0.50 ng/mL using 100 μl of sample. The intra-assay and interassay coefficient of variation (CV) were 5.2% and 11.1%, respectively. Plasma concentrations of IGF-I were determined in the evening samples or, if not available, the morning samples. Although concentrations of IGF-I decrease after the morning meal, differences between morning and evening samples are less than 8% (M. E. Wilson and S. Lackey, unpublished observations). Total IGF-I levels were measured using a previously validated RIA following removal of IGF binding proteins (41). The assay has a sensitivity of 50 ng/mL using 0.25-μL equivalents of sample. The intra-assay and interassay CV were 4.1% and 12.1%, respectively. GH concentrations were determined in the sequential nocturnal samples. Since these samples had been assayed previously (40), volumes were limited. Consequently, some of the early (<day −40) and later age points (>day +30) were not available for every subject. The assay was performed using a commercially available kit (Diagnostic Products Corp. Los Angeles, CA). The intra-assay and interassay CV were 3.8% and 10.6%, respectively. For the purpose of the present analysis, the mean GH level across each sequential bleed was determined for each animal.

Data were aligned to the onset of puberty, day 0, in 10-day increments from day −60 through day +50 for IGF-I and leptin. Sample volumes at the youngest ages, however, were limited for the GH assay. Therefore, GH data are expressed in 10-day increments from day −20 onward and 20-day increments before day −20. Data were expressed as mean ± SEM. Data were grouped in three intervals for analysis: early prepubertal (EPP) (day −60 through Day −40), late prepubertal (LPP), and postpubertal (PP) [day 0 through day +40 (GH) or 50 (leptin and IGF-I)]. The LPP interval is the period in which the acceleration of nocturnal LH pulse frequency seems to be initiated (40). All animals contributed data points to the analysis of these interval data. Given the small sample size, overall developmental changes were evaluated with the Friedman nonparametric statistic, which tests the hypothesis that the values come from the same population. To test the hypothesis that these growth-related signals increased before puberty, concentrations from the EPP were compared with the LPP period, whereas concentrations from the LPP were compared with either the EPP or PP using paired *t* tests or Wilcoxon matched pair tests. Statistical values having a probability of 0.05 or less were considered significant.

Results

As reported earlier (40), ages of the five subjects at the increase in nocturnal LH pulses from the GnRH-sensitized pituitary, defining the onset of puberty (day 0), were 23.9, 25.5, 24.1, 28.9, and 27.9 (26.2 ± 0.9) months. The relationship between day and nighttime levels of leptin and the onset of puberty are shown in Fig. 1. Daytime leptin concentrations (ng/mL) decreased significantly (Friedman statistic, $P = 0.02$) from the EPP interval (0.88 ± 0.08) to the LPP interval (0.70 ± 0.04; $P = 0.04$, Wilcoxon test). However, this difference was not significant when evaluated with a *t* test ($t_3 = 2.36$). After the onset of puberty, daytime leptin concentrations (PP; 1.17 ± 0.20) were similar to values observed during the EPP ($t_3 = 1.07$ or Wilcoxon $P = 0.34$). In contrast, nocturnal leptin concentrations (ng/mL) increased significantly from the EPP (0.82 ± 0.10) to LPP period (1.23 ± 0.16; Friedman statistic, $P = 0.02$ and $t_4 = 4.31$). This prepubertal elevation in nocturnal leptin was sustained following puberty (1.90 ± 0.47) with values statistically similar between LPP and PP ($t_4 = 1.25$). The developmental change in nocturnal leptin showed large individual variations (*e.g.* one animal's values ranged from 0.78–2.64 ng/mL, whereas another's ranged from 0.60–7.15 ng/mL. Consequently, individual mean concentrations during LPP and PP were also expressed

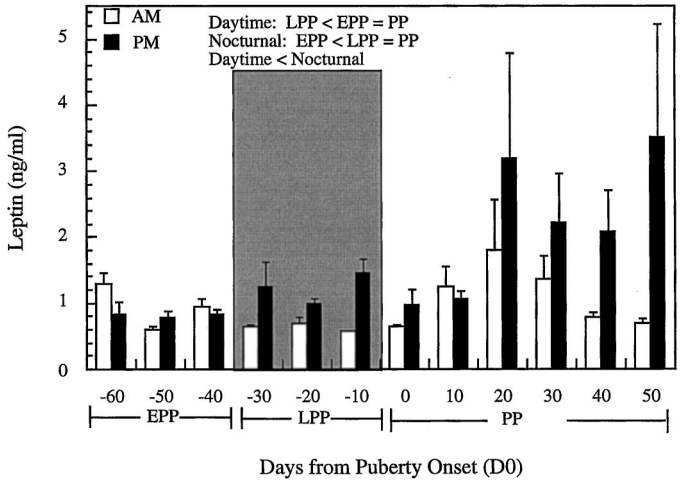

FIG. 1. Mean ± SEM daytime (□) and nocturnal leptin concentrations (■) from 60 days before and 50 days after the onset of puberty (day 0). For analysis, data were grouped in three intervals: early EPP, LPP, and PP. The *shaded area* encompasses the LPP period in which the prepubertal acceleration in pulsatile LH occurs (Ref. 41). Differences between intervals that are significant indicated by "<" ($P < 0.05$), whereas those that are not significant are indicated by " = ."

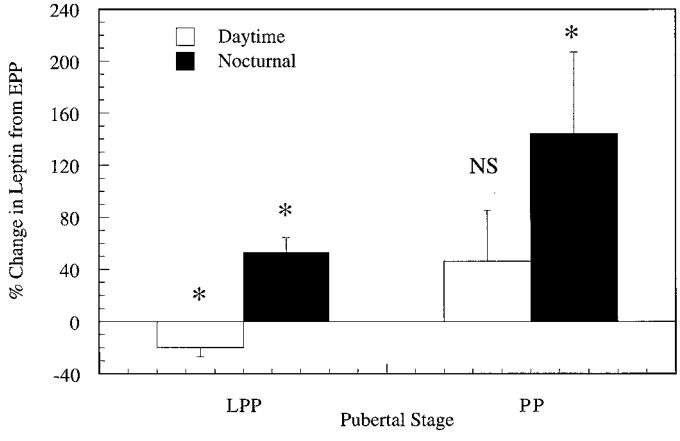

FIG. 2. The percentage change (mean ± SEM) in daytime and nocturnal leptin concentrations from the EPP to the LPP, and PP. Intervals with a significant percentage change from "0" are indicated by "<" ($P < 0.05$), whereas those that are not significant are indicated by "NS".

as the percentage change from EPP (Fig. 2). The percentage change in daytime leptin from EPP to LPP was significant ($t_3 = 2.76$), and the change from EPP to PP was not significant ($t_3 = 1.18$). Importantly, the percentage increase in nocturnal leptin from EPP to LPP and from EPP to PP was significant ($t_4 = 4.39$; $t_4 = 3.42$, respectively), whereas the change from LPP compared to that of PP was not significant ($t_4 = 1.39$). Finally, daytime values (ng/mL) of leptin (0.92 ± 0.07) were significantly lower than nocturnal concentrations (1.60 ± 0.33; $P = 0.04$, Wilcoxon test).

The relationship between plasma GH and IGF-I concentrations aligned to the onset of puberty are illustrated in Fig. 3. There was a significant increase in mean nocturnal GH concentrations from prepuberty through the postpubertal period (Friedman statistic, $P = 0.02$). GH levels (ng/mL) increased significantly from the EPP (1.20 ± 0.10) to the LPP

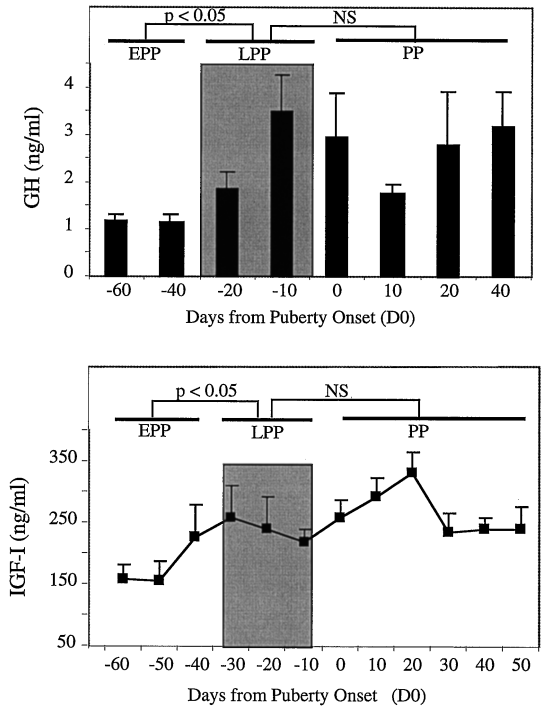

FIG. 3. Mean ± SEM concentrations of nocturnal GH (*top*) and IGF-I (*bottom*) from prepuberty (representing two successive age points) through the PP period. For analysis, data were grouped in three intervals: early EPP, LPP, and PP. The *shaded area* encompasses the LPP period in which the prepubertal acceleration in pulsatile LH occurs (Ref. 41). Differences between intervals that are significant are indicated by $P < 0.05$.

interval (2.15 ± 0.36; $t_4 = 3.42$), and this increase was sustained through the PP period (2.50 ± 0.76; $t_4 = 1.86$). The developmental change in circulating IGF-I concentrations also followed a similar pattern (Fig. 3; Friedman statistic, $P = 0.00$). Levels (ng/mL) increased significantly from the EPP (178 ± 30) to the LPP interval (238 ± 36), and this increase, too, was sustained through the PP period (266 ± 26; $t_4 = 0.87$).

The average percentage maximum increase from day 60 for leptin was 89% (±36) and for IGF-I was 92% (±38). For two males, this maximum increase for both IGF-I and leptin occurred on day −10, for one male on day −20, and for one male on day −30. For the remaining male, the maximum increase in leptin occurred on day −30 and for IGF-I on day −10. For GH, the average maximum percentage change from day −60 was 157% (±46), and this occurred on day −10 for four males and day −20 for the male who contributed data at 20-day intervals. Schematic representations of the developmental changes in nocturnal pulsatile LH secretion from two males (40) are shown in Fig. 4. Indicated in each panel are concentrations of leptin, GH, and IGF-I at the same time points. These data illustrate that all three growth-related signals increase prior to the defined day of the onset of puberty.

Weekly measures of body weight in these animals during the 100 days preceding and following the pubertal increase in nocturnal LH are shown in Fig. 5. Increments in weight did not differ significantly between day −30 and day +40 ($P = 0.52$).

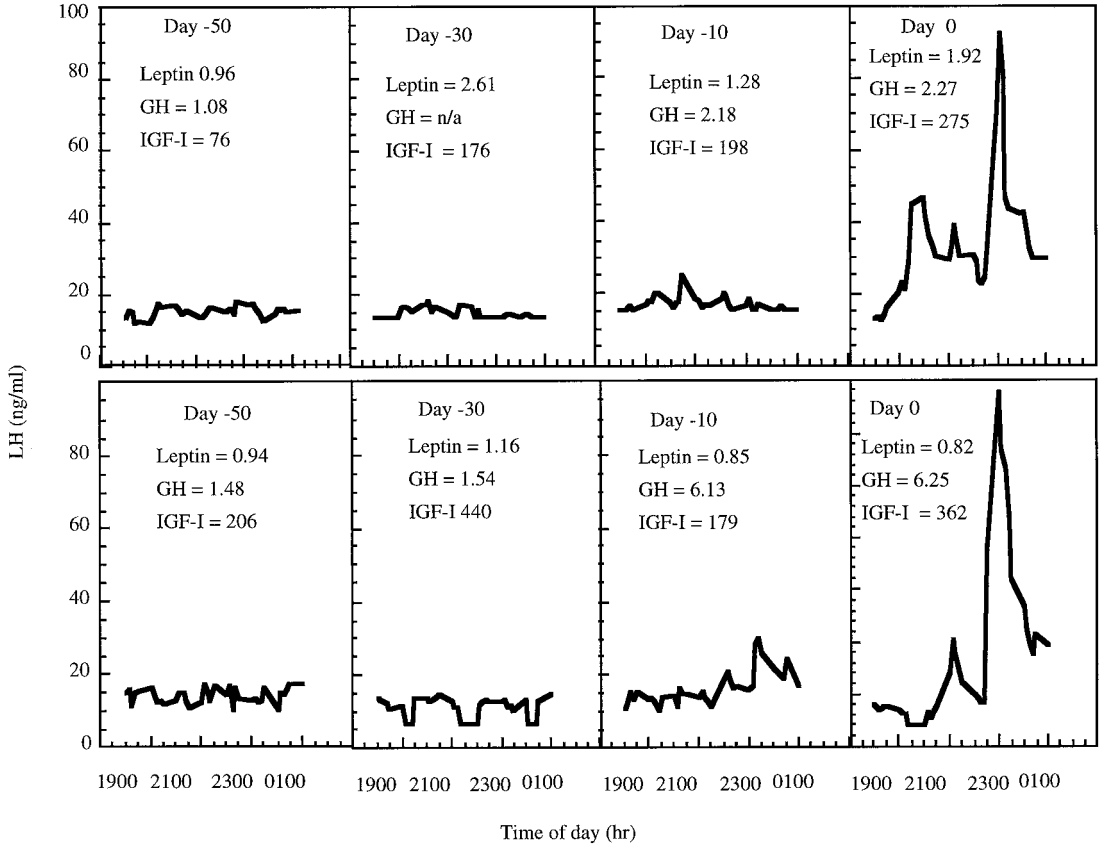

FIG. 4. Developmental changes in nocturnal pulsatile LH release up to the onset of puberty (D0) in two representative males, as described previously (Ref. 40). Indicated in each *panel* are the corresponding plasma concentrations of nocturnal leptin, GH, and IGF-I on each day. Units for each hormone are ng/mL. The GH value for for D-30 in the *top panel* was not available.

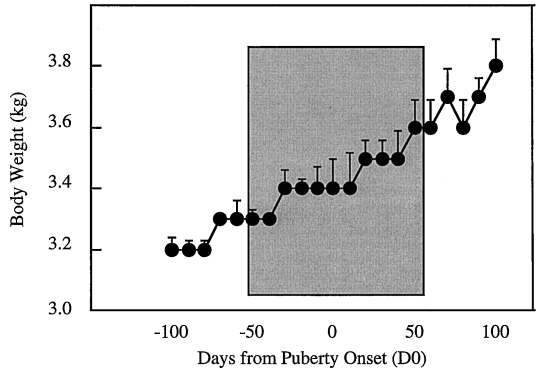

FIG. 5. Mean ± SEM weekly body weights in subject males, as reported previously (40). The *shaded area* indicates the 100 days surrounding the onset of puberty (D0).

Discussion

The data derived from this analysis of the plasma from agonadal male monkeys support the hypothesis that the developmental increase in nocturnal leptin, GH, and IGF-I precedes the increase in nocturnal pulsatile LH secretion at the onset of puberty. Although the prepubertal elevations in circulating GH and IGF-I were more robust than observed for nocturnal leptin concentrations, increases were nonetheless significant compared to younger developmental ages. These data from agonadal males provide evidence that one or all of these growth-derived signals may comprise the peripheral component of the "somatometer," providing critical information regarding body size or composition and, in this way, regulate developmental changes in GnRH secretion (17).

The identification of leptin has provided a mechanism to account for the long-standing hypothesis that puberty is initiated by an accumulation of body fat (42, 43). Accumulating fat mass during maturation results in an increased secretion of leptin from the adipocyte (44), and correlational studies in children suggest that a developmental increase in leptin secretion initiates puberty by signaling the brain that energy stores, composed in part by fat depots, are sufficient to support reproduction (18–23). Although body fat was not measured in the present analysis, the subjects were actively growing and showed a significant increment in body weight from early prepuberty to the period prior to the increase in pulsatile LH secretion. Accordingly, the increase in leptin observed in the present study, may reflect changes in body composition.

The finding in the present study of a significant prepubertal elevation in nocturnal leptin levels supports the hypothesis that leptin may be an important signal to the brain for the initiation of puberty. Other analyses suggest that leptin acts as a gate, permitting puberty to proceed (45). Indeed, the first significant rise in leptin, when normalized to prepubertal levels, occurred at the time when the acceleration in LH pulse frequency seemed to be initiated (*i.e.* days

−30 through −10; Ref. 40). Moreover, it is interesting that the robust increase in nocturnal leptin concentrations after the onset of puberty in the present study is associated with the transition to rapid, high amplitude LH pulses (40). Data from women (46) indicate that nocturnal pulsatile leptin secretion may induce and synchronize LH and, presumably, GnRH pulses. If this were the case, then the prepubertal and early postpubertal rises in leptin observed in the present study might also underlie a similar synchronization of GnRH release during this critical phase of development.

In contrast to the findings of the present study, previous studies of juvenile monkeys have not found a relationship between daytime leptin levels and LH (28) or testosterone (29). The discrepancy between these data and the present results may reflect methodological differences. In the one study (28), puberty was defined by the increase in daytime LH levels. However, the onset of puberty as assessed with nocturnal LH pulses by sequential sampling from a GnRH-sensitized pituitary, precedes the daytime increase as assessed from a single sample by as much as 5 months (40, 47). These data suggest that increases in daytime LH secretion do not accurately reflect, as do nocturnal estimates, the underlying hypothalamic event initiating puberty. Accordingly, reliance on daytime samples or down-stream gonadal responses (28, 29) may over-estimate the age of the onset of puberty and miss any transient changes in leptin secretion, such as those associated with puberty in boys (19).

The present study found a diurnal variation in leptin secretion during the 110 days surrounding the onset of puberty. This diurnal variation in leptin concentrations during the pre- and early pubertal period in the agonadal monkeys supports previous data from monkeys (28) in children (48–50). Other studies suggest that the diurnal leptin rhythm occurs independent of age and may be the result of the daytime pattern of food intake (51, 52); however, the absolute increase in leptin within this rhythm reflects the degree of sc fat (53). The absence of a more robust diurnal rhythm in the present study may reflect the limited number of animals and the high variability in leptin levels among individuals.

If, indeed, leptin is an important signal initiating puberty, the mechanism of action has yet to be defined. Leptin receptors are found in the lining of the third ventricle, as well as in the MBH, and are localized on NPY (54–57) and opioid (55). Leptin receptors are expressed in a model GnRH neuronal system (*i.e.* GT1–7 cells; 56, 58), and leptin application in this system results in GnRH release (58). However, hypothalamic GnRH neurons do not express leptin receptors (55). Accordingly, it is likely that any effect of leptin on GnRH release is mediated through an intermediary system such as NPY neurons. In this regard, some data from monkeys suggest that NPY regulates GnRH after but not before puberty (59), whereas most recent data suggest that a diminution in NPY inhibition of GnRH cell bodies may be responsible for the transitional increase in GnRH at the onset of puberty (10, 11). The observation that leptin decreases NPY gene expression and activity in the MBH of rodents (60) provides a possible functional link in the prepubertal increase in leptin observed in the present study and the concomitant pubertal shift in episodic LH release (40). In contrast to a direct effect on neuromodulators, other evidence suggests that leptin may increase GnRH indirectly by increasing intracellular metabolic fuel oxidation within the MBH (17, 61), as well as insulin sensitivity and glucose utilization (62). Thus, it remains to be determined whether changes in diurnal leptin secretion drive diurnal changes in LH (and GnRH) and whether leptin stimulates LH secretion in prepubertal monkeys, regardless of degree of adiposity and metabolic fuel stores (17).

In addition to a significant prepubertal elevation in nocturnal leptin secretion, the present analysis also shows that nocturnal GH and IGF-I rise before the onset of puberty. Data from children indicate the developmental rise in GH-induced IGF-I secretion is a post-onset phenomenon, resulting from an increase of gonadal steroid secretion (36, 37). Previous data from ovariectomized monkeys indicate that GH and IGF-I levels increase developmentally and that estradiol replacement enhances this increase (38, 39). The present analysis unequivocally demonstrates that the rise in GH and IGF-I is a prepubertal event that occurs before the developmental increase in nocturnal LH secretion and is largely independent of gonadal influence. At this time, it is not known what accounts for this prepubertal increase in GH, which ultimately controls IGF-I release (63). Although differences in the regulation of GH pulsatility between prepubescent and pubertal children have been extensively investigated (see Ref. 64 for review), little attention has focused on the prepubertal interval itself. It is tempting to speculate that leptin may be involved as data from rodents suggest that leptin stimulates GH release in a number of contexts (65, 66). However, leptin may actually inhibit GH secretion in humans (67), analogous to other differences in metabolic regulation between rodents and primates (66). Consequently, the prepubertal increase in leptin and GH may not be causally linked. Finally, it is not clear what would account for the earlier pre-onset rise in IGF-I compared to GH observed in the present study. As previously noted, only limited plasma was available for GH analysis at the youngest age, preventing us from performing a robust analysis of this issue. It is likely that a prospective analysis of dedicated samples would show a coincident rise in pulsatile GH secretion and IGF-I during the prepubertal interval. A better understanding of what factors regulate prepubertal changes in GH and IGF-I secretion would not only elucidate their potential role in the onset of puberty but would also help account for the significant increments in skeletal growth and acquisition of bone mineral density that occur prior to puberty (16).

This prepubertal increase in GH and IGF-I secretion in male monkeys supports data from other species that this axis, in particular IGF-I, is important for the onset of puberty. GH-induced hepatic expression of IGF-I increases before puberty, resulting in an increase in IGF-I mRNA in the median eminence (33). Although some studies report that IGF-I infusion does not affect puberty onset in female rats (35), intracerebroventricular administration of IGF-I increases serum LH via GnRH and initiates puberty in juvenile rats (33). These effects are supported by other rodent studies indicating IGF-I stimulates GnRH from the median eminence *in vitro* (68), the expression of GnRH mRNA in cell cultures (69, 70), and increases process extensions of GnRH neurons and cell-cell contacts (71). A series of studies using a female monkey

model indicates that a somatostatin analog delays age at first ovulation (72), whereas GH or IGF-I accelerates age at first ovulation (73, 74) by decreasing the sensitivity to gonadal negative feedback inhibition of LH secretion (75). Because these manipulations did not affect age at menarche, one could conclude that the GH axis may regulate the tempo of sexual maturation but not the onset of puberty. However, these treatments in monkeys were initiated at an age after the increase in GnRH pulsatility had likely occurred (76, 77). Manipulations of the GH axis at juvenile ages will produce a more definitive test of the hypothesis that IGF-I initiates puberty.

In conclusion, the findings of the present study are the first to demonstrate an increase in nocturnal leptin and GH-induced IGF-I secretion before the onset of puberty in the agonadal male monkey, providing justification for further investigation of the role of leptin and the GH axis, in particular IGF-I, in regulating developmental increases in pulsatile GnRH secretion. It is not clear whether one signal may have a more prominent role or whether redundancy is present with respect to growth-related signals to guarantee fertility is attained. Even in this limited analysis, it is intriguing that the maximum prepubertal increase in leptin and IGF-I occurred simultaneously in four of the five animals studied. The observation that this maximum increase varied between 10 and 30 days before the onset of puberty among the animals emphasizes the need to define more carefully the temporal relationship between the secretion of GnRH and these growth-related cues. Unlike other mammals, the interval between the onset of puberty and fertility in monkeys and children is extended and is susceptible to perturbations. Accordingly, what maintains the developmental drive in GnRH secretion is as important as what is responsible for its initiation, marking the transition to puberty. This underscores the need to not only identify the signal(s) that initiates puberty, but also how these signals sustain the developmental increase in GnRH secretion that ultimately results in fertility.

Acknowledgments

The samples used for the present analysis were collected during the course of another study (40), which was performed at the University of Pittsburgh, Center for Research in Reproductive Physiology (Pittsburgh, PA), and supported by Grants HD13254 and HD08610 (to T. M. Plant). We thank T. M. Plant, W. S. Bell, and V. L. Gay for facilitating the transfer of these samples to the Yerkes Primate Research Center, Emory University (Lawrenceville, GA). The reagents for the IGF-I assay were a generous gift from the National Hormone Pituitary Program, NIDDK, USDA, NICHD, and Dr. A. F. Parlow (University of California–Los Angeles, Los Angeles, CA).

References

1. **Wildt L, Marshall G, Knobil E.** 1980 Experimental induction of puberty in the infantile female rhesus monkey. Science. 207:1373–1375.
2. **Plant TM, Gay VL, Marshall GR, Arslan M.** 1989 Puberty is triggered by chemical stimulation of the hypothalamus. Proc Natl Acad Sci USA. 86:2506–2510.
3. **Bourguignon JP, Gerard A, Gonzales MLA, Purnelle G, Franchimont P.** 1995 Endogenous glutamate involvement of GnRH: evidence from effect of glutamine and developmental changes. Endocrinology. 136:911–916.
4. **Zamorano PI, Mehesh VB, De Sevilla L, Brann D.** 1998 Excitatory amino acids receptors and puberty. Steroids. 63:268–270.
5. **Gore AC, Wu TJ, Rosenburg JJ, Roberts JL.** 1996 GnRH and NMDA receptor gene expression and co-localization change during puberty in female rats. J Neurosci. 16:5281–5289.
6. **Urbanski H, Ojeda SR.** 1990 A role for NMDA receptors in the control of LH secretion and the initiation of female puberty. Endocrinology. 126:1774–1776.
7. **Mitsushima D, Hei DL, Terasawa E.** 1994 GABA is an inhibitory neurotransmitter restricting the release of LHRH before the onset of puberty. Proc Natl Acad Sci USA. 91:395–399.
8. **Mitsushima D, Marzban F, Luchansky LL, et al.** 1996 Role of glutamic acid decarboxylase in the prepubertal inhibition of the LHRH release in female rhesus monkeys. J Neurosci. 16:2563–2573.
9. **Roth C, Jung H, Kim K, et al.** 1997 Involvement of GABA in the post natal function of the GnRH pulse generator as determined on the basis of GnRH and GnRH-receptor gene expression in the hypothalamus and pituitary. Exp Clin Endocrinol Diabetes. 105:353–358.
10. **El Majdoubi M, Sahu A, Plant TM.** 1998 Pubertal changes in hypothalamic GnRH and related peptide gene expression in the monkey. Soc Neurosci Abstr. 25(Part 1):796.
11. **Plant TM, El Majdoubi M, Sahu A, Ramaswamy S.** Hypothalamic gene expression during puberty in the monkey. Proc 81st Meeting of The Endocrine Society, San Diego, CA, 1999, abstract S61-1.
12. **Ojeda SR, Ma YJ, Rage F.** 1995 A role for TGF-α in the neuroendocrine control of female puberty. In: Plant TM, Lee PA, eds. The neurobiology of puberty. Bristol, UK: J Endocrinol Ltd.; 103–117.
13. **Ma YJ, von der Emde KB, Rage F, Wetsel WC, Ojeda SR.** 1997 Hypothalamic astrocytes respond to TGF-α with the secretion of neuroactive substances that stimulate the release of LHRH. Endocrinology. 138:19–25.
14. **Perera AD, Suter KJ, Pohl CR, Plant TM.** 1995 The neurobiology of the prepubertal restraint of pulsatile GnRH release in the monkey. In: Plant TM, Lee PA, eds. The neurobiology of puberty. Bristol, UK: J Endocrinol Ltd; 175–184.
15. **Bourguignon JP.** 1991 Growth and the timing of puberty: reciprocal effects. Horm Res. 36:131–135.
16. **Plant TM, Fraser MO, Medhamurthy R, Gay VL.** 1989 Somatogenic control of GnRH neuronal synchronization during development in primates: a speculation. In: Delemarre-van de Waal HA, Plant TM, van Rees GP, Schoemaker J, eds. Control of the onset of puberty III. Amsterdam: Excerpta Medica; 111–121.
17. **Foster DL, Nagatani S.** 1998 Physiological perspectives on leptin as a regulator of reproduction: role in timing puberty. Biol Reprod. 60:205–215.
18. **Matkovic V, Ilich JZ, Skugor M, et al.** 1997 Leptin is inversely related to age at menarche in human females. J Clin Endocrinol Metab. 82:3239–3245.
19. **Mantzoros CS, Flier JS, Rogol AD.** 1997 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. 82:1066–1070.
20. **Arslanian S, Suprasongsin C, Kalhan SC, Drash AL, Brna R, Janosky JE.** 1998 Plasma leptin in children: relationship to puberty, gender, body composition, insulin sensitivity and energy expenditure. Metabolism. 47:309–312.
21. **Blum WF, Englaro P, Hekman M, Anttanasio AM, Kiess W, Rascher W.** 1997 Plasma leptin levels in healthy children and adolescents. J Clin Endocrinol Metab. 82:2904–2910.
22. **Kiess W, Blum WF, Aubert MT.** 1998 Leptin, puberty and reproductive function. Eur J Endocrinol. 138:26–29.
23. **Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF.** 1997 Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary gonadal hormones and pubertal stage. J Clin Endocrinol Metab. 82:2849–2855.
24. **Clayton PE, Tillmann V, Hall CM, Gill MS.** Constitutional delay in growth and puberty is associated with hypoleptinemia in boys. Proc 80th Meeting of The Endocrine Society, New Orleans, LA, 1998, abstract P1-248.
25. **Chehab FF, Mounzih K, Lu R, Lin ME.** 1997 Early onset of reproductive function in normal female mice treated with leptin. Science. 275:88–90.
26. **Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS.** 1997 Leptin accelerates the onset of puberty in normal female mice. J Clin Invest. 99:391–395.
27. **Cheung CC, Thorton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA.** 1997 Leptin as a metabolic gate for the onset of puberty in the female rat. Endocrinology. 138:855–858.
28. **Plant TM, Durrant AR.** 1997 Circulating leptin does not appear to provide a signal for triggering the initiation of puberty in the male rhesus monkey (*Macaca mulatta*). Endocrinology. 138:4505–4508.
29. **Urbanski HF, Pau KYF.** 1998 A biphasic developmental pattern of circulating leptin in the male rhesus macaque. Endocrinology. 139:2284–2286.
30. **Ogilvy-Stuart AL, Shalet SM.** 1992 GH and puberty. J Endocrinol. 135:405–406.
31. **Darendelier F, Hindmarsh PC, Preece MA, Cox L, Brook CGD.** 1990 GH increases the rate of sexual maturation. Acta Endocrinol. 122:414–416.
32. **Stanhope R, Albanese A, Hindmarsh P, Brook CDG.** 1992 The effects of GH therapy on spontaneous sexual development. Horm Res. 38:9–13.
33. **Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL.** 1996 IGF-I of peripheral origin acts centrally to accelerate the initiation of female puberty. Endocrinology. 137:3717–3728.
34. **Tanner JM, Whitehouse RH.** 1975 A note on the bone age at which patients

with true isolated GH deficiency enter puberty. J Clin Endocrinol Metab. 41:788–790.
35. **Gruaz NM, d'Alleves V, Charnay Y, et al.** 1997 Effects of constant infusion with IGF-I to immature female rats on body weight gain, tissue growth, and sexual function. Endocrine. 6:11–19.
36. **Mauras N, Blizzard RM, Link K, Johnson ML, Rogal AD, Veldhuis JD.** 1987 Augmentation of GH secretion during puberty. J Clin Endocrinol Metab. 64:596–601.
37. **Link K, Blizzard RM, Evans WS, Kaiser DL, Parker MW, Rogol AD.** 1986 The effect of androgens on the pulsatile release and twenty four hour mean concentration of growth hormone in peripubertal meales. J Clin Endocrinol Metab. 62:159–164.
38. **Wilson ME, Gordon TP, Tanner JM.** 1993 Constant low-dose oestradiol replacement accelerates skeletal maturation and growth in ovariectomized adolescent rhesus monkeys. J Endocrinol. 137:519–527.
39. **Wilson ME.** 1998 Regulation of the growth hormone 0 insulin-like growth factor I axis in developing and adults monkeys is affected by estradiol replacement and supplementation with insulin like growth factor I. J Clin Endocrinol Metab. 83:2018–2028.
40. **Suter KJ, Pohl CR, Plant TM.** 1998 The pattern and tempo of the pubertal re-augmentation of open-loop pulsatile GnRH release in the male rhesus monkey as assessed using the *in situ* pituitary. Endocrinology. 139:2774–2783.
41. **Osterud EL, Lackey S, Wilson ME.** 1986 estradiol increases somatomedin-C concentrations in adolescent rhesus macaques. Am J Primatol. 11:53–62.
42. **Frisch RE.** 1972 Weight at menarche: similarity for well nourished and undernourished girls at differing ages and evidence for historical constancy. Pediatrics. 50:445–450.
43. **Kennedy GC, Mitra J.** 1963 Hypothalamic control of energy balance and the reproductive cycle in the rat. J Physiol. 166:395–407.
44. **Mantzoros CS, Moschos SJ.** 1998 Leptin: in search of role(s) in human physiology and pathophysiology. Clin Endocrinol. 49:551–567.
45. **Cunningham MJ, Clifton DK, Steiner RA.** 1998 Leptin's action on the reproductive axis: perspectives and mechanisms. Biol Reprod. 60:216–222.
46. **Licinio J, Negro AB, Mantrzoros C, et al.** 1998 Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc Natl Acad Sci USA. 95:2541–2546.
47. **Plant TM.** 1985 A study of the role of the postnatal testes in determining the ontogeny of gonadotropin secretion in the male rhesus monkey (*Macaca mulatta*). Endocrinology. 116:1341–1350.
48. **Matkovic V, Ilich JZ, Badenhop NE, et al.** 1997 Gain in body fat is inversely related to nocturnal rise in serum leptin in young females. J Clin Endocrinol Metab. 83:1368–1372.
49. **Palmert MR, Radovick S, Boepple PA.** 1998 The impact of reversible gonadal sex steroids suppression on serum leptin concentrations in children with central precocious puberty. J Clin Endocrinol Metab. 83:1091–1096.
50. **Pombo M, Herrera-Justiniano E, Considine RV, et al.** 1997 Nocturnal rise of leptin in normal prepubertal and pubertal children and in patients with perinatal stalk-transection syndrome. J Clin Endocrinol Metab. 82:2751–2754.
51. **Ahima RS, Prabakaran D, Flier JS.** 1998 Postnatal leptin surge and regulation of circadian rhythm by feeding. J Clin Invest. 101:1020–1027.
52. **Fruhbeck G, Jebb SA, Prentice AM.** 1998 Leptin: physiology and pathophysiology. Clin Physiol. 18:399–419.
53. **Stoving RK, Vinten J, Handberg A, et al.** 1998 Diurnal variation of the serum leptin concentration in patients with anorexia nervosa. Clin Endocrinol. 48:761–768.
54. **Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM.** 1997 Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci USA. 94:1023–1028.
55. **Finn PD, Cunninghjam MJ, Pau KYF, Spies HG, Clifton DK, Steiner RA.** 1998 The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology. 139:4652–4662.
56. **Zamorano PL, Mahesh VB, De Sevilla LM, Chorich LP, Bhat GK, Brann DW.** 1997 Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology. 65:223–228.
57. **Guan XM, Yu H, van der Ploeg LHT, Smith RG.** 1997 Preparation of functionally active [^{35}S] leptin for mapping its receptors in the brain. Anal Biochem. 247:175–177.
58. **Magni P, Vettor R, Pagano C, et al.** 1999 Expression of a leptin receptor in immortalized gonadotropin-releasing hormone secreting cells. Endocrinology. 140:1582–1585.
59. **Gore AC, Mitsushima D, Terasawa E.** 1993 A possible role of neuropeptide Y in the control of the onset of puberty in female rhesus monkeys. Neuroendocrinology. 58:23–34.
60. **Schwartz MW, Seely RJ, Campfield LA, Burn P, Baskin DG.** 1996 Indentification of targets of leptin action in the rat hypothalamus. J Clin Invest. 98:1101–1106.
61. **Schneider JE, Goldman MD, Tang S, Bean B, Ji H, Friedman MI.** 1998 Leptin indirectly affects estrous cycles by increasing metabolic fuel oxidation. Horm Behav. 33:217–228.
62. **Shi ZQ, Nelson A, Whitcomb L, Wang J, Cohen AM.** 1998 ICV administration of leptin markedly enhances insulin sensitivity and systemic glucose utilization in conscious rats. Metabolism. 47:1274–1280.
63. **Sara VR, Hall K.** 1990 Insulin like growth factors and their binding proteins. Physiol Rev. 70:591–614.
64. **Giustina A, Veldhuis JD.** 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev. 19:717–797.
65. **Carro E, Considine RV, Casanueva FF, Dieguez C.** 1997 Regulation of *in vivo* growth hormone secretion by leptin. Endocrinology. 138:2203–2206.
66. **Tannenbaum GS, Gurd W, Lapointe M.** 1998 Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. Endocrinology. 139:3871–3875.
67. **Bianda TL, Glatz Y, Boeni-Schnetzler M, Froesch ER, Scmid C.** 1997 Effects of growth hormone (GH) and insulin-like growth factor-I on serum leptin in GH deficient adults. Diabetologica. 40:363–364.
68. **Hiney JK, Ojeda SR, Dees LW.** 1991 IGF-I: a possible metabolic signal involved in the regulation of female puberty. Neuroendocrinology. 54:420–423.
69. **Zhen S, Zakaria M, Wolfe A, Radovick S.** 1997 Regulation of GnRH gene expression by IGF-I in a cultured GnRH-expressing neuronal cell line. Mol Endocrinol. 11:1145–1155.
70. **Longo KM, Sun Y, Gore AC.** 1998 IGF-I effects on GnRH biosynthesis in GT1–7 cells. Endocrinology. 139:1125–1132.
71. **Castro-Alamancos MA, Arevalo MA, Torres-Aleman I.** 1996 Involvement of protein kinase-C and nitric oxide in the modulation by IGF-I of glutamate-induced GABA release in the cerebellum. Neuroscience. 70:843–847.
72. **Wilson ME, Tanner JM.** 1994 Somatostatin analog treatment slows growth and the tempo of reproductive maturation in female rhesus monkeys. J Clin Endocrinol Metab. 79:495–501.
73. **Wilson ME.** 1998 Premature elevation in serum IGF-I advances first ovulation in rhesus monkeys. J Endocrinol. 158:247–257.
74. **Wilson ME, Gordon TP, Rudman CG, Tanner JM.** 1989 Effects of GH on the tempo of sexual maturation in female rhesus monkeys. J Clin Endocrinol Metab. 68:29–38.
75. **Wilson ME.** 1995 IGF-I administration advances the decrease in hypersensitivity to oestradiol negative feedback inhibition of serum LH in adolescent female rhesus monkeys. J Endocrinol. 145:121–130.
76. **Pohl CR, DeRidder CM, Plant TM.** 1995 Gonadal and non-gonadal mechanisms contribute to the prepubertal hiatus in gonadotropin secretion in the female rhesus monkeys (*Macaca mulatta*). J Clin Endocrinol Metab. 80:2094–2101.
77. **Suter KJ, Pohl CR, Plant TM.** 1999 Pulsatile GnRH released assessed indirectly in agonadal prepubertal aged female rhesus monkeys and a comparison with GnRH pulse generator activity in the castrate male monkey of comparable age. J Endocrinol. 160:35–41.