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ORIGINAL ARTICLE *Reproductive endocrinology*

Thyroid-stimulating hormone is associated with insulin resistance independently of body mass index and age in women with polycystic ovary syndrome

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BACKGROUND: The aim of this study was to evaluate the association between thyroid function, reflected by thyroid-stimulating hormone (TSH) levels, and insulin resistance (IR) in 337 women suffering from polycystic ovary syndrome (PCOS).

METHODS: Clinical, metabolic and endocrine parameters were obtained and an oral glucose tolerance test was performed, with calculation of IR indices. The association between thyroid function and IR was evaluated with classification analysis using logistic regression and 10-fold cross-validation to identify a possible TSH threshold for IR. Parameters were then compared between women above and below the TSH threshold using two-sample tests. One-way analyses of covariance were performed to explore whether the impact of TSH on IR is independent of other variables.

RESULTS: A TSH cut-off value around 2 mIU/l had the best sensitivity and specificity for identifying women with IR. Women with TSH $\geq$ 2 mIU/l were younger, had a higher body mass index (BMI) and were more insulin-resistant compared with women with TSH $<$ 2 mIU/l. This effect of TSH on IR was independent of age and BMI.

CONCLUSIONS: In women with PCOS, a significant association between thyroid function, as reflected by TSH $\geq$ 2 mIU/l, and IR was found and the association appeared to be independent of age and BMI.

Key words: thyroid-stimulating hormone / insulin resistance / hyperandrogenic syndrome / PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most frequent androgen excess disorder in women and has been defined by the Androgen Excess Society (AES) as a predominantly hyperandrogenic syndrome (Azziz *et al*., 2006). Women with PCOS are at risk for chronic anovulation, infertility and early pregnancy loss. In addition, many are obese, and the numbers of obese individuals among patients with PCOS are increasing (Yildiz *et al*., 2008). In women with PCOS, approximately 50–70% has been reported to have hyperinsulinaemic insulin resistance (IR) (Dunaif, 1997) and metabolic syndrome (MS), which increase the risk for type 2 diabetes mellitus and cardiovascular disease (Dunaif, 1997; Azziz *et al*., 2004). Several groups have reported that women with PCOS are more insulin-resistant than would be expected on the basis of their age and body mass index (BMI) (Barber *et al*., 2006). The mechanisms underlying this phenomenon are not fully understood. Although hyperandrogenaemia may play a role (Moghetti *et al*., 1996; Möhlig *et al*., 2006), other factors such as thyroid function may also be involved.

In hypothyroidism, glucose uptake in muscle and adipose tissue is resistant to insulin, resulting in higher levels of insulin in these patients (Dimitriadis *et al*., 2006). Whereas these changes are well recognized

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in patients with hypothyroidism, the evidence regarding such a relationship in subclinical hypothyroidism (SCH) is inconsistent (Canaris *et al*., 2000; Bakker *et al*., 2001). [SCH is defined as elevated serum TSH in association with normal thyroid hormones (Gharib *et al*., 2005).] However, the results reported by Maratou *et al*. (2009) have recently supported this hypothesis.

In addition, women with PCOS have a high prevalence of increased thyroid-stimulating hormone (TSH) levels (Janssen *et al*., 2004), which may additionally contribute to their phenotype (Poppe and Glinoer, 2003; Poppe *et al*., 2007). An association between TSH and fasting insulin levels and insulin sensitivity has been previously reported (Chubb *et al*., 2005; Michalaki *et al*., 2006), as has a higher prevalence of SCH in women with MS (Uzunlulu *et al*., 2007). The diagnosis of SCH critically depends, of course, on the definition of the upper limit of 'normal' for TSH in serum. Two studies have proposed that there should be a TSH cut-off point at 2.0–2.5 mIU/l, in contrast to the conventionally used levels of 4–5 mIU/l (Baloch *et al*., 2003; Völzke *et al*., 2003). TSH has also been described as the most sensitive test for detecting minor degrees of primary thyroid dysfunction (Larson *et al*., 2000; Demers and Spencer, 2003).

In women with PCOS, who frequently have IR and MS, SCH developing in addition may aggravate IR and other risk factors. Women with PCOS might therefore be candidates for screening in order to identify disturbances in thyroid function and changes in metabolic parameters, as well as IR indices. The aim of this study was therefore to evaluate whether thyroid function, as reflected by TSH levels, may be associated with IR in women with PCOS. The intention was to identify a possible cut-off point for TSH at which an association between thyroid function and IR in the women studied persists, and to clarify whether the association between thyroid function and IR is independent of other parameters that are known to influence IR, such as age and BMI.

Patients and Methods

Patients

The study was performed from January 2005 to December 2007. All of the women included had to meet the definition of PCOS in accordance with the Rotterdam criteria, reflecting the potential phenotypes A–I (with exception of phenotype J; these women were not included) as described by the AES (Zawadzki and Dunaif, 1992; Rotterdam, 2004; Azziz *et al*., 2006). However, the Rotterdam criteria have been modified in some points, as recently suggested in detail by Geisthövel and Rabe (2007).

The exclusion criteria were: 21-hydroxylase-deficient non-classical adrenal hyperplasia; hyperandrogenism, insulin resistance and acanthosis nigricans syndrome; an androgen-secreting neoplasm; elevated levels of prolactin; Cushing syndrome; any history of manifest hypothyroidism or hyperthyroidism or any history of thyroid surgery; or use of thyroid hormone or iodine medication. Women who had been receiving hormonal therapy, including oral contraceptive pills or steroid medications, within 6 months of their initial visit were also excluded. The study was approved by the local institutional review board. All of the patients provided written informed consent and completed a standard medical history questionnaire with an emphasis on menstrual dating and regularity, hirsutism, acne, gynaecological history, history of infertility, medications and family history.

Procedures and definitions

All of the women underwent a complete screening panel, including physical examination, weight and height measurement and ultrasound examination of the ovaries, and their BMI was calculated.

Hirsutism

Hirsutism was scored in accordance with the modified Ferriman-Gallwey (mF-G) score, and an mF-G score $\geq$6 was classified as hirsutism (Archer and Chang, 2004; Souter *et al*., 2004; Azziz *et al*., 2006).

Hyperandrogenaemia

Measuring serum total testosterone (TT) as the only marker for biochemical hyperandrogenism in women is challenging (Vermeulen *et al*., 1999; Azziz *et al*., 2006; Rosner *et al*., 2007). Therefore the free androgen index (FAI) was used additionally (see detailed description below). Hyperandrogenaemia was defined as TT $\geq$ 1.90 nmol/l and FAI $\geq$ 4.0 (Cupisti *et al*., 2007a; Mueller *et al*., 2008).

Assessment of ovulation status

The interval between bleeding episodes was assessed. The major clinical signs, oligomenorrhoea and amenorrhoea, vary in duration, but are generally unambiguous (Geisthövel and Rabe, 2007; Norman *et al*., 2007). Women with regular bleeding episodes between 26 and 35 days were categorized as eumenorrhoeic although women with cycles longer than 35 days were categorized as oligomenorrhoeic. In these women, serum was obtained between Days 3 and 5 of the cycle. In women who had amenorrhoea within the previous 6 months, blood was taken for hormonal analysis at random (Mueller *et al*., 2006a, b, c, 2007).

Polycystic ovaries

Polycystic ovaries were diagnosed on ultrasound when there were 12 or more follicles measuring 2–9 mm in maximum diameter (Rotterdam, 2004).

Calculation of IR

All of the patients were on an unrestricted diet. After an overnight fasting period of 12 h, glucose (mg/dl) and insulin (μIU/ml) levels were measured and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used to assess IR, as previously described in detail (Matthews *et al*., 1985; Kajaia *et al*., 2007). Defined cut-off points were used at HOMA-IR $\geq$ 2.5 (Ascaso *et al*., 2003; Yilmaz *et al*., 2005).

Exclusion of related disorders

The method used to exclude related disorders has been described in detail elsewhere (Cupisti *et al*., 2007b). Briefly, for evaluation of an androgen-secreting neoplasm, the TT cut-off value used was above 7 nmol/l, at which point computed tomography of the adrenal gland is normally carried out to exclude an androgen-secreting neoplasm. To exclude 21-hydroxylase deficiency in patients with a 17-hydroxyprogesterone (17-OHP) level above 6 nmol/l, 17-OHP levels stimulated by adrenocorticotrophic hormone were measured. If the stimulated 17-OHP levels were $>$30 nmol/l the women were considered to have 21-hydroxylase deficiency and further genotyping of CYP 21 was initiated (Azziz *et al*., 2004, Mueller *et al*., 2006c, 2007).

Biochemical measurements

All of the assays were carried out in a routine diagnostic endocrine laboratory using established commercial assays routinely monitored by participation in external quality-control programs. All samples were obtained between 0800 and 1000 hours.

TT, dehydroepiandrosterone sulfate, sex hormone–binding globulin (SHBG), estradiol, prolactin, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and insulin were measured with chemiluminescent enzyme immunoassays (Immulite 2000, Siemens Medical Solutions Diagnostics Ltd, Bad Nauheim, Germany), as described in detail previously (Mueller *et al*., 2006a, b, c, 2007; Cupisti *et al*., 2007a, b, 2008; Kajaia *et al*., 2007).

TSH was determined using an immunoradiometric assay (Brahms, Henningsdorf, Germany). The calibration range of the assay was up to 16 mIU/l, with an analytical sensitivity of 0.02 mIU/l with a reference range from 0.3 to 4.5 mIU/l. The intra-assay coefficients of variation (CVs) were 5.1, 2.5 and 1.5% at the levels of 0.16, 1.48 and 16.6 mIU/l. The corresponding inter-assay CVs were 13.6, 4.1 and 2.3%. The cross-reactivity with FSH and LH was $<0.1\%$.

Free triiodothyronine was measured using the solid-phase antigen radioimmunometric technique (SPART; Brahms, Henningsdorf, Germany). The analytical sensitivity of the assay was 0.7 pmol/l with a reference range from 3.50 to 8.10 pmol/l. The intra-assay CVs were 5.16, 5.30 and 6.45% at the levels of 4.07, 6.79 and 17.2 pmol/l, respectively. The inter-assay CVs were 7.9, 9.5 and 9.2% at the levels of 3.4, 9.56 and 15.9 pmol/l, respectively. The cross-reactivity with L- and D-thyroxine was 0.15 and 0.07%.

Free thyroxine was also measured using SPART (Brahms, Henningsdorf, Germany). The analytical sensitivity of the assay was 1.25 pmol/l with a reference range from 10 to 25 pmol/l. The intra-assay CVs were 5.5, 3.4 and 3.6% at the levels of 11, 20.6 and 101.5 pmol/L, respectively. The inter-assay CVs were 8.7, 5.1 and 3.0% at the levels of 8.0, 17.6 and 51 pmol/L, respectively. The cross-reactivity with triiodo-L-thyronine was 0.6%.

Total cholesterol (reference range from 92 to 234 mg/dl), low-density lipoprotein (LDL) (reference range from 45 to 300 mg/dl), high-density lipoprotein (HDL) (reference range from 25 to 90 mg/dl), and triglycerides (TG) (reference range from 100 to 400 mg/dl) were regularly measured after an overnight fasting period of 12 h, using routine clinical chemistry methods, and documented. The intra-assay CVs and inter-assay CVs were always below 4% at mid range concentrations for all lipid parameters. Calculation of the FAI: the FAI was calculated as the quotient $100 \times$ TT/SHBG (Mathur *et al*., 1981).

Statistical analysis

All of the data are presented as medians and 95% central range. In order to identify a cut-off value for TSH at which TSH is associated with IR, a classification analysis with logistic regression as classifier and 10-fold cross-validation was carried out for different cut-off points of TSH from 1.5 to 3.5 in 0.1 steps. In accordance with the results of the classification analysis, two-sample tests were performed to compare several parameters between women with TSH <2.0 mIU/l and women with TSH ≥ 2.0 mIU/l. The *t*-test was used for normally distributed data after log transformation, and the Wilcoxon rank-sum test was used for non-normally distributed data. The *P*-values for these multiple tests were adjusted using the Bonferroni–Holm method. To explore whether the impact of TSH on IR is independent of other variables, one-way analyses of covariance (ANCOVA) with one significant variable each as a continuous variable and HOMA-IR as a response variable were performed. All of the tests were two-sided, and a *P*-value <0.05 was considered statistically significant. All statistical analyses were carried out using the R system for statistical computing (version 2.8.0; R Development Core Team, Vienna, Austria; 2008).

Results

A total of 337 women who presented at our outpatient clinic for evaluation of possible hyperandrogenism fulfilled the inclusion criteria. Complete datasets for their endocrine and metabolic parameters were available for analysis. The women had elevated androgenic parameters and were on average overweight.

Classification analysis: TSH ≥ 2 mIU/l is associated with IR

The association of TSH and IR using HOMA-IR is shown in Fig. 1. The scatter plot of HOMA-IR versus TSH shows a high frequency of women with TSH < 2 mIU/l and low HOMA-IR values, whereas women with higher TSH levels tend to also reveal higher HOMA-IR values. To explore the impact of TSH on IR, a classification analysis based on logistic regression was carried out for various TSH thresholds. The analysis was performed for different TSH cut-off points from 1.5 to 3.5 in steps of 0.1 and different parameters that directly describe IR (HOMA-IR), or parameters that are associated with IR (i.e. BMI and age), or parameters that are not associated with IR but are important for the syndrome. The results are shown in Fig. 2. The calculation was also performed with all other parameters that are not correlated with IR, without improving the analysis (data not shown). In this process, sensitivity, specificity and the Youden index were calculated for the above-mentioned TSH thresholds. The Youden index describes the performance of the analyses and is defined as Youden index = sensitivity + specificity − 1; and can vary from −1 to +1. A test with a perfect accuracy would have a Youden Index of +1. The classification analysis shows how well TSH values are associated with IR (Fig. 2). TSH thresholds around 2 mIU/l had the best sensitivity and specificity for identifying women with IR. The use of other parameters did not result in any improvement in the analysis (data not shown).

Comparison of hormonal and metabolic parameters in accordance with the threshold of the classification analysis

A total of 154 women had TSH ≥ 2.0 mIU/l and 183 women had TSH < 2.0 mIU/l. The biochemical characteristics of the women were compared and are shown in Table I. Women with TSH ≥ 2.0 mIU/l had significantly increased BMI and HOMA-IR values and lower age in comparison with women with TSH < 2.0 mIU/l. After ANCOVAs had been performed, the significant difference for HOMA-IR between the two groups of women was confirmed as being independent of BMI and age ($P < 0.01$ and $P < 0.0001$, respectively).

Discussion

So far as we are aware, this is the first study that has aimed to evaluate whether thyroid function, as reflected by TSH levels, is associated with IR in women with PCOS. We identified an association between TSH ≥ 2 mIU/l and increasing IR in women with PCOS, an aspect that has not previously been evaluated. Thyroid dysfunction is common in women of reproductive age, with a prevalence of elevated TSH ranging from 4 to 9% in this population (Canaris *et al*., 2000; Hollowell *et al*., 2002).

There has recently been increasing interest in the relationship between thyroid function and weight and metabolic status. Thyroid function is also related to weight status, as reported by Reinehr and

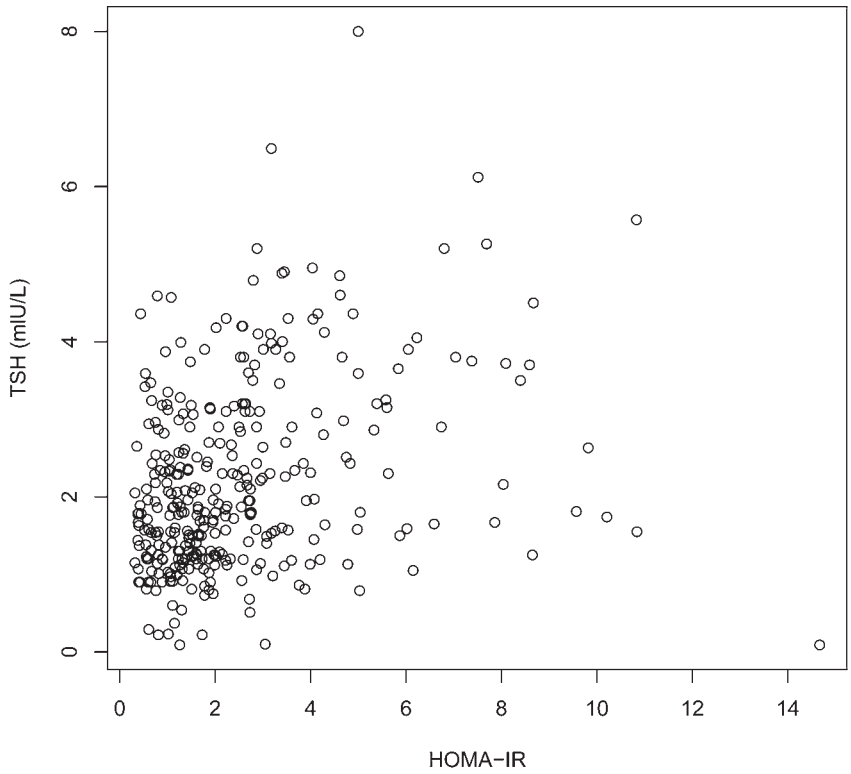

Figure 1 The scatter plot of the HOMA-IR versus TSH shows a high frequency of women with TSH < 2 mIU/L and low HOMA-IR values, whereas women with higher TSH levels tend to higher HOMA-IR values.

Andler (2002), with increased TSH levels in obese women. The increase in TSH showed a tendency to normalize again with weight loss (Reinehr *et al*., 2006, 2008). In addition, there is a close correlation between obesity and the degree of IR in women with PCOS, and obesity itself has been reported to be a risk factor for the development of IR in these women (Nestler, 2000; Gambineri *et al*., 2002; Barber *et al*., 2006).

In agreement with the results reported by Reinehr and Andler (2002), increased BMI was also found in the women with elevated TSH levels ≥2mIU/l in the present study. However, waists to hip ratios were not measured in the women studied. This is one of the possible limitations of the study. Furthermore, a methodical lack of this study is that no control group of non-PCOS women was evaluated. In order to clarify whether the relationship between TSH and IR is independent of age and BMI, an analysis of covariance was performed, and the association between TSH and IR was found to be independent of age and BMI. It may therefore be speculated that in some women with PCOS, IR may be aggravated by disturbed thyroid function, as reflected by elevated TSH levels. In agreement with this finding, it has also been postulated that insulin and IR may be related to TSH, with leptin as a possible key factor (Ortiga-Carvalho *et al*., 2002; Reinehr and Andler, 2002; Chubb *et al*., 2005). However, leptin levels were not investigated in the present study. One minor limitation of the study is that progesterone values are missing. Changes in thyroid function are known to influence SHBG levels and may therefore also cause indirect changes in the estrogene/progesterone ratio. On the other hand due to missing progesterone values, we were not able to identify amenorrhoeic women in their luteal phase. Endocrine investigations should be performed under stable conditions with hormonal analysis in the early follicle phase. However, this limitation may affect only a minority of the women studied. Furthermore, autoimmune thyroid disease is the most common cause of hypothyroidism in women at this age (Poppe *et al*., 2007). We have not evaluated the role of autoimmune thyroid disease because thyroid function itself was the main objective of our study. For complete endocrine evaluation of those women with subclinical or manifest hypothyroidism, evaluation of thyroid antibodies should be implemented in the diagnostic setup. However, the therapeutic consequences may be limited, despite the possibility of L-Thyroxin supplementation.

Experimental studies have shown that in normal conditions, thyroid hormones may influence the expression or activation of uncoupling protein, β-adrenergic receptor, and peroxisome proliferator-activated receptor-gamma, all of which are involved in regulating insulin sensitivity (Frederiksen *et al*., 2002; Dallongeville *et al*., 2003; Wang *et al*., 2004). It has been reported that in obese diabetic rodents, treatment with thyroid hormone enhances insulin sensitivity and reduces hyperglycaemia and hyperinsulinaemia (Koritschoner *et al*., 2001). Thyroid hormones also co-operate with catecholamines to enhance lipolysis and reduce visceral fat mass, with resultant improvement in IR (Torrance *et al*., 1997). Thyroid hormone deficiency or mutation of deiodinase, which is the regulator of thyroid hormone

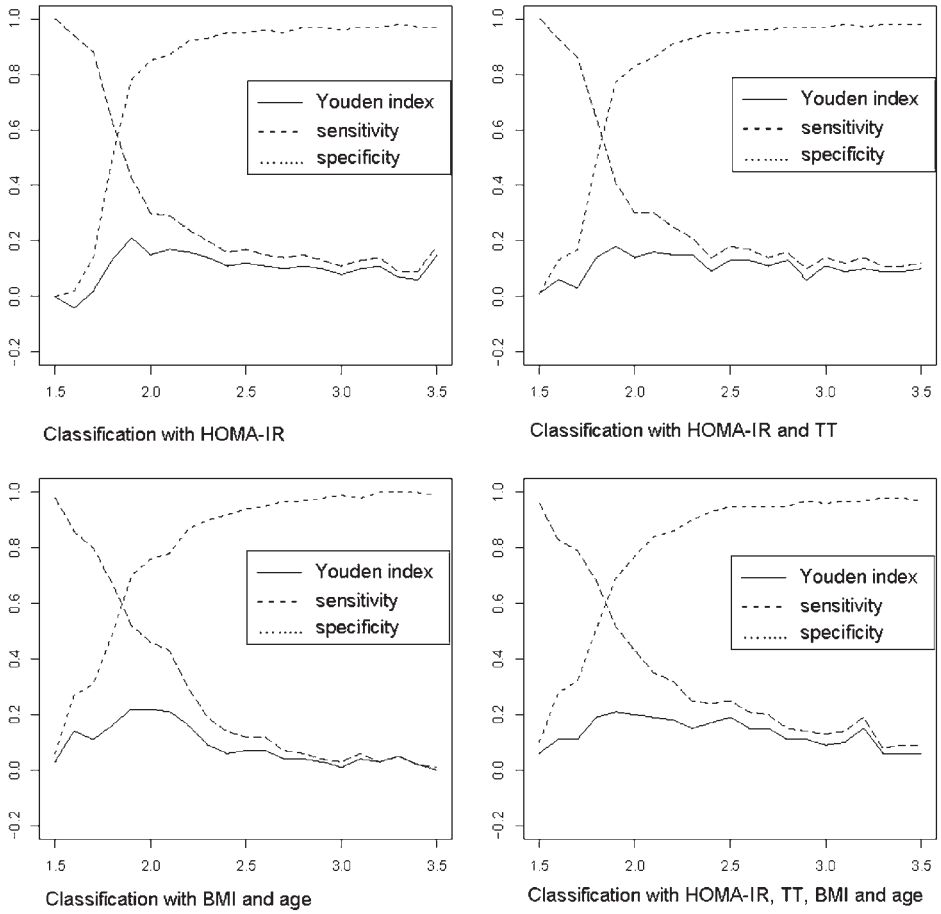

Figure 2 Classification analyses based on logistic regression with different variables and 10-fold cross-validation relative to different cut-off values for TSH (cut-off values = *x*-axis), and measures for identifying IR in the women studied.

The binary outcome of the logistic regression is defined by the cut-off value. The *y*-axis shows sensitivity, specificity and the Youden index of each classification analysis.

Table I Comparison of women with TSH < 2.0 mIU/l and with TSH ≥ 2.0 mIU/l

Parameter (unit)	TSH < 2.0 mIU/l (*n* = 183)	TSH ≥ 2.0 mIU/l (*n* = 154)	Raw *P*-value	Adjusted *P*-value
Age (year)	29.00 (17.00–42.00)	26.00 (13.82–40.17)	<0.01[a]	0.02
BMI (kg/m^2)	25.56 (17.51–46.95)	29.40 (18.35–43.47)	<0.001[a]	<0.01[a]
HOMA-IR	1.61 (0.40–8.22)	2.56 (0.54–8.43)	<0.0001[b]	<0.001[b]
TT (nmol/l)	2.11 (0.63–5.36)	2.17 (0.82–5.14)	0.36[a]	1.00[a]
SHBG (nmol/l)	37.67 (11.00–96.15)	39.50 (12.00–96.67)	0.19[a]	1.00[a]
FAI	5.56 (0.88–25.03)	5.89 (1.17–26.44)	0.95[a]	1.00[a]
Lipid parameters	(*n* = 92)	(*n* = 68)		
Total cholesterol (mg/dl)	197.50 (138.30–253.93)	199.00 (135.10–280.18)	0.69[b]	1.00[b]
LDL (mg/dl)	122.00 (69.80–185.60)	123.50 (84.65–183.15)	0.43[b]	1.00[b]
HDL (mg/dl)	56.00 (33.80–85.40)	49.00 (34.65–82.67)	<0.01[a]	0.05[a]
TG (mg/dl)	94.50 (35.23–275.88)	131.00 (52.50–423.00)	0.02[a]	0.10[a]

Data are shown as medians with 95% central range, with raw and adjusted *P*-values.

BMI, body mass index; FAI, Free Androgen Index; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model for Assessment of Insulin Resistance; LDL, low-density lipoprotein; SHBG, sex hormone–binding globulin; TG, triglycerides; TSH, thyroid-stimulating hormone; TT, total testosterone.

[a]Wilcoxon rank-sum test.

[b]*T*-test with log-transformed data.

metabolism, has also been shown to induce obesity and IR (Mentuccia *et al*., 2002; Levine *et al*., 2003).

Specific changes associated with manifest hypothyroidism are well recognized. However, the evidence for such a relationship in less severe forms of hypothyroidism such as SCH is inconsistent (Canaris *et al*., 2000; Bakker *et al*., 2001). The diagnosis of SCH is critically dependent, of course, on the definition of the upper limit of 'normal' for serum TSH and is still a matter of controversy. Some authors argue that the TSH reference interval should remain at 0.4–4.0 mIU/l, as there is at present insufficient justification for lowering the upper limit of 'normal' for TSH (Brabant *et al*., 2006). The classification analysis conducted in this study showed that a TSH threshold of 2 mIU/l was associated with the best sensitivity and specificity in the model used to identify women with IR. However, the maximum Youden index achieved here is at best a little more than 0.2. This is a relatively weak Youden index and further development of the model used is needed to explore this association.

Thyroid function, as reflected by TSH levels, is associated with IR in women with PCOS independent of age and BMI. Therefore the relation between thyroid function and IR needs to be investigated further in PCOS women and in non-PCOS women. The potential effect of thyroid medication on restoring insulin sensitivity should be limited to well-controlled trials (Surks *et al*., 2004).

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