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Testosterone treatment increases androgen receptor and aromatase gene expression in myotubes from patients with PCOS and controls, but does not induce insulin resistance

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ABSTRACT

Polycystic ovary syndrome (PCOS) is associated with insulin resistance and increased risk of type 2 diabetes. Skeletal muscle is the major site of insulin mediated glucose disposal and the skeletal muscle tissue is capable to synthesize, convert and degrade androgens. Insulin sensitivity is conserved in cultured myotubes (*in vitro*) from patients with PCOS, but the effect of testosterone on this insulin sensitivity is unknown. We investigated the effect of 7 days testosterone treatment (100 nmol/l) on glucose transport and gene expression levels of hormone receptors and enzymes involved in the synthesis and conversion of testosterone (*HSD17B1*, *HSD17B2*, *CYP19A1*, *SRD5A1-2*, *AR*, *ER-α*, *HSD17B6* and *AKR1-3*) in myotubes from ten patients with PCOS and ten matched controls.

Testosterone treatment significantly increased aromatase and androgen receptor gene expression levels in patients and controls. Glucose transport in myotubes was comparable in patients with PCOS vs. controls and was unchanged by testosterone treatment ($p = 0.21$ PCOS vs. controls). These results suggest that testosterone treatment of myotubes increases the aromatase and androgen receptor gene expression without affecting insulin sensitivity and if testosterone is implicated in muscular insulin resistance in PCOS, this is by and indirect mechanism.

1. Introduction

Polycystic ovary syndrome (PCOS) is characterized by anovulation, hyperandrogenemia and/or polycystic ovaries [1], and affects 5–8% of premenopausal women [2,3]. Insulin resistance is present in more than 50% of patients with PCOS [4] but the pathogenesis behind this insulin resistance remains to be understood. We previously reported comparable glucose transport, glycogen synthesis, glycogen synthase activity and additional metabolic pathways in myotubes from patients with PCOS compared to controls [5]. Skeletal muscle biopsies from these patients did however display significantly impaired insulin activation of glycogen synthase [6]. This led us to suggest that the mechanisms governing insulin resistance is not primary, but rather adaptive [5]. A possible factor contributing to what seems to be an adaptive induction of muscular insulin resistance is hyperandrogenemia. The Androgen Excess Society considers androgen excess to be a crucial point in the PCOS pathogenesis [7]. However, a possible mechanism by which androgens as testosterone could be implicated in the muscular insulin resistance in PCOS has not been clearly established. Testosterone may affect the insulin sensitivity of several tissues and cell types, hence testosterone reduced glucose uptake in cultured female adipocytes and endometrial cells at basal and maximal insulin stimulation [8,9].

Abbreviations: DHT, dihydrotestosterone; NOGD, non-oxidative glucose disposal; HI, high insulin; I, insulin.

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In this context, the peripheral steroidogenesis, conversion and degradation of testosterone and related precursors, may be relevant in relation to disease. Genes encoding steroid hormone enzymes were differentially expressed in subcutaneous adipose tissue from patients with PCOS vs. controls [10]. A modified intracellular steroidogenesis in myotubes could potentially result in altered intracellular levels of androgens and estrogens, which then again could affect glucose metabolism. If patients with PCOS harbour changes in the intracellular steroidogenesis in myotubes, androgen excess may stress the effect of these changes. Skeletal muscle expresses steroidogenic and androgen converting enzymes, and conversion of testosterone to dihydrotestosterone (DHT) [11] along with the activation of glucose metabolism related pathway by DHT, was detected in cultured rat skeletal muscle cells [12].

To our knowledge, the gene expression of steroidogenic enzymes has not been investigated in myotubes from PCOS. It is therefore unknown, whether the expression of these genes responds differently to testosterone treatment in patients with PCOS vs. healthy control subjects. It is furthermore not established, if testosterone treatment induces insulin resistance in myotubes from patients with PCOS. Cultured human myotubes are considered a well-established model when discriminating between genetic and environmental factors in the etiology of insulin resistance [13].

In the present study we aimed to investigate if testosterone treatment of myotubes from patients with PCOS and controls altered glucose transport and induced insulin resistance. In addition, we aimed to investigate if testosterone treatment affected the gene expression of steroid hormone receptors and steroidogenic enzymes in myotubes established from patients with PCOS compared to controls.

2. Materials and methods

2.1. Patients and controls

Ten white patients diagnosed with PCOS according to the Rotterdam criteria. Seven of the ten patients fulfilled the Rotterdam criteria for hyperandrogenemia (clinical or biochemical hyperandrogenism) [1]. Ten healthy, weight- and age-matched white women were included as controls. Patients and controls were previously described [5,6,14–17]. All controls had regular menses (period lengths 28–34 days) and did not suffer from hyperandrogenemia or hirsutism.

Patients and controls paused oral contraceptives for at least three months before evaluation, and did not use any medication known to affect hormonal or metabolic parameters. The study was approved by the local ethics committee (The Scientific Ethical Committee for Vejle and Funen Counties now referred to as The Scientific Ethical Committee of the Region of Southern Denmark) and all subjects gave written informed consent. The work was carried out in accordance with the Helsinki Declaration.

2.2. Clinical and biochemical analyses

Blood sampling and transvaginal ultrasound were performed during the follicular phase in patients with oligomenorrhea and in healthy controls. Patients with amenorrhea (period length > 3 - months) were examined randomly. Patients with diabetes (fasting plasma glucose $\geqslant$ 7.0 mmol/l), hypertension, elevated liver enzymes, s-prolactin or s-TSH outside reference interval, renal dysfunction, and congestive heart disease were not included in the study.

The free testosterone levels were evaluated as previously described [18] and analyzed at Statens Serum Institut (Copenhagen, Denmark). Free testosterone was calculated from total testosterone and SHBG levels according to Vermeulen et al. [19]. The analyses of total testosterone and SHBG levels along with the inter- and intra-assay variability were previously described [5,20].

2.3. Euglycemic hyperinsulinemic clamp

The clamp protocol and calculations was previously described [5,15]. Muscle biopsies were obtained from the vastus lateralis muscle of patients with PCOS and controls using a modified Bergström needle with suction under local anaesthesia. Biopsies were immediately blotted free of blood, fat and connective tissue, and cells were immediately isolated.

2.4. Calculations

The rates of glucose appearance and glucose disposal were calculated as described in [5,15], with the stated assumptions (a glucose distribution volume of 200 ml/kg body weight and a pool fraction of 0.65).

2.5. Myotube cultures

The establishment of cell cultures from muscle biopsies and cell culture conditions were described in [21,22]. Muscle tissue was minced, washed and dissociated for 60 min by three treatments with 0.05% trypsin-EDTA (Life Technologies, Naerum, Denmark). The cells obtained were seeded for up-scaling on extracellular matrix (ECM) gel (Sigma–Aldrich, Broendby, Denmark) coated dishes after 30 min of pre-plating. Growth medium contained Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Naerum, Denmark) supplemented with 2% heat inactivated fetal bovine serum (FBS) (Sigma–Aldrich), 2% Ultroser G (Pall Biopharmaceuticals, Cedex, France), 50 U/ml penicillin (Life Technologies), 50 mg/ml streptomycin (Life Technologies) and 1.25 mg/ml amphotericin B (Life Technologies). Cells were sub cultured twice before final seeding. At 75% confluence, growth medium was replaced by basal medium (DMEM without phenol red supplemented with 2% charcoal-stripped FBS (Sigma–Aldrich), 50 U/ml penicillin, 50 mg/ml streptomycin, 1.25 mg/ml amphotericin B, 25.03 mM HEPES (Life Technologies), 3.97 mM GlutaMAX (Life Technologies), supplemented with 25 pmol/l insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) in order to induce differentiation. After 4 days of differentiation, cell cultures were added either testosterone (Sigma–Aldrich) (100 nmol/l; total ethanol concentration: 0.1%) or ethanol alone (0.1%) for 7 days. This testosterone concentration has previously been demonstrated to induce insulin resistance in human adipocytes and endometrial cells [8,9]. During the 7 days testosterone treatment, cell cultures were constantly kept under physiological conditions of insulin (25 pmol/l) and glucose (5.5 mmol/l) and the medium was changed every 2–3 day.

Throughout the study, DMEM without phenol red (Life Technologies, Naerum, Denmark) and charcoal-stripped FBS (Sigma–Aldrich, Broendby, Denmark) were used to avoid estrogenic effects from phenol red and exogenous hormones from FBS.

2.6. Glucose transport

Glucose uptake was measured by capturing 2-[1-14C]-deoxyglucose, as previously described [13,23]. 25 pmol/l and 1 μmol/l insulin (Actrapid, Novo Nordisk) was used to study baseline and high insulin stimulated glucose transport, respectively. Radioactivity was determined with a Microbeta counter (PerkinElmer, Finland).