ISSN 0025-7680

ORIGINAL ARTICLE MEDICINA (Buenos Aires) 2008; 68: 120-124

SEX HORMONE BINDING GLOBULIN DECREASE AS A POTENTIAL PATHOGENETIC FACTOR FOR HIRSUTISM IN ADOLESCENT GIRLS

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Abstract We investigated 252 non-obese female subjects aged 13-39 years to evaluate if an exaggerated descent of sex hormone binding globulin (SHBG) levels during adolescence can play a role in the development of hirsutism. Body hair was assessed according to Ferriman and Gallwey (FG), with a stringent criterion of normality of ≤ 4. In 13-14 years girls, SHBG and free testosterone (FT) levels were similar in "hirsute" girls (FG > 4) and controls (FG ≤ 4, regular menstrual cycles, no acne). In 15-18 years girls, SHBG values were lower in "hirsute" girls, FT levels were similar in both groups, FG correlated inversely with SHBG. In 19-39 yr women, FT levels were higher in "hirsute" subjects, SHBG values were similar in both groups, FG correlated positively with FT. Lowest SHBG values were observed at 15-18 years, but the slope of the decrease from 13-14 years values was greater in the "hirsute" group. FT values increased progressively with age, but the increase was greater in the "hirsute" group. Those results suggest an important role of SHBG decrease in adolescence *vs.* a more accentuated testosterone increase in adults, as factors conditioning the development of hirsutism in these two different periods of life.

Key words*:* hirsutism, puberty, SHBG, testosterone, adolescence

Resumen ***Disminución de la globulina transportadora de hormonas sexuales como factor patogénico de hirsutismo en la adolescencia***. Se investigaron 252 mujeres con peso normal, de 13 a 39 años de edad, para evaluar si un descenso exagerado en los niveles de la globulina transportadora de hormonas sexuales ("sex hormone binding globulin"; SHBG) puede tener un rol en el desarrollo de hirsutismo. Este signo fue evaluado con la escala de Ferriman y Gallwey (FG), empleando un criterio riguroso de normalidad ≤ 4. En niñas de 13-14 años, tanto SHBG como la testosterona libre ("free testosterone"; FT) fueron similares en niñas "hirsutas" (FG > 4) y controles (FG ≤ 4, ciclos menstruales regulares, sin acné). En adolescentes de 15-18 años, los valores de SHBG fueron menores en las "hirsutas", los niveles de FT fueron similares en ambos grupos y el índice de FG correlacionó inversamente con SHBG. En las mujeres de 19-39 años, los niveles de FT fueron mayores en las "hirsutas", los valores de SHBG fueron similares en ambos grupos y FG correlacionó positivamente con FT. Los valores más bajos de SHBG se observaron entre 15 y 18 años, pero la pendiente de disminución a partir de los valores de 13-14 años fue mayor en el grupo de "hirsutas". Los valores de FT se incrementaron progresivamente con la edad, pero el aumento fue mayor en el grupo de "hirsutas". Estos resultados sugieren un rol importante del descenso de SHBG en la adolescencia *vs.* un incremento más acentuado de los niveles de testosterona en las adultas, como factores que condicionan el desarrollo del hirsutismo en esos dos diferentes periodos de la vida.

Palabras clave*:* hirsutismo, pubertad, SHBG, testosterona, adolescencia

Hyperandrogenism is one of the most frequent endocrine disorders in young women[1]. Hirsutism, acne, seborrhea, androgenic alopecia, menstrual disturbances and/or ovulatory dysfunction as isolated signs, or in different combinations, are possible clinical expressions of this syndrome. Other endocrine and metabolic alterations, including insulin resistance and obesity, are frequently associated with hyperandrogenic syndrome in polycystic ovary syndrome[2].

The development of androgenic features may result from androgen overproduction and/or from increased androgen action in specific tissues. Androgen actions depend in particular on their bioavailability at cellular level, thus on circulating sex hormone-binding globulin (SHBG) levels[3, 4]. Indeed, androgenic features correlate better with free testosterone and bioavailable testosterone (i.e. non-

Received: 7-V-2007 Accepted: 23-XI-2007

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SHBG bound testosterone) than with total testosterone levels[4]. We have previously reported that SHBG level is a sensitive marker of hirsutism in adolescent girls[5].

The aim of the present study was to evaluate if an exaggeration in the normal descent of circulating SHBG levels during puberty and adolescence can play a role in the development of hirsutism.

Materials and Methods

During routine school medical examination at the Carlos Pellegrini school and at the *Universidad de Buenos Aires*, Buenos Aires, Argentina, blood samples were obtained from 252 post-menarcheal (cut-off time: one year) female, non-obese subjects, in a prospective study design. The protocol was approved by the ethical review committee of the *Hospital de Clínicas*, University of Buenos Aires, and a written informed consent was obtained from each participant. Subjects with overt neurological, renal, hepatic or thyroid disease, personal history of psychological illness, subjects receiving any kind of medication (including hormonal contraception), and subjects with a body mass index (BMI) > 24 kg/m^2 were excluded from the study.

During the visit, information regarding the duration and the regularity of the menstrual cycles as well as the date of the last menses was recorded for each subject. Physical examination included a standardized evaluation of body hair according to the scoring scale of Ferriman and Gallwey (FG score)[6]. For each site, hair growth was rated from 0 (absence of terminal hair) to 4 (complete and heavy cover). A stringent FG cutoff value (upper limit) of 4 was used to characterize a normal control group. In all subjects, blood samples were obtained at 07:00-10:00 h. In menstruating subjects, blood samples were drawn either before day 11 or after day 18 of the menstrual cycle in order to avoid the periovulatory period; the proportion of samples obtained in follicular and luteal phases (60/40%) was similar for all groups studied (see below). Serum was kept at –20 °C until assay. Testosterone was measured by RIA with a lower limit of sensitivity of 0.02 nmol/l (50 mg/ml) and a mean interassay coefficient of variation of 13%[7]. SHBG was determined by a commercially available IRMA (Farmos Diagnostica, Finland) with a lower limit of sensitivity of 0.5 nmol/l (4.5 µg/dl) and a mean interassay coefficient of variation of 5.3%. Free testosterone (FT) levels were calculated according to Södergard formula[8, 9].

A total of 252 subjects, aged 13-39 yr (mean 19 yr), with a BMI of 20.8 ± 0.1 kg/m^2 (mean ± SEM) were included in the study. Their menarcheal age averaged 12.2 ± 0.1 yr. Subjects were divided into three age groups according to their chronological age: 13-14 yr; 15-18 yr; 19-39 yr (referred to as adult group). Within each age group, we compared data obtained in normal control subjects (regular menstrual cycles 26-34 days long, no acne, no seborrhea, FG score ≤ 4) and in subjects with a FG score > 4 (referred to as hirsute subjects). Subjects with a FG score ≤ 4 but with irregular menstrual cycles and/or acne or seborrhea (n = 107) were excluded from group comparisons, but were included in a global analysis of the relationship of FG score to biological variables.

Statistical calculations were performed by non-parametric tests using the Statview^{SE+} software (Abacus concepts, Berkeley, CA, USA) for Macintosh computers. All group values are expressed as means ± SEM.

Results

Relationship of FG score to biological variables

In subjects 13-14 yr old, no relationship (Spearman test) was evidenced between FG score and circulating SHBG or androgen levels ($P \geq 0.89$; n = 48). In 15-18 yr old girls, FG score was inversely correlated with SHBG levels (r = -0.42; $P < 0.004$; n = 49) but there was no significant relation between FG score and androgen levels ($P \geq 0.10$). In contrast, in adult women (19-39 yr old), FG score was positively associated with total testosterone (r = 0.18; $P < 0.03$; n = 155) and free testosterone (r = 0.23; $P = 0.005$; n = 155) while there was no relationship between FG score and SHBG levels ($P > 0.30$).

Androgen and SHBG levels in hirsute subjects and normal controls

Pertinent clinical data are summarized in Table 1. For each age group, chronological age, menarcheal age, weight and BMI were similar in hirsute subjects and normal controls.

TABLE 1.– *Clinical characteristics of each group of subjects*

Age group	13-14 yr		15-18 yr		19-39 yr	
Clinical group	NC	Hirsute	NC	Hirsute	NC	Hirsute
Number of subjects	18	9	16	17	36	49
Chronological age (yr)	13.1 ± 0.1	13.0 ± 0.1	16.3± 0.3	16.5 ± 0.3	21.3 ± 0.5	21.2 ± 0.5
Menarcheal age (yr)	11.7 ± 0.2	12.1 ± 0.2	12.5 ± 0.2	12.1 ± 0.2	12.1 ± 0.2	12.4 ± 0.2
Weight (kg)	50.6 ± 1.6	47.8 ± 1.6	55.6 ± 1.2	54.1 ± 1.2	52.6 ± 0.9	52.3 ± 0.8
BMI (kg/m^2)	19.9 ± 0.5	19.9 ± 0.6	20.6 ± 0.5	20.6 ± 0.4	21.0 ± 0.3	20.8 ± 0.3
FG score	1.4 ± 0.4	5.9 ± 0.6	2.3 ± 0.4	8.9 ± 1.1	2.3 ± 0.2	7.0 ± 0.3

Values are means ± SEM. NC: normal controls; yr: years

Androgen and SHBG values (means ± SEM) for each age group are shown in Table 2. In 13-14 yr old girls, circulating androgen and SHBG values were similar in hirsute subjects and normal controls. In 15-18 yr old girls, SHBG levels were significantly lower in hirsute subjects than in normal controls, averaging 40.1 ± 3.8 nmol/l and 55.3 ± 4.8 nmol/l, respectively ($P < 0.03$), while androgen values were similar in both groups. In contrast, in adult women, SHBG values were only slightly, but not significantly, lower in hirsute subjects than in normal controls (54.6 ± 3.4 nmol/l *vs.* 64.4 ± 5.4 nmol/l; $P > 0.20$), while free testosterone levels were significantly elevated in the hirsute group ($P < 0.03$).

Age-related changes in SHBG levels are further illustrated in Fig.1. A biphasic curve was observed, both in normal and hirsute groups, with lowest SHBG levels found in 15-18 yr girls. The decrease from the values recorded in the 13-14 yr age group was statistically significant in normal ($P < 0.03$) and in hirsute ($P < 0.001$) subjects. However, SHBG levels were significantly lower in the hirsute group at 15-18 yr ($P<0.03$). The magnitude of this decrease was far more pronounced in hirsute girls than in normal controls, averaging 49% and 21%, respectively.

In both normal and hirsute groups, total testosterone levels were similar in the 13-14 and the 15-18 yr age groups ($P = 0.30$), but increased significantly thereafter. In contrast, free testosterone values increased progressively with age, both in normal and hirsute groups, as illustrated in Fig. 1. However, the magnitude of this increase was considerably lower in normal than in hirsute girls, averaging 53% and 127%, respectively.

Discussion

Although hirsutism is the most frequent clinical feature of androgen excess, not all patients with hirsutism have overt evidence of androgen excess. Androgen effects depend on their availability at cellular level in specific tissues, and clinical signs of hyperandrogenism correlate better with free than with total testosterone[3,4], as observed in adult women in the present study. Thus, the regulation of SHBG levels appears to be an important factor in the possible

TABLE 2.– *Comparison of SHBG and androgen values between normal controls and hirsute subjects in each age group*

Age group		13-14 yr			15-18 yr			19-39 yr		
Clinical group		NC	Hirsute	*P*	NC	Hirsute	*P*	NC	Hirsute	*P*
Number of subjects		18	9		16	17		36	49	
SHBG	nmol/l	69.6 ± 4.8	79.3 ± 4.1	ns	55.3 ± 4.8	40.1 ± 3.8	<0.03	64.4 ± 5.4	54.6 ± 3.4	ns
Total T	nmol/l	1.35 ± 0.07	1.39 ± 0.10	ns	1.52 ± 0.14	1.32 ± 0.10	ns	1.91 ± 0.10	2.05 ± 0.52	ns
Free T	pmol/l	11.1 ± 1.0	9.4 ± 0.3	ns	14.6 ± 1.4	16.6 ± 1.7	ns	17.0 ± 1.0	21.1 ± 1.4	<0.03

Values are means ± SEM. NC: normal controls. P values were calculated by the Wilcoxon signed-rank test. All ns values were ≥ 0.20.

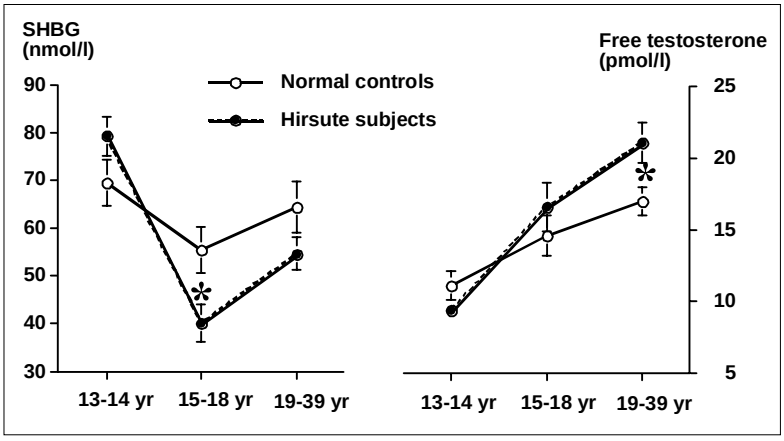

Fig. 1.– Plasma levels of SHBG (left panel) and free testosterone (right panel) in normal controls and hirsute subjects in the three age groups. Values shown are means ± SEM. * P < 0.03 *vs.* age-matched controls (Wilcoxon signed-rank test).

expression of hyperandrogenism. This study confirms that SHBG level could be a sensitive marker of hirsutism in adolescent girls.

In our study, we found a significant negative correlation between SHBG levels and FG score in adolescent but not in prepubertal or adult women whereas in the last, FG score positively correlated with testosterone concentrations. Those findings point to the eventual role that decreased levels of SHBG could play in the development of hirsutism during adolescence.

Sex hormone binding globulin levels have been shown to decrease from infancy to late prepuberty in healthy girls, and it has been suggested that this change could play a role in pubertal development through an increase in free fractions of sexual steroids[10]. In a longitudinal study of puberty in girls, a slow but significant decrease in SHBG levels was reported from 8-10 yrs of age to after 15 yrs of age[11]. On the other hand, insulin resistance occurs at the onset of puberty in normal children[12], inducing compensatory hyperinsulinemia[13]. Insulin resistance returns to near prepubertal levels by the end of puberty[14]. It has been suggested that in normal prepubertal and pubertal girls, the GH/IGF-1 axis and insulin resistance might be involved in the mechanism of adrenarche in prepubertal girls[15]. A strong relationship has been evidenced in non-obese women with polycystic ovary syndrome between insulin sensitivity and SHBG levels[16], and it has been suggested that SHBG level could be a marker of insulin secretion and/or insulin action[17]. The above mentioned normal decrease in insulin resistance together with an increased production of estrogens could explain why the differences observed in SHBG levels during adolescence are not seen in adult women.

The present study confirms the decrease of SHBG from early to late puberty: a decrease of circulating SHBG levels from the 13-14 yr age group to the 15-18 yr age group was observed both in normal and in hirsute girls. However, this decrease was more than twice as pronounced in our normal weight hirsute subjects than in our normal controls, suggesting that an exaggeration of the normal descent of circulating SHBG levels during puberty and adolescence could play a role in the development of hirsutism, possibly by inducing slight, and sometimes undetectable, elevations of free testosterone. Because of the design of the study (one single blood sample in non-fasted subjects during routine school medical examination), the relationship between insulin and SHBG could not be investigated in the present study.

In our subjects, free testosterone levels increased progressively with age, both in normal and in hirsute subjects, but the magnitude of this increase was much higher in hirsute girls, resulting in higher free testosterone values in hirsute than in normal adults.

In conclusion, the present results strongly suggest a major, though subtle, role for the decrease of SHBG in adolescence as against a more accentuated increase of testosterone in adults, as factors conditioning the development of hirsutism signs in these two different periods of life.

Acknowledgements: We thank Dr. Fabienne Mestrez for valuable collaboration, and the volunteers for generously participating in this study. This work was supported in part by research grants from the Secretary of Science and Technology, *Universidad de Buenos Aires*, from the Belgian Fonds de la *Recherche Scientifique Médicale*, and from the *Université Libre de Bruxelles.*

This study was presented in part at the 11th International Congress of Endocrinology, Sydney, Australia, October-November 2000.

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Defining conflicts of interest

Conflict of interest has been defined as 'a set of conditions on which professional judgment concerning a primary interest (such as a patients' welfare or the validity of research) tends to be unduly influenced by a secondary interest (such as financial gain)'*. *It is important to understand that it is a* condition *not a* behaviour*. Many doctors fail to declare a conflict of interest because they are confident that the conflict has not caused them to behave in a different way. This is to misunderstand conflict of interest. It is hard –perhaps impossible– to know whether the conflict of interest has caused us to behave in a different way. We do not always understand our own motivations. Double blind ramdomized trials are so, not because researchers are consciously dishonest, but because bias is pervasive and unconscious. It is the same, I suggest, with conflicts of interest.*

Definiendo conflictos de interés

Un conflicto de interés ha sido definido como 'un grupo de condiciones en las cuales el juicio profesional concerniente a un interés primario (tal como el bienestar de un paciente o la validez de una investigación) tiende a estar indebidamente influenciado por un interés secundario (tal como un beneficio financiero)'*. Es importante entender que esto es una *condición* no una *conducta.* Muchos médicos dejan de declarar un conflicto de interés porque confían que el conflicto no los ha llevado a comportarse de una manera diferente. Esto es no entender lo que es un conflicto de interés. Es difícil –tal vez imposible– para nosotros saber si un conflicto de interés nos ha llevado a comportarnos de una manera diferente. No siempre entendemos nuestras propias motivaciones. Los ensayos clínicos doble ciego aleatorizados son tan importantes, no porque los investigadores sean conscientemente deshonestos, sino porque el sesgo es penetrante e inconsciente. Es lo mismo, sugiero, que los conflictos de interés.

Richard Smith

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