Sex Steroids Affect Triglyceride Handling, Glucose-Dependent Insulinotropic Polypeptide, and Insulin Sensitivity

A 1-week randomized clinical trial in healthy young men

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OBJECTIVE — To evaluate metabolic effects of sex steroids in nonfasting and fasting conditions, independent from changes in body composition.

RESEARCH DESIGN AND METHODS — A randomized clinical trial was performed to create contrasting sex steroid levels in healthy young men: by letrozole (aromatase inhibitor) to lower estradiol (E_2) and increase testosterone (group T, $n = 10$) versus letrozole plus E_2 patches to lower T and raise E_2 (group E, $n = 10$). Mixed meals and hyperinsulinemic-euglycemic clamps were performed before and after a 1-week treatment period.

RESULTS — Following intervention, the postprandial triglyceride response displayed a diverging response with a decline in group T and an increase in group E; the postprandial glucose-dependent insulinotropic polypeptide (GIP) response increased in group T. Insulin sensitivity increased in group T but 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.

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Low testosterone has been shown to be a strong predictor of metabolic syndrome in men aged 20–40 years, although underlying biological mechanisms are poorly understood (1,2). Typically, low testosterone in the presence of disturbed glucose metabolism is combined with high estradiol (E_2) (3). Modifying steroid levels into low E_2 and high testosterone, by an aromatase inhibitor (AI) reducing conversion of testosterone into E_2 through aromatase (P450a), results in decreased fasting glucose and insulin levels in young men (4). Yet, nonfasting conditions might be relevant as well since 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 nonfasting conditions; men were randomly treated with an AI to lower E_2 and increase testosterone (group T) or an AI and E_2 patches to lower testosterone and raise E_2 (group E). E_2 patches in men cause downregulation of testosterone 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 years) were randomized to receive either 2.5 mg letrozole (Femara; Novartis, Basel, Switzerland) (group T, $n = 10$) or letrozole plus Dermestril, exemption 75 μg/day (Besins, Brussels, 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 1-week treatment period were performed in similar conditions, starting at 0800 h after overnight fasting and a 10-min bed rest at the hospital. Hyperinsulinemic-euglycemic clamps were initiated by a primed-continuous insulin infusion and fixed at 40 $mIU/m^2_{body\ surface\ area}$/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 min, 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 min and corrected for lean body mass (dual-energy X-ray absorptiometry). Thirty minutes after the clamp, a standard mixed meal (bread, margarine, cheese, and milk), providing a caloric content of 1,000 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 min after ingestion. Triglycerides, glucose, and C-peptide serum concentrations were determined using standard laboratory assays (modular immunoassay; Roche Diagnostics, Penzberg, Germany). Intra- and interassay coefficients of variation for all parameters were <3 and 6%, respec-

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Table 1—*A 1-week hormonal intervention and effects on postprandial and fasting metabolic parameters in healthy young men*

	Group T (letrozole only) (n = 10)			Group E (letrozole + E_2 patches) (n = 10)		
Sex steroids	Before	After	P value*	Before	After	P value*
Testosterone (ng/dl)	495 ± 138	988 ± 137	<0.001	425 ± 137	246 ± 127	<0.001
Free testosterone (ng/dl)	8.8 ± 2.0	21.5 ± 4.9	<0.001	9.5 ± 2.2	5.3 ± 2.7	<0.001
Sex hormone–binding globulin (nmol/l)	41 ± 18	39 ± 16	0.11	32 (22–40)	32 (26–43)	0.33
Estradiol (pg/ml)†	20.5 (16.8–23.0)	8.9 (8.5–9.4)	0.005	16.3 (15.1–19.8)	19.4 (15.9–41.3)	0.059
Free E_2 (pg/ml)†	0.37 (0.30–0.42)	0.18 (0.17–0.19)	0.005	0.30 (0.28–0.38)	0.36 (0.28–0.76)	0.074
Postprandial response						
Glucose response (mg/dl/min)‡	1.20	1.25	0.11	1.20	1.19	0.65
C-peptide response (ng/ml/min)‡	0.070	0.069	0.71	0.070	0.069	0.62
Glucagon-like peptide 1 response (pM/min)‡	0.57	0.59	0.55	0.57	0.60	0.37
GIP response (pM/min)‡	1.19	1.24	0.047	1.19	1.17	0.37
Triglyceride response (mg/dl/min)‡	0.50	0.44	0.036	0.50	0.54	0.010
Fasting measurements						
Glucose (mg/dl)	0.88 ± 0.15	0.84 ± 0.08	0.40	0.84 ± 0.06	0.86 ± 0.12	0.56
Insulin (IU/l)†	5.1 (3.2–8.7)	4.3 (2.5–7.1)	0.17	5.0 (3.7–8.4)	5.8 (4.5–7.2)	0.58
Triglycerides (mg/dl)†	79 (61–139)	75 (56–92)	0.12	82 (56–140)	81(70–145)	0.68
Euglycemic-hyperinsulinemic clamp						
Mean glycemic level (mmol/l)	5.1 ± 0.27	4.9 ± 0.15	0.11	4.9 ± 0.27	4.9 ± 0.15	0.70
GIR (ml/h)	167 ± 65	199 ± 65	0.051	194 ± 53	194 ± 49	0.99
M value$_{LBM}$ (μmol/min/kg$_{LBM}$)	51.3 ± 21.5	61.3 ± 21.9	0.042	60.4 ± 16.4	60.3 ± 14.5	0.99

Data are means ± SD or median (1st–3rd quartile) in case of non-Gaussian distribution. *According to paired Student t test. †According to Wilcoxon signed-rank test. ‡Longitudinal mixed-effects modeling.

tively. Commercial immunoassays were used to determine serum E_2 (Incstar, Stillwater, MN), testosterone, and sex hormone–binding globulin (Orion Diagnostica, Espoo, 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 postmeal responses and were performed using the SPSS 12.0 software package (SPSS, Chicago, IL) and SAS 9.1.3 (SAS Institute, Cary, North Carolina).

RESULTS — All subjects were Caucasian, and no anthropometric differences were observed between both groups (age 34 ± 7 and 31 ± 5 years; BMI 22.4 ± 2.4 and 23.4 ± 2.2 kg/m^2; waist-to-hip ratio 0.87 ± 0.05 and 0.86 ± 0.04, group T and E, respectively) (Table 1).

Sex steroid changes

Following intervention, E_2 levels decreased by 56% and testosterone levels increased by 114% in group T, whereas in group E, testosterone levels declined by 44% and total E_2 levels increased by 43%. Adverse events or side effects did not occur.

Nonfasting measurements

Following intervention, postprandial responses of glucose, C-peptide, and glucagon-like peptide 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 triglyceride 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 nonfasting conditions in healthy young men.

Concomitantly increasing testosterone and decreasing E_2 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 weeks of AI in young men (4) and corroborates a former report (10) showing acute reduction in insulin sensitivity 2 weeks after discontinuing testosterone replacement in hypogonadal men. It remains unclear whether these observations result from changes in lipid metabolism or altered postreceptor insulin signaling in muscle (11) and whether improved insulin sensitivity enhances muscle lipid uptake (12). Further, this same intervention increased postprandial glucose-dependent insulinotropic polypeptide (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 testosterone and relatively high E_2 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 E_2. These findings were demonstrated in a limited

number of healthy male subjects, necessitating confirmation and extension to populations at risk (those who are obese, those with disturbed glucose metabolism, and the 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.

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