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Diabetes & Metabolism 40 (2014) 439–444

Original article

Serum sex steroids and steroidogenesis-related enzyme expression in skeletal muscle during experimental weight gain in men

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Received 29 August 2013; received in revised form 24 March 2014; accepted 25 March 2014
Available online 30 April 2014

Abstract

Objectives. – Low-circulating testosterone is associated with development of type 2 diabetes in obese men. In this study, we examined the effects of experimental overfeeding and weight gain on serum levels of sex hormones and skeletal muscle expression of steroidogenic enzymes in healthy men with (FH+) and without (FH–) a family history of type 2 diabetes.

Methods. – Following a 3-day lead in energy balanced diet, FH+ ($n=9$) and FH– men ($n=11$) were overfed by 5200 kJ/day (45% fat) for 28 days. Body weight, fasting glucose, insulin, sex steroid, sex hormone binding globulin (SHBG) levels, insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) and body fat (DXA) were assessed in all individuals at baseline and day 28, and sex steroidogenesis-related enzyme expression in *vastus lateralis* biopsies was examined in a subset ($n=11$).

Results. – Body weight, fat mass and fasting insulin levels were increased by overfeeding ($P<0.01$) and insulin was increased significantly more in FH+ men ($P<0.01$). Serum sex hormone binding globulin (SHBG) and 5α-dihydrotestosterone (DHT) were reduced with overfeeding ($P<0.05$), and serum testosterone and DHT were reduced to a greater extent in FH+ men ($P<0.05$). Overfeeding reduced mRNA expression of 3β-hydroxysteroid dehydrogenase (HSD) and 17βHSD ($P\leq 0.007$), independently of group. 5α-Reductase (SRD5A1) mRNA expression was not changed overall, but a time by group interaction was observed ($P=0.04$).

Conclusion. – Overfeeding reduced SHBG and muscle expression of enzymes involved in the formation of testosterone in skeletal muscle. Men with a family history of T2DM were more susceptible to deleterious outcomes of overfeeding with greater reductions in serum testosterone and DHT and greater increases in markers of insulin resistance, which may contribute to increased risk of developing type 2 diabetes.

Keywords: Insulin resistance; Overfeeding, Serum testosterone; Steroidogenic enzymes

1. Introduction

Obesity, and particularly visceral adiposity in conjunction with reduced muscle mass, is associated with the metabolic syndrome and an increased risk of type 2 diabetes mellitus (T2DM) [1]. In men, obesity is associated with reduced serum sex hormone binding globulin (SHBG) and testosterone concentrations while those of serum estradiol and estrone are increased [2]. A meta-analysis of 52 studies showed that men in the lowest quartile for serum testosterone or SHBG had higher risk of metabolic syndrome versus men in the highest quartile [3]. In the majority of these studies, the relationship holds following adjustment with BMI, although typically adjustments were not made for visceral adiposity. Low serum testosterone also independently predicts progression to T2DM in overweight men [4,5]. Thus, increasing evidence supports a role for low testosterone in pro-

Abbreviations: T2DM, Type 2 diabetes mellitus; FH, Family History; DHEA, Dehydroepiandrosterone; DHEA-S, Dehydroepiandrosterone and its sulfate derivate; DHT, 5α-Dihydrotestosterone; 3β-HSD, 3β-Hydroxysteroid dehydrogenase; 17β-HSD, 17β-Hydroxysteroid dehydrogenase; DHT, 5α-Dihydrotestosterone; SRD5A1, 5α-reductase type 1.

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http://dx.doi.org/10.1016/j.diabet.2014.03.006
1262-3636/

moting insulin resistance and type 2 diabetes, although causal evidence is lacking.

Obese men with T2DM also have lower serum levels of dehydroepiandrosterone (DHEA) and its sulfate derivate (DHEA-S) [6,7]. DHEA is primarily produced by the adrenal gland, but several studies have demonstrated that other tissues, including human and rodent skeletal muscle can produce some DHEA [8–10]. DHEA is converted to testosterone by 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD. DHEA-S is a terminal steroid that cannot be further biotransformed into active steroid metabolites. Testosterone is converted in a tissue-specific manner to either 5α-dihydrotestosterone (DHT), a more potent ligand for the androgen receptor by 5α-reductase (SRD5A) [11], or oestradiol by aromatase. There are two isoforms of SRD5A (designated type 1 and type 2) that exhibit tissue-specific expression patterns with the type 1 isoform broadly distributed and the type 2 isoform found predominantly in male sex-accessory tissue, such as epididymis, the prostate and seminal vesicles. Aromatase is also found in extragonadal sites, including brain, bone and adipose tissue and its activity in adipose tissue is elevated in obesity [12]. In skeletal muscle, metabolic enzymes 3β-HSD, 17β-HSD, and SRD5A1 as well as synthesis of DHT and testosterone from DHEA have all been reported [10,13]. In skeletal muscle, reduced levels of DHEA and SRD5A1 are observed in obese men with T2DM and in rats fed with a high sucrose diet, which can be reversed with DHEA treatment [6,7]. DHEA treatment also activates the insulin signalling pathway and reduces blood glucose levels in streptozotocin-induced diabetic mice [14]. These studies suggest that local sex steroidogenesis may have direct effects on insulin sensitivity, at least in rodents.

First-degree relatives of individuals with a family history of T2DM (FH+) are at increased risk of developing T2DM [15,16] and have a greater tendency towards insulin resistance [16,17], central adiposity [18], inflammation [19], and reduced mitochondrial function [20,21]. Low serum testosterone is also associated with greater risk of developing metabolic syndrome in men with a family history of type 2 diabetes [22]. The effects of short term experimental overfeeding on testosterone and other sex steroid hormone levels as well as local muscle steroidogenesis in men with and without a family history of T2DM has not previously been tested. Here, we hypothesised that overfeeding would reduce serum testosterone levels as well as local expression of muscular steroidogenesis-related enzymes 3βHSD, 17β-HSD and SRD5A1, and that these effects would be greater in men with a family history of T2DM.

2. Methods

2.1. Subjects and metabolic tests

This study protocol was approved by the Human Research and Ethics Committee at Saint-Vincent's Hospital, Sydney and subjects provided informed written consent before commencement of the study, which were conformed to the standards set by the Declaration of Helskinki. The original study design is described in detail previously [23], and included 20 women and 20 men. This analysis includes all 20 men that were studied in the original cohort, and 9 reported at least one first-degree relative with type 2 diabetes [23]. Subjects were excluded if their weight had changed by > 2 kg in the preceding 6 months, if they exercised more than 60 min per week, if they were taking medications known to affect insulin sensitivity, lipid metabolism or blood pressure, or if they had a personal history of type 2 diabetes or cardiovascular disease. Insulin sensitivity was measured as the glucose infusion rate (GIR) necessary to maintain euglycaemia for the final 30 min of a 2 h hyperinsulinaemic clamp (60 $mUm^{-2}min^{-1}$) and was adjusted for fat free mass by dual X-ray absorptiometry (Hologics, USA), and abdominal fat distribution and liver fat was assessed by computed tomography (GE Healthcare) as previously described [23].

2.2. Diets

Estimated energy requirements were calculated for each participant using equations previously generated by doubly labeled water and intake balance techniques [24]. A trained dietitian then planned individual menus for the participants. All foods were provided at baseline energy requirements with a nutrient composition of 30% of energy as fat, 15% as protein and 55% as carbohydrate for 3 days prior to baseline metabolic testing. All foods were also provided from day 0–3 and day 25–28 of the study. On days 3–25 of overfeeding, participants were instructed to consume their regular diets and were provided with high fat snacks to achieve an intake of 5200 KJ/day above baseline. They were required to complete a checklist every day, reporting which snacks were consumed, complete 3-day diet diaries once before study commencement and twice during the overfeeding phase, and to meet with the study dietitian weekly. Diets were analysed for macronutrients and fatty acid composition using FoodWorks 2007 based on the Australian foods database (Xyris Software, QLD, Australia).

2.3. Gene expression

Muscle biopsies were carried out according to previously described techniques in a subset of men ($n = 11$, 4 FH–and 7 FH+) [25]. Total tissue RNA was isolated using Trizol according to previously described studies [25] and the quality and amounts were assessed by nanodrop. Total tissue RNA was reverse transcribed by omniscript reverse transcriptase using a Quantitec cDNA synthesis kit according to the manufactures instructions (Qiagen, California, USA). qPCR was carried out using a PCR thermal cycler with Fast Universal Master Mix according to the manufactures recommendations (Applied Biosystems, CA). The gene pecific primers for 3β-HSD [Hs04194787_g1], 17β-HSD type 3 [Hs00609319_m1], SRD5A1 type 1 [Hs00602691_mH] were purchased (Applied Biosystems, CA). Results are normalised to GAPDH [Hs02758991_g1], which was not changed by overfeeding and was not significantly different between groups.