Serum soluble glycoprotein 130 concentration is inversely related to insulin sensitivity in women with polycystic ovary syndrome

Short running title: sgp30 and insulin sensitivity

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Submitted 3 September 2009 and accepted 17 January 2010.

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Objective: Insulin resistance might play a role in the pathogenesis of polycystic ovary syndrome (PCOS). The family of glycoprotein 130 (gp130) cytokines could influence insulin action. One of these cytokines is interleukin 6 (IL-6), which exerts short-term insulin-sensitizing effect, whereas in long-term period it might induce insulin resistance. Some other gp130 activators are supposed to have beneficial metabolic effects. Gp130 is present in the circulation in soluble form (sgp130), which inhibits intracellular gp130 signaling. The aim of the present study was to estimate relation between sgp130 and insulin sensitivity in women with PCOS.

Research Design and Methods: We studied 78 women with PCOS (35 lean and 43 obese) and 34 healthy women (18 lean and 16 obese). Euglycemic hyperinsulinemic clamp and the measurements of serum sgp130, IL-6, soluble IL-6 receptor (sIL-6R) and sex hormones were performed.

Results: Both obesity and PCOS were characterized by an increased sgp130 (p<0.0001 and p=0.0002, respectively). Concentration sIL-6R was lower (p=0.0036) in women with PCOS independently of obesity. Serum sgp130 was negatively correlated with insulin sensitivity when control and PCOS women were analyzed together (r=-0.36, p<0.0001) and in the PCOS subjects separately (r=-0.34, p=0.002). In multiple regression analysis, this correlation was significant after adjustment for BMI, waist, percent of body fat, postload glucose and insulin, TG and high sensitive C-reactive protein.

Conclusions: Serum sgp130 is inversely and independently associated with insulin sensitivity in women with PCOS. An increased serum sgp130 in obesity and PCOS suggests an inhibition of intracellular gp130 signaling in insulin resistant conditions.
Poly cystic ovary syndrome (PCOS) is a common endocrine disorder with diverse and heterogeneous nature, which is present in 5-10% of reproductive age women. PCOS is characterized by hyperandrogenism and chronic anovulation (1,2). Insulin resistance is a common feature of PCOS and might be the cause of significant metabolic and cardiovascular complications, observed in this condition (3).

The family of glycoprotein 130 (gp130) cytokines might influence insulin action (4). This family includes interleukin 6 (IL-6), interleukin 11, leukemia inhibitory factor, ciliary neurotrophic factor (CNTF), oncostatin and cardioprotin-1 (5). Gp130 cytokines play a pivotal role in the regulation of the immune, hematopoietic, nervous, cardiovascular and endocrine system (5). These cytokines exert their actions through a heterodimeric or homodimeric receptor consisting of two membrane-bound glycoproteins: a cytokine binding subunit and a glycoprotein termed IL-6 transducer or IL6ST, also known as gp130, which is responsible for signal transduction (6).

IL-6 was thought to be an insulin-desensitizing factor (7). However, it might have some beneficial metabolic actions and it was hypothesized that it exerts short-term insulin-sensitizing effect, whereas in long-term period it might induce insulin resistance (8). IL-6 increased glycogen synthesis and enhanced lipid oxidation via an AMP activated protein kinase (AMPK)-dependent mechanism in skeletal muscle (9). Another gp130 cytokine, CNTF, prevented weight gain and stimulated AMPK in skeletal muscle (10). Therefore, it is supposed that gp130 activators might be useful in the treatment of obesity and insulin resistance (4). Gp130 is expressed in most cells of the body (6) and is present in the circulation in soluble form (sgp130), which inhibits intracellular gp130 signaling (11). The role of sgp130 in the pathogenesis of human insulin resistance has not been studied so far.

Soluble form of the IL-6R (sIL-6R) occurs in various body fluids and might induce gp130 dimerization and signaling when complexed with IL-6. The presence of sIL-6R leads to sensitization of IL-6-responsive cells towards the ligand (12). The IL-6/sIL-6R complex stimulates several type of cells, which only express gp130 and are unresponsive to IL-6 alone. This process is called transsignaling (13). Sgp130 exerts also inhibitory effects toward the IL-6/sIL-6R complex (14).

We hypothesized that serum sgp130 might be altered in insulin resistant conditions in humans and might be related to insulin sensitivity. To test this hypothesis, we studied serum sgp130, IL-6 and sIL-6R in relation to insulin sensitivity in lean and obese women with PCOS and BMI-matched healthy controls.

**RESEARCH DESIGN AND METHODS**

The study group consisted of 78 women with PCOS (35 lean, BMI<25kg/m$^2$ and 43 overweight or obese, BMI >25kg/m$^2$) and 34 healthy, normally menstruating women (18 lean and 16 overweight or obese). The subjects’ recruitment and diagnosis of PCOS according to Rotterdam criteria (15) was described in detail previously (16). Before entering the study, a physical examination and appropriate laboratory test were performed. A standard 75g oral glucose tolerance test (OGTT) was performed and all participants had normal glucose tolerance. None of women had morbid obesity (BMI>40 kg/m$^2$), cardiovascular disease, hypertension, infections or other serious medical problems, all were non-smokers and they were not taking any anti-inflammatory drugs and drugs known to affect glucose and lipid metabolism. Studies were performed in the PCOS group,
3-5 days after a spontaneous menses, or independent of cycle phase in the presence of amenorrhea and in regularly cycling women during the early follicular phase (3-5 day) of their menstrual cycle. All analyses were performed after an overnight fast. The study protocol was approved by Ethics Committee of Medical University of Białystok, Poland. All participant gave written informed consent before entering the study. Anthropometric measurements were performed as previously described (16).

**Insulin sensitivity:** Insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp technique as previously described (16). The rate of whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate from 80-120 min, corrected for glucose space and normalized per kilogram of fat free mass (M/ffm).

**Biochemical analyses:** Plasma glucose was measured immediately by the enzymatic method using glucose analyzer (YSI 2300 STAT Plus, Yellow Springs, OH, USA). The serum insulin was measured with immunoradiometric assay with the sensitivity 1 µIU/ml and intra- and interassay coefficients of variation (CVs) less than 2.2% and 6.5%, respectively (BioSource Europe, Nivelles, Belgium). Serum lipids were measured as previously described (16).

Before estimation of serum insulin, sgp130, IL-6 and sIL6-R, samples were kept frozen at -80°C. Serum sgp130 was measured with quantitative sandwich enzyme immunoassay kit (ELISA) (R&D Systems, Minneapolis, MN, USA) with a lowest detectable limit of 0.08 ng/ml and with intraassay and interassay CVs below 5.5% and 5.2% respectively. Serum high sensitivity IL-6 (hsIL-6) and sIL-6R concentrations were determined with ELISA kits (R&D Systems, Minneapolis, MN, USA). The minimum detectable concentration was 0.039 pg/ml for hsIL-6 and 6.5 pg/ml for sIL-6R. Intra- and interassay CVs were, respectively, below 7.8% and 9.6% for hsIL-6 and below 8.6% and 9.6% for sIL-6R. Serum high-sensitive C-reactive protein (hsCRP) was measured by means of particle enhanced immunonephelometry using CardioPhase kit (Dade Behring, Marburg, Germany) with sensitivity of 0.175 mg/l and intra- and interassay CVs below 4.5% and 5.8%, respectively.

Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and estradiol were measured by chemiluminescence method (ACS Chirone 180) and serum sex hormone-binding globulin (SHBG) – by the IRMA method (ZenTech, Angleur, Belgium). The sensitivity and CVs for all these assays were identical as reported previously (16). Free androgen index (FAI) was calculated as serum testosterone (nmol/l) x 100/SHBG (nmol/l) ratio (17).

**Statistical analysis:** The statistics were performed with STATISTICA 8.0 software. The variables, which did not have normal distribution (fasting and postload insulin, TG, IL-6, sIL-6R, sgp130, SHBG, testosterone, FAI, estradiol, hsCRP) were log-transformed prior to analyzes. For the purpose of the data presentation, these variables were transformed to absolute values in the Results. The differences between the groups were estimated with factorial ANOVA, with PCOS status and obesity as categorical factors and the studied parameters as dependent variables. The relationships between variables were studied with the Pearson product-moment correlation analysis and with multiple regression analysis. The level of significance was accepted at p<0.05.

**RESULTS**

The clinical characteristics of the studied groups is shown in Table 1. In the factorial ANOVA, we observed that the PCOS women had higher serum sgp130 concentration in comparison with the control
group (p=0.0002). We also found that the obese women had higher serum sgp130 concentration in comparison with the lean women (p<0.0001). The interaction between PCOS status and obesity was not significant (p=0.29), which suggests that an increase in sgp130 in PCOS was independent of obesity and an increase in sgp130 in obesity was independent of PCOS status. Additionally, PCOS women had significantly lower serum sIL-6R (p=0.0036). In contrast, serum sIL-6R did not differ between lean and obese women (p=0.93). Serum IL-6 and hsCRP did not differ between PCOS and control subjects, whereas they were increased in obese in comparison to lean group (p=0.007 and p<0.001, respectively).

Both the presence of PCOS and obesity were independently characterized by lower insulin sensitivity (p=0.0021 and p=0.0033, respectively; p=0.62 for the interaction), lower serum SHBG (p=0.011 and p<0.0001, respectively; p=0.22 for the interaction) and higher FAI (both p<0.0001, p=0.34 for the interaction). PCOS women had also higher serum LH and testosterone concentrations (both p<0.0001).

Cross-sectionally, serum sgp130 was related to BMI (r=0.35, p<0.001), waist (r=0.36 p<0.001), body fat (r=0.28, p=0.005), postload glucose and insulin (r=0.29, p=0.002 and r=0.29, p=0.002, respectively), TG (r=0.20, p=0.032), hsCRP (r=0.24, p=0.012) and FAI (r=0.28, p=0.002). We also observed the significant inverse correlation of sgp130 with insulin sensitivity (r=-0.36, p<0.0001) (Fig. 1) and SHBG (r=-0.23, p=0.015). In the subgroup analysis, the correlation between serum sgp130 and insulin sensitivity was present in the PCOS subjects (r=-0.34, p=0.002) but not in the control group (r=-0.16, p=0.35).

Using M value normalized for body weight (M/bw) instead of M/ffm increase the effect of obesity (Table 1) and the correlation with sgp130 was slightly stronger (r=-0.40, p<0.001).

Serum sIL-6R was related to FSH in the entire study group (r=0.23, p=0.017) and in the subgroup of PCOS women (r=0.24, p=0.041).

In multiple regression analysis, we observed that the relationship between sgp130 and insulin sensitivity was independent of BMI, waist, percent of body fat, postload glucose and insulin, TG and hsCRP (all adjusted β values between -0.23 and -0.33, all p<0.05), whereas correlations of sgp130 with SHBG and FAI disappeared after controlling for insulin sensitivity.

Exclusion of subjects with the highest values of postload insulin did not change any of the results.

**DISCUSSION**

The main finding of the present study is an increased serum sgp130 concentration in obesity and PCOS and an inverse relationship between sgp130 and insulin sensitivity in PCOS women.

So far, serum sgp130 has not been determined in obesity and PCOS. Escobar-Morreale et al (18) did not find the difference in circulating sgp130 between control and hyperandrogenic women, however, almost 40% of subjects in the hyperandrogenic group did not have PCOS. The frontier between PCOS and hyperandrogenism is diffuse, however, due to the large heterogeneity of PCOS itself, differences in characteristics of the studied groups are likely to influence the results. There are studies showing the association between polymorphisms in IL-6 or IL-6R and gp130 genes with clinical characteristics of PCOS and hyperandrogenism (18,19). Regarding the studied polymorphism of gp130 gene, it did not affect circulating level of this factor. In another study, polymorphism at the gp130 locus was associated with traits of the metabolic syndrome, however, circulating sgp130 was not reported (20).
Serum sgp130 was inversely related to insulin sensitivity in PCOS women. As mentioned, it was hypothesized that the gp130 cytokine family might exert beneficial effects and might be useful in the treatment of obesity and insulin resistance (4). Most gp130 cytokines occur in circulation in a very small, usually undetectable concentrations. It is possible that they might exert auto- and paracrine effects in insulin sensitive tissues, as in experimental studies CNTF was demonstrated to regulate metabolic processes in adipocytes and skeletal muscle cells (4,10). Given the fact that circulating sgp130 acts as an inhibitor of intracellular gp130 signaling (11), the correlation observed in our study might reflect this inhibitory effect.

The question arises, whether an association between sgp130 and insulin sensitivity is specific only for PCOS. We did not observe significant correlation in the control group. Other populations with different clinical characteristics should probably be investigated. However, sgp130 was also increased in obese insulin resistant women without PCOS.

Serum sgp130 was also related to SHBG and FAI, however, these associations were no longer significant after controlling for insulin sensitivity. IL-6, sIL-6R and sgp130 might also regulate other reproductive functions, such as granulosa cell survival, follicular growth, development of human preovulatory processes and blastocyst implantation (21). We cannot exclude the possibility that sgp130 influences other features of PCOS phenotype, not assessed in the present study. Nevertheless, these are mainly local actions of sgp130. In our study estimation of sgp130 serum concentration was performed during the 3-5 day of menstrual cycle and the influence of sgp130 on endometrium in proliferative phase of menstrual cycle seems to be relatively week (21).

There are studies, which demonstrate an increase in circulating IL-6 and hsCRP in PCOS (22). However, our results on the lack of an increase in these parameters in PCOS are in agreement with other data (23,24).

To our knowledge, decrease in sIL-6R in PCOS was not reported previously. Polymorphisms in IL-6R gene were associated with BMI and characteristics of the metabolic syndrome (18,25), but not with hyperandrogenism (18). The balance between sIL-6R and sgp130 in PCOS seems to be directed toward an inhibition of IL-6 action, however the significance of this finding remains unclear.

In conclusion, our data indicate that serum sgp130 is inversely and independently associated with insulin sensitivity in women with PCOS. An increased serum sp130 in obesity and PCOS suggest an inhibition of intracellular gp130 signaling in insulin resistant conditions.

ACKNOWLEDGMENTS

This study was supported by the grants 3-50815L and 3-50816L from the Medical University of Bialystok.
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<table>
<thead>
<tr>
<th>Table 1. Clinical and biochemical characteristics of the studied groups</th>
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<tbody>
<tr>
<td>Lean (Control (n=18)</td>
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<tr>
<td>-----------------------</td>
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<tr>
<td>Age (year)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Waist (cm)</td>
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<tr>
<td>Body fat (%)</td>
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<tr>
<td>Fasting glucose (mg/dl)</td>
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<td>Postload glucose (mg/dl)</td>
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<td>Fasting insulin (µIU/ml))</td>
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<td>Postload insulin (µIU/ml))</td>
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<td>hsIL-6 (pg/ml)</td>
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<td>sIL-6R (ng/ml)</td>
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<tr>
<td>sgp130 (ng/ml)</td>
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<td>hsCRP (mg/l)</td>
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</tbody>
</table>

Data are presented as mean±SD. P values are derived from factorial ANOVA. All interactions between PCOS status and obesity are nonsignificant. Postload glucose and insulin refer to value at 120 minute of the OGTT.
Table 2. Hormonal characteristics of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Lean Control (n=18)</th>
<th>PCOS (n=35)</th>
<th>Obese Control (n=16)</th>
<th>PCOS (n=43)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pmol/l)</td>
<td>299.4±303.78</td>
<td>282.00±267.18</td>
<td>163.98±113.98</td>
<td>245.26±242.32</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>5.99±1.25</td>
<td>5.78±1.48</td>
<td>5.56±1.73</td>
<td>5.65±1.39</td>
<td>NS</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>6.94±5.56</td>
<td>10.21±5.30</td>
<td>4.25±2.24</td>
<td>8.93±4.01</td>
<td>Obesity – NS PCOS – p&lt;0.001</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.79±0.38</td>
<td>2.93±1.11</td>
<td>1.75±0.51</td>
<td>2.83±1.06</td>
<td>Obesity – NS PCOS – p&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>89.51±56.28</td>
<td>59.91±46.74</td>
<td>41.50±18.35</td>
<td>36.20±17.96</td>
<td>Obesity – p&lt;0.001 PCOS – p=0.011</td>
</tr>
<tr>
<td>FAI</td>
<td>2.79±1.73</td>
<td>6.95±4.49</td>
<td>4.69±1.70</td>
<td>9.74±6.19</td>
<td>Obesity – p&lt;0.001 PCOS – p&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. P values are derived from factorial ANOVA. All interactions between PCOS status and obesity are nonsignificant.

Figure legend

Fig. 1. Correlations between sgp130 and insulin sensitivity in the whole studied group (n=112). Values of sgp130 are shown on log-transformed scale. Circles, PCOS group; squares, control group.
sgp30 and insulin sensitivity

\[ r = -0.36, \ p < 0.001 \]