Sex Hormone-Binding Globulin Levels Predict Insulin Sensitivity, Disposition Index and Cardiovascular Risk During Puberty

Kaspar Sørensen MD1, Lise Aksglæde MD1; Thor Munch-Andersen2; Niels Jacob Aachmann-Andersen2, Joergen Holm Petersen PhD1,3, Linda Hilsted MD, DMSc4, Jørn Wulff Helge PhD5, Anders Juul MD, DMSc1

1Department of Growth and Reproduction, Copenhagen University Hospital, Denmark
2Copenhagen Muscle Research Centre, Copenhagen University Hospital, University of Copenhagen, Denmark
3Department of Biostatistics, University of Copenhagen, Denmark
4Department of Clinical Biochemistry, Copenhagen University Hospital, Denmark
5Copenhagen Muscle Research Centre, Department of Biomedical Sciences, University of Copenhagen, Denmark

Correspondences to:
Kaspar Sørensen MD
Email: kaspar.soerensen@rh.regionh.dk


This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**Objective:** Early puberty is associated with increased risk of subsequent cardiovascular disease. Low sex hormone-binding globulin (SHBG) levels are a feature of early puberty as well as conditions associated with increased cardiovascular risk. The aim of the present study was to evaluate SHBG as predictor of glucose metabolism and metabolic risk during puberty.

**Research design and methods:** Cross-sectional study on 132 healthy Caucasian children and adolescents evaluated by oral glucose tolerance test, dual energy X-ray absorptiometry scan, direct oxygen uptake measurement during cycle ergometry and fasting blood samples.

**Results:** SHBG levels declined with advancement of puberty in both boys (p < 0.001) and girls (p = 0.019). SHBG was statistically significantly positively associated with insulin sensitivity in boys (p < 0.001) and girls (p < 0.001). In addition, SHBG was a strong predictor of insulin sensitivity (p = 0.001) and the only predictor of the disposition index (p = 0.031) after adjusting for puberty, fat mass and aerobic fitness. SHBG was significantly negatively associated with metabolic risk (p = 0.032) as well as hypersensitive CRP levels (p = 0.030) after adjustment for relevant confounders.

**Conclusions:** SHBG was a strong predictor of insulin sensitivity and metabolic risk during puberty. Thus, we hypothesize that SHBG integrates the marked changes in glucose metabolism and body composition that occur during pubertal transition.
Early puberty has been associated with increased cardiovascular risk in adulthood (1-3), the underlying mechanism of which is unknown. However, a shared feature of both early puberty and cardiovascular risk is low serum levels of sex hormone-binding globulin (SHBG).

In adults, SHBG levels are negatively associated with a cluster of conditions, all of which have a strong association with obesity and insulin resistance. Thus, low levels of SHBG have consistently been associated with a wide array of cardiovascular risk factors including visceral and subcutaneous adiposity, hypertension, dyslipidemia and insulin resistance (4,5). Consequently, SHBG levels are low in overt type 2 diabetes, and have more recently been associated with the metabolic syndrome (6-8). In addition, SHBG levels have been found to be a determinant of cardiovascular risk independently of obesity and insulin resistance (9), and able to predict future type 2 diabetes and metabolic syndrome in adults (7,10).

Despite the increasing focus on SHBG as a marker of cardiovascular risk in adults, few studies have examined the relation to glucose metabolism and metabolic risk during puberty. SHBG levels have been negatively associated with fasting insulin levels (11), body composition and sex steroid levels (12) during puberty. We have recently shown that early pubertal timing in girls is associated with lower SHBG levels than predicted by pubertal stage and body mass index (12), suggesting that differences in glucose metabolism could be at least partly responsible. However, no study to date has evaluated the predictive value of SHBG on glucose metabolism during puberty.

The aim of the present study was to investigate SHBG as a predictor of insulin sensitivity, insulin secretion and metabolic risk after correction for other known influential confounders such as puberty, adiposity and aerobic fitness.

RESEARCH DESIGN AND METHODS

Subjects. All participants were recruited as a part of The COPENHAGEN Puberty Study from three primary schools in the Copenhagen Community. Hundred and thirty-two healthy Caucasian children and adolescents (70 girls) aged 8.5 – 16.1 years volunteered. No prior or present medical history of confounding conditions was reported. Four of the adolescent girls were excluded for analysis on SHBG due to intake of contraceptive pills. No other intakes of medications were reported. All participants filled out a lifestyle questionnaire. None of the participants were smokers or reported consuming alcohol. The median time spent on physical activity was 5-6 hours per week. Median hours spent per day in front of the television and computer was 1-2 hours and less than 1 hour, respectively.

Pubertal development. Pubertal development was described according to the Tanner classification. Date of last menstrual bleeding was recorded in post-menarcheal girls (n = 21).

Body composition. A whole body DEXA scanning was performed in all subjects using a Hologic CDR 1000/W densitometer (Hologic Inc, Bedford, MA, USA) with software version 6.2. Waist circumference (CW) was measured 3 times at the mid-axillary line at the level mid-point between the anterior superior iliac spine and the 12th rib.

Aerobic fitness. Maximal oxygen uptake (VO₂max) was assessed during a cycle ergometry using an electronically braked cycle ergometer (Ergomedic 839; Monark, Varberg, Sweden). VO₂max was measured directly using an online pulmonary gas analyzer System (Quark CPET, Cosmed, Rome, Italy). Initial and incremental
workloads were 20 W for children weighing less than 30 kg and 25 W for children weighing 30 kg or more. For adolescents over 15 years of age initial workload was 70 W for girls and for boys 100 w and incremental workload was 35 W. Workload was increased every third minute until exhaustion for children and adolescents below 15 years of age, and every second minute until exhaustion for adolescents over 15 years of age. Heart rate (HR) was recorded continuously throughout the test using a HR monitor (Polar electro, Oulu, Finland). Criteria for a maximal effort were HR of 185 beats per minute or greater, and a subjective judgement by the observer that the individual could no longer continue, even after encouragement. For a valid directly measured test, the oxygen consumption curve should show signs of levelling off. The cycle ergometer was electronically calibrated on every test day. Maximal oxygen consumption per kilogram body weight is an expression of aerobic fitness.

Two subjects could not participate in cycle ergometry due to foot injury. One hundred and twenty-two (92.4%) subject fulfilled the criteria for a valid cycle ergometry, of which 113 (85.6%) had a valid directly measured VO$_2$max due to technical problems with the online pulmonary gas analyzer system. In order to include all participants with a valid cycle ergometry test, the predicted VO$_2$max was estimated by linear regression analysis from the maximal power output from all participants with valid direct VO$_2$max.

$$\text{VO}_2\text{max boys} = (\text{maximal power output} \times 12.2) + 368.9$$

$$\text{VO}_2\text{max Girls} = (\text{maximal power output} \times 12.4) + 303.9$$

The estimated values were highly significantly correlated to the direct measurements in both boys ($r = 0.938$) and girls ($r = 0.895$).

**Blood sampling.** An intravenous cannula was inserted into an antecubital vein, from which fasting venous blood samples were drawn into standard vacuum tubes. Blood was centrifuged (3000 G at 10 min) and plasma samples isolated and stored at –20° Celsius until analysis.

**Oral Glucose Tolerance Test (OGTT).** Standard 2-hours OGTT with an oral glucose load of 1.75 grams of glucose per kg BW (maximum 75 gram glucose) were carried out on all subjects. Blood samples were drawn with 30 minutes intervals. Blood was centrifuged within 30 minutes and plasma immediately stored at -20° Celsius until determination of insulin and glucose levels. The area under curve for plasma glucose and insulin were calculated by the trapezoidal rule. Whole body insulin sensitivity index (WBISI) was calculated by the formula developed by Matsuda et al (13), and subsequently converted to pmol/l insulin per mmol/l glucose. First phase insulin release (1$^{st}$ PH) was calculated by the formula developed by Stumvoll et al (14). β-cell compensatory capacity was evaluated by the disposition index (DI), as the product of WBISI and 1$^{st}$ PH, which has recently been validated from OGTT in adults (15). Application of the same statistical model (see statistics) on the values from the present children confirmed the rectangular hyperbolic relationship with the following formula: 1347.8/WBISI + 516.9 = 1$^{st}$ PH ($R^2 = 0.512$, $p < 0.001$).

**Analyses.** SHBG was determined by a time-resolved immunofluoresence assay (Delfia, Wallac Oy, Turku, Finland) with a detection limit of 0.23 nmol/l. Intra- and interassay coefficients of variation (CV) were 5.8% and 6.4%, respectively. Insulin was determined by an ElectroChemiLuminiscens Immunoassay (ECLIA) (Elecys insulin reagents kit, Roche diagnostics, Mannheim, Germany) on automated Roche Modular Analytics Module E170 (Roche, Mannheim,
Germany). The detection limit was 2 pmol/l. The intra- and interassay CV was 4.2 % and 8.2 %, respectively. Glucose, Lipids (HDL-C, triglycerides (TG) and hypersensitive C-reactive Protein (hsCRP) were all determined on a Roche Modular Analytics (SWA) Module P (Roche Diagnostics, Mannheim, Germany). Glucose was determined by enzymatic absorption photometry and the intra- and interassay CV was 1.1 and 1.7%, respectively. HDL-C and TG were determined by enzymatic colorimetric analyses (CFAS HDL plus, TG GPO-PAP, Roche, Mannheim, Germany). For HDL the intra- and interassay CV was 0.9% and 1.85%, respectively. For TG the intra- and interassay CV was 1.5% and 1.8%, respectively. hsCRP were determined by immunturbidimetric analyses (Cobas, Roche, Mannheim, Germany) and the intra- and interassay CV were 1.0% and 2.4%, respectively.

**Blood Pressure.** Blood pressure (BP) was measured with a conventional sphygmomanometer (Heine Gamma G5, Heine Optotechnik GmbH & Co. KG, Herrsching am Ammersee, Germany) in the left arm after 10 minutes rest in supine position.

**Metabolic syndrome score.** Metabolic syndrome is defined as a cluster of dyslipidemia, hypertension, glucose intolerance and obesity in adults. Until recently no definition of the metabolic syndrome existed in children and adolescents, but the international Diabetes Federation (IDF) came out with a consensus in 2007 (16). No participant met the diagnostic criteria of metabolic syndrome based on the IDF criteria. In order to evaluate the metabolic parameters as a continuous variable we generated a combined Z-score based on the five variables included in the IDF consensus. Age- and gender-specific Z-scores for WC were done using combined cross-sectional and longitudinal data from The COPENHAGEN Puberty Study with 2103 observations on 1093 girls and 765 boys (unpublished data). The Z-score for HDL-C was inversed due to the negative association to metabolic syndrome. All five Z-scores were added and subsequently divided by 5 generating a joint Z-score with positive and negative scores meaning higher and lower than mean risk profile for this specific population, respectively.

**Statistics.** No differences in outcome variables were found between post-menarcheal girls tested during the luteal phase versus the follicular phase and data were pooled before analyses. Descriptive statistics are shown as medians (10th percentile; 90th percentile). Mann Whitney U-tests were used for non-parametric comparison between groups. SHBG, fat mass, insulin sensitivity, insulin secretion, disposition index and hypersensitive CRP were log-transformed to obtain approximate normal Gaussian distribution of the residuals as well as to obtain a residual variance which did not depend on the level. General linear models were used to assess the main determinants of insulin sensitivity, insulin secretion, disposition index, metabolic risk score and hsCRP, respectively. No interactions between puberty and sex were observed in any of these models. Analyses with insulin sensitivity, insulin secretion and DI as dependent variables, respectively, included the following covariates: sex, age, puberty, fat mass, SHBG and aerobic fitness.

To confirm the inverse relationship between insulin secretion and insulin sensitivity a regression analysis was done using the following model: $1^{st} \text{PH} = \text{constant x WBISI}^\beta \Rightarrow \log(1^{st} \text{PH}) = \text{constant} + \beta \log(\text{WBISI})$. The inverse relationship was confirmed with $\beta = -0.583$ and a 95% CI of $\beta$ from $-0.691$ to $-0.474$. A linear regression analysis was done with direct measured VO$_2$max as the response and maximal power output as explanatory variable, in order to predict the VO$_2$max in all subjects with a
valid cycle ergometry test, including those with invalid direct measurements of VO2max. No other variables contributed significantly in this regression.

**Ethics.** The study was in accordance with the ethical principles of the Helsinki II declaration. The study protocol was approved by the local ethics committee (Ref no. KF 01 282214 and KF 11 2006-2033). All children and parents gave their informed written consent.

**RESULTS**

Descriptive characteristics according to sex are shown in Table 1.

*SHBG levels* decreased with increasing stage of puberty in boys (p < 0.001) and girls (p = 0.019), respectively. However, SHBG levels in girls tended to plateau during mid-puberty after which a slight non-significant increase was noted. Girls had statistically significantly higher SHBG levels (p = 0.023) compared with boys after adjustment for age, puberty, fat mass and insulin sensitivity.

*Insulin sensitivity* during puberty in relation to SHBG levels above and below the median is illustrated in Figure 1A. In univariate analyses insulin sensitivity was significantly positively associated with SHBG and aerobic fitness and negatively associated with fat mass, in boys (p < 0.001, p = 0.042 and p < 0.001, respectively) as well as in girls (all p < 0.001). SHBG remained a significant predictor of insulin sensitivity after adjustment for fat mass and aerobic fitness (see Table 2).

*Insulin secretion* during puberty in relation to SHBG levels above and below the median is illustrated in Figure 1B. In univariate analyses insulin secretion was significantly positively associated with SHBG and fat mass as well as negatively associated with aerobic fitness in boys (p = 0.009, p < 0.001 and p = 0.013, respectively) as well as in girls (all p < 0.001). However, SHBG was no longer a significant predictor of insulin secretion after adjustment for insulin sensitivity.

*Disposition index* during puberty in relation to SHBG levels above and below the median is shown in Figure 1C. SHBG was significantly negatively associated with DI (p = 0.005). Neither fat mass nor aerobic fitness were significantly associated with DI in univariate analyses. SHBG and puberty contributed the most to the variance in DI (p = 0.004, R2 = 0.08). Predictors of disposition index are shown in Table 2.

*Metabolic risk* during puberty was evaluated by the combined Z-score on risk factor derived from the recent IDF consensus on childhood metabolic syndrome. Metabolic risk during puberty in relation to SHBG levels above and below the median is shown in Figure 1D. Metabolic risk was negatively associated with SHBG and aerobic fitness and positively associated with fat mass in boys (p < 0.001, p = 0.002 and p < 0.001, respectively) as well as in girls (p = 0.003, p < 0.001 and p < 0.001, respectively). Predictors of metabolic risk are shown in Table 2.

*Hypersensitive CRP* levels were not associated with puberty per se. HsCRP was positively associated with metabolic risk and fat mass as well as negatively associated with SHBG and aerobic fitness in univariate analyses in both boys (p = 0.002, p < 0.001, p = 0.003 and p < 0.001, respectively) and girls (p = 0.006, p = 0.035, p = 0.018 and p = 0.006, respectively). After adjustment for fat mass and aerobic fitness, both SHBG (p = 0.030) and metabolic risk (p = 0.019) remained significant predictors of hsCRP during puberty.

**CONCLUSIONS**

In the present study of healthy children and adolescents we found strong associations between SHBG levels and markers of glucose metabolism and metabolic risk. SHBG was a significant positive
predictor of insulin sensitivity and disposition index during puberty independent of fat mass and aerobic fitness. In addition, SHBG negatively predicted childhood metabolic risk and low-grade inflammation.

Early puberty has been associated with an increased risk of insulin resistance, dyslipidemia and adiposity in adulthood (1-3). The underlying mechanism responsible for this association is unknown, but a shared feature of both conditions is low SHBG levels.

In accordance with previous studies (11,12), SHBG levels declined during puberty in boys and girls, although levels in girls tended to plateau from around mid-puberty. During puberty SHBG levels has been associated with body composition, sex steroids (12) as well as with fasting insulin levels (11). Interestingly, girls with precocious puberty have lower SHBG levels than predicted by pubertal stage and body mass index (12), indicating that some of the residual variation in SHBG in such patients might be related to differences in insulin sensitivity and secretion.

Puberty is characterized by a marked physiological decline in insulin sensitivity leading to a compensatory increase in insulin secretion both of which recover by late puberty (17). In the present study, a similar curvilinear pattern with declining insulin sensitivity and increasing insulin responses until mid-puberty was confirmed. Due to the inverse relationship between insulin sensitivity and insulin secretion, it has been speculated that risk assessment for future development of type 2 diabetes is most accurately measured during periods of β-cells challenge (18). Pregnancy may constitute such an example in that β-cells are challenged by low insulin sensitivity. Low SHBG levels during first trimester of pregnancy predict gestational diabetes (19), which have been found to be strongly associated with subsequent development of overt type 2 diabetes (18). In analogy, puberty might constitute a comparable sensitive period during childhood and adolescence in which low SHBG might predict increased risk of type 2 diabetes and related metabolic changes.

Previous studies in adults have consistently shown strong correlations between SHBG and different indices of insulin sensitivity (8,20). A similar positive correlation have been found in children and adolescents during puberty, although this has previously only been studied in relation to fasting insulin levels (11). In the present study, we found insulin sensitivity assessed by OGTT to be strongly associated with SHBG levels in both boys and girls during puberty. In addition, SHBG was a strong predictor of insulin sensitivity independently of fat mass and aerobic fitness. In accordance with previous studies (21), insulin sensitivity was strongly associated with both fat mass and aerobic fitness. However, due to the strong interrelation between aerobic fitness and fat mass, these covariates explain much of the same variability in insulin sensitivity, and therefore end up non-significant when both are included in the same variance analysis.

Insulin secretion was strongly positively associated with SHBG, but not when adjusted for insulin sensitivity. In order to evaluate the predictive value of SHBG on β-cell function we calculated the disposition index by a method which has recently been validated during oral glucose tolerance test (15). DI was lowest during mid-puberty, which is in accordance with previous studies (17). As reported in adults (22), we found that SHBG was significantly associated with the DI. In addition, SHBG was the only significant predictor of the DI during puberty. However, it should be noted that SHBG and puberty only accounted for approximately 8% of the variance in DI.

Obesity and insulin resistance are major risk factors for development of the
SHBG, insulin and metabolic risk during puberty

metabolic syndrome in children and adolescents (16,23). In light of the strong associations between SHBG levels, adiposity and insulin resistance (8,20), it is not surprising that SHBG levels have been shown to be strongly associated with the metabolic syndrome as well as overt type 2 diabetes in adults (6-8). In the present study, we found SHBG to be a significant predictor of the combined Z-score on all variables derived from the IDF consensus on childhood metabolic syndrome even after adjustment for fat mass and aerobic fitness. In accordance with previous studies (24), aerobic fitness did not independently predict metabolic risk in these children, indicating that aerobic fitness influence metabolic risk indirectly through effects on fat mass. However, the metabolic syndrome score is partially based on waist circumference, an abdominal fat mass estimate, and thus fitted to be strongly associated with fat mass per se. Thus, coexistence of adverse metabolic characteristics was independently associated with low SHBG levels, indicating that SHBG might be valuable in the assessment of cardiovascular risk during puberty. High metabolic risk was independently associated with high hsCRP levels in the present study. Elevated hsCRP have been associated with the metabolic syndrome in obese children and adolescents in previous studies (25), and the present finding extends this to include non-obese children without overt metabolic syndrome. In addition, high hsCRP levels were associated with low SHBG independent of fat mass and aerobic fitness. Thus, SHBG might serve as an independent marker of low-grade systemic inflammation during puberty, which further strengthens its candidature as a predictor of cardiovascular risk during childhood and adolescence.

In conclusion, strong associations were found between SHBG and markers of glucose metabolism in healthy non-obese children during puberty. Importantly, SHBG was a strong predictor of insulin sensitivity as well as a predictor of disposition index independently of total fat mass and aerobic fitness. In addition, the clustering of adverse metabolic characteristics as well as elevated hypersensitive CRP levels was independently associated with low SHBG levels. We hypothesize that SHBG integrates the marked changes in glucose metabolism and body composition that occur during pubertal transition.

ACKNOWLEDGEMENTS

The COPENHAGEN Puberty Study received financial support by The Kirsten and Freddy Johansen’s Foundation. JWH received financial support by The 1991 Pharmacy Foundation.
REFERENCES


Table 1 - Descriptive characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Boys (n = 62)</th>
<th>Girls (n = 70)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pubertal stage 1-5 (%)</td>
<td>34; 24; 11; 11; 19</td>
<td>14; 11; 19; 43; 13</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.4 (9.2; 14.9)</td>
<td>12.3 (9.1; 15.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>7.5 (5.1; 15.8)</td>
<td>9.6 (5.2; 15.0)</td>
<td>0.042</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>34.1 (23.7; 52.8)</td>
<td>32.5 (22.4; 44.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting Insulin (pmol/l)</td>
<td>43 (21; 72)</td>
<td>49 (32; 91)</td>
<td>0.010</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.8 (4.1; 5.5)</td>
<td>4.8 (4.2; 5.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean AUCinsulin (pmol/l)</td>
<td>242 (118; 379)</td>
<td>248 (155; 458)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean AUCglucose (mmol/l)</td>
<td>5.8 (4.5; 7.3)</td>
<td>5.5 (4.6; 6.7)</td>
<td>NS</td>
</tr>
<tr>
<td>WBISI</td>
<td>2.1 (1.3; 4.3)</td>
<td>1.9 (1.0; 3.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin release (pmol)</td>
<td>1002 (635; 1647)</td>
<td>1305 (876; 2088)</td>
<td>0.003</td>
</tr>
<tr>
<td>Disposition Index</td>
<td>2260 (1377; 3924)</td>
<td>2492 (1524; 3555)</td>
<td>NS</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>46.8 (35.6; 55.0)</td>
<td>40.0 (33.4; 48.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.61 (0.43; 1.11)</td>
<td>0.79 (0.53; 1.36)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.47 (1.15; 1.88)</td>
<td>1.52 (1.04; 1.84)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ABP (mmHg)</td>
<td>78.3 (70.0; 93.3)</td>
<td>76.7 (71.7; 90.0)</td>
<td>NS</td>
</tr>
<tr>
<td>WC Z-score</td>
<td>0.22 (-0.99; 1.34)</td>
<td>0.07 (-1.02; 1.32)</td>
<td>NS</td>
</tr>
<tr>
<td>Met Z-score</td>
<td>-0.13 (-0.56; 0.78)</td>
<td>0.04 (-0.53; 0.77)</td>
<td>NS</td>
</tr>
<tr>
<td>HsCRP (mg/l)</td>
<td>0.28 (0.16; 1.40)</td>
<td>0.28 (0.16; 2.68)</td>
<td>NS</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>63.0 (23.0; 122.0)</td>
<td>81.5 (34.5; 130.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 1. Results are presented as medians (10th percentile; 90th percentile). Comparisons between boys and girls by Mann-Whitney U-test*. AUC, area under curve. WBISI, insulin sensitivity a.m. Matsuda (13). Insulin release as 1st phase a.m. Stumvoll (14). VO₂max, predicted aerobic fitness. ABP, arterial blood pressure. WC, waist circumference. Met, metabolic risk score. Z-score, standard deviations score. HsCRP, hypersensitive CRP. To convert glucose from nmol/l to mg/dl, multiply by a factor 18. To convert insulin from pmol/l to µU/ml divide by a factor 6. NS, non-significant (P > 0.05).
Table 2 – Predictors of insulin sensitivity, disposition index and metabolic risk in healthy non-obese children and adolescents

<table>
<thead>
<tr>
<th></th>
<th>Insulin Sensitivity (WBISI)</th>
<th>Disposition Index (DI)</th>
<th>Metabolic Risk (Met Z-score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est (95% CI)</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>21.4% (7.9 – 35.7)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-6.7% (-24.2 – 14.9)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>VO2max (ml/kg/min)</td>
<td>38.5% (-12.3 – 120.4)</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

Puberty

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>P</th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner II vs. I</td>
<td>-13.9% (-30.9 – 7.3)</td>
<td>0.004</td>
<td>0.18</td>
<td>-6.8% (25.9 – 17.4)</td>
<td>0.07</td>
<td>-0.20 – 0.34</td>
</tr>
<tr>
<td>Tanner III vs. I</td>
<td>-26.7% (-42.9 – -5.8)</td>
<td></td>
<td></td>
<td>-4.9% (-26.7 – 23.4)</td>
<td>0.14</td>
<td>-0.17 – 0.45</td>
</tr>
<tr>
<td>Tanner IV vs. I</td>
<td>-11.3% (-32.3 – 17.4)</td>
<td></td>
<td></td>
<td>9.4% (-18.1 – 44.8)</td>
<td>0.05</td>
<td>-0.28 – 0.39</td>
</tr>
<tr>
<td>Tanner V vs. I</td>
<td>15.0% (-18.9 – 64.9)</td>
<td></td>
<td></td>
<td>29.7% (-9.5 – 87.8)</td>
<td>0.05</td>
<td>-0.39 – 0.49</td>
</tr>
</tbody>
</table>

Adjusted R² = 0.48 Adjusted R² = 0.04 Adjusted R² = 0.38

All models were adjusted for sex and age none of which contributed significantly to any of the models. For the continuous covariates the parameter estimates (Est) and 95% confidence intervals (CI) are presented as the changes in the response resulting from a 100% increase in the covariate, i.e. a doubling of SHBG results in a 21.4% increase in WBISI and 0.15 standard deviations reduction in the metabolic risk score. To calculate the change (d) in the dependent variable of a given change (c) in covariates the following formulas can be used: For WBISI and DI (log-transformed) the change is \( d = c \log_2(\text{Est}) \). For the Met Z-score (untransformed) the change is \( d = (\text{Est}/\ln(2)) \times \ln(c) \). As an example an isolated (i.e not changing other covariates) 50% increase in SHBG levels will lead to a 12% \((1.5^{\log_2(1.21)} = 1.5^{0.28})\) increase in WBISI, and a 0.09 \((-0.15/\ln(2)) \times \ln(1.5) = -0.22 \times \ln(1.5)\) decrease in Met Z-score. For pubertal stages the changes in relation to Tanner stage I are presented as relative changes for WBISI and DI, and as absolute changes for Met Z-score. As an example, the SHBG level is 26.7% lower and Met Z-score 0.14 higher in Tanner stage III than in stage I. Significant P-values (P < 0.05) are in bold.
Legend to Figure 1

Figure 1. Insulin sensitivity (A), insulin secretion (B), disposition index (C) and metabolic Z-score (D) during puberty based on oral glucose tolerance test in healthy children and adolescents. For each pubertal stage, the effect of low vs. high SHBG levels is illustrated by grouping all children according to the median SHBG level for pubertal stage and sex. The below median SHBG group is presented by solid bars (■) and the above median group as open bars (□). The whiskers represent the 90th percentiles. Insulin sensitivity (WBISI a.m. Matsuda (13)) and insulin secretion (1st phase release a.m. Stumvoll (14)) are calculated using glucose in mmol/l and insulin in pmol/l concentrations. To convert WBISI to glucose in mg/dl and insulin in µU/ml, multiply by a factor 3. To convert insulin secretion from pmol/l to µU/ml divide by a factor 6. Disposition index is calculated as the product of insulin sensitivity and insulin secretion. Metabolic Z-score is generated from combining Z-scores from fasting glucose, triglyceride levels, inverse HDL-C levels, waist circumference and mean BP levels divided by 5. An increase in the combined Z-score indicates an increase in metabolic risk.