SEX HORMONE-BINDING GLOBULIN AS A MARKER OF THE EFFECT OF HORMONAL TREATMENT IN TURNER'S SYNDROME

Richard Hampl , [1]Marta Snajderova , [2]Jan Lebl , Lidka Lisa , Marcela Dvorakova, Martin Hill , Jarmila Sulcova , Luboslav Starka

Institute of Endocrinology, 119 94 Praha, Czech Republic; [1]2nd Pediatric Department, 2nd Faculty of Medicine, Charles University, Praha; [2]Department of Pediatrics, 3rd Faculty of Medicine, Charles University, Praha
E-mail: rhampl@endo.cz

Objective. In girls with Turner's syndrome a positive effect of growth hormone (GH) therapy on statutal growth has been well documented, in spite of the fact that the levels of GH and IGF-I in these patients are usually within the normal range. It is known that plasma levels of sex hormone-binding globulin (SHBG) are negatively associated with growth hormone (GH) and with IGF-I. Limited data on SHBG levels in these patients and only scarce reports on other than gonadal steroids in these patients are available so far. The major aim of this study was to find out whether SHBG does respond to GH treatment.

Methods. Plasma levels of SHBG, estradiol, progesterone, testosterone and the adrenal steroids and their precursors, namely cortisol, 17α-hydroxyprogesterone, androstenedione, dehydroepiandrosterone and its sulfate were determined in 65 patients aged 2-23 years with Turner's syndrome. The patients were divided into 4 groups according to their actual treatment (untreated, sex steroids only, GH only, GH and sex steroids) and the differences between the groups were evaluated. With respect to dependence of SHBG and of steroids studied on age (with exception of cortisol), their values were expressed as per cents of medians of the physiological values.

Results. As expected, low levels of sex steroids were found. As to the adrenal steroids, in general, their average levels were lower than in healthy subjects but still within the physiological limits. With one exception (androstenedione), no significant differences were found between the groups. The levels of cortisol were undistinguishable from healthy subjects. Absolute as well as relative SHBG levels in untreated group and in the group treated with estrogens were normal or even lower than those reported for normal subjects of the same chronological age. Significantly reduced absolute as well as relative SHBG levels in comparison to all other groups were found in GH treated children.

Conclusion. The results indicate that SHBG may serve as a sensitive biochemical marker of the response to the GH administration.

Key words: Turner's syndrome – Growth hormone treatment – Sex hormone binding globulin (SHBG)

Though there is no convincing evidence that growth hormone (GH) deficiency is involved in the growth disorder in Turner's syndrome, and normal or only insignificantly lowered levels of GH and insulin-like growth factor I (IGF-I) and also of its binding protein 3 have been found in these patients at least till puberty, they usually respond well to GH treatment (Ranke et al. 1983, 1987; Ranke 1995).

Since the response to GH alone an/or in combination with estrogens varies considerably from patient to patient, suitable laboratory markers are searched which, eventually, could reflect the different response

Table 1
Number of patients in individual groups according to the treatment and chronological age. A – untreated, B – treated with estrogens only, C – treated with GH only, D – treated with a combination of estrogens and GH

Age group (years)	Group according to the treatment				Total
	A	B	C	D	
below 10	4	0	14	0	18
10, 11	1	0	5	0	6
12, 13	2	3	8	0	13
14	3	1	4	4	12
15 – 25	2	7	3	4	16
Total	12	11	34	8	65

to the treatment. In hormonal spectrum, however, no striking abnormalities other than those related to gonadal dysgenesis have been found (Ranke et al. 1987; Lippe 1991).

One of the proteins, intimately associated with many hormones, besides steroid and thyroid hormones including also insulin and some growth factors, is sex hormone-binding globulin (SHBG) (Hampl and Starka, 1996). It has been demonstrated that the levels of this hepatic protein negatively correlate with GH and also with IGF-I: Decreased levels of SHBG were reported in patients with acromegaly whereas significantly increased levels were found in subjects with GH deficiency (Rosner 1990). Addition of GH as well as of IGF-I to the SHBG-producing hepatoma cells (Hep-G2) in culture caused suppression of SHBG production (Leukovaara et al. 1995).

Only a few reports deal with SHBG levels and other than gonadal steroids in patients with Turner's syndrome (Heyns 1977; Hauesler et al. 1996; Gravholt et al. 1999). It is also known, in spite that the levels of thyroid hormones do not differ much from euthyroid ones, that thyroid abnormalities, especially autoimmune thyroiditis as detected by by presence of thyroid peroxidase and/or thyreoglobulin antibodies, are much more frequent in these subject (Germain and Plotnick 1986; Fleming et al. 1988; Ivarsson et al. 1995; Radetti et al. 1995; Chiovato et al. 1996; Wilson et al. 1996; Madeiros et al. 2000). The fact that SHBG is closely associated with thyroid status may be an additional reason to evaluate its levels in these patients.

In this study, SHBG levels, along with the main adrenal and gonadal steroids are reported in patients with Turner's syndrome under various treatment regimes.

Materials and Methods

Patients. 65 females with Turner's syndrome, aged 2-23 years (mean 12.3) were investigated. 44 had karyotype 45,X, 21 a chromosomal mosaic and/or a structural abnormality of X chromosome. The patients were divided into 4 groups according to their actual treatment as follows: A – untretaed, B – treated with sex steroids only (estrogens, eventually in combination with gestagen), C – treated with GH only, D – treated with a combination of sex steroids and GH. Micronized ethinyl estradiol, 100 μg (Schering A.G., FRG), mestranol, 50 μg (Jenapharm, FRG) or estradiol patches (Estraderm TTS, 25 μg, Novartis, Svitzerland) were used as estrogens, in patients above 13 years usually together with a gestagen (medroxyprogesterone acetate, 5 mg, Provera, Pharmacia-Upjohn, Belgium). The patients treated with GH received human recombinant GH (Norditropin 24, Novo-Nordisk A/S, Danemark), in a dose 1 IU/kg body weight/week for at least one year. The number of patients in each group according to the age is shown in the Table 1.

Hormone analyses. Serum SHBG was measured by using immunoradiometric SHBG-IRMA kit from ORION (Finnland). All steroids were determined by radioimmunoassay (RIA). Estradiol was measured by the kit from HUMALAB, Kosice (Slovakia), dehydroepiandrosterone (DHEA), its sulphate (DHEAS) and 17α-hydroxyprogesterone by RIA kits purchased from IMMUNOTECH (France). Androstenedione was determined by the method of Putz et al. (1982), testosterone and cortisol were measured by using rabbit antisera against steroid-3-O-(carboxymethyloxime)-bovine serum albumin (BSA) conjugates and radioiodinated homologous tyrosine methyl ester derivatives as tracers (Hampl 1994; Bicíkova et al. 1988), progesterone by the method of Langer et al. (1978) using antiserum against progesterone-11α-hemisuccinate:BSA conjugate and tritiated tracer from Radiochemical Center, Amersham (UK). Since the normal levels of SHBG and the steroids with exception of cortisol are age dependent,

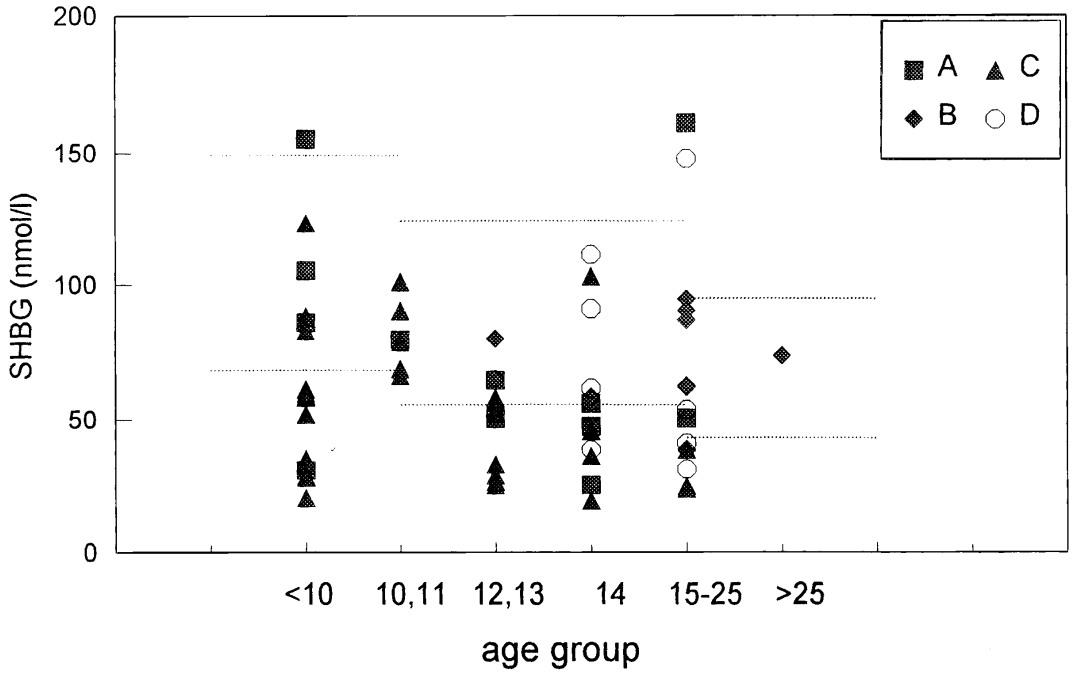

Figure 1
SHBG levels in patients with Turner's syndrome under various treatment regimes according to age. Group A untreated, group B treated with sex steroids only, group C treated with GH only, group D treated with a combination of sex steroids and GH. Horizontal lines represent the range of physiological levels.

the relative values of analyzed compounds were calculated as the per cents of medians of the physiological values reported in authors' laboratory.

Statistical evaluation. The normal distribution of the measured levels in individual groups was tested by Kolmogorov-Smirnov test. The significance of differences among the groups was determined by analysis of variance (ANOVA), the significance of differences between the individual pairs of groups was determined by the least square difference (LSD) method. The linear correlation of the corresponding data was performed by the least square method using Statgraphic software Version.3.3 (Statistical Graphic Co., Kaysville, Utah, USA).

Results

The individual values of serum SHBG in the groups of patients divided according to the treatment are shown in Figure 1. In untreated patients (group A) the SHBG levels did not differ from those reported for healthy subjects of the same chronological age. The means, standard deviations and medians of absolute as well as of relative SHBG levels are summarized in Table 2, together with statistical evaluation of differences between the groups. Significantly lower relative SHBG levels than in other groups have been found in patients treated with GH only (group C).

Absolute and relative levels of estradiol and progesterone are shown in Table 3. The lowest relative estradiol levels have been found in both groups of GH-treated patients included that receiving also estradiol supplementation (group D). The differences from the first two groups (A, B) were significant. Lower than normal average progesterone levels have been found in all the patients. The highest serum progesterone concentrations when compared to other groups have been recorded in the group receiving GH only (C). The difference from the other patients receiving hormones (groups B and D) was significant.

Absolute and relative levels of dehydroepiandrosterone and its sulfate are shown in Table 4, those of

Table 2
Absolute and relative values of serum SHBG in the groups of patients divided according to the treatment (see Table 1). The significance of differences of the relative SHBG values between individual groups of patients was determined by least square difference (LSD) test

Group	n	Absolute SHBG levels (nmol/l)		Relative SHBG levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences **between groups** [a)]	Median
A	12	76.3 ± 44.5	60.3	84.8 ± 55.8	C*	81.4
B	11	65.3 ± 18.5	62.7	92.2 ± 27.5	C*	89.3
C	34	54.2 ± 27.4	52.6	61.4 ± 30.6	A*, B*, D**	56.9
D	8	71.9 ± 41.2	57.6	103 ± 38.9	C**	82.4

* $p < 0.05$, ** $p < 0.01$
[a)] Only relative values were considered

Table 3
Absolute and relative values of serum female sex hormones in patients divided according to treatment (see Table 1). The significance of between group differences was determined by LSD test., * $p < 0.05$, ** $p < 0.01$

Estradiol

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	12	0.22 ± 0.22	0.15	186 ± 288	C**, D**	40.1
B	11	0.34 ± 0.27	0.16	47.8 ± 39.5	-	23.2
C	32	0.10 ± 0.12	0.07	51.5 ± 72.5	A**	37.7
D	8	0.06 ± 0.04	0.04	8.08 ± 5.48	A**	6.30

Progesterone

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	7	0.96 ± 0.55	0.82	35.5 ± 45.4	-	10.9
B	9	1.32 ± 0.84	1.81	9.34 ± 5.94	C**	12.8
C	28	1.39 ± 0.44	1.39	60.5 ± 51.6	B**, D**	48.0
D	7	1.42 ± 0.58	1.30	10.0 ± 4.10	C**	9.19

* $p < 0.05$, ** $p < 0.01$
[a)] Only relative values were considered

17α-hydroxyprogesterone, androstenedione and testosterone in Tables 5 and 6, respectively. In general, the average levels of all these steroids were lower than in healthy subjects but still within the range of normal values of the corresponding age. With one exception (significantly higher relative androstene-

Table 4
Absolute and relative values of serum dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) in patients divided according to treatment (see Table 1). The significance of between group differences was determined by LSD test

DHEA

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	9	22.1 ± 11.4	21.0	113 ± 55.6	–	101
B	8	22.1 ± 8.88	21.0	82.7 ± 29.3	–	83.9
C	30	15.7 ± 12.6	13.1	93.8 ± 50.0	–	93.2
D	8	23.5 ± 14.5	22.0	94.3 ± 57.5	–	89.4

DHEAS

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	9	2.78 ± 1.32	3.60	53.7 ± 21.0	–	49.0
B	9	4.39 ± 2.24	4.80	59.3 ± 29.1	–	59.2
C	32	2.77 ± 2.35	2.20	63.1 ± 62.3	–	43.8
D	8	5.34 ± 3.48	4.85	80.3 ± 46.4	–	63.1

[a)] Only relative values were considered

dione levels in the group B than in the group A, no other statistically significant differences were found between the groups. The levels of cortisol were undistinguishable from those of healthy subjects (mean ± SD = 401 ± 190 nmol/l).

All the data were mutually correlated to find out whether some relationship does exist between the hormonal parameters studied. The correlations were calculated for each group as well as for cumulated data from all the patients. With respect to dependence of the hormonal levels on age, only relative values were taken into consideration.

No correlation has been found between SHBG and any of the hormones under study. When all the patients' data were cumulated, the only significant correlations have been found between the pairs estradiol – 17α-hydroxyprogesterone, DHEAS – DHEA and 17α-hydroxyprogesterone – androstenedione. The respective correlation coefficients and P values are shown in Table 7.

Discussion

From the point of view of endocrine regulations SHBG represents an important hormonal marker, the formation of which is closely associated with at least three hormonal systems: 1. the hypothalamo-pituitary-gonadal axis, 2. insular-growth hormone-IGF-1 axis and 3. the thyroid axis (Sinnecker 1993; Hampl and Starka 1996). So far only a few reports concern SHBG in patients suffering from Turner's syndrome, known to be accompanied with abnormalities in all the mentioned systems (Heyns 1977; Hauesler et al. 1996; Gravholt et al. 1999).

In a cross-sectional study on 40 girls with Turner's syndrome aged 3.7-16.4 years Hauesler et al. (1996) investigated the changes of fasting insulin, insulin-like growth factor binding protein 1 (IGFBP-1) (known to act as an inhibitor of IGF-1) and also of SHBG before, and after 1-2 year GH monotherapy, followed by treatment with anabolic steroid oxan-

Table 5
Absolute and relative values of serum 17a-hydroxyprogesterone and androstenedione in patients divided according to treatment (see Table 1). The significance of between group differences was determined by LSD test

17a-Hydroxyprogesterone

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	10	2.57 ± 1.72	1.80	99.6 ± 77.6	–	79.8
B	9	2.33 ± 1.16	2.50	90.2 ± 53.4	–	97.9
C	29	1.60 ± 0.92	1.40	57.9 ± 43.5	–	44.4
D	8	1.92 ± 1.24	1.75	63.1 ± 38.6	–	48.6

Androstenedione

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	7	2.36 ± 1.11	2.41	51.9 ± 22.7	B*	52.4
B	9	3.78 ± 1.25	4.05	96.7 ± 41.3	A*	96.3
C	32	3.55 ± 1.83	3.16	83.3 ± 43.5	–	69.5
D	7	3.74 ± 1.52	3.45	81.0 ± 33.2	–	74.4

[a)] Only relative values were considered
* $p < 0.05$

Table 6
Absolute and relative values of serum testosterone in patients divided according to treatment (see Table 1). The significance of between group differences was determined by LSD test

Group	n	Absolute levels (nmol/l)		Relative levels (% of medians of physiological values)		
		Mean ± SD	Median	Mean ± SD	Significance of differences between groups [a)]	Median
A	7	1.23 ± 1.30	0.89	118 ± 111	–	74.2
B	7	1.06 ± 0.82	0.77	63.0 ± 47.0	–	45.3
C	32	0.58 ± 0.40	0.48	88.6 ± 95.2	–	53.5
D	8	0.80 ± 0.30	0.74	58.1 ± 27.3	–	52.0

[a)] Only relative values were considered

drolone. They found that pretreatment values of all the parameters studied were within the normal range, and decreased with chronological age. After GH administration insulin concentration increased, while IGFBP-1 and SHBG decreased, followed by a further decrease after introduction of oxandrolone.

Our study differed from that of HAUESLER et al. (1996) in that various groups of patients were com-

Table 7
Correlation coefficients (r) and p-values for cumulated data of the levels of six hormonal steroids in patients with Turner´s syndrome, irrespective to treatment. The right part of the diagonal shows the p-values, the left one the respective correlation coefficients. The upper values for each pair of steroids were calculated for the absolute hormonal levels, the lower ones for the relative values expressed as per cents of medians of the physiological levels at the corresponding chronological age. The data are shown only for those pairs of steroids, where significant correlation occurred at least in one instance. NS = not significant

r \ p	Estradiol	17α-OH-P	DHEAS	DHEA	Androstenedione	Testosterone
Estradiol	–	0.006				
		0.001				
17α-OH-P	0.458	–		0.001	0.001	
	0.528			NS	0.008	
DHEAS			–	0.002		0.025
				0.005		NS
DHEA		0.577	0.535	–	0.045	0.023
		0.242	0.487		NS	NS
Androstenedione		0.504		0.375	–	
		0.478		0.164		
Testosterone			0.416	0.435		–
			0.180	0.112		

pared according to treatment. In accordance with the Austrian authors, in all the patients including the untreated girls (group A) the relative SHBG levels were within the range or even lower than in normal subjects of the same chronological age. In agreement with well known fact that administration of estradiol results in an increase of circulating SHBG (Rosner 1990; Sinnecker 1993), the highest relative levels of this protein have been found in both groups receiving estrogens (B, D). Significantly lowered relative as well as absolute SHBG levels were found in the group of girls treated with GH only (C), confirming thus that GH administration led to a decrease of this protein.

Gravholt et al. (1999) compared the levels of SHBG and major adrenal and gonadal steroids, namely testosterone (total and free), dihydrotestosterone, androstenedione, DHEAS, estradiol, estrone and its sulfate in 27 adult patients with Turner's syndrome with those of 24 age-matched healthy controls. With exception of DHEAS, they have found significantly reduced levels of all the steroids and even of SHBG in the patients. In our study on children the average levels of steroids of predominantly adrenal origin were lower than in normal subjects, but still within the physiological range, apparently due to a lack of gonadal contribution, in accordance with previous reports (Zachman et al. 1984). The concentrations of circulating sex steroids, especially in older girls in comparison with physiological values of the same chronological age, reflect the unimpaired adrenal production and peripheral conversion as well as the known fact that not all patients with Turner's syndrome should have gonadal dysgenesis (see LIPPE 1991 and the literature therein).

In conclusion, our results demonstrate that SHBG in these patients does respond well to GH administration and thus may serve as a sensitive and independent biochemical marker of the hormonal treatment.

Acknowledgement

This study has been supported by the Grant No. 6313-of the Internal Grant Agency of the Czech Ministry of Health.

References

Bicikova M, Hampl R, Putz Z, Starka L: Comparison of isotope and nonisotope variants of direct cortisol immunoassay. In: Advances in steroid analysis 1987, ed. S. Görög, pp. 101-106, Akadémiai Kiadó, Budapest 1988

Chiovato L, Larizza D, Bendinelli G, Tonacchera M, Marino M, Mammoli C, Lorini R, Severi F,

Pinchera A: Autoimmune hypothyroidism and hyperthyroidism in patients with Turner's syndrome. Eur J Endocriol **134**, 568-575, 1996

Fleming S, Cowell C, Bailey J, Burrow Gn: Hashimoto's disease in Turner's syndrome. Clin Invest Med **11**, 243-246, 1988

Germain El, Plotnick Lp: Age-related anti-thyroid antibodies and thyroid abnormalities in Turner's syndrome. Acta Pediatr Scand **75**, 750-755, 1986

Gravholt Ch, Svenstrup B, Bennett P, Christiansen Js: Reducedandrogen levels in adult Turner syndrome: influence of female sex steroids and growth hormone status. Clin. Endocrinology **50**, 791-800, 1999

Hampl R: Comparison of three immunoassays for testosterone determination. In: Advances in steroid analysis '93, ed. S Görög, pp. 163-169, Akadémiai Kiadó, Budapest 1994

Hampl R Starka L: Sex-hormone-binding globulin in endocrine regulation. Endocrine Regul **30**, 56-66, 1996

Heusler G, Schmitt K, Bluemel P, Ploechl E, Waldhoer T, Frisch H: Insulin, insulin-like growth factor-binding protein-1, and sex hormone-binding globulin in patients with Turner's syndrome: course over age in untreated patients and effect of therapy with growth hormone alone and in combination with oxandrolone. J Clin Endocr Metab **81**, 536-541, 1996

Heyns W: The steroid-binding β-globulin of human plasma. Adv Steroid Biochem Pharmacol **6**, 59-79, 1977

Ivarsson Sa, Ericsson Ub, Nilsson Ko, Gustafsson J, Hagenas L, Hager A, Moell C, Tuvemo T, Westphal O, Albertsson-wikland K: Thyroid antibodies, Turner's syndrome and growth hormone therapy. Acta Paediatr **84**, 63-65, 1995

Langer L, Veleminsky J, Hampl R, Starka L, Holan J: Radioimmunoassay of plasma progesterone. Radiochem Radioanal Letters **34**, 267-272, 1978

Leukovaara M, Carson M, Adlercreutz H: Regulation of production and secretion of sex hormone-binding globulin in HepG2 cell cultures by hormones and growth factors. J Clin Endocr Metab **80**, 160-164, 1995

Lippe B: Turner's syndrome. Endocrinology and Metabolism Clinics of North America **20**, 121-152, 1991

Madeiros Cc, Marini Sh, Baptista Mt, Guerra G Jr, Maciel-Guerra At: Turner's syndrome and thyroid disease: a transverse study of pediatric patients in Brazil. J Pediatr Endocrinol Metab **13**, 357-362, 2000

Putz Z, Hampl R, Vanuga A, Veleminsky J, Starka L: A selective radioimmunoassay of androstenedione in plasma and saliva. J Clin Chem Clin Biochem **20**, 761-764, 1982

Radetti G, Mazzanti L, Paganini C, Bernasconi S, Russo G, Rigon F, Cacciari E: Frequency, clinical and laboratory features of thyroiditis in girls with Turner's syndrome. Acta Paediatr **84**, 909-912, 1995

Ranke Mb: Growth hormone therapy in Turner's syndrome. Horm Res **44** (suppl), 35-41, 1995

Ranke Mb, Blum Bf, Hang Fh, Rosendahl W, Attanasio A, Enders H, Gupta D, Bierich Jg: Growth hormone, somatomedin levels and growth regulation in Turner's's sydrome. Acta Endocrin (Copenh) **116**, 305-313, 1987

Ranke Mb, Pflueger H, Rosendahl W Stubbe P, Enders M, Bierich Jr, Majewski F: Turner's syndrome: Spontaneous growth in 150 cases and review of the literature. Eur J Pediatr **141**, :81-88 1983

Rosner W The function of CBG and SHBG: recent advances. Endocrine Rev **11**, 80-91, 1990

Sinnecker Ghg: Sexualhormon bindendes Globulin. Georg Thieme Verlag, Stuttgart New York, pp 103-106, 1993

Wilson R, Chu Ce, Donaldson Md, Thomson Ja, Mckillop Jh, Connor Jm: An increased incidence of thyroid antibodies in patients with Turner's syndrome and their first degree relatives. Autoimmunity **25**, 47-52, 1996

Zachmann M, Manella B, Eiholzer U, Bucher H, Prader A: Influence of estrogen in high and low doses on plasma steroid concentrations in girls with tall stature and Turner's syndrome. Acta Endocrin (Copenh) **106**, 368-373, 1984

Corresponding author: Doc. RNDr. R. Hampl, DrSc.,
Institute of Endocrinology
Národní 8
CZ-116 94 Praha 1
Czech Republic
Fax: +42 2 24905 325
E-mail: rhampl@endo.cz