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Serum Levels of Tryptophan, 5-Hydroxytryptophan and Serotonin in Patients Affected with Different Forms of Amenorrhea

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Abstract: Tryptophan (Trp) is present in the serum, partly bound to albumine and in the free form. The unbound portion of circulating tryptophan has the property of crossing the hematoencephalic barrier and being converted within the brain into serotonin (5-HT) through the enzymatic processes of hydroxylation and decarboxylation. The serotoninergic system plays an important role in neuroendocrine control of reproductive hormone secretion, and in particular, it may influence GnRH pulsatility, a function essential for reproductive processes. In this study, we analysed serum levels of tryptophan, serotonin and 5-hydroxytryptophan (5-HTP) in women with three different forms of amenorrhea: 16 patients were diagnosed with anorexia nervosa, 60 patients with functional hypothalamic amenorrhea, and 14 patients with hyperprolactinemia. Data were compared with those of a group of 25 healthy women. Serum Trp levels were significantly ($P \leq 0.05$) lower in the anorexic (11.64 ± 0.53 µg/ml, mean ± S.E.) than in the control (12.98 ± 0.37 µg/ml) groups. In addition, in the anorexic group a statistical dispersion of Trp values was shown indicating a bimodal data distribution suggesting the existence of two different subgroups of patients. Regarding 5-HTP, an increase of its serum level was observed in all the groups with amenorrhea with the highest value in hyperprolactinemic patients. On the contrary, no statistical differences in serum 5-HT levels among the four analyzed groups were observed.
This study shows that women affected by various forms of amenorrhea present an altered metabolism of tryptophan *via* serotonin and, in particular, markedly high differences are observed between the two subgroups of anorexic patients.

Keywords: tryptophan, 5-hydroxytryptophan, serotonin, amenorrhea, anorexia nervosa

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Introduction

Amenorrhea, a term deriving from Greek, a = negative, men = month, and rhoia = flow, is the absence or abnormal cessation of the menses for a period longer than three months in a woman during the reproductive age. Amenorrhea can be primary or secondary, and the distinction is made by the occurrence of amenorrhea before and after menarche, respectively.[1]

The normal menstrual cycle is of 28 days during which the different hypothalamic-pituitary-ovarian hormones influence their synthesis and secretion each other giving rise to the follicular ovulation and luteinic phases of the cycle. The gonadotropin-releasing hormone (GnRH) is pulsatively secreted by the hypothalamus and it controls the synthesis and secretion by the pituitary gland of the follicle-stimulating (FSH) and luteinizing (LH) hormones which stimulate the synthesis of estrogen and progesterone mainly by the ovary. Each of these hormones produced by the hypothalamic-pituitary-ovary system controls the synthesis of the others by not still well understood positive and negative feedback mechanisms.[2]

The causes giving amenorrhea can be divided in five groups[3] and among these, functional hypothalamic amenorrhea is the most prevalent, being responsible for approximately 35% of the cases.[4] Also eating disorders can lead to amenorrhea and in fact, it is one of the features necessary for the diagnosis of anorexia nervosa. Hyperprolactinemia, a condition in which the pituitary gland produces more prolactine, is considered another cause of amenorrhea since prolactine interferes with the normal GnRH pulsatility giving rise to a suppression of gonadotropins release and thus, to a decrease of the estrogen levels.[5]

Secretion of GnRH is also regulated by a lot of neurotransmitters among which we have recently reported a pivotal role of serotonin (5-HT), deriving from tryptophan (Trp),[6,7] the only amino acid presents in the serum partly bound to albumine and in the free form.

The unbound portion of circulating tryptophan has the property of crossing the hematoencephalic barrier and being converted within the brain into serotonin (5-HT) through the enzymatic processes of hydroxylation and decarboxylation.[8,9]

Therefore, tryptophan levels in serum might reflect its utilization in the central nervous system (CNS), particularly in the hypothalamus, where Trp and 5-HT concentrations are higher than in other regions of the brain.[10,11]

We have demonstrated that tryptophan has cyclical variation during the menstrual cycle showing a negative correlation with gonadotropins concentration in a highly significant way.[6] It was demonstrated that also 5-hydroxytryptophan, representing the immediate precursor of 5-HT along the "serotonin pathway", can influence LH secretion in women in the follicular phase acting on GnRH release stimulation by hypothalamus or increasing the sensitivity of the pituitary to GnRH.[12]

In this study, we aimed to analyse serum levels of tryptophan (Trp), 5-hydroxytryptophan (5-HTP) and serotonin (5-HT) in women affected with differently caused amenorrhea: anorexia nervosa, functional hypothalamic amenorrhea, and hyperprolactinemia. Data were also compared with those of a group of healthy women.

Materials and Methods

Study population

115 women (aged between 14 and 32 years old) were recruited from the Department of Gynecological Endocrinology, Medical University of Poznan, Poland and divided in four groups: 16 patients with anorexia nervosa (according to the Diagnostic and Statistical Manual of Mental Disorders IV criteria), 60 patients with functional hypothalamic amenorrhea, 14 patients with hyperprolactinemia and 25 healthy women. Detailed data for each group of patients are reported in Table 1.

Patients suffering from functional hypothalamic amenorrhea (stress-related, exercise-related or weight loss-related amenorrhea) met following requirements:

- at least a 3 months history of amenorrhea not due to pregnancy,
- at least one documented less then 5 U/ml LH and FSH serum levels.

Patients suffering from pituitary amenorrhea were diagnosed with hyperprolactinemia (serum prolactin levels higher than 40 ng/ml).

The control group consisted of 25 healthy non-hospitalized women. They were enrolled according to the following criteria:

- normal menstrual cycles (28–32 days),
- normal body mass index (BMI) (18.5–24.9 kg/m^2).
- no endocrine diseases.
- no consumption of hormonal treatment in the last 3 months.

Table 1. Characteristics of study population. Data are reported as mean ± S.D.; number of cases is indicated in parentheses.

	Control (25)	Hypotalamic amenorrhea (60)	Anorexia nervosa (16)	Hyperprolactinemia (14)
Age (years)	20.3 ± 4.3	21.8 ± 4.6	19.9 ± 3.9	21.1 ± 9.6
BMI	22.7 ± 1.1	19.1 ± 2.7	17.2 ± 1.8	23 ± 1.7
Onset of amenorrhea (years)	–	18.7 ± 4.0	17.1 ± 2.1	21.8 ± 4.4
Duration of amenorrhea (month range)	0	3–190	6–120	3–180

Abbreviation: BMI, body mass index.

All the procedures were in compliance to the principles of the Helsinki Declaration, the Council of Europe, and the Universal Declaration of UNESCO on human rights, biomedicine, and human genome, and all individuals, after adequate information about the study plan, gave their consent to the blood sampling.

Sample collection and analysis of tryptophan and its metabolites

Blood samples were collected after an overnight fast, allowed to clot at room temperature, centrifuged at 3000 g for 10 minutes. The obtained serum was then stored at –35 °C.

Tryptophan and its metabolites were measured according to the method of Comai et al[13] using a high performance liquid chromatography (HPLC) system equipped with a Shimadzu RF-10 AXL fluorometer detector set at excitation and emission wavelengths of 285 and 345 nm, respectively. The method briefly consists of online HPLC retention of the protein fraction in a LiChroprep RP-8 (25–40 μm) precolumn system (Merck, Darmstadt, Germany), elution with isocratic gradient of acetonitrile-phosphate buffer 0.004 M, pH 3.5 and chromatographic separation carried out by an analytical Platinum EPS C18 100A column (5 μm; 250 mm × 4.6 mm; Alltech, Deerfield, IL).

Data analysis

Data are expressed as mean ± S.E. and the differences between groups are determined by using Student's *t* test with Bonferroni's correction for unpaired or paired data (when possible). Statistically significant difference was accepted for $P \leq 0.05$.

Results

Table 2 reports the serum levels of tryptophan, 5-hydroxytryptophan and serotonin from three groups of women affected by different forms of amenorrhea: i.e. patients diagnosed with anorexia nervosa, with functional hypothalamic amenorrhea, or with hyperprolactinemia, respectively. For comparison, data from the control group are also shown.

Trp concentration was significantly lower in the anorexic than in the control groups (11.64 ± 0.53 vs. 12.98 ± 0.37 μg/ml; $P \leq 0.05$) and also in the hypothalamic amenorrhea women, even though, in this case, not significantly. No difference was found between hyperprolactinemic and control groups.

Table 2. Serum levels of tryptophan (Trp), 5-hydroxytryptophan (5-HTP) and serotonin (5-HT) in controls and in patients affected by hypothalamic amenorrhea, hyperprolactinemia and anorexia nervosa. Data are mean ± S.E.; number of cases is indicated in parentheses.

Group	Trp (μg/ml)	5-HTP (ng/ml)	5-HT (ng/ml)
Controls (25)	12.98 ± 0.37	44.97 ± 2.77	97.29 ± 9.95
Hypothalamic amenorrhea (60)	12.13 ± 0.29	50.24 ± 1.55**	101.36 ± 7.37
Hyperprolactinemia (14)	12.89 ± 0.32	72.71 ± 5.16***	98.93 ± 11.05
Anorexia nervosa (16)	11.64 ± 0.53*	57.99 ± 3.30*	73.78 ± 10.52

*$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.001$ vs. control group.

The hyperprolactinemic patients showed the highest levels of 5-HTP (72.71 ± 4.91 ng/ml) which were statistically different with respect to the other three groups. Also 5-HTP serum levels of patients with anorexia and hypothalamic amenorrhea were significantly higher than controls ($P \leq 0.05$) indicating that this metabolic pathway is highly induced in all types of amenorrhea.

Even though a 5-HT concentration decrease was observed in the anorexic group with respect to controls, no statistical differences were present among the four groups.

Our previous data[6,7] showed that during the fertile years Trp levels are negatively correlated to gonadotropins concentration and its release at midcycle and that this correlation is lost after menopause. For this reason, we focused our attention on tryptophan data: the question was to understand the mechanism through which anorexia determines lack of gonadotropins release and amenorrhea. First of all we analysed the quartiles distribution of Trp values for each group of subjects.

The dispersion statistical parameters of serum tryptophan levels of the four analysed groups showed different results (Fig. 1).

In particular, the interquartile intervals of the data of anorexic and hyperprolactinemic patients were wider than those of control and hypothalamic amenorrhea groups as shown by the expansion of the rectangles reported on the top of each diagram of the Figure 1. The rectangles represent the data distribution between the 25th and 75th quartiles.

Furthermore, the statistical dispersion parameters of Trp levels in the anorexic group put in evidence a bimodal data distribution perfectly symmetric to the mean value; actually, this mean value, even

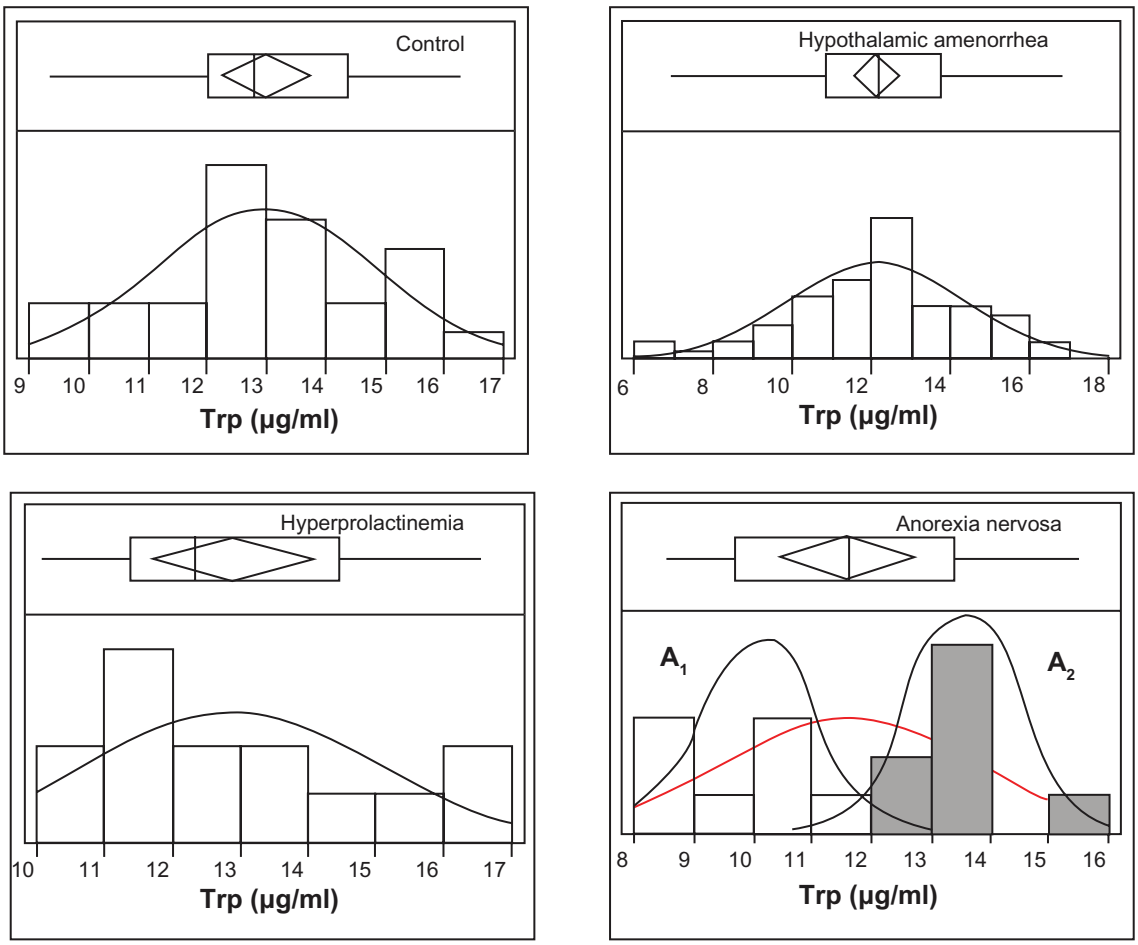

Figure 1. Statistical dispersion parameters of serum TRP values in the four analyzed groups: controls, hypothalamic amenorrhea, hyperprolactinemia and anorexia nervosa.

though coincident with the median value, was in a median quartile not representing the most abundant and for this reason not giving rise to a typical Gaussian curve. Therefore, it was possible to individuate the presence of two Gaussian curves corresponding to two subgroups of anorexic patients: A_1 with a serum Trp mean value statistically lower than that of the control group (9.81 ± 0.97 vs. 12.98 ± 0.37 µg/ml, respectively), and A_2 with a mean value of 13.48 ± 0.93 µg/ml not significantly different from the controls.

On the basis of these findings, we wanted to investigate possible differences on serum contents of 5-HTP and 5-HT in the two anorexic subgroups.

Figure 2 shows the serum levels of 5-HTP (left) and 5-HT (right) in A_1 and A_2 subgroups compared with control and anorexic group values. In the A_1 subgroup, serum 5-HTP levels were markedly higher than those of A_2 subgroup which presented a mean value of 54.02 ng/ml not statistically different from controls.

Similarly, differences of 5-HT serum levels between A_1 and A_2 could be noticed. As observed for 5-HTP, in this case serum 5-HT content was significantly lower in the A_2 subgroup than control, whereas, 5-HT levels of A_1 reached values similar to those of controls and higher than those of the total anorexic group.

Besides, serum Trp levels of the two subgroups of anorexic patients (A_1 and A_2) were related with Body Mass Index (BMI) and weight loss as shown in Table 3.

Regarding BMI no difference was observed between A_1 and A_2 subgroups; instead, a significant variation ($P \leq 0.05$) was observed in weight loss values: in particular A_1, with the lower serum Trp content, presented a significantly lower loss of weight than the A_2 subgroup (14.25 ± 2.05 kg vs. 18.71 ± 4.23 kg, $P < 0.05$).

Discussion

In this study we found that serum Trp levels were unchanged in patients affected by hypothalamic amenorrhea or hyperprolactinemia with respect to the healthy subjects, whereas anorexic group showed a statistically lower value of Trp than the control group ($P < 0.05$) in agreement with a previous report.[14] Furthermore, we pointed out the presence of two different subgroups of anorexic patients, one characterized by a markedly lower content of Trp and correspondent higher levels of 5-HTP and 5-HT. Some authors have proposed the existence of two types of anorexia nervosa characterized by the presence or absence of binge-eating; they showed one group of anorexic patients with reduced serotoninergic central activity but with high impulsivity, and one presenting normal or even increased 5-HT activity but without binge-eating.[15,16]

Regarding 5-HTP levels, an increase of this metabolite in all the groups with amenorrhea was observed with the highest value in hyperprolactinemic patients. Lado-Abeal et al[12] found that pulsatile administration of L-5-HTP amplifies LH secretion in fertile women, while Scacchi et al[17] reported an increase of LH and FSH plasma levels in prepubertal female rats after 5-HTP intake. Consequently, the increase of 5-HTP we found in different forms of amenorrhea, might be explained as a self-regulating mechanism with the aim to reactivate altered gonadotropins system.

We have previously reported[6] that the conversion of free tryptophan to 5-HTP occurring in the brain through the action of tryptophan-5-hydroxylase, represents a key mechanism in determining the release of gonadotropins at midcycle and successive ovulation.

This mechanism might be altered in the anorexic patients as we found a critical decrease of Trp serum levels (subgroup A_1) or a lack of activity of brain

Table 3. Tryptophan levels, BMI and weight loss of the two subgroups of anorexic patients A_1 and A_2.

Subgroup	Trp (µg/ml) mean ± S.E.	BMI mean ± S.D.	Weight loss (Kg) mean ± S.D.
A_1	9.81 ± 0.97*	16.90 ± 1.80	14.25 ± 2.05*
A_2	13.48 ± 0.93	17.69 ± 1.93	18.71 ± 4.23

*A_1 vs. A_2 $P \leq 0.05$.

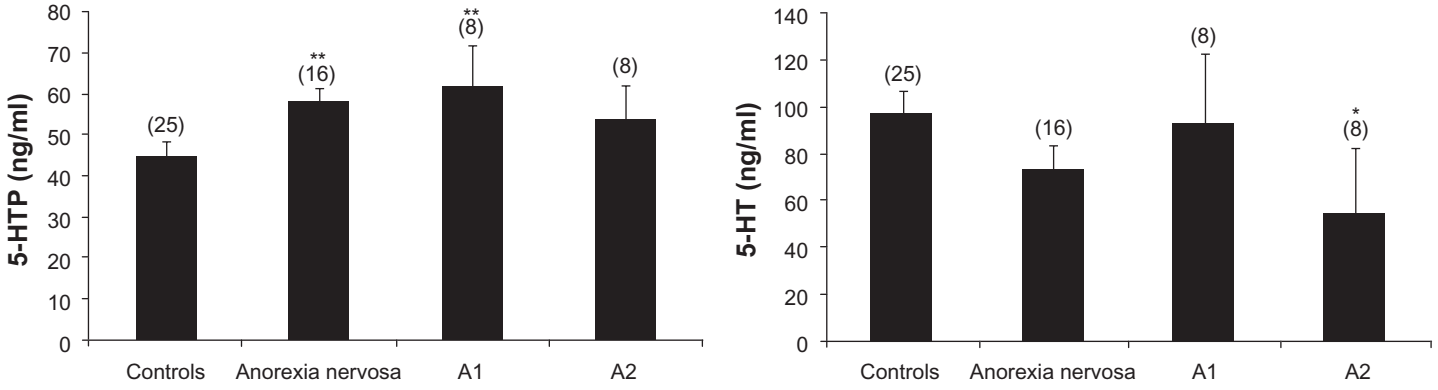

Figure 2. Serum levels (mean ± S.E. (ng/ml)) of 5-HTP (left) and serotonin (right) in controls, patients affected by anorexia nervosa and in the two subgroups of anorexic patients A_1 and A_2.
*P ≤ 0.05, **P ≤ 0.005 vs. control group.

tryptophan 5-hydroxylase (subgroups $A_1 + A_2$). These alterations would determine the lack of gonadotropins release and, consequently, the lack of ovulation explaining the onset of amenorrhea.

For these reasons, anorexic patients showing a decrease of serum Trp, as the case of A_1 subgroup, may benefit not only of a hormonal therapy with estrogens which are important for tryptophan 5-hydroxylase function[18] but also of a tryptophan supplementation.

In conclusion, this work provides the evidence of altered metabolism of tryptophan *via* serotonin in patients affected by different forms of amenorrhea offering the basis for further studies required to better evaluate the utility of a tryptophan supplementation in some forms of amenorrhea as well as the role of increased 5-HTP levels in restoring the release of gonadotropins.

Disclosures

The authors report no conflicts of interest.

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