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Gender-related differences in urinary 6-sulfatoxymelatonin levels in obese pubertal individuals

Abstract: The objective of this study was to measure the urinary excretion of the main melatonin metabolite 6-sulfatoxymelatonin in obese and normal weight (wt) boys and girls. The study included 94 subjects, aged 4–15.7 yr (50 obese and 44 normal wt; 48 boys) classified as: mid-childhood (4–7.99 yr), late-childhood (8–12 yr) and pubertal (10.1–15.7 yr, Tanner II–IV). Normal wt subjects were children with a body mass index (BMI) between the 25th and 75th percentiles, and the group of obese subjects included children whose BMI was above the 97th percentile. A 24-hr urine sample was collected during two intervals: (i) 18:00–08:00 hr, and (ii) 08:00–18:00 hr. Analysis of urinary 6-sulfatoxymelatonin levels was performed by radioimmunoassay. Excretion of 6-sulfatoxymelatonin was expressed as: (i) total amount excreted (μg); (ii) μg excreted per time interval, nocturnal or diurnal; and (iii) the difference between nocturnal and diurnal samples (μg, estimated amplitude). A factorial analysis of variance indicated that nocturnal 6-sulfatoxymelatonin excretion and amplitude were significantly higher in the obese individuals. A significant interaction 'BMI × age' was detected, i.e. the effect of BMI was significant in the pubertal group only. Total, nocturnal and diurnal 6-sulfatoxymelatonin excretion was significantly higher in girls. The increase in 6-sulfatoxymelatonin excretion found in obesity occurred only in boys and at the pubertal age. To what extent this increase in melatonin production contributes to a delayed puberty in some pubertal obese males remains to be established.

Hugo L. Fideleff[1], Hugo Boquete[1], Gabriel Fideleff[1], Liliana Albornoz[2], Santiago Pérez Lloret[2], Martha Suarez[1], Ana I. Esquifino[2], Margarita Honfi[3] and Daniel P. Cardinali[2]

[1]Departmento de Endocrinología, Hospital T. Alvarez; [2]Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires; [3]Centro de Endocrinología, Hospital Francés, Buenos Aires, Argentina

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Address reprint requests to Dr Hugo L. Fideleff, Departmento de Endocrinología, Hospital T. Alvarez, Aranguren 2701, 1406 Buenos Aires, Argentina.
E-mail: hugofideleff@arnet.com.ar

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Introduction

Melatonin is produced in most organisms from algae to mammals, and its role varies considerably across the phylogenetic spectra [1]. In humans, it plays a major function in the coordination of circadian rhythmicity, including the sleep/wake cycle [2]. Circulating melatonin is produced and secreted mostly at night by the pineal gland. Its secretion is proportional to the duration of darkness, and it thus acts as a chemical code of scotophase duration.

Melatonin secretion is an 'arm' of the biologic clock in the sense that it responds to signals from the central pacemaker located in the suprachiasmatic nuclei (SCN) and in that the timing of the melatonin rhythm indicates the status of the clock, both in terms of phase (i.e. internal clock time relative to external clock time) and amplitude. Melatonin also plays a role in energy expenditure and body mass regulation in mammals [3]. Visceral fat levels increase with age [4, 5] whereas melatonin production declines [6]. Daily melatonin supplementation to middle-aged rats restored melatonin levels to those observed in young rats and suppressed the age-related gain in visceral fat [7, 8]. Melatonin treatment also has been shown to prevent the increase in body fat caused by ovariectomy in rats [9]. Melatonin partly mediates its effects through MT_2 receptors present in adipose tissue [10].

In human adults, obesity was not accompanied by significant modifications of melatonin production [11]. In childhood and adolescence, significant changes in body composition take place [12]. However, the possible correlation of obesity in prepubertal children and adolescents with melatonin production has not been examined so far. To better characterize a possible melatonin alteration in prepubertal and pubertal obesity, we studied diurnal, nocturnal and total melatonin production in 50 obese children and adolescents, and 44 normal controls matched on age, sex and maturational stage. Melatonin production was assessed by measuring the 24-hr urinary output of the predominant melatonin metabolite, 6-sulfatoxymelatonin. It is well established that urinary excretion of 6-sulfatoxymelatonin is an accurate reflection of melatonin production.

Materials and methods

Population examined

The study included 94 subjects of a Caucasic-Hispanic origin, aged 4–15.7 yr: 50 obese and 44 with normal weight

(wt) (48 boys). The sample was divided into the following groups: mid-childhood (4–7.9 yr), including eight obese and eight normal wt boys, and nine obese and 10 normal wt girls; late-childhood (8–12 yr), including eight obese and eight normal wt boys, and 10 obese and four normal wt girls; pubertal (10.1–15.7 yr, Tanner II–IV): including eight obese and eight normal wt boys, and seven obese and six normal wt girls. All pubertal girls were premenarchal.

Normal wt subjects belonged to a cohort of children and adolescents participating in a follow-up study of normal growth and development. Obese subjects were derived from the Department of Pediatrics for diagnosis and treatment of obesity; in all of them obesity was related to overfeeding and familiar history of obesity. Obese individuals had a body mass index (BMI) above the 95th percentile according to sex and age (Table 1). BMI of normal wt subjects was between 25th and 75th percentiles according to sex and age [13]. All children slept from 21:30–22:30 to 07:00–08:00 hr daily; nobody showed evidence of sleep apnea or any other sleep disorder. Subjects with any clinical or endocrine pathology or those receiving medication were excluded from the sample: all subjects had normal liver function tests. They lived in Buenos Aires or its surroundings (34°37′S, 58°25′W).

Informed consent was obtained from all subjects and their parents. The study was conducted according to the Declaration of Helsinki II and the Guidelines for Good Clinical Practice. The protocol was approved by the Ethical and Research Committees of the participating centers.

Urine collection

A 24-hr urine sample was collected at home during two intervals: (i) a 14-hr nocturnal sample (from 18:00 to 08:00 hr), and (ii) a 10-hr diurnal sample (from 08:00 to 18:00 hr). Detailed verbal and written instructions were given to the parents to assure complete collection of samples. All collections were made on Sunday to avoid possible interference with school activities.

Collected urines were stored in a refrigerator until delivered to the laboratory within 24 hr of the urine collection. The volume of each urine collection was measured and aliquots were conserved in plastic bottles without preservatives and stored frozen (−20°C) until assay. A trained laboratory technician was in charge to receive the samples and to verify their completeness. Urine samples that were lesser than the expected volume by body wt were discarded.

Table 1. Body mass index (BMI) (range) in the population examined

Group	Age	BMI (range)
Normal boys	Mid-childhood	12.2–17.4
	Late-childhood	15.2–18.9
	Puberty	19.2–26.0
Normal girls	Mid-childhood	14.0–17.4
	Late-childhood	14.8–17.0
	Puberty	16.0–21.0
Obese boys	Mid-childhood	19.0–25.2
	Late-childhood	22.8–30.5
	Puberty	25.0–34.9
Obese girls	Mid-childhood	17.3–26.5
	Late-childhood	22.3–32.0
	Puberty	23.0–34.5

Determination of urinary 6-sulfatoxymelatonin

Blinded analysis of urine 6-sulfatoxymelatonin levels was performed by radioimmunoassay using an assay kit from Stockgrand Ltd. (Guildford, UK) as previously described [14]. The urine samples were diluted prior to assay (1/250). The intra- and interassay coefficients of variation were 4% and 7%, respectively. Excretion of 6-sulfatoxymelatonin was expressed as: (i) total amount excreted (μg); (ii) μg excreted per time interval; and (iii) estimated amplitude: the difference between nocturnal and diurnal samples.

Statistical analysis

Results were statistically analyzed after log transformation of data by a factorial analysis of variance (ANOVA), Pearson's test for correlations and Student's *t*-test. An initial analysis included the whole data and gender, weight and age as variables. Subsequently, female and male data were assessed separately. SPSS software, version 10.1 (SPSS Inc., Chicago, IL, USA) was employed. Results are expressed as mean ± S.D. *P*-values < 0.05 were considered evidence for statistical significance.

Results

Nocturnal (18:00–08:00 hr) and diurnal (08:00–18:00 hr) 6-sulfatoxymelatonin excretion in the 24-hr urine collection are depicted in Fig. 1. A factorial ANOVA of the whole set of data indicated that nocturnal 6-sulfatoxymelatonin excretion and amplitude were significantly higher in the obese individuals ($F_{1.82} = 7.5$, $P = 0.008$ and $F_{1.82} = 4.0$, $P = 0.048$, respectively). A significant interaction 'BMI × age' was detected, i.e. the effect of BMI was seen mainly at the pubertal age ($F_{1.82} = 3.8$, $P = 0.027$ and $F_{1.82} = 3.1$, $P = 0.038$, for nocturnal 6-sulfatoxymelatonin excretion and the estimated amplitude of 6-sulfatoxymelatonin excretion, respectively). Total, nocturnal and diurnal 6-sulfatoxymelatonin excretion was significantly higher in girls than in boys ($F_{1.82} = 29.8$, $F_{1.82} = 25.8$ and $F_{1.82} = 19.0$, respectively, $P = 0.001$, factorial ANOVA). The effect of age did not attain significance for any parameter tested. A Pearson's test indicated significance for the following correlations: total and nocturnal 6-sulfatoxymelatonin excretion ($r = 0.98$, $P < 0.001$), total and diurnal 6-sulfatoxymelatonin excretion ($r = 0.46$, $P < 0.001$), nocturnal sulfatoxymelatonin excretion and estimated amplitude of 6-sulfatoxymelatonin excretion ($r = 0.92$, $P < 0.001$), total 6-sulfatoxymelatonin excretion and estimated amplitude of 6-sulfatoxymelatonin excretion ($r = 0.85$, $P < 0.001$), and diurnal and nocturnal 6-sulfatoxymelatonin excretion ($r = 0.36$, $P < 0.001$). Diurnal 6-sulfatoxymelatonin excretion did not correlate with amplitude of 6-sulfatoxymelatonin excretion ($r = 0.13$, $P = 0.2$).

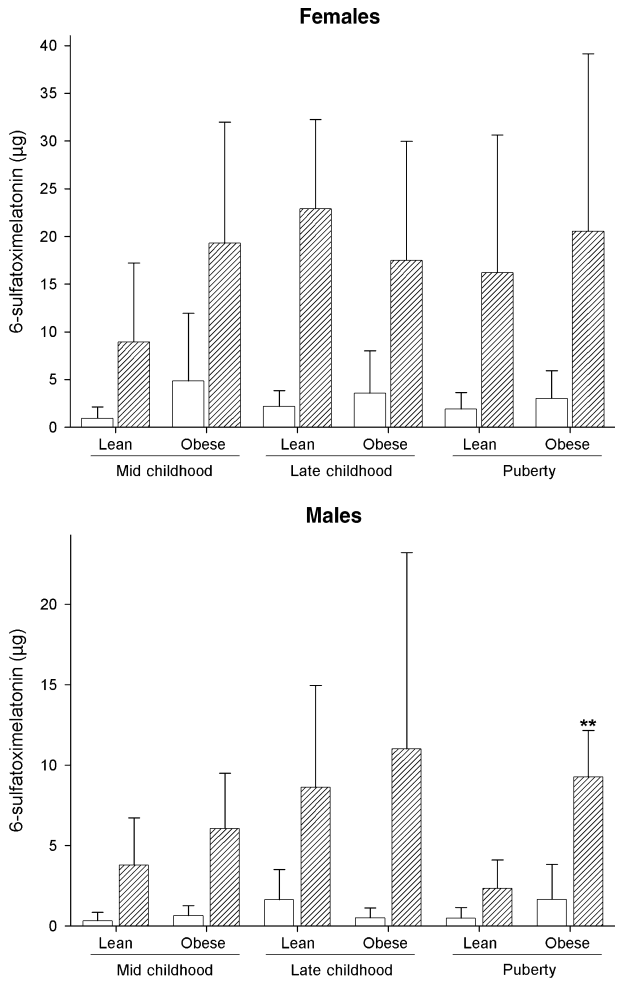

Fig. 1. Nocturnal (i.e. 18:00–08:00 hr collection) (hatched bars) and diurnal (i.e. 08:00–18:00 hr collection) (open bars) urinary levels of 6-sulfatoxymelatonin (μg) in lean and obese girls, and boys at mid-childhood (4–7.9 yr), late-childhood (8–12 yr) and puberty (10.1–15.7 yr, Tanner II–IV) (mean ± S.D.). $^{**}P < 0.0001$ when compared with nocturnal values of lean puberal boys, Student's *t*-test. For further statistical analysis see text.

Further analysis of results segregated by sex indicated that the difference in nocturnal 6-sulfatoxymelatonin excretion between pubertal obese and normal BMI boys was significant (Student's *t*-test, $P < 0.0001$; Fig. 1, lower panel) whereas differences in nocturnal 6-sulfatoxymelatonin excretion between obese and normal BMI girls did not attain significance (Fig. 1, upper panel). The differences in diurnal values of urinary 6-sulfatoxymelatonin of obese and normal BMI boys or girls did not attain significance.

Total 6-sulfatoxymelatonin excretion (μg/24 hr) in mid-childhood, late-childhood and puberty of boys was 6.7 (±3.7), 11.5 (±11.9) and 10.9 (±4.0) (obese) and 4.2 (±2.8), 10.3 (±7.6) and 2.8 (±2.1) (normal BMI), respectively. The difference in total 6-sulfatoxymelatonin excretion between obese and normal BMI boys found at puberty was significant (Student's *t*-test, $P < 0.0001$).

Total 6-sulfatoxymelatonin excretion in girls (μg/24 hr) at mid-childhood, late-childhood and puberty was 24.1 (±16.0), 21.0 (±14.0) and 22.4 (±20.9) (obese) and 9.9 (±9.0), 25.0 (±10.6) and 18.1 (±14.6) (normal BMI), respectively. The differences in total 6-sulfatoxymelatonin excretion between obese and normal BMI girls did not attain significance.

Estimated amplitudes (i.e. the difference between nocturnal and diurnal urinary 6-sulfatoxymelatonin excretion) at mid-childhood, late-childhood and puberty (μg) were, in obese boys, 5.4 (±3.3), 10.5 (±12.5) and 7.6 (±3.1) and, in normal BMI boys, 3.4 (±3.2), 7.0 (±5.3) and 1.9 (±1.6), respectively. The difference in amplitude of 6-sulfatoxymelatonin excretion between pubertal obese and normal BMI boys was significant (Student's *t*-test, $P < 0.0001$). In mid-childhood, late-childhood and puberty, amplitude of 6-sulfatoxymelatonin (μg) in girls was 14.5 (±13.2), 13.8 (±12.6) and 16.0 (±17.5) (obese) and 8.0 (±7.6), 20.6 (±8.1) and 14.3 (± 14.5) (normal BMI), respectively. The differences between obese and normal BMI girls did not attain significance.

Discussion

Normal melatonin rhythms are closely related to those of reproductive hormones during infancy and reciprocally correlated during puberty. The demonstration of melatonin receptors in reproductive organs [15, 16], and the localization of sex hormone receptors in the pineal gland [17–20], further strengthen these inter-relationships. However, it is not yet clear whether such correlations are functionally meaningful and a regulatory role of melatonin in puberty has yet to be established.

The foregoing results indicate that the urinary excretion of the major melatonin metabolite, 6-sulfatoxymelatonin, is higher in obese than in lean boys at a pubertal age, a difference not seen in girls. Puberty is a slow evolutionary process that starts years before the first signs or biochemical changes are detectable [21]. In the female, calorie intake, body composition, or adipose tissue reserves control the hypothalamic secretion of gonadotropin releasing hormone and presumably exerts a permissive action in the initiation of puberty [21]. Such a link between fat reserves and gonadal function is not seen in males [22]. Rather a delay in pubertal development has been reported in a subpopulation of obese boys [23].

Serum melatonin concentration is undetectable in humans for the first 3 months of life, increases to a peak value at 1–3 yr of age and declines thereafter. During childhood, serum melatonin concentrations drop by about 80% [24]. The decrease in nocturnal serum melatonin in children and adolescents correlated with body weight and body surface area [6, 25]. A progressive decrease in nocturnal serum melatonin or in urinary excretion rate of 6-sulfatoxymentonin has been reported with advancing age, compatible with a reduction in the amplitude of the circadian rhythm with maturation [6, 26–30]. Indeed, prepubertal children metabolize melatonin faster than adults as shown by examining melatonin in serum and saliva, and 6-sulfatoxymelatonin in urine after an i.v. infusion of melatonin [31].

Although a trend of decreasing urinary 6-sulfatoxymelatonin excretion with age was seen in the present study, the differences found did not attain significance, presumably

because of the small number of individuals examined. Indeed, although day-to-day melatonin production is remarkably similar, individually a large inter-individual variability exists, as it can be seen in the present study. Others [32] reported that despite huge inter-individual differences, melatonin production remains constant in one and the same individual during childhood and adolescence.

The huge inter-individual variability of melatonin production makes it difficult to conclude that what is found in this small sample of normal boys reflects the changes in the population. This applies to the low values of urinary 6-sulfatoxymelatonin (2.8 μg/24 hr) found in normal puberal boys. Kennaway et al. [33] reported that 20-yr-old subjects who were obese but were thin at birth produced approximately 50% less melatonin than normally proportioned subjects. The small sample of subjects examined in the present study ruled out any possible correlation with weight at birth.

The trend in melatonin observed as a function of pubertal stage is altered in the presence of pubertal disorders [27]. Nocturnal melatonin concentration increased in male patients with hypogonadotropic hypogonadism or delayed puberty compared with those in healthy controls [34]. By contrast, plasma melatonin concentrations of a girl with central precocious puberty were reportedly low for chronological age, but appropriate for pubertal status [35]. In addition, nocturnal serum melatonin (between 23:00 and 01:00 hr) was statistically significantly lower in 1- to 5-yr-old patients with central precocious puberty when compared with healthy controls, whereas pubertal patients aged 5–9 yr had circulating melatonin concentrations in the same range as healthy subjects approaching pubertal age [36].

Few observations have been published on the levels of melatonin in obese, endocrinologically normal, individuals. Shafii et al. [37] described an increase in serum melatonin and in urinary melatonin and 6-sulfatoxymelatonin in a severely obese 15-yr-old girl, which was not found in her 12-yr-old sister with a mild overweight. In the present study, in which obese and normal individuals were clearly separated by selecting quite apart BMI, i.e. percentile 25–75 for normal and percentile >95 for obese, total, nocturnal and diurnal, 6-sulfatoxymelatonin excretion was significantly higher in girls.

Indeed, melatonin levels tend to be higher in females throughout the life span [38]. Likewise, differences in body fat distribution between males and females are detectable as prepubertal age [39]. Fat distribution of late-pubertal boys is more 'male' or 'android' than prepubertal boys, but late-pubertal girls do not differ consistently from prepubertal girls [40]. Therefore, the higher levels of 6-sulfatoxymelatonin melatonin found in girls and the pubertal differences between obese and lean boys could be partially explained by differences in composition and distribution of body fat.

In conclusion, the results show that obese pubertal males have a greater urinary excretion of 6-sulfatoxymelatonin and therefore a greater production of melatonin. To what extent the increase in melatonin in pubertal obese males accounts for delayed puberty in some of these subjects deserves to be further explored.

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