Sex steroids affect triglyceride-handling, glucose-dependent insulinotropic polypeptide and insulin sensitivity: a one-week randomized clinical trial in healthy young men

Short Title: Sex steroids and glucose metabolism in men

Bruno Lapauw, MD1; Margriet Ouwens, PhD3; Leen M. ‘t Hart, PhD4; Birgitte Wuyts, PhD2; Jens J. Holst, PhD5; Guy T’Sjoen, MD1; Jean-Marc Kaufman, MD1 and Johannes B. Ruige, MD1

From the 1Department of Endocrinology and 2Laboratory for Metabolic Diseases, Ghent University Hospital, Ghent, Belgium; 3Deutsches Diabetes Zentrum, Institut für Klinische Biochemie und Pathobiochemie, Düsseldorf, Germany; 4Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands; 5Department of Biomedical Sciences, University of Copenhagen, the Panum Institute, Copenhagen, Denmark.

Corresponding author:
Johannes Ruige
e-mail: johannes.ruige@ugent.be

Clinical Trial Registry No.: NCT00740194; clinicaltrials.gov

Submitted 19 March and accepted 4 May 2010.

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 - To evaluate metabolic effects of sex steroids in non-fasting and fasting conditions, independent from changes in body composition.

Research Design and Methods - A randomized clinical trial (RCT) was performed to create contrasting sex steroid levels in healthy young men; by letrozole (aromatase inhibitor) to lower estradiol (E2) and increase testosterone (T) (group T, n=10) versus letrozole plus E2 patches to lower T and raise E2 (group E, n=10). Mixed-meals and hyperinsulinemic-euglycemic clamps were performed before and after a one-week treatment period.

Results – Following intervention, the postprandial triglyceride response displayed a diverging response with decline in group T and increase in group E. The postprandial glucose-dependent insulinoropic polypeptide (GIP) response increased in group T. Insulin sensitivity increased in group T, though remained unaltered in group E.

Conclusions - In healthy young men, short-term changes in sex steroids affect postprandial triglyceride and GIP response, and insulin sensitivity.
Low testosterone (T) has been shown to be a strong predictor of metabolic syndrome in men aged 20-40 yrs, though underlying biological mechanisms are poorly understood (1, 2). Typically, low T in presence of disturbed glucose metabolism is combined with high estradiol (E₂) (3). Modifying steroid levels into low E₂ and high T, by an aromatase inhibitor (AI) reducing conversion of T into E₂ through aromatase (P450a), results in decreased fasting glucose and insulin levels in young men (4). Yet, non-fasting conditions might be relevant as well as humans spend the majority of time in this state which might better predict cardiovascular risk (5). In the present study, short-term sex steroid effects were explored in both fasting and non-fasting conditions; men were randomly treated with an AI to lower E₂ and increase T (group T), or an AI and E₂ patches to lower T and raise E₂ (group E). E₂ patches in men cause down-regulation of T secretion through the feedback mechanism of the hypothalamic-pituitary-gonadal axis. A week is supposed to be sufficient to reach a steady state.

**RESEARCH DESIGN AND METHODS**

Twenty healthy young men (aged 20-40 yrs) were randomized to receive either letrozole 2.5 mg, Femara®, Novartis AG, Switzerland (group T; n=10), or letrozole plus Dermestril®, exemption 75µg/day, Besins, Belgium (group E; n=10). All subjects gave written informed consent and completed the trial, which was approved by the Ethical Review Board of the Ghent University Hospital, conducted according to the principles of the Declaration of Helsinki, and registered at ClinicalTrials.gov (NCT00740194).

Measurements before and after a one-week treatment period were performed in similar conditions, starting at 8.00h am after overnight fasting and 10-min bed rest at the hospital. Hyperinsulinemic-euglycemic clamps were initiated by a primed-continuous insulin infusion and fixed at 40 mU/m²body surface area/min throughout the 120 min clamp to completely suppress endogenous glucose production (6). The insulin infusion rate was similar in both groups. Variable infusion of glucose, adjusted every 5 minutes, was used to maintain euglycemia (5 mmol/L). Venous blood was arterialized through retrograde cannulation of a wrist vein while heating the hand at 60–70°C using a custom-made heating box. The glucose disposal rate (insulin sensitivity; M-value) was measured during the last 30 minutes and corrected for lean body mass (DXA). Thirty minutes after the clamp, a standard mixed-meal (bread, margarine, cheese and milk), providing a caloric content of 1000 kcal (45% fat, 36% carbohydrates and 19% proteins), was served and blood samples were taken before and 10, 30, 60, 120, 180, 240 and 300 minutes after ingestion. Triglycerides, glucose, and c-peptide serum concentrations were determined using standard laboratory assays (Modular immunoassay, Roche Diagnostics, Germany). Intra- and interassay coefficients of variation for all parameters were less than 3 and 6%, respectively. Commercial immunoassays were used to determine serum E₂ (Incstar, USA), T and SHBG (Orion Diagnostica, Finland). Total glucagon-like peptide-1 and intact glucose-dependent insulinotropic polypeptide were determined as previously described (7). Apart from standard statistical analyses, longitudinal mixed-effects modeling was used to assess differences in post-meal responses and were performed using SPSS 12.0 software package (SPSS Inc., USA) and SAS 9.1.3 (SAS Institute, USA).

**RESULTS**

All subjects were Caucasian, and no anthropometric differences were observed...
between both groups (age 34±7 and 31± 5 yrs; BMI 22.4±2.4 and 23.4±2.2 kg/m²; WHR 0.87±0.05 and 0.86±0.04, group T and E, respectively).

**Sex-steroid changes** - Following intervention, E2 levels decreased by 56% and T levels increased by 114% in group T, whereas in group E T levels declined by 44% and total E2 levels increased by 43%. Adverse events or side effects did not occur.

**Non-fasting measurements** - Following intervention, postprandial responses of glucose, c-peptide and GLP-1 remained unchanged, though triglycerides displayed a diverging response, declining in group T and increasing in group E and the glucose-dependent insulinotropic polypeptide response increased in group T.

**Fasting measurements** - Following intervention, no differences in fasting glucose, insulin levels or triglycerides levels were revealed. Yet, an increase in insulin sensitivity (M-value LBM) was observed in group T, whereas no change was observed after intervention in group E. The observation remained similar when adjusting for mean glucose levels during steady-state (data not shown; P = 0.018).

**CONCLUSIONS**
The study shows that short-term changes in sex steroids affect glucose and lipid metabolism in fasting and non-fasting conditions in healthy young men.

Concomitantly increasing T and decreasing E2 levels has positive effects on both postprandial triglyceride handling and insulin sensitivity. Effects on postprandial triglyceride-handling are in line with previous reports (8), and seem relevant for metabolic risk (9). The improvement in insulin sensitivity explains our previous findings of reduced fasting glucose and insulin levels after 4 wks of AI in young men (4), and corroborates a former report showing acute reduction in insulin sensitivity 2 wks after discontinuing T replacement in hypogonadal men (10). It remains unclear whether these observations result from changes in lipid metabolism, altered post-receptor insulin signaling in muscle (11), and whether improved insulin sensitivity enhances muscle lipid uptake (12). Further, this same intervention increased postprandial GIP response, though future research is needed to establish metabolic consequences. Effects of sex steroids on GIP have not been reported before. Action of GIP is not limited to pancreatic cells, and may affect lipid homeostasis (13), and intestinal glucose transport (14).

The hormonally contrasting group with decreased T and relatively high E2 levels displayed a larger postprandial triglyceride response without effects on insulin sensitivity, though possible effects might have been masked due to variation in transdermal delivery of E2.

These findings were demonstrated in a limited number of healthy male subjects, necessitating confirmation and extension to populations at risk (obesity, disturbed glucose metabolism, elderly) to evaluate potential clinical implications, e.g. related to lipid-handling or buffering of adipocytes for prevention of lipotoxicity (9, 13, 15).

In summary, changing sex steroid levels in a largely physiological range influences postprandial triglyceride handling, GIP and insulin sensitivity in healthy young men. Given the short-term of this intervention, these effects can be assumed to occur independently from changes in body composition.

**Author Contributions.** B.L. researched data and wrote the manuscript. M.O. reviewed/edited the manuscript. L.M.H. contributed to discussion. B.W. researched data. J.J.H. researched data and reviewed/edited the manuscript. G.T. contributed to discussion and reviewed/edited
the manuscript. J.M.K. reviewed/edited the manuscript. J.B.R. researched data and reviewed/edited the manuscript.

ACKNOWLEDGEMENTS
The authors are indebted to Prof. Dr. S. Vansteelandt, Ghent University, Belgium, for statistical consulting and to employees of the Dept. of Endocrinology; Kaatje Toye, Bea Vervinckt and Nera Steyaert for the meticulous realization of the study protocol and to Inge Bocquaert and Kathelijne Mertens for performing the immunoassays. We also would like to thank Eric Matthys for using his skills to create the custom-made heating box. We thank all volunteers who participated as study subjects.

This study was supported in part by Grant G0066207 of the Flemish Fund for Scientific Research (FWO Vlaanderen).

No potential conflicts of interest relevant to this article were reported.

REFERENCES
9 Ruige JB, Van Gaal LF. Low Fasting Triglycerides: Hallmark of the Healthy Large Hip? Obesity (Silver Spring) 2009; 17: 1621-1626
15. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia. 2002; 45: 1201-1210
### Table – A one-week hormonal intervention and effects on postprandial and fasting metabolic parameters in healthy young men.

<table>
<thead>
<tr>
<th>Sex steroids</th>
<th>Before</th>
<th>After</th>
<th>P-value*</th>
<th>Before</th>
<th>After</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/dL)</td>
<td>495 ± 138</td>
<td>988 ± 137</td>
<td>&lt;0.001</td>
<td>425 ± 137</td>
<td>246 ± 127</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free T (ng/dL)</td>
<td>8.8 ± 2.0</td>
<td>21.5 ± 4.9</td>
<td>&lt;0.001</td>
<td>9.5 ± 2.2</td>
<td>5.3 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>41 ± 18</td>
<td>39 ± 16</td>
<td>0.11</td>
<td>32 [22 ; 40]</td>
<td>32 [26 ; 43]</td>
<td>0.33</td>
</tr>
<tr>
<td>Estradiol (pg/mL)†</td>
<td>20.5 [16.8 ; 23.0]</td>
<td>8.9 [8.5 ; 9.4]</td>
<td>0.005</td>
<td>16.3 [15.1 ; 19.8]</td>
<td>19.4 [15.9 ; 41.3]</td>
<td>0.059</td>
</tr>
<tr>
<td>Free E2 (pg/mL)†</td>
<td>0.37 [0.30 ; 0.42]</td>
<td>0.18 [0.17 ; 0.19]</td>
<td>0.005</td>
<td>0.30 [0.28 ; 0.38]</td>
<td>0.36 [0.28 ; 0.76]</td>
<td>0.074</td>
</tr>
<tr>
<td>Postprandial response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose response (mg/dL/min)‡</td>
<td>1.20</td>
<td>1.25</td>
<td>0.11</td>
<td>1.20</td>
<td>1.19</td>
<td>0.65</td>
</tr>
<tr>
<td>C-peptide response (ng/mL/min)‡</td>
<td>0.070</td>
<td>0.069</td>
<td>0.71</td>
<td>0.070</td>
<td>0.069</td>
<td>0.62</td>
</tr>
<tr>
<td>GLP-1 response (pM/min)‡</td>
<td>0.57</td>
<td>0.59</td>
<td>0.55</td>
<td>0.57</td>
<td>0.60</td>
<td>0.37</td>
</tr>
<tr>
<td>GIP response (pM/min)‡</td>
<td>1.19</td>
<td>1.24</td>
<td>0.047</td>
<td>1.19</td>
<td>1.17</td>
<td>0.37</td>
</tr>
<tr>
<td>Triglyceride response (mg/dL/min)‡</td>
<td>0.50</td>
<td>0.44</td>
<td>0.036</td>
<td>0.50</td>
<td>0.54</td>
<td>0.010</td>
</tr>
<tr>
<td>Fasting measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>0.88 ± 0.15</td>
<td>0.84 ± 0.08</td>
<td>0.40</td>
<td>0.84 ± 0.06</td>
<td>0.86 ± 0.12</td>
<td>0.56</td>
</tr>
<tr>
<td>Insulin (IU/L)†</td>
<td>5.1 [3.2 ; 8.7]</td>
<td>4.3 [2.5 ; 7.1]</td>
<td>0.17</td>
<td>5.0 [3.7 ; 8.4]</td>
<td>5.8 [4.5 ; 7.2]</td>
<td>0.58</td>
</tr>
<tr>
<td>TG (mg/dL)†</td>
<td>79 [61; 139]</td>
<td>75 [56; 92]</td>
<td>0.12</td>
<td>82 [56; 140]</td>
<td>81 [70; 145]</td>
<td>0.68</td>
</tr>
<tr>
<td>Euglycemic hyperinsulinemic clamp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean glycemic level (mmol/L)</td>
<td>5.1 ± 0.27</td>
<td>4.9 ± 0.15</td>
<td>0.11</td>
<td>4.9 ± 0.27</td>
<td>4.9 ± 0.15</td>
<td>0.70</td>
</tr>
<tr>
<td>G.I.R. (ml/h)</td>
<td>167 ± 65</td>
<td>199 ± 65</td>
<td>0.051</td>
<td>194 ± 53</td>
<td>194 ± 49</td>
<td>0.99</td>
</tr>
<tr>
<td>M-valueLBM (µmol/min/kgLBM)</td>
<td>51.3 ± 21.5</td>
<td>61.3 ± 21.9</td>
<td>0.042</td>
<td>60.4 ± 16.4</td>
<td>60.3 ± 14.5</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Data are means ± SD or median [1st ; 3rd quartile] in case of non-Gaussian distribution. *According to paired student’s t-test; †According to Wilcoxon signed rank test; ‡Longitudinal mixed-effects modeling