CLINICAL STUDY

Low sex hormone-binding globulin as a predictive marker for insulin resistance in women with hyperandrogenic syndrome

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Abstract

Background: The aim of the present study is to assess insulin resistance (IR) in women with hyperandrogenic syndrome, which was suggested to replace the term polycystic ovary syndrome by the Androgen Excess Society, and to evaluate whether sex hormone-binding globulin (SHBG) can be used as a predictive marker of IR in hyperandrogenic women.

Methods: Clinical, metabolic, and endocrine parameters were measured, and an oral glucose tolerance test was carried out. The women were classified as IR group or non-IR group, in accordance with defined cutoff points for the homeostatic model assessment of IR (HOMA-IR) at ≥ 2.5, the quantitative insulin sensitivity check index at ≤ 0.33, and the Matsuda insulin sensitivity index (ISI) at ≤ 5.

Results: The women classified as having IR had a significantly higher body mass index (BMI) and free androgen index (FAI) and showed significantly lower SHBG and high-density lipoprotein (HDL) levels, regardless of the indices used. However, with the Matsuda ISI, generally more women were diagnosed as having IR, and this group had significantly higher total testosterone and triglyceride values, as well as a higher incidence of hirsutism.

Conclusions: Women who were classified as being insulin resistant using insulin sensitivity indices showed significantly higher BMI and FAI values and lower SHBG and HDL levels. However, the Matsuda ISI may be more favorable for identifying IR in hyperandrogenic women. SHBG may serve as a predictive marker of IR in these women, particularly in those who are obese.

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Introduction

The most common endocrine disorder with the clinical and biochemical features of hyperandrogenemia seen in gynecological practice is polycystic ovary syndrome (PCOS) (1). The syndrome is associated with gynecological, dermatological, and metabolic changes. It is a collection of signs and features in which no single phenomenon is diagnostically relevant on its own (2). The definition of the syndrome has continued to generate significant controversy (2, 3).

On the basis of the definition of PCOS, it is possible that completely different phenotypes of the condition exist (2, 3). Recently, the Androgen Excess Society suggested that the original National Institutes of Health criteria should be accepted with a few modifications, defining PCOS as an androgen excess syndrome or ‘hyperandrogenic syndrome’ (2).

Approximately 50–70% of women with the condition known as PCOS were described as showing hyperinsulinemic insulin resistance (IR), which may play a major pathological role in the development of the syndrome (4). IR also has a causative role in the development of the metabolic syndrome (MS) (5, 6), with severe endocrine and metabolic disturbances leading to several complications later in life, such as cardiovascular diseases (7) and type 2 diabetes (8) and resulting in higher morbidity and mortality, placing a high financial burden on healthcare costs in the 21st century (9, 10). A diagnosis of PCOS therefore implies an increased risk of these complications developing later in life.

The earliest diagnosis of IR in women may be possible at the time when hyperandrogenemia-associated symptoms first become manifest. Women with hyperandrogenic syndrome are thus an ideal group for identifying IR at an early stage and preventing its complications later in life (11, 12). There are several methods of diagnosing IR. The reference measurement procedure is the hyperinsulinemic euglycemic clamp (13), but this is a complex and time-consuming method of assessing IR (12). Several calculation models using insulin sensitivity indices have therefore been developed for diagnosing IR in clinical practice, and these may also be more useful for epidemiological studies. Despite these
calculation models, which allow easier and faster assessment, some investigators have advocated the use of sex hormone-binding globulin (SHBG) as a predictive marker of IR in women with hyperandrogenic syndrome (14). However, some groups have only described very small populations of women with PCOS (15, 16), or used the Rotterdam criteria to define PCOS (17), while other authors have described the phenomenon additionally in postmenopausal women (18) or in obese children without changes in their androgenic status (19). Recently, cutoff points for insulin sensitivity indices for the simple identification of insulin-resistant patients have been defined (20).

The aims of the present study were to evaluate IR in women with the hyperandrogenic syndrome (2), using different mathematical models and indices for assessing IR, and to evaluate whether SHBG may be able to serve as a predictive marker of IR in hyperandrogenic women.

Materials and methods

Patients

During the study period, 214 women were referred to the Division of Gynecological Endocrinology and Reproductive Medicine for the evaluation of possible androgen excess or hyperandrogenism. According to the definition of the Androgen Excess Society, PCOS is above all a disorder of androgen excess in women. As such, with currently available evidence, the diagnosis of PCOS cannot be clearly established without evidence of either clinical or biochemical hyperandrogenism (2), so the inclusion criteria were:

1. Androgen excess and/or
2. Clinical hyperandrogenism (e.g. hirsutism, acne, seborrhea, and/or menstrual dysfunction, e.g. oligomenorrhea or amenorrhea in the absence of other endocrine abnormalities also affecting ovulatory function, such as hyperprolactinemia, functional hypothalamic amenorrhea, or thyroid dysfunction).

Androgen excess was defined by the laboratory as total testosterone (TT) > 2.08 nmol/l or DHEAS > 6.6 µmol/l. Ovulatory and menstrual dysfunction was defined as oligomenorrhea in women with menstrual bleeding intervals longer than 35 days and as amenorrhea in women with absence of any bleeding within the last 6 months (2, 21). Polycystic ovarian morphology on ultrasound was not used to enroll the women into the study.

The criteria for exclusion were 21-hydroxylase-deficient nonclassical adrenal hyperplasia (NCAH), hyperandrogenic insulin-resistance acanthosis nigricans (HAIRAN) syndrome, or an androgen-secreting neoplasm (ASN). Women who had been receiving hormonal therapy, including oral contraceptive pills or steroid medications, within 3 months of their initial visit were not included. The study was approved by the local ethics committee. All the patients provided written informed consent and completed a standard medical history questionnaire, with an emphasis on menstrual dates and regularity, hirsutism, acne, gynecological history, history of infertility, medications, and family medical history.

Procedures

All the women underwent a complete screening panel, including physical examination, weight and height measurement, ultrasound examination of the ovaries, and calculation of the body mass index (BMI). The interval between menstrual periods was assessed. Women with amenorrhea within the previous year were categorized as anovulatory without further testing, and blood was taken for hormonal analysis immediately. In women with menstrual cycles, serum was obtained between days 3 and 5 of their menstrual cycle. Women with cycles of less than 26 days were not included in the study.

Calculation of IR

All the patients were on an unrestricted diet. An oral glucose tolerance test (75 g) was carried out, with glucose (mg/dl) and insulin (µU/ml) measured at 0, 60, and 120 min. After the glucose and insulin levels had been measured, the following mathematical models were used to assess IR:

The homeostatic model assessment of IR (HOMA-IR) was calculated using the following formula (22):

$$\text{Fasting glucose (FG, mmol/l)} \times \text{fasting insulin (FI, µU/ml)}$$

$$22.5$$

The HOMA of β-cell function (HOMA-B) was calculated using the following formula (22):

$$20 \times \text{FI (µU/ml)} \div (\text{FG (mmol/l)} - 3.5)$$

The quantitative insulin sensitivity check index (QUICKI) was calculated using the following formula (23):

$$\frac{1}{\log(\text{FI}) + \log(\text{FG})}$$

The insulin sensitivity index (ISI) was calculated using the following formula (24):

$$\frac{1}{\log(\text{FI})}$$
where $G$ = mean plasma glucose concentration and $I$ = mean insulin concentration during oral glucose tolerance test (OGTT).

Women were classified as being insulin resistant (IR group) or not insulin resistant (non-IR group) in accordance with defined cutoff points for HOMA-IR $\geq 2.5$ (25, 26), QUICKI $\leq 0.33$ (26, 27), and ISI $\leq 5$ (20, 24).

**Exclusion of related disorders**

For evaluation of an ASN, the TT cutoff value used was above 7 nmol/l, at which point computed tomography of the adrenal gland is normally carried out at our institution to exclude an ASN. To exclude 21-hydroxylase deficiency NCAH, the 17-HP level was greater than 30 nmol/l, the woman was considered to have 21-hydroxylase deficiency NCAH.

**Biochemical measurements**

All the assays were carried out in our routine diagnostic endocrinology laboratory, using established commercial assays routinely monitored by participation in external quality control programs. All samples were obtained between 0800 and 1000 h (30).

TT, DHEAS, and SHBG were measured with chemiluminescent enzyme immunoassays (Immulet 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). The calibration range of the TT assay was 0.7–55 nmol/l, with an analytical sensitivity of 0.5 nmol/l. The cross-reaction with 5-α-dihydrotestosterone was 2%. The calibration range of the DHEAS assay was 0.41–27 μmol/l, with an analytical sensitivity of 0.08 μmol/l. No cross-reactivity with other compounds was known. The calibration range of the SHBG assay was up to 180 nmol/l, with an analytical sensitivity of 0.02 nmol/l. The inter-assay and intra-assay coefficients of variation (CV) were always below 11% at mid-range concentrations. No cross-reactivity with other compounds is known.

Estradiol was measured using a solid-phase competitive chemiluminescent enzyme immunoassay (Immulet 2000, Diagnostic Products Corporation). The calibration range of the assay was 73–7342 pmol/l with an analytical sensitivity of 55 pmol/l. The intra-assay CV values were 9.9, 7.8, and 4.3% at the levels of 327, 660, and 1692 pmol/l respectively. The corresponding inter-assay CV values were 16, 11, and 6.7%. The cross-reactivity with 17β-estradiol valerate was 1.14%.

Prolactin (PRL) was measured using an immunometric assay (Immulet 2000, Diagnostic Products Corporation). The calibration range of the assay was up to 3180 mIU/l, with an analytical sensitivity of 3.4 mIU/l. The intra-assay CV values were 2.8, 3.6, and 2.3% at the levels of 186.6, 402.6, and 466.6 mIU/l. The corresponding inter-assay CV values were 8.2, 7.4, and 5.9%. No cross-reactivity with other compounds is known.

Luteinizing hormone (LH) was measured with an immunometric assay (Immulet 2000, Diagnostic Products Corporation). The calibration range of the assay was up to 200 mIU/ml, with an analytical sensitivity of 0.05 mIU/ml. The intra-assay CV values were 3.04, 3.71, and 3.6% at the levels of 1.04, 1.89, and 8.7 mIU/ml. The corresponding inter-assay CV values were 6.6, 6.2, and 6.7%. The cross-reactivity with human chorionic gonadotropin was 0.20%.

Follicle-stimulating hormone (FSH) was measured with an immunometric assay (Immulet 2000, Diagnostic Products Corporation). The calibration range of the assay was up to 170 mIU/ml, with an analytical sensitivity of 0.1 mIU/ml. The intra-assay CV values were 2.5, 2.9, and 2.1% at the levels of 4, 9.1, and 40 mIU/ml. The corresponding inter-assay CV values were 6.3, 5.5, and 4.3%. The cross-reactivity with thyroid-stimulating hormone was 0.01%.

Plasma insulin was determined using a solid-phase two-site chemiluminescent immunometric assay (Immulet 2000, Diagnostic Products Corporation). The calibration range of the assay was up to 300 μIU/ml, with an analytical sensitivity of 2 μIU/ml. The intra-assay CV values were 5.5, 4.0, 3.3, 3.9, 3.8, and 3.7% at the levels of 7.67, 12.5, 17.2, 26.4, 100, and 291 μIU/ml. The corresponding inter-assay CV values were 7.3, 4.9, 4.1, 5.0, 4.2, and 5.3%. The cross-reactivity with proinsulin was 8%. The plasma concentration was measured with the glucose oxidase method, using an automatic biochemical analyzer (Immulet 2000, Diagnostic Products Corporation). Total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG) were regularly measured after an overnight fasting period of 12 h, using routine clinical chemistry methods, and documented.

**Calculation of the free androgen index (FAI)**

The FAI was calculated as the quotient 100 × TT/SHBG (31).

**Statistical analysis**

All the data are presented as means ± s.d. unless otherwise stated. We employed nonparametric statistical
tests, which are based on ranks of observations and require no assumptions about the underlying distribution of data. All hypothesis tests were two sided and conducted at the 0.05 significance level. Two-sample Wilcoxon tests (i.e., Wilcoxon rank-sum tests) were used to compare parameters between women in the IR group and women in the non-IR group. We explored the association of SHBG with HOMA-IR, QUICKI, and Matsuda ISI using simple Spearman’s rank-order correlation procedures; the resulting estimates of rho are reported as $r_s$ values. In addition, it was assessed whether clinical manifestations of hyperandrogenemia (hirsutism, acne, and ultrasound evidence of polycystic ovaries) were more frequently found in the IR group or non-IR group using the $\chi^2$-test of independence. All the statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS, version 13.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

A total of 214 women with clinical or biochemical signs of hyperandrogenemia presented for the evaluation of possible androgen excess or hyperandrogenism at the Division of Gynecological Endocrinology and Reproductive Medicine in Erlangen University Hospital between January 2005 and December 2006. Nineteen women were not included, as they had been receiving hormonal treatment within 3 months of their initial visit. Eight women had hyperprolactinemic oligo-/anovulation, and two women showed evidence of 21-OH-deficient NCAH. All the women included in the study had at least one raised androgen level. No ovarian tumors were identified using ultrasonography in any of the women, and none of them had HAIRAN syndrome. The study population thus consisted of 185 women with a hyperandrogenic syndrome.

Comparison of women in the IR group and non-IR group

The anthropometric and metabolic parameters, steroid hormone levels, SHBG levels, and lipid parameters in the IR group and non-IR group using the different mathematical models and indices for assessing IR, and to evaluate whether SHBG might be able to serve as a predictive marker of IR in hyperandrogenic women. In this study, hyperandrogenic women

HOMA-IR

A total of 71 women were classified as having IR in accordance with a HOMA-IR value of $\geq 2.5$. There were no significant differences between the groups with regard to age or levels of LH, FSH, PRL, estradiol (E), TT, DHEAS, TC, TG, or LDL. Women classified as having IR had significantly higher values for BMI, FG, G-1h, G-2h, FI, I-1h, I-2h, and HOMA-B, and significantly lower values for the glucose infusion rate (GIR), QUICKI, and Matsuda ISI. Women classified as having IR had significantly lower SHBG and HDL values and higher FAI values. No differences were found between the women in the two groups with regard to possible clinical manifestations of hyperandrogenemia.

QUICKI

A total of 74 women were classified as having IR in accordance with a QUICKI value of $\leq 0.33$. There were no significant differences in age, LH, FSH, PRL, E, TT, DHEAS, TC, TG, or LDL between the two groups. Women classified as having IR had significantly higher values for BMI, FG, G-1h, G-2h, FI, I-1h, I-2h, HOMA IR, and HOMA B, and significantly lower values for GIR and Matsuda ISI. Women classified as having IR had significantly lower SHBG and HDL and higher FAI values. No differences were found between the women in the two groups with regard to possible clinical manifestations of hyperandrogenemia.

ISI

A total of 91 women were classified as having IR in accordance with an ISI value of $\leq 5$. There were no significant differences in age, LH, FSH, PRL, E, DHEAS, TC, or LDL between the two groups. Women classified as having IR had significantly higher values for BMI, FG, G-1h, G-2h, FI, I-1h, I-2h, HOMA IR, and HOMA B, and significantly lower values for GIR and QUICKI. Women classified as having IR had significantly lower SHBG and HDL and higher FAI, TT, and TG values. Women in the IR group had hirsutism significantly more often.

Spearman’s rank-order correlations

There was a negative correlation between SHBG and HOMA-IR, with estimated rho ($r_s$) values of $-0.491$ for all the women (Fig. 1), $-0.324$ for women in the IR group, and $-0.309$ for women in the non-IR group. There was a positive correlation between SHBG and QUICKI, with estimated rho ($r_s$) values of $0.423$ for all the women (Fig. 2), $0.352$ for women in the IR group, and $0.310$ for women in the non-IR group. There was a positive correlation between SHBG and Matsuda ISI, with estimated rho ($r_s$) values of $0.539$ for all the women (Fig. 3), $0.413$ for women in the IR group, and $0.285$ for women in the non-IR group (Table 2).

Discussion

The study was conducted in order to assess IR among women with the hyperandrogenic syndrome (2), using different mathematical models and indices for assessing IR, and to evaluate whether SHBG might be able to serve as a predictive marker of IR in hyperandrogenic women. In this study, hyperandrogenic women
who were identified as having IR were found to have significantly increased BMI levels, and fasting and stimulated glucose and insulin levels, while SHBG and HDL were significantly decreased, regardless of the insulin sensitivity indices used. However, the Matsuda ISI method identified more women as being insulin resistant, and hirsutism was more evident as a major clinical symptom of androgen excess. In general, there were few or no differences between the two groups with regard to acne, hirsutism, and the ultrasound appearance of the ovaries.

The urgent need for a simple method of measuring IR has led to the development of a large number of insulin sensitivity indices, which have been reviewed elsewhere.
Recently, reference values have also been established for the Matsuda ISI to distinguish between insulin-resistant and insulin-sensitive patients (20). However, it may be difficult to use the cutoff points for the selected indices, and it should be noted that these indices are not absolutely strict for the diagnosis of impaired carbohydrate metabolism, although they may help identify women who are at the highest risk of developing diabetes (20). Diagnosing IR in women with hyperandrogenic syndrome or androgen excess may help identify endocrine and metabolic risk factors and reveal individuals suitable for targeted treatment with insulin sensitizers, to improve therapeutic approaches and prevent complications later in life (11, 12).

SHBG is the main serum transporter of testosterone, while insulin is one of the main modulators of serum SHBG levels (35). The increased concentrations of insulin and androgens suppress SHBG and insulin-like growth factor-binding protein production in the liver (36). This process is enhanced in patients with raised BMI levels (37). A reduced SHBG level is thus not only a possible marker for hyperandrogenism, but has also been postulated as a predictive marker of IR (38, 39). Some studies have also suggested using SHBG as a marker of IR in women with PCOS, but these only included small groups of patients (14, 15); another study reported no advantage in using SHBG as a predictive marker of IR in 21 women with PCOS, in comparison with 17 control individuals (40). Dahan & Goldstein conclude that there is significant variability and overlap in SHBG levels among lean insulin-sensitive control individuals and patients with IR, and despite the statistically significant correlation coefficient, the relationship between the groups was weak. This was explained by the multifactorial regulation of the SHBG concentration and its genetic variability (40).

Some studies have emphasized the influence of genetics on circulating SHBG levels. Recently, it was postulated that polymorphisms within the coding sequence and in the regulatory sequence of the SHBG gene were associated with circulating SHBG levels, which may be a possible genetic background of sex hormone activity in humans (41, 42). On the other hand, Bendlova et al. reported no significant differences in the genotype distribution between PCOS and controls and no association with plasma SHBG levels (43). Nevertheless, there may be a genetic determination of low SHBG levels contributing to an increase in biologically active androgen levels during fetal, pubertal, and later in life, resulting in different phenotypes of androgen excess (42).

Ducluzeau et al. (44) reported that the GIR is a better marker for assessing IR than SHBG levels in nonobese women with PCOS. The authors also reported that low SHBG levels in PCOS are associated with the BMI, suggesting that certain signals from adipose tissue may regulate the hepatic production of SHBG. It has been reported that SHBG may constitute an index of IR only in the hyperinsulinemic state (45). These results are similar to those of the present study: Spearman’s rank correlations for SHBG versus the insulin sensitivity indices used were lowest in the non-IR group and reached a lower level of significance both in comparison with the IR group and also when all women were taken into account. It should be noted that women in the non-IR group had significantly lower BMI values. In general, the results using the HOMA-IR and QUICKI indices were similar. It was only using the Matsuda ISI method that more women were identified as being insulin resistant, and hirsutism as a major clinical symptom of androgen excess was more evident in this group. In addition, women identified as having IR using the Matsuda ISI had significantly higher testosterone levels in comparison with non-IR women.

It has been postulated that insulin sensitivity indices using values obtained during an OGTT may provide greater objectivity in assessing IR than indices calculated from the fasting glucose and insulin values alone (20, 46, 47). It has also been reported that the Matsuda ISI can best predict the risk of IR, and that it provides the best relative sensitivity and specificity rates in comparison with HOMA-IR (20, 46).

SHBG also shares many features with HDL; both are reduced in women with PCOS (48). It has been hypothesized that SHBG affects atherogenesis both directly and indirectly, through the lipoprotein...
metabolism or by affecting the estradiol–testosterone balance (49). In the present study, women identified as having IR had significantly lower HDL concentrations, regardless of the calculation model used. The TG concentration was also higher in women in the IR group, but the difference was only significant with the Matsuda ISI test. Chen et al. conclude that the coexistence of low SHBG and reduced HDL levels is associated with the occurrence of MS, and that it may increase the risk of cardiovascular disease in women with PCOS (17). It was also postulated that in women with type 2 diabetes, androgen excess may contribute to greater burden of coronary heart disease risk factors (50).

Since SHBG binds testosterone, a decrease in SHBG results in a more pronounced increase in circulating free androgens, independently of albumin levels (51, 52). In the revised 2003 consensus, it was postulated that calculating free testosterone from measurements of SHBG and TT and calculating the FAI are more sensitive methods of assessing hyperandrogenemia in women with PCOS (53). Whether they meet the Rotterdam criteria or not, hirsute women have significantly increased calculated free and bioavailable testosterone levels in comparison with nonhirsute hyperandrogenic control individuals (54). However, this diagnostic advantage for calculated free and bioavailable testosterone levels was not apparent in PCOS women in comparison with hirsute controls (55). These phenomena support the hypothesis that the definition of PCOS in the revised 2003 Rotterdam criteria identifies a different subset of women who have different endocrine profiles, whether they are hyperandrogenic or not. We recently reported that the BMI is associated with significantly lower SHBG levels in women with hirsutism and PCOS, resulting in significantly increased calculated free testosterone and FAI levels, which were appropriate markers for assessing hyperandrogenemia in these women (54, 56).

This study investigated the endocrine and metabolic changes in women with hyperandrogenic syndrome who showed evidence of clinical or biochemical hyperandrogenism. Furthermore, most women met also the Rotterdam criteria for PCOS. Therefore, these findings are not contradictory to the conclusion that PCOS should be understood as a disorder of androgen excess or hyperandrogenism considering the possibility of the different phenotypes.

In summary, despite the controversy over the definition, this study shows that women with hyperandrogenic syndrome who are classified as being insulin resistant using insulin sensitivity indices have significantly higher BMI levels and lower HDL and SHBG levels, resulting in higher FAI values. There appears to be no obvious association with the clinical manifestations of hyperandrogenemia, such as hirsutism, acne, and ultrasound evidence of polycystic ovaries. In addition, the Matsuda ISI may be preferable for identifying IR, and SHBG may serve as predictive marker of IR in all hyperandrogenic women with a better correlation in obese women.

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