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# Melatonin and Gonadotropin Hormones in Pubertal Sportsgirls*

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In order to determine the influence of physical training on menstrual disturbances in sportsgirls, the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and melatonin have been studied in young athletes of track and field speciality using the Cooper test. Basal hormone levels and anthropometric data were also studied in age matched control girls. No significant differences in LH, FSH and melatonin hormone concentrations were observed between the PRE and POST Cooper test. However, significantly lower basal levels of LH were found in the early follicular phase or luteal phase of sportsgirls when contrasted with the control girls. No differences in FSH levels were observed in the early follicular phase of sportsgirls but higher FSH levels were found in the luteal phase. Daytime melatonin levels of sportsgirls were significantly higher than those in control girls. Age and anthropometric parameters studied showed no differences in height, weight, tricipital skinfold and percentage of body fat, but abdominal and subescapular skinfold measures were greater in control girls than in sportsgirls. It appears that continuous physical training can produce alterations in antireproductive hormone secretion such as melatonin, which can play an inhibitory role on the menstrual cycle hormone patterns in sportsgirls.

Key words: Sportsgirls, LH, FSH, Melatonin.

Menstrual alterations with less frequent menses are higher in sportswomen than in sedentary women. Menstrual irregularity as well as the frequency of oligomenorrhea and amenorrhea (20) is not consistent across sports. Some sports cause little dys-

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function while others show more (e. g. track) (14). Causes of menstrual alterations are not completely understood in sportswomen. They may be produced by hypothalamic dysfunction, incorrect feedback or other endocrine or metabolic alteration (11). Among the menstrual alteration producing causes, nutrition and stress (19) as well as physical training may be considered. Physical training can induce an increase in antireproductive hormones such as beta-endorphin, dopamine, melatonin, which can inhibit the normal pulsatil secretion pattern of the gonadotropin, producing secondary amenorrhea and a shortening of the luteal phase and secondary amenorrhea (8) or the inhibition of the stimulatory effect of noradrenaline on GnRH (13).

In relation to body composition, both the absolute and relative amounts of fat have been considered (6) to be important for the onset and maintenance of regular ovulatory menstrual cycles. However, the validity of this theory has been challenged by many, and different investigators have not found significant differences in the relative fat content between eumenorrheic, oligomenorrheic and amenorrheic college athletes (20) or eumenorrheic and amenorrheic runners (10, 18).

In an attempt to clarify the neuroendocrine processes of sexual menstrual cycles in sportswomen and bearing in mind the antigonadotropic effect of melatonin in mammals (16) and possibly in humans (12, 5) the influence of physical exercise on the levels of LH, FSH and melatonin has been studied and the baseline levels of these hormones have been compared with those of age-matched sedentary girls.

## Materials and Methods

Seventeen young female athletes with an average age of 14.27 were studied. Their average weight and height were 51.7 Kg and 161.5 cm respectively. Seven were in the early follicular phase, eight in the luteal phase and two with amenorrhea. They had an average of 3-5 years of training (5-7 training sessions of 1.5 h each per week), related to their track and field speciality. Sportsgirls were submitted to the Cooper test (12 min running to maximal speed). This test implies a maximum anaerobic effort as can be deduced from the pulsations of the sportsgirls at the end of the test (190.588 ± 6.38 pulsations/min) as well as from the post exercise blood lactic acid values (5.94 ± 0.33 mM/l) which were measured in post exercise blood samples by means of a micro-sample in an ANALOX-L-34 using an electroenzymatic method.

Sixteen sedentary girls with an average age of 14.7 years were also studied. Their average weight and height were 55.2 kg and 165.5 cm respectively.

Blood samples were taken between 10.00-12.00 a.m. from the prominent arm vein, collected in a vacutainer and immediately placed on ice. The blood was centrifugated and serum distributed into separate storage vials, and frozen at −20 °C until assay. Blood samples were obtained before starting the test (PRE) and 5 minutes after finishing the test (POST). To avoid time differences in blood sampling, groups were reduced to a total of 3-4 girls.

The percentage of body fat composition was calculated according to the formula of Lohemen using skinfold parameters. These were measured with a lipometre (Holtain skinfold caliper).

TBF = 0.135 (B/W) + 0.373(T. skf) + 0.389(S. skf) - 3.967; TBF (Total Body Fat), B/W (Body Weight), T. skf. (Tricipital skinfold), S. skf. (Subescapular skinfold) and Abdominal skinfold were measured.

*Hormone assays.* — Gonadotropin hormones LH and FSH were measured by RIA using commercial $^{125}$I-LH and $^{125}$I-FSH immunoradiometric assay, according

to the manufacturer's instructions (Biomerieux). Antibody 1 was coated on the inner wall of the assay tube, and antibody 2 labelled with $^{125}I$. Therefore the independent development of the immunological reactions led to the formation of the complex $^{125}I$-$Ab_2$-Ag-$Ab_1$ on the assay tube wall. The minimum amount of LH significantly different from the zero concentration for a probability of 95 % was 0.4 mUI/ml, and for the FSH this value was 0.15 mUI/ml.

Melatonin was determined in serum after diethyleter extraction, at a proportion of 0.5 ml of serum to 3 ml of diethyleter, and the recovery was between 80-95 % as measured with $^3H$-Melatonin. Serum melatonin was determined by radioimmunoassay using a commercially available kit (Tecova AG, Wholen, Switzerland). This method had a CV of 6.6-6.9 %, and the sensitivity of the assay was 3 pg/ml. Radioactivity was determined in a 1271 RIA-GAMMA (Automatic Gamma Counter, LKB Wallac).

Statistical analysis was carried out with a SIGMA Statistical Programme and the Student «t» test.

## Results

Age, height and weight were similar between the two groups studied. The values for tricipital skinfold in both sportsgirls 12.6 ± 0.88 (mm) and in the control girls 12.99 ± 0.82 (mm) were similar as was also the value for percentage of body fat (21.12 ± 0.70 and 21.82 ± 0.17, respectively). However, the value for subescapular skinfold in sportsgirls 8.34 ± 0.35 (mm) was significantly lower ($P < 0.025$) than in control girls 9.96 ± 0.6 (mm). Similarly the value for abdominal skinfold in sportsgirls 8.15 ± 0.62 (mm) was also significantly lower ($P < 0.05$) than that in control girls 10.32 ± 0.84 (mm).

Hormonal responses in sportsgirls to the Cooper test did not show significant differences in the concentrations of LH, FSH and melatonin in terms of the pre and post test values. Thus the pre exercise values for LH serum concentrations were 2.26 ± 0.35 mIU/ml (n = 6) in the follicular phase, and 2.54 ± 0.46 mIU/ml (n = 7) in the luteal phase. The post values were similar. Pre and post exercise values for FSH serum concentrations were 3.49 ± 1.07 mIU/ml (n = 7) in the early follicular

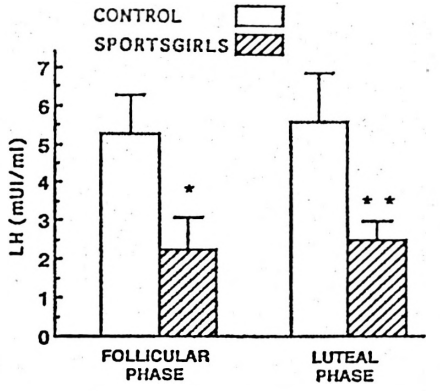


Fig. 1. *Basal LH values (mean ± SEM) in control and sportsgirls in the early follicular phase and in the luteal phase.*
* $P < 0.005$ and ** $P < 0.025$ vs. control group.

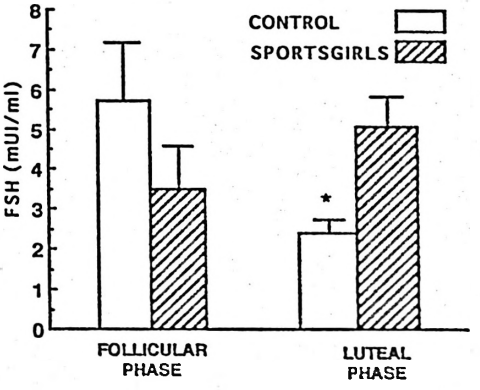


Fig. 2. *Basal FSH values (mean ± SEM) in control and sportsgirls in the early follicular phase and in the luteal phase.*
* $P < 0.005$ vs. control group.

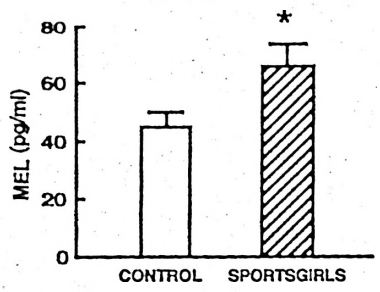


Fig. 3. *Basal daytime melatonin values (mean ± SEM) in control and sportsgirls.*
* P < 0.025 vs. control group.

phase, and 5.04 ± 0.75 mIU/ml (n = 8) in the luteal phase; post values were also similar. As there were no significant effects on daytime serum melatonin levels (9) depending on the day of the menstrual cycle, daytime melatonin baseline concentrations are shown independently of the menstrual cycle phase, pre and post exercise melatonin serum values were 66.50 ± 7.05 and 64.89 ± 8.05 (pg/ml, n = 8), respectively.

Baseline concentrations of LH were significantly lower in sportsgirls in both the early follicular phase and the luteal phase (fig. 1) than in those of age-matched control girls. No significant differences in FSH concentrations were found between the control and sportsgirls in the early follicular phase, but significantly higher FSH levels were found in sportsgirls during the luteal phase (fig. 2). In relation to baseline melatonin concentrations, sportsgirls showed significantly higher concentrations than the age-matched sedentary controls (fig. 3).

## Discussion

The data on the acute effect of physical exercise on gonadotropin hormone concentrations are variable. Bonen and Keizer (2) found no differences in the LH serum concentrations and higher FSH concentrations in five women after a marathon race. Other results show that exercise practice in 8 highly trained women marathon runners and 13 eumenorrheic untrained women resulted in decreased LH levels whereas FSH levels remained unchanged (7). However, in contrast LH and FSH were found to be elevated in seven normal menstruating women during 60 minutes of resistance exercise after a two month training period (4). In our sportsgirls no changes were found in the LH and FSH serum concentrations in the early follicular phase or in the luteal phase (post Cooper test). In early pubertal sportsgirls, pre and post Cooper test values for LH serum concentrations were 1.73 ± 0.25 mIU/ml (n = 7) and 1.74 ± 0.31 mIU/ml (n = 7), and for FSH serum concentrations were 3.88 ± 0.48 mIU/ml (n = 8), and 4.00 ± 0.56 mIU/ml (n = 8), and in agreement with pubertal sportsgirls no statistically significant differences were found. The different results may be due to differences in the experimental procedure, i.e. the fact that our sportsgirls run for a shorter period and also that the actual training programme followed by them is much longer than those found in other investigations.

Nevertheless, when the baseline LH concentrations of sportsgirls are compared with those of control girls, the former group showed significantly lower LH levels in both the follicular and luteal phase (fig. 1). This agrees with data available in the literature which states that women distance runner were the first to be recognised as having menstrual dysfunction, and that these have a higher incidence of menstrual dysfunction than swimmers, whose incidence is higher than the general population (10). In agreement with our studies significantly reduced LH values and a reduced number of secretion episodes have been observed in female athletes in relation to the age-matched con-

trols (15). Similarly in early pubertal girls significantly lower ($P < 0.025$) baseline LH values in sportsgirls 2.12 ± 0.24 mIU/ml (n = 10) in comparison to those found in the control girls 3.48 ± 0.48 mIU/ml (n = 10) were found. No significant differences in the baseline FSH levels were found during the early follicular phase when sportswomen with sedentary control girls. Nevertheless higher FSH concentrations were found in the luteal phase in sportsgirls when compared with age-matched control girls, a fact which confirms the menstrual disturbances of sportswomen with a luteal deficiency and shorter luteal phase (8). Previous investigations have shown that exercise increases melatonin concentrations in women after long distance running (3, 17) but conflicting results have also been reported (22). Melatonin has also been found (21) to rise significantly from baseline values after a run but a progressive diminution of this increase is observed as training progresses, which suggests that vigorous exercise training may attenuate rather than augment the secretion of pineal melatonin. According to this theory, the lack of variation in melatonin concentrations may be attributed to the training programme followed by the young athletes (90 km/week). However, significantly higher values were observed in athletes compared to controls which could indicate hormonal alteration due to physical exercise practice. The persistence of a certain melatonin secretion during daytime also could induce an inhibitory action on the neuroendocrine reproductive axis, as has been observed in LH levels. In this way functional hypothalamic amenorrhea secretion (1) has been associated with amplification of nocturnal melatonin secretion. Our results show high concentrations of melatonin associated with decreased LH concentrations in sportsgirls which can imply that the melatonin hormone is one of the antireproductive hormones produced during physical exercise practise, and is involved in the menstrual disturbances of sportsgirls.

## Resumen

Se estudia el efecto del ejercicio físico sobre las alteraciones menstruales en muchachas deportistas determinando las concentraciones de hormonas gonadotrópicas LH y FSH y de melatonina, antes y después de realizar el test de Cooper. No hay diferencias en los valores de hormonas pre y post ejercicio tanto en la fase folicular temprana como en la luteal. Sin embargo, en las atletas se observan valores significativamente más bajos de LH en esas mismas fases, y valores de FSH significativamente más alto en la fase luteal. No hay diferencias entre ambos grupos en las concentraciones de FSH en fase folicular temprana. Los valores diurnos de melatonina, en las atletas, son significativamente más elevados. Del estudio antropométrico se destaca una mayor medida de los pliegues abdominal y subescapular en los controles, respecto de las atletas, aunque no hay diferencia en la altura, peso, pliegue tricipital y porcentaje de grasa corporal. Parece que el entrenamiento físico continuo puede producir alteraciones en la secreción de hormonas antireproductivas como la melatonina la cual puede jugar un papel inhibitorio en las hormonas de los ciclos menstruales de las deportistas.

**Palabras clave:** Atletas, LH, FSH, Melatonina.

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