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Longitudinal Study of the Simultaneous Secretion of Melatonin and Leptin during Normal Puberty

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Key Words
Puberty · Development, pubertal · Melatonin · Leptin

Abstract
Background/Aims: Pubertal changes are a consequence of the activation of the hypothalamic-pituitary-gonadal axis due to an increase in the frequency and magnitude of pulses of gonadotropin-releasing hormone (GnRH), which may depend on the intrinsic properties of the neurons of the hypothalamic arcuatus nucleus, or on the influence of neurotransmitters and/or neuromodulators. We evaluated the serum concentrations of melatonin and leptin in healthy prepubertal and adolescent subjects of both sexes, to define their participation at the initial stages and during the progression of pubertal development. ***Methods:*** 80 pediatric subjects (47 females and 33 males), aged 6–18 years, were divided into 2 groups, prepubertal (n = 25) and adolescent (n = 55), according to the absence or presence, respectively, of physical signs of pubertal development. The subjects were assessed on two occasions: at the time of their inclusion in the study, and 12–18 months later when the subject had advanced one pubertal stage according to the Tanner classification. Blood was obtained in fasting for clinical purposes and for the hormonal study. Melatonin and leptin were measured by radioimmunoanalysis. ***Results:*** As described previously, melatonin decreases at the onset of puberty and during pubertal development. Both the absolute melatonin value and the decrease between evaluations tended to be greater in females; the variations were correlated with neither an increase in body weight nor with the degree of pubertal development. The concentration of leptin increased in both sexes with the progression of puberty, this value being 40% greater in women, and correlated with the indicators of an increase in body volume and fat accumulation. Although its concentration remained stable between evaluations for both sexes, among the males the association between leptin and pubertal development took place at the start of the process, while for the females we observed a significant overall association between pubertal stage and leptin concentration, this association being stronger at more advanced Tanner stages. Neither at the onset of puberty nor during its course did we observe any significant relation between melatonin concentration and any of the Tanner stages, whether for males or for females. Neither was there any correlation between the absolute values or rates of modification of melatonin and leptin. ***Conclusion:*** According to the evolutionary dynamics of their respective concentrations, both initially and during pubertal progress, melatonin and leptin do not interact in the initiation or progression of human pubertal development, and do not seem to play a key role in this process.

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Introduction

During puberty the secondary sexual characteristics appear, the speed of growth increases until final height is reached, and fertility is achieved. These changes are the consequences of the activation of the hypothalamic-pituitary-gonadal axis for an increase in the frequency and magnitude of pulses of gonadotropin-releasing hormone (GnRH) [1, 2]. It is not known if the pulsatile nature of GnRH secretion depends on the intrinsic properties of the neurons of the hypothalamic arcuate nucleus or if it reflects the influence of neurotransmitters and/or neuromodulators [3]. Among the neuromodulators involved, different products of the pineal gland can be found, including melatonin, 5-methoxytryptophol [4] and 5-methoxytryptamine. In animals with seasonal reproduction, the pineal gland synchronizes the reproductive functions with the optimum environmental conditions [5].

Another neuromodulator, leptin, which conveys information about the nutritional state to the hypothalamic generator of pulses of GnRH, seems necessary for the beginning of puberty and the establishment of the secondary sexual characteristics, although the liberation of gonadotropins during early life is independent of the presence of leptin [6]. Menarche needs a minimum of 17% body fat and at least 22% for the maintenance of regular menstruation in girls aged more than 16 years [7, 8]. Since leptin levels reflect the stores of body fat, in situations of extreme thinness or in the presence of menstrual anomalies (e.g., among athletes, or in cases of anorexia nervosa), the fall in leptin levels below a critical threshold interrupts the reproductive processes.

During sexual maturation, leptin presents a dimorphic feature for both sexes: in boys, a brief peak in the serum levels of leptin seems to precede the beginning of puberty [9, 10], whereas in female adolescents there is a significant correlation between the progressive increase in the mean concentration with total body fat; however, the percentage increase in the nocturnal concentration of leptin is inversely related to the percentage body weight increase [11, 12]. In women there is a sustained increase in leptin during pubertal development, whereas in males it is unchanged [13] or decreases from Tanner stage II [14, 15]. This pattern is the inverse to that of the levels of testosterone, thereby suggesting a 'feedback' between leptin and the gonadotropin-testosterone axis.

We tested the hypothesis that during pubertal development melatonin and leptin may interact as key signals that can provide information for the control of GnRH secretion. Consequently, in the present study, we analyzed the longitudinal evolution of melatonin and leptin concentrations in healthy subjects of both sexes and the behavior and role of these neurohormones in pubertal development. Relationships with body weight or body fat were also examined to assess their relative intervention and interaction in the initiation and progression of normal pubertal development.

Subjects and Methods

80 pediatric patients (47 females and 33 males) aged 6–18 years were recruited from among those treated at the outpatient department of the Materno-Infantil Hospital and the San Cecilio University Hospital (both in Granada, Spain) for mild pathologies (nonspecific abdominal pain, headache, overweight, rhinoconjunctivitis, mild asthma, thoracic pain, etc.), without organic aftereffects (following normal clinical examination). None were receiving medical treatment for this pathology. The recruitment of these pediatric patients was the result of their clinical demands, and was not performed on the basis of an ad hoc prospective study; for this reason, and for ethical arguments (the patients suffered mild pathologies), we did not obtain samples during nocturnal hours.

The samples obtained were classified into 2 groups: group I, pre-pubertal (n = 25), and group II, adolescent (n = 55), according to the absence or presence, respectively, of initial physical signs of pubertal development; this was regardless of whether the samples were from the first or the second analytical evaluation, because after analyzing the information there were 12 cases that remained fully in stage I in the second evaluation. The mean age difference between groups I and II was 55.80 months for the males, and 61.65 months for the females. In none of the subjects in group I was there any physical sign of pubertal development according to the Tanner criteria (pubic or axillary hair in both sexes, increased penis size or testicle volume among the males, or the beginning of breast growth among the females). The testicle volume of the males was less than 4 ml in every case. In group II, on the initial physical examination, 18 subjects were classified as Tanner stage II (4 males and 14 females), 16 were in stage III (8 and 8), 13 in stage IV (5 and 8) and 8 in stage V (1 and 7). By the second examination, each subject in group II had advanced by just one pubertal stage, except for obvious reasons the 8 subjects in stage V.

In all cases, both the person involved and their parents or guardians were informed verbally, and written consent was obtained.

Exclusion criteria were: (a) low probability of completing the protocol questionnaire; (b) acute severe illness; (c) chronic severe pathology, such as diabetes, morbid obesity, moderate-severe asthma, complex cardiopathy, polymalformative syndrome, psychomotor or mental retardation, delayed growth, thyroid pathology, sexual and/or adrenal hormone pathology, intestinal malabsorption, and (d) medical treatment with corticoids and/or immunosuppressors, or any medical treatment continued during the 3 months previous to inclusion in the study. The presence of family antecedents of the above-mentioned criteria was not a reason for exclusion.

Table 1. Data are represented as mean ± SD, with mean standard deviation score (SDS) within brackets for somatometric variables

	Boys (n = 33)				Girls (n = 47)			
	onset of puberty (n = 15)		adolescents (n = 18)		onset of puberty (n = 10)		adolescents (n = 37)	
	evaluation 1	evaluation 2	evaluation 1	evaluation 2	evaluation 1	evaluation 2	evaluation 1	evaluation 2
Age, years	8.54 ± 1.49	9.59 ± 1.52	13.08 ± 1.64	14.02 ± 1.58	7.88 ± 1.34	8.77 ± 1.21	12.58 ± 2.23	13.52 ± 2.17
Weight, kg	31.1 ± 7.8 (–0.098)	33.6 ± 8.6 (–0.241)	57.6 ± 16.1 (0.420)	62.9 ± 17.1 (0.422)	26.5 ± 4.8 (–0.371)	28 ± 4.3 (–0.676)	49.7 ± 10.6 (–0.085)	52.1 ± 11.1 (–0.162)
Height, cm	129.7 ± 7.8 (–0.359)	134.7 ± 7.6 (–0.373)	157.5 ± 12.9 (0.056)	163.6 ± 12.6 (0.039)	122.5 ± 6.5 (0.0031)	127 ± 5.6 (0.0071)	151 ± 11 (0.0027)	154.5 ± 9.3 (0.0032)
BMI, kg/m^2	18.3 ± 3.5 (0.174)	18.3 ± 3.6 (–0.130)	22.9 ± 4 (0.446)	23.2 ± 4.3 (0.456)	17.7 ± 3 (–0.894)	17.4 ± 2.4 (–0.979)	21.6 ± 3.2 (–0.493)	21.6 ± 3.3 (–0.382)
4 cf sum, mm	44.8 ± 29.2	43.1 ± 27.6	66.2 ± 31.9	62.9 ± 34.2	41.1 ± 13.3	36.7 ± 13	65.7 ± 24.6	62 ± 23.7
Melatonin, pg/ml	12.18 ± 19.3	9.75 ± 10.5	9.7 ± 10.5	5.6 ± 5.1	24.1 ± 25.3	13.1 ± 10.5	24.8 ± 38.8	14.5 ± 27.7
Leptin, ng/ml	4.76 ± 2.7	5.6 ± 3.4	8.7 ± 5.3	8.5 ± 5.5	6.2 ± 3	6.12 ± 3.6	11.2 ± 5.3	11.1 ± 4.5
DHEAS, μg/dl	532.1 ± 460.3	1,096.9 ± 977.7	1,334.3 ± 1,079.8	1,529.7 ± 1,077	312.2 ± 265.5	498 ± 343.9	1,055.2 ± 749.1	1,226.6 ± 658.5
FSH, UI/l	0.83 ± 0.53	0.43 ± 0,36	1.15 ± 0.82	1.18 ± 0.87	1.6 ± 1.08	1.3 ± 1.16	3.12 ± 2.34	2.39 ± 1.13
LH, UI/l	0.19 ± 0.29	0.3 ± 0.36	0.39 ± 0.88	0.1 ± 0.22	1.88 ± 1.15	2.99 ± 4.46	3.34 ± 3.32	5.98 ± 7.77
Testosterone, ng/dl	0.27 ± 0.25	0.21 ± 0.09	2.43 ± 1.79	3.06 ± 1.72	–	–	–	–
Estradiol, pg/ml	–	–	–	–	6.54 ± 3.49	3.6 ± 6.6	55.6 ± 68.7	43.5 ± 46.1

BMI = Body mass index, 4 cf sum = sum of four cutaneous folds, DHEAS = dehydroepiandrosterone sulfate, FSH = follicle-stimulating hormone, LH = luteinizing hormone. Data obtained from statistical analysis of BMI, melatonin and leptin are included in their respective figures (numbers 3, 1 and 2, respectively): these variables are included in this table only to record the values of their mean ± SD. For SDS calculations, we tested the individual values of our patients against the comparable populational mean age ± SD obtained from the Andalusian transversal study of growth 2004–2005 (Estudio transversal de crecimiento andaluz 2004–2005).

Clinical Methods

Every patient was evaluated on two occasions: at the time of selection and 12–18 months later when they had advanced one pubertal stage according to Tanner's classification of pubertal development. As mentioned, 12 subjects remained in Tanner stage I even at the second evaluation.

A detailed clinical history and physical exploration was carried out, compiling the pertinent information about physical development (weight, height), nutritional state (skin folds of left side: bicipital, tricipital, subscapular and suprailiac skin fold thickness, obtained with a Holtain compass) and sexual maturation (Tanner stages I–V). The body mass index (BMI) was calculated as weight (kg)/height (m^2); arterial tension was measured, and the average of two determinations was annotated. Part of this information is shown in table 1.

Analytical Methods

Blood samples were obtained after a 12-hour overnight fast, at 9.00 a.m., for the routine evaluation of global health state and to measure the hormonal parameters that were the object of the study, i.e. melatonin and leptin. DHEAS, testosterone (only in males) and estradiol (only in females) were included as internal controls of the normality of pubertal evolution among our subjects, and of the validity of the samples obtained. Menstruating female adolescents were assayed in the first 5 days after menstruation. The serum samples obtained were divided into four aliquots of 0.5 ml in Eppendorf tubes and frozen at –20°C until the time of analysis.

Melatonin and leptin were measured by radioimmunoanalysis (RIA). For melatonin, we used the kit made by Labor Diagnostika Nord GmbH and Co KG, (Nordhorn, Germany; Cat. No. BA 0800), which possesses a detection range for melatonin of 0.75–320 pg/ml. For leptin, we used a kit prepared by LINCO Research, Inc. (St. Charles, Mo., USA; Cat. No. *HL-81HK), with a detection limit of 0.5–100 ng/ml. For both RIA assays, the specificity was 100% and the intra- and inter-assay variation coefficients were less than 10%.

Statistical Methods

Although, a priori, the set of variables included might be considered typical biological variables, the Shapiro and Willis test showed that most of the distributions did not fit criteria of normality. Consequently, we employed the Friedman test to compare two variables that were not parametric, with paired data and two factors (factor I, the evaluation number, and factor II, sex). For correlation studies (Pearson) and linear simple regression analyses, variables were normalized by calculating the neperian logarithm (Ln). In order to compare melatonin and leptin (the continuous quantitative variables) with the Tanner stage (the ordinal variable), we carried out a multinomial logistic regression analysis, taking the Tanner classification as the dependent variable and stage I as the reference category. The analysis was individualized for males and females, and separately for each of the two evaluations. In each case, the variables 'concentration of leptin' and 'concentration of melatonin' were assumed to be a predictor. SPSS for Windows, version 13 (SPSS Inc., 1999–2004, Chicago, Ill., USA) was used for data entry and statistical analysis.

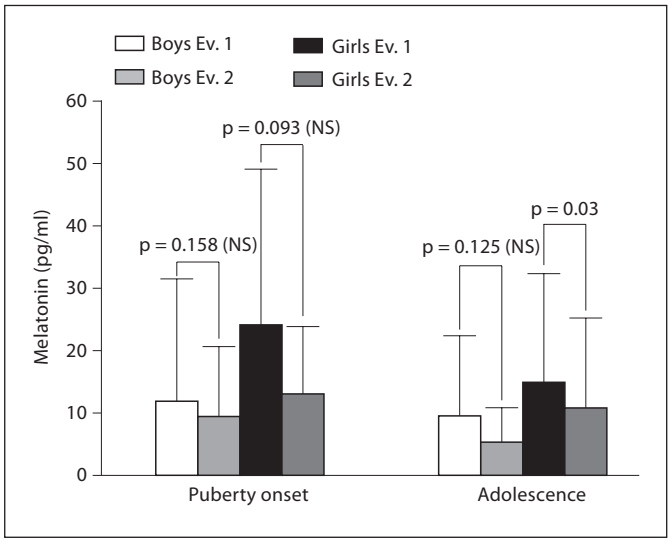

Fig. 1. Serum concentration of melatonin in the morning for both sexes in the 2 study groups (onset of puberty and adolescence). Also shown are the numerical data of the statistical analysis. NS = Not significant; Ev. = evaluation number.

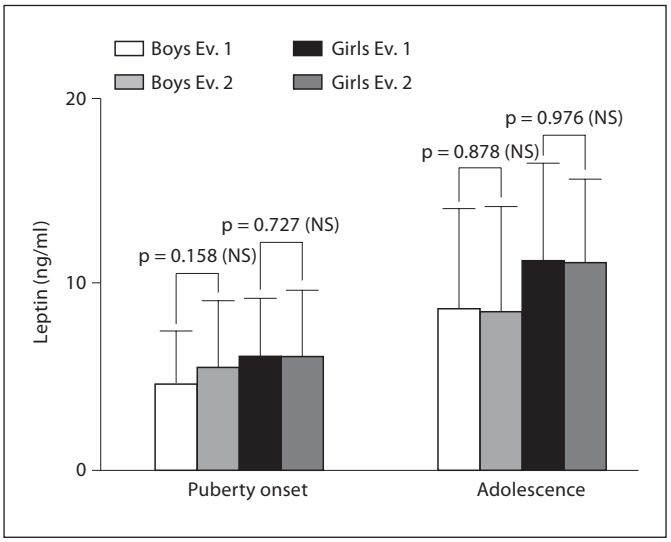

Fig. 2. Serum concentration of leptin in the morning for both sexes in the 2 study groups (onset of puberty and adolescence). Also shown are the numerical data of the statistical analysis. NS = Not significant; Ev. = evaluation number.

Results

The pubertal modifications of the concentrations of melatonin and leptin are shown in figures 1 and 2, with the evaluation number (first or second) and gender as classification variables. A trend for BMI and skin folds to decrease was observed in all groups. Melatonin levels decreased in both sexes between the first and the second evaluation, both in those beginning puberty (group I) and in the adolescents (group II), with values close to statistical significance, which was only reached in the group of adolescent females ($p < 0.05$; fig. 1). Moreover, the concentration of melatonin tended to be greater in females, although the differences were not statistically significant.

One year after the first evaluation, the levels of leptin remained almost unchanged in both groups. The overall concentrations were greater in females, especially in the group of adolescents (fig. 2). Between evaluations, the serum concentration of DHEAS showed a significant increase, which was greater in males (table 1). In the inter-evaluation results, the BMI remained practically unaltered in both groups, although a slight increase was observed in the group of adolescent boys, this difference being close to statistical significance (fig. 3).

The sum of the four skin folds (table 1), which represents a good index of fat accumulation, tended to decrease, reaching statistical significance only in the group of females beginning puberty ($p < 0.01$) and in adolescent boys ($p < 0.05$). The wide age difference between the 2 groups, in both sexes, explains the important increase in

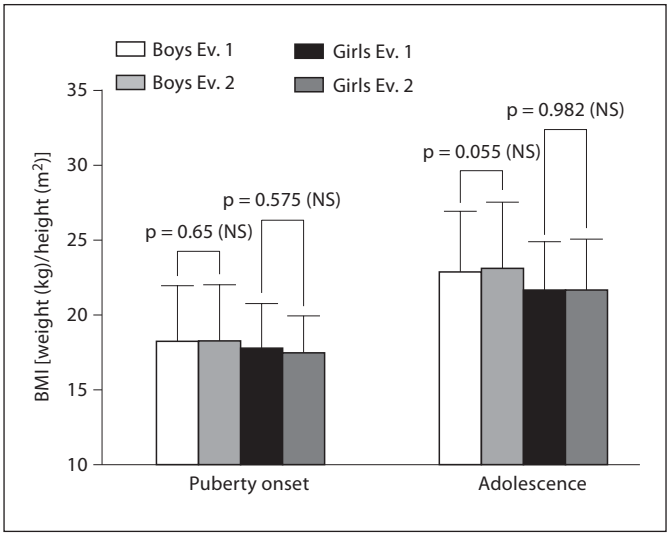

Fig. 3. Body mass index (BMI) values for both sexes in the 2 study groups (onset of puberty and adolescence). Also shown are the numerical data of the statistical analysis. NS = Not significant; Ev. = evaluation number.

the values of leptin, DHEAS and BMI and the sum of the four skin folds.

The measured inter-evaluation modifications to the variables only approach statistical significance for the decrease in melatonin ($F = 7.267$, $p < 0.1$); this modification was greater in males than in females, but did not occur between the groups of prepubertal subjects and adolescents. The increase in weight did not influence the decrease in melatonin. We did not measure any modification in the levels of leptin or DHEAS; the only indication of an interaction was observed between gender and pubertal stage for leptin.

In our overall set of samples, leptin showed a strong positive correlation with estradiol and DHEAS in females ($p < 0.001$) and with FSH ($r = 0.384$, $p < 0.001$) in males. In both sexes, leptin correlated with the BMI and with body weight.

At the onset of puberty, in general the blood concentration of leptin is associated with the Tanner stage among both males ($\chi^2 = 24.9$; $p < 0.001$) and females ($\chi^2 = 16.9$; $p < 0.01$). Among the males, the concentration of leptin discriminates between Tanner stages II and III with respect to stage I; thus, for each unit of increase in plasma leptin, the OR of being in Tanner stage II with respect to the males in stage I is multiplied by 1.8 (p = 0.01; 95% CI 1.15–2.91). Similarly, for each unit of increase in the plasma concentration of leptin, the possibility of being in Tanner stage III, with respect to males in Tanner stage I, is multiplied by 1.55 (p = 0.01; 95% CI 1.10–2.17). Among the females, the leptin concentration at the onset of puberty is more strongly associated with the more advanced Tanner stages; thus, for each unit of increase in plasma leptin concentration, the OR of being in Tanner stage II, with respect to females in stage I, is not significant (OR = 1.28; 95% CI 0.98–1.68). On the other hand, for each unit of increase in the plasma leptin concentration, the OR of being in Tanner stage III is 1.37 (p = 0.03; 95% CI 1.02–1.84); the OR of being in Tanner stage IV, for each unit of increase in plasma leptin concentration is 1.48 (48 (p = 0.009; 95% CI 1.1–1.99). As was to be expected, and in accordance with the results presented above, among the young males for whom puberty had already started, no significant association of the concentration of plasma leptin with Tanner stages was seen ($\chi^2 = 2.33$; p = NS); in other words this confirms thatamong the male subjects, the association of leptin and pubertal development takes place at the onset of the latter. Among the females, we observed a significant overall association between the pubertal stage and leptin concentrations ($\chi^2 = 13.01$; p = 0.01). For each unit of increase in the plasma concentration of leptin, the possibility of being in Tanner stage II was multiplied by 1.08 (p = NS; 95% CI 0.77–1.52), while that of being in Tanner stage III, with respect to the female subjects in stage I, is multiplied by 1.47 (p = 0.02; 95% CI 1.06–2.06) for each unit of increase in the plasma concentration of leptin. Similarly, for Tanner stage IV, we obtained an OR of 1.45 (p = 0.03; 95% CI 1.04–2.03), and for Tanner stage V an OR of 1.41 (p = 0.03; 95% CI 1.02–1.93).

Neither at the onset of puberty nor when it was in course did we observe any significant association between the concentration of melatonin and any of the Tanner stages, in males or in females.

Discussion

As far as we know, this is the first longitudinal study in which the serum levels of melatonin and leptin have been evaluated simultaneously in healthy preadolescents and adolescents of both sexes, to quantify the relative and conjoint participation of these two neuromodulators at the initial stages and during the progression of human pubertal development. While high concentrations of melatonin seem to inhibit puberty [16, 17], increased levels of leptin (related with the increase in fat apposition) would favor it [18–20].

In the present study, we confirm the previously documented drop in the levels of melatonin in both sexes and study groups [4, 21, 22]. This reduction only reaches statistical significance in females who have initiated puberty, a fact that contradicts the findings of Cavallo and Dolan [23] about the stability of the concentration from pubertal stage II up to the adult age, as well as those of our own group about a slight increase between ages 11 and 14 years [4]. Nevertheless, the values included here, obtained from samples taken in the early morning, are longitudinal (two measurements in the same subject, separated by one pubertal stage, approximately 1 year). Although melatonin levels tend to be higher in females throughout the lifespan [24, 25], only one previous paper from our group recorded differences during adolescence in the concentration of melatonin according to gender [4]; the results of the present study confirm that during puberty the values of serum melatonin tend to be higher in females. In addition, the variation between measurements was also greater among females, although without reaching statistical significance. An article published by Fideleff et al. [26] indicates that urinary excretion, nocturnal and diurnal, of the major melatonin metabolite,

6-sulfatoxymelatonin, is higher in obese than in lean boys at pubertal age, a difference not seen in girls. This difference could be partially explained by differences in the composition and distribution of body fat.

Melatonin levels decrease in both sexes when pubertal development begins [27], reaching statistical significance in females who have begun puberty. In contrast to another article [28], in our study the decrease in melatonin was not correlated with an increase in body weight; nor were the absolute levels or the variation between the two evaluations in our longitudinal study correlated with the rate of advance of pubertal development. Neither at the onset of puberty nor during its course did we observe any significant association between melatonin concentration and any of the Tanner stages in males or in females. Therefore, our results do not demonstrate a significant participation of melatonin at the beginning of and/or during the progression of human pubertal development, nor do they confirm the 'theory of dilution' in this respect.

Leptin shows a sexual dimorphism in children and adults; women produce greater quantities of leptin than do men with a similar BMI, and display a circadian rhythm for leptin production [29] similar to that of melatonin, with acrophase to midnight, nadir after midday and a pulsatile secretion profile, which disappears in anorexic women [30]. Before the start of puberty, the concentration of leptin increases and that of melatonin diminishes [21, 22, 31, 32]. In pubertal girls, who produce approximately twice as much leptin per kilogram of fat as do boys, there is a significant correlation between the average levels of leptin and overall body fat. Males, however, produce lower quantities of leptin per kilogram of fat after puberty than before it [18].

The concentration of leptin increases during infancy, in parallel with the deposits of energy, up to a concentration threshold that informs the central nervous system of the preparation of the body for the reproductive function and growth. Nevertheless, in primates the prepubertal increase in leptin does not stimulate the liberation of GnRH [33, 34] and, therefore, does not participate in the onset of puberty [35–37]. In humans leptin also plays an important role, since the ontogenic signals of the hypothalamic-pituitary-gonadal axis in the monkey and in man seem to be qualitatively identical [33, 34]. The modifications induced by increased levels of leptin would thus enable the operative capacity of other critical factors, the real 'puberty triggers'. Leptin is a necessary mediator between the deposits of energy and the hypothalamic-pituitary-gonadal axis; it correlates with BMI, the fat mass and the tricipital skin fold [38], and one of its fundamental roles is the regulation of body weight.

A strong inverse correlation has been documented between the nocturnal concentration of melatonin and age, Tanner stage and the LH concentration [39, 40]. Nevertheless, in our cases with morning samples, no correlation was found in the overall group (with two evaluations for every subject) between melatonin and LH, in either sex. Neither did we find any correlation between melatonin and BMI or body weight, in either sex. In agreement with our findings, Luboshitzky et al. reported that: (1) there is no relation between the secretions of melatonin and testosterone, LH, FSH or DHEAS [41]; (2) the secretion of LH is independent of the night pulses of melatonin [42], and (3) the modifications in the secretion of melatonin might be a consequence of the changes in the concentrations of pituitary gonadotropins and gonadal hormones [41]. Only in the overall set of samples from females included in our study did melatonin show a strong positive correlation with estradiol.

In the overall sample set, leptin levels were 41.73% higher in females than in males (10.12 $\pm$ 5 vs. 7.14 $\pm$ 4.8 ng/ml), although it should be borne in mind that the females were, on average, 6 months older than the males (144.59 $\pm$ 34.07 vs. 138.12 $\pm$ 33.2 months).

Although some studies do not find a significant correlation between the levels of leptin and those of estradiol in girls [43, 44], García-Mayor et al. [15] reported a positive correlation between leptin and FSH, LH and estradiol in girls aged 5–15 years, and a negative one with testosterone, FSH and LH in boys of the same age. In our overall set of samples, leptin showed a strong positive correlation with estradiol and DHEAS in females and with FSH in males. In both sexes, leptin correlated with the BMI and with body weight. When we examined each pubertal stage, the only correlations that persisted were those of BMI and body weight. The small number of cases in the subgroups comprised of the subjects in each Tanner stage might be the reason for the absence of other correlations between these hormones.

Our overall set of samples confirmed that: (1) leptin increases in both sexes with advancing puberty; (2) its concentration is greater in girls, and (3) the differences increase as pubertal development advances. Considering the interval between the two measurements, leptin only increased in the group of males who showed physical signs of pubertal development (although the difference was not statistically significant; fig. 3), suggesting a possible active role in this process. Nevertheless, in males with constitutional growth delay, the above-mentioned

increase does not take place at Tanner stage II and puberty progresses, which suggests that it is not necessary for the correct progression of puberty [45].

During the day, acute modifications in the levels of melatonin do not influence those of leptin in menopausal women, whether they are being treated with estradiol [46] or not, which confirms our findings about the absence of a correlation between these two hormones. While melatonin levels decreased in both sexes in the interval between the two evaluations made in our study, those of leptin remained practically unchanged during the interval of time in which puberty advanced one Tanner stage. Nevertheless, other studies have found an inverse relation between melatonin and leptin, and it has been suggested that the rhythm of leptin might be pineal or photoperiod dependent [47] and that the liberation of melatonin generates a decrease in that of leptin [48].

Although leptin has been proposed as an initiator of sexual maturation, together with gonadotropins and the GH axis [49, 50], our study does not support such an essential contributory role. The concentration of leptin does increase in both sexes as puberty advances, but our longitudinal study did not reveal any significant modification in leptin levels at one additional stage in pubertal development progression. Moreover, except for a slight increase in leptin at the beginning of puberty in males (an observation that has been published previously [10]), in the remaining comparisons the concentration of leptin presented a noteworthy degree of stability. We must also remember that, in the study groups entering puberty, pubertal physical signs began between the two evaluations made, and thus we cannot attribute a significant contribution to leptin, not having been able to measure possible modifications in serum levels. In the comparisons between the groups in which puberty had already begun (boys/girls in pubertal stages II–V) no change in the leptin concentration was observed. This fact was confirmed when it was shown that during the interval of the study, the BMI either remained unchanged or tended to fall. Only in the group of girls initiating pubertal development was a slight increase in the BMI observed; this was statistically significant, but may not be so in clinical terms. Moreover, this increased BMI in the group of girls entering puberty corresponds to an increase in lean mass, since the sum of the four skin folds (a good indicator of fat accumulation) decreased in all the groups, and in the groups that were entering puberty (girls) and those that had entered it (boys), the value was statistically significant. Our study group included normal boys/girls who were seen at the Department of Pediatrics at the request of the parents for minor problems. The general advice recommending a healthy diet and taking sufficient exercise might explain the decrease in the sum of the four skin folds, reflecting a decrease in the fat mass which might mask an increase in leptin levels. These levels remained unaltered (whereas the pubertal development begins or progresses) although the total adipose mass decreased or tended to decrease in some groups. This observation cannot be due merely to the inclusion of overweight subjects, because only 10 subjects (12% of the sample) were overweight and, consequently, only this small group was encouraged to change their lifestyle and so would not have confounded our results.

As limitations to our study, we recognize that, as in other studies of this nature, samples were taken in the morning, although the levels of the substances measured at this time may reflect nocturnal concentrations. In addition, the number of subjects in each subgroup was quite low, after dividing the overall sample by sex and pubertal stage. The follow-up period was designed to ensure that the subjects had advanced only one pubertal stage, while the main objective of the study was to define the longitudinal relationship between melatonin and leptin during puberty more than to establish normal reference values for these hormones.

In summary, the levels of leptin increased in both sexes at a similar rate during puberty, and were on average 40% greater in women, with this difference increasing over time. The increase in leptin levels correlated with the indicators of greater body volume and fat accumulation. Among the males, the association of leptin with pubertal development occurred at the onset of puberty. On the contrary, the females presented a significant overall association between pubertal stage and leptin concentrations. However, leptin levels remained virtually stable between evaluations, in both sexes, while the BMI behaved as a confounding variable, which leads us to believe that its direct participation in the onset and/or progress of puberty, must be very slight.

In conclusion, judging by the dynamics of their respective concentrations, melatonin and leptin do not have an influence on the initiation or progression of pubertal development, and due to the slight (but different) behavior of their concentrations they probably act more as permissive factors for the onset of puberty; individually, they are not capable of initiating puberty. These different behavioral patterns of melatonin and leptin may be necessary for pubertal development.

Our study supports the notion by which multiple factors cooperating over time are involved in the onset of

puberty, in accordance with the theory of a change in the hypothalamic sensibility that liberates the generating center of hourly pulses of GnRH, of the arcuate nucleus of the hypothalamus. Consequently, the truly important event in sexual maturation must be the interaction of the different hormonal axes and not their individual effects, which are better known. The changes in body composition and fat distribution constitute signs of the correct functioning of the hypothalamic-pituitary-hormonal axes and the secretion of insulin throughout life, this effect being even greater during puberty [51].

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