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ORIGINAL ARTICLE

The effect of intrauterine and oral levonorgestrel administration on serum concentrations of sex hormone-binding globulin, insulin and insulin-like growth factor binding protein-1

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Background. The concentrations of sex hormone-binding globulin (SHBG) have been shown to decrease during the use of levonorgestrel (LNG)-containing contraception. This decrease has been thought to be due to the androgenic action of LNG. In endogenously hyperandrogenic women, particularly in those with increased body weight, serum SHBG correlates with circulating insulin-like growth factor binding protein-1 (IGFBP-1) concentration, and both are inversely related to insulin. LNG-containing combined contraceptives have also been reported to increase the pancreatic insulin secretion.
Objective. To examine whether serum insulin and IGFBP-1 levels are related to SHBG during the use of intrauterine or oral levonorgestrel contraception.
Methods. Thirty-one fertile women were divided into three study groups: A copper-releasing intrauterine device (IUD) was inserted in control group (n=10), and the LNG-releasing intrauterine contraceptive system (LNG-IUS) in group II (n=10), and 30 μg LNG-containing contraceptive minipills were given in group III (n=11). Twenty-nine women completed the study and one woman was excluded because of a high body mass index. Fasting concentrations of blood glucose, insulin, SHBG, IGFBP-1, testosterone and LNG before and after three-months-use of contraception were measured.
Results. SHBG concentrations decreased slightly during oral LNG contraception, but not during the use of the LNG-IUS. No change was found in blood glucose, serum insulin, serum IGFBP-1 and serum total testosterone concentrations in either group. In our study group, including women with normal body weight, no correlation was detected between insulin and SHBG concentrations before or after LNG contraception, whereas an inverse correlation was found between insulin and IGFBP-1 levels at the baseline as well as after LNG-IUS use (R^2= 0.578; p=0.001). Multiple regression analysis showed no significant association between the levels of SHBG and IGFBP-1 as dependent factors, and glucose, insulin, LNG, age, waist-hip ratio and body mass index as dependent factors.
Conclusions. Our data imply that the effect of levonorgestrel on variables associated with endogenous hyperandrogenism remains borderline in women with normal body mass index.

Key words: contraception; insulin; insulin-like growth factor-binding protein-1; levonorgestrel; sex hormone-binding globulin

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The results of previous studies have shown that the concentrations of sex hormone-binding globulin (SHBG) as measured by the androgen binding capacity are decreased during the use of either the levonorgestrel-releasing intrauterine contraceptive system (LNG-IUS))1–4) or vaginal or oral contraceptives containing levonorgestrel (LNG) (5, 6). In a study by van Kammen et al. (7) as little as 30 μg of LNG daily induced an approximately 30% decrease in circulating SHBG, as measured by its androgen binding capacity. Although several hormones influence the circulating levels of SHBG, the estrogen/androgen balance has been thought to be the major factor determining serum SHBG concentration. The decrease in SHBG concentrations during LNG-treatment has been considered to reflect the androgenicity of 19-norprogestins, i.e., either a direct effect of LNG on the synthesis of SHBG or an indirect effect arising from its displacement of testosterone from the carrier protein and consequent depression of SHBG concentrations, or a combination of both factors (6). In endogenous hyperandrogenism, such as associated with obesity and polycystic ovarian disease, hyperinsulinemia is believed to be the primary metabolic disorder and insulin, by inhibiting hepatic SHBG production, is a primary cause of decreased serum SHBG levels (8–10). LNG-containing combined contraceptives have also been reported to increase the pancreatic insulin secretion. If this is the case, it might be reflected it serum insulin concentrations.

Insulin-like growth factor-binding protein-1 (IGFBP-1) is another protein which is synthesized by the liver and whose gene expression and secretion are inhibited by insulin (11, 12). In hyperandrogenic women, particularly in those with an increased body mass index (BMI), serum IGFBP-1 and insulin levels are inversely correlated, whereas SHBG and IGFBP-1 show parallel alterations (13–17). IGFBP-1 is also produced by decidualized endometrial stromal cells, where progesterone and LNG induce IGFBP-1 mRNA expression as well as protein secretion (18–21). However, changes in endometrial IGFBP-1 production during normal menstrual cycle or during the use of LNG-IUS are not reflected in serum IGFBP-1 concentrations.

To clarify whether LNG contraception also affects serum insulin concentrations, the fasting levels of SHBG, insulin and IGFBP-1 before and after three months' use of oral and intrauterine LNG contraception were measured. These different routes of administration were chosen to see whether an oral hormone administration has a stronger liver effect. In addition, total testosterone and fasting glucose concentrations were measured.

Materials and methods

Subjects and inclusion criteria

Thirty-one healthy women of reproductive age who requested contraception were enrolled in the randomized study, and 29 completed the study. The women were assigned to three groups using a random-number Table with group allocation predetermined. In group I (control group, $n=10$), a copper-releasing IUD (NovaT380, Leiras, Turku, Finland) was inserted, and in group II ($n=10$) the LNG-IUS (Levonova, Leiras, Turku, Finland) was inserted. Women in group III ($n=11$) started taking 30 μg LNG-only-containing contraceptive pills (Microluton, Leiras, Turku, Finland). One woman in group I and one in group III discontinued the study, and one woman in group II was excluded because of a high body mass index. The LNG-IUS has a steroid reservoir around a vertical stem, releasing 20 μg LNG/24 h. The copper IUD has the same plastic core, but a copper wire around the vertical stem. Blood samples were drawn between 0730 and 0900h after an overnight fast on cycle days 5–7 before contraception. The second sample was obtained after three months' use of contraception (cycle day not determined). Serum samples were stored at −20°C until analyzed. The copper IUDs and LNG-IUSs were inserted at the beginning of the follicular phase on cycle days 5–7 and the women in group III started taking their pills on the first day of the next cycle. The pills were instructed to be taken at the same time in the morning every day. None of the women had used hormonal injectables or implants for contraception in the previous six months, or oral contraceptives or other hormonal preparations in the month before entering the study, and none of the subjects were on any medication. Physical examinations, including blood pressure, showed normal results. The medical histories of the subjects and those of their close relatives did not reveal any previous significant disease. Transvaginal ultrasonography was performed at baseline to exclude any abnormality such as polycystic ovaries (PCO). According to the study protocol, there were no restrictions concerning women's weight, but since the results from one extremely obese woman (BMI 35 kg/m^2) in group II with an exceptionally low serum SHBG concentration (26 nmol/l) and a high insulin level (21 mU/l) distorted the data, this subject was excluded. Thereafter, the three groups were comparable with

Abbreviations:
SHBG: sex hormone-binding globulin; LNG: levonorgestrel; IGFBP-1: insulin-like growth factor binding protein-1; IUD: intrauterine device; IUS: intrauterine system; BMI: body mass index; PCO: polycystic ovaries; WHR: waist-to-hip ratio.

regard to demographic data, with subjects equally matched for age, parity, abortions, BMI and waist-to-hip ratio (WHR) (Table I).

All subjects gave their informed consent, and the study was approved by the Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Central Hospital.

Assays

The concentrations of serum LNG were determined by radioimmunoassay (22). This assay utilizes tritium-labeled LNG as a tracer. Serum IGFBP-1 concentrations were determined using a monoclonal immunoenzymometric assay, as previously described (23). Monoclonal IGFBP-1 antibody (MAb 6305; Medix Biochemica) was used as a catching antibody, and [^{125}I] MAb 6303 as a detecting antibody (24). The sensitivity of the assay was 0.25 ng/ml, and the intra- and interassay coefficients of variation were 6.2% and 9.8%, respectively. Serum insulin concentrations were measured by using the Phadeseph Insulin RIA method (Pharmacia AG, Uppsala, Sweden). The upper limit of normal fasting insulin concentrations is 20 mU/l. Plasma glucose concentrations were measured by a glucose hexokinase method. The upper limit of normal fasting glucose concentrations in our laboratory is 5.5 mmol/l. Serum testosterone concentrations were measured by using radioimmunoassay kits employing steroid extraction and ^{125}I-testosterone (Farmos Diagnostica, Turku, Finland). Serum SHBG concentrations were measured by immunofluorometric assay (DELFIA SHBG kit) kits (Wallac OY, Oulunsalo, Finland), with 1.4%, 1.3% and 1.8% intra-assay and 8.2%, 5.1% and 10.1% interassay coefficients of variation at SHBG levels of 19.7, 57.0 and 130 nmol/l respectively, and a sensitivity of 0.5 nmol/l.

Table I. Clinical and endocrinological characteristics of study groups at baseline (mean±s.d.)

	Copper IUD [a] (n=9)	LNG IUD [a] (n=9)	LNG pills[a] (n=10)
Age (year)	32±3.8	30.7±5.0	31±4.6
Body mass index (BMI) (kg/m^2)	23.1±1.4	23.4±2.6	22.1±2.7
Waist-to-hip ratio (WHR)	0.83±0.03	0.83±0.07	0.8±0.03
Births	1.78±1.1	1.7±0.9	1.4±1.4
Abortions	0.4±0.5	0.2±0.4	0.6±1.1
Fasting blood glucose (mmol/l)	4.8±0.4	4.7±0.3	4.8±0.3
Serum insulin (mU/l)	6.8±3.5	4.6±2.1	5.1±1.4
Serum testosterone (nmol/l)	1.6±0.5	1.3±0.5	1.3±0.2
Serum SHBG (nmol/l)	62.7±2.15	60.8±10.3	55.1±13.6
Serum IGFBP-1 (ng/ml)	3.4±2.2	5.9±4.4	4.7±2.7

[a] Includes the women who completed the study.

Statistical methods

All results are expressed as mean±s.d. Data comparisons at baseline and after three months between groups were made by one-way analysis of variance. Analyses of circulating glucose, insulin, SHBG, IGFBP-1 and testosterone concentrations within and between the study groups were made by 2-way repeated measure analysis of variance. The relationships between variables such as SHBG and insulin, LNG, glucose, IGFBP-1 and testosterone at baseline and after three months in each group were evaluated by first using simple regression and then linear multiple regression with SHBG and IGFBP-1 as dependent variables. Independent variables included age, BMI, WHR, fasting glucose, insulin and testosterone. p-values below 0.05 were considered statistically significant.

Results

The circulating levels of glucose, insulin, SHBG, IGFBP-1 and testosterone before and during contraception are shown in Fig. 1. At baseline, the study groups did not differ from each other as regards any of the measured parameters.

Before hormone administration, there were stat-

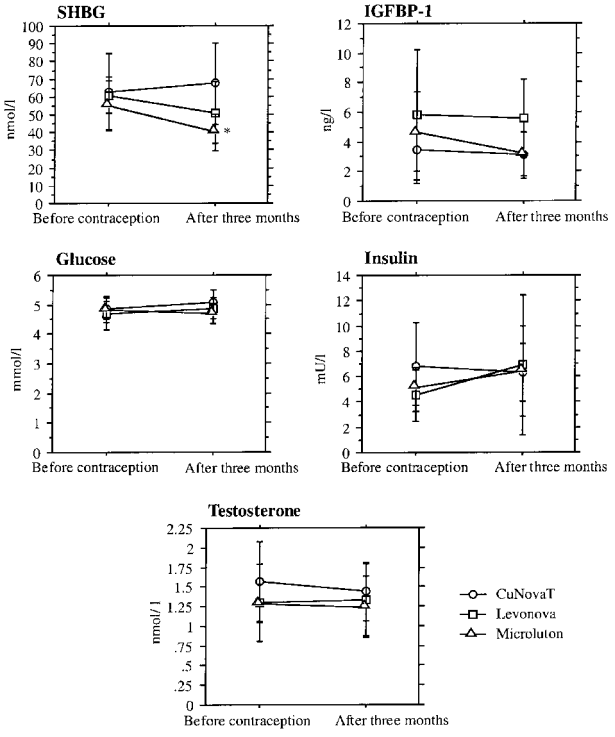

Fig. 1. Circulating fasting concentrations (mean±s.d.) of SHBG, IGFBP-1, glucose, insulin, total testosterone before and after three months' use of a copper IUD (group I), intrauterine LNG (group II) and oral LNG (group III). *=change from baseline statistically significant (p<0.05).

Table II. Simple regression analysis between SHBG and age, BMI, WHR, glucose, insulin and testosterone, and between IGFBP-1 and age, BMI, WHR, glucose, insulin and testosterone at baseline

	SHBG R^2	IGFBP-1 R^2
Age	−0.053	0.108
BMI	−0.076	−0.366
WHR	−0.238	−0.284
Fasting glucose	−0.402*	−0.519*
Testosterone	0.317	−0.312
SHBG		0.228
Insulin	−0.336	−0.578*

R^2=the coefficient of determination. *=$p<0.05$.

Table III. Simple regression analysis between SHBG and age, BMI, WHR, glucose, insulin and testosterone, and between IGFBP-1 and age, BMI, WHR, glucose, insulin and testosterone after three months in controls (group I) and of use of intrauterine (group II) or oral (group III) LNG

	SHBG R^2	IGFBP-1 R^2
Group I (using a copper-releasing IUD)		
Age	−0.087	0.040
BMI	−0.462	0.446
WHR	−0.513	0.449
Fasting glucose	0.230	0.169
Testosterone	0.847*	0.144
SHBG		0.869*
Insulin	−0.494	0.363
Group II (using LNG-IUS)		
Age	−0.638	0.160
BMI	0.062	−0.726*
WHR	0.346	−0.630
Fasting glucose	−0.084	−0.424
Testosterone	0.951*	0.454
SHBG		0.130
Insulin	−0.290	−0.780*
LNG	0.890*	0.335
Group III (taking 30 μg LNG only oral contraceptive pills)		
Age	0.151	0.464
BMI	−0.417	−0.152
WHR	−0.872*	−0.696*
Fasting glucose	−0.229	−0.327
Testosterone	0.959*	0.209
SHBG		0.741*
Insulin	0.424	0.113
LNG	0.161	0.243

R^2=the coefficient of determination. *=$p<0.05$.

istically significant inverse correlations between blood glucose and SHBG, between glucose and IGFBP-1, and between insulin and IGFBP-1, whereas no significant correlation was found between insulin and SHBG or between SHBG and IGFBP-1 concentrations (Table II).

After three months' use of LNG, the mean (±s.d.) serum LNG concentrations in groups II and III were 309 pg/ml (±115) and 361 pg/ml (±245), respectively. In group II, there was a significant positive correlation between serum LNG and SHBG (R^2=0.890, p=0.0005) (Table III). The glucose levels remained unchanged in each group (Fig. 1). There was also no significant change in fasting insulin concentrations after three months use of either oral or intrauterine LNG (Fig. 1).

The mean SHBG concentration decreased from 60.8 nmol/l to 50.9 nmol/l (16.3%) in women using the LNG-IUS; in group III (oral LNG) the corresponding values were 55.1 nmol/l and 39.9 nmol/l (decrease 27.6%), respectively. In group I (control) the mean SHBG level was 62.7 nmol/l at baseline and 67.3 nmol/l (7.3% increase) three months later. The decrease in SHBG concentrations was significant in group III (p=0.0202) but not in group II (in 2-way repeated measure analysis of variance) (Fig. 1).

In women having the LNG-IUS or the copper releasing IUD, no change was found in serum IGFBP-1 levels in three months and the decrease during the use of oral LNG was not significant (Fig. 1).

Serum testosterone concentrations remained unchanged during the study period (Fig. 1). At three months, significant correlations were found between SHBG and IGFBP-1 in group I and group III (Table III). An inverse correlation was found between insulin and IGFBP-1 in group II (Table III), whereas no correlation was found between serum insulin and SHBG concentrations in oral or intrauterine LNG groups.

The results of multiple regression showed non-significant association between the serum concentrations of SHBG and IGFBP-1 as a dependent factors, and age, BMI, WHR, fasting glucose, testosterone, insulin or LNG as independent factors.

Discussion

Confirming the data from previous studies, the present results show that serum SHBG concentrations slightly decrease during the use of oral LNG-containing contraception (1–6), in spite of relatively low levels of circulating LNG in this group. The LNG-induced decrease in SHBG concentration correlated with serum LNG concentration, which is in agreement with previous studies. The time period between the last pill intake and the blood sample collection (24 hours) may be an explanation for the small LNG concentrations and, consequently, small changes in SHBG levels in these women. In earlier studies SHBG concentrations were determined by androgen binding assay (4, 5). The decrease has been thought to be due to the androgenic action of LNG (25). However, decreased SHBG levels might also reflect increased insulin secretion, because in-

sulin has been shown to inhibit hepatic production of SHBG (8), and a negative correlation has been described between fasting insulin and SHBG levels in obese and hyperandrogenic women (26, 27). In support of this hypothesis, Godsland and co-workers have shown that LNG-containing combined contraceptives increase pancreatic insulin secretion (28). In their study the progestin-only preparations had no such effect. In keeping with the results of Godsland and co-workers, no significant change was found in fasting insulin during LNG-only contraception, and after excluding one obese woman, who had abnormally low serum SHBG levels and high insulin levels, no correlation was found between serum insulin and SHBG concentrations before or after three months contraception in any of the study groups. The lack of any correlation between insulin and SHBG even at baseline contrasts the results published by Preziosi et al. (29). The reason for this discrepancy remains unclear but the small number of subjects with normal BMI may partly account for the results in the present study. A stimulating effect of endogenous estradiol on SHBG secretion may account for the difference in SHBG response during oral and intrauterine LNG administration, since most of the menstrual cycles remain ovulatory during the use of LNG-IUS, and serum estradiol levels correspond to those during the normal menstrual cycle (30, 31). Moreover, oral administration of steroids is more likely to affect the liver function altogether more than parenteral administration. Further, a possible difference in SHBG levels as measured by androgen binding assay and immunoassay may be a factor at least partly explaining why the decrease in SHBG in this study was less than expected.

In contrast to SHBG, serum concentration of IGFBP-1, another protein whose liver synthesis is regulated by insulin, was inversely correlated with insulin at baseline and also after three months use of intrauterine LNG, although no significant alterations were detected in either protein during the study period. This suggests that the decrease of serum SHBG during LNG contraception does not occur through insulin action.

In summary, confirming data from previous studies, a slight decrease was found in serum SHBG levels after three months use of oral LNG contraception. Serum glucose, insulin and testosterone levels remained unchanged. This, as well as the lack of any correlation between SHBG and insulin before or after three months use of LNG, with simultaneously detected inverse correlation between IGFBP-1 and insulin, implies that LNG contraception has no significant effect on insulin production and insulin is not the major regulator of circulating SHBG in normal weight women without or with LNG contraception.

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