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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).

2.7. RNA isolation and cDNA preparation

Cells were washed twice in Dulbecco's PBS (without Ca/Mg) (Fisher Scientific, Slagerup, Denmark) before total RNA extraction with TRIzol (TRIzol® Reagent, Life Technologies). RNA concentration, purity and integrity was determined using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and BioAnalyzer 2100 (Agilent Technologies, Santa Clara, USA). cDNA was synthesized according to manufactures manual from 1 μg of isolated RNA with the AffinityScript QPCR cDNA Synthesis Kit (Agilent Technologies, Aarhus, Denmark). No reverse transcriptase (no RT) controls were included for each study subject.

2.8. Reverse transcriptase quantitative PCR (RT-qPCR)

The gene expression of 11 hormone receptors or steroidogenic enzymes was determined by qPCR. The qPCR reactions were carried out with SYBR green (Life Technologies) according to manufactures manual and run at Applied Biosystems 7300 Real-Time PCR System (Life Technologies), using the following PCR conditions: 48 °C for 2 min and 95 °C for 10 min. and 40 cycles of 95 °C for 15 s and 60–65 °C for 1 min. Reactions were run in duplicates with a no RT control. *HPRT1* and *ATP50* were used as reference genes for normalization of gene expression. Information on the primers is listed in Supplementary Table 1. For each gene, the individual sample with the lowest expression was set to 1. qPCR results were analyzed with the qBase program.

2.9. Statistical analyses

Statistical analyses were performed with SPSS version 19 and free software package R [24]. The non-parametric Mann–Whitney *U* test was used to compare clinical and biochemical characteristics. The generalized estimation equation (GEE) model was applied to determine the overall effects of disease status, insulin stimulation and testosterone treatment on absolute glucose transport with each individual as one cluster. The GEE model estimated the influences of testosterone treatment and disease status, together with their interaction, on the levels of gene expression. Log_{10} transformation was applied to the normalized gene expression data. Results were presented as means ± s.e.m. *p*-values < 0.05 were considered significant.

3. Results

3.1. Clinical and biochemical characteristics

Table 1 shows the clinical and biochemical characteristics of patients with PCOS compared to controls. Patients with PCOS differed significantly from controls in free testosterone levels, fasting insulin, glucose oxidation and non-oxidative glucose disposal (NOGD).

3.2. Glucose transport

High insulin (HI) stimulation overall significantly increased the absolute glucose transport in patients and controls ($p = 0.034$), whereas testosterone treatment or disease status had no significant effects ($p = 0.21$ and $p = 0.08$, respectively) (Fig. 1A). The absolute glucose transport in testosterone untreated myotubes was comparable between patients and controls during baseline insulin (I) ($p = 0.26$) and HI stimulation ($p = 0.19$).

Table 1
Clinical and biochemical characteristics of patients with PCOS and controls.

Characteristic	Controls ($n = 10$)	PCOS ($n = 10$)
Age (years)	37 (29–41)	32 (23–35.5)
BMI (kg/m^2)	32.1 (28.7–37.9)	31.6 (27.5–37)
Free testosterone (nmol/l)	0.024 (0.017–0.03)	0.034 (0.027–0.05)*
Fasting insulin (pmol/l)	35 (23–49)	64 (55–76)*
Fasting glucose (mmol/l)	5.5 (5.2–5.9)	5.4 (5.2–5.9)
Clamp		
Glucose oxidation (mg/min/m^2)	122 (113–134)	91 (84–111)*
NOGD (mg/min/m^2)	164 (97–252)	81 (54–96)*

Data are presented as medians (25–75% quartiles). *p*-values were calculated by Mann–Whitney *U*-test.
* $p < 0.05$ vs. controls.

3.3. Gene expression of steroidogenic and degradation enzymes in myotubes

The relative gene expression levels were detected for the following genes: *HSD17B1*, *HSD17B2*, *CYP19A1*, *SRD5A1*, *SRD5A2*, *AR*, *ER-α*, *HSD17B6*, *AKR1C1*, *AKR1C2* and *AKR1C3* (Fig. 1B).

In both patients and controls, testosterone significantly increased the gene expression levels of *CYP19A1* ($p = 0.03$) and the androgen receptor (*AR*) ($p = 0.013$). Testosterone treatment had no significant effect on the expression of *HSD17B1*, *HSD17B2*, *SRD5A1*, *SRD5A2*, *ER-α*, *HSD17B6*, *AKR1C1*, *AKR1C2* and *AKR1C3*. Disease status had no significant impact on the gene expression of any genes and no significant interactions between disease status and testosterone treatment was found for any of the genes (data not shown).

4. Discussion

In the present study, we investigated the effect of testosterone treatment on glucose transport and gene expression of key enzymes involved in the synthesis, conversion and degradation of testosterone in myotubes established from patient with PCOS and healthy, matched controls.

We found that testosterone treatment significantly increased gene expression levels of the androgen receptor and the aromatase in both patients and controls. The expressions of the additional genes investigated were unaffected by testosterone and did not differ between patients and controls. These findings suggest that the gene expressions of the key steroidogenic enzymes were independent of disease status and may indicate that the conversion of testosterone in myotubes from patients with PCOS and healthy controls does not differ. It therefore seems unlikely that the *in vivo* detected muscular insulin resistance in our patients (Table 1) – and in PCOS patients in general – is caused by an altered intramuscular steroidogenesis and thus changes in the intramuscular levels of steroid hormones.

In agreement with our previous findings [5] and reported by others [25,26], we found that the *in vivo* detected insulin resistance was not conserved in testosterone untreated myotubes *in vitro*. We found no evidence that testosterone treatment induced insulin resistance in myotubes from neither patients with PCOS nor controls. Previous studies found that testosterone treatment at a lower concentration (10 nmol/l) and at concentrations similar to our study (100 nmol/l), induced insulin resistance in cultured adipocytes from healthy women [8]. In addition, treatment with 100 nmol/l testosterone significantly reduced insulin-stimulated glucose uptake in cultured endometrial cells [9]. In contrast, testosterone treatment (10 nmol/l) of myotubes *in vitro* significantly increased insulin stimulated glucose incorporation into glycogen in healthy female, but not male myotubes [27]. Previous studies reported that treatment with androgen receptor antagonist

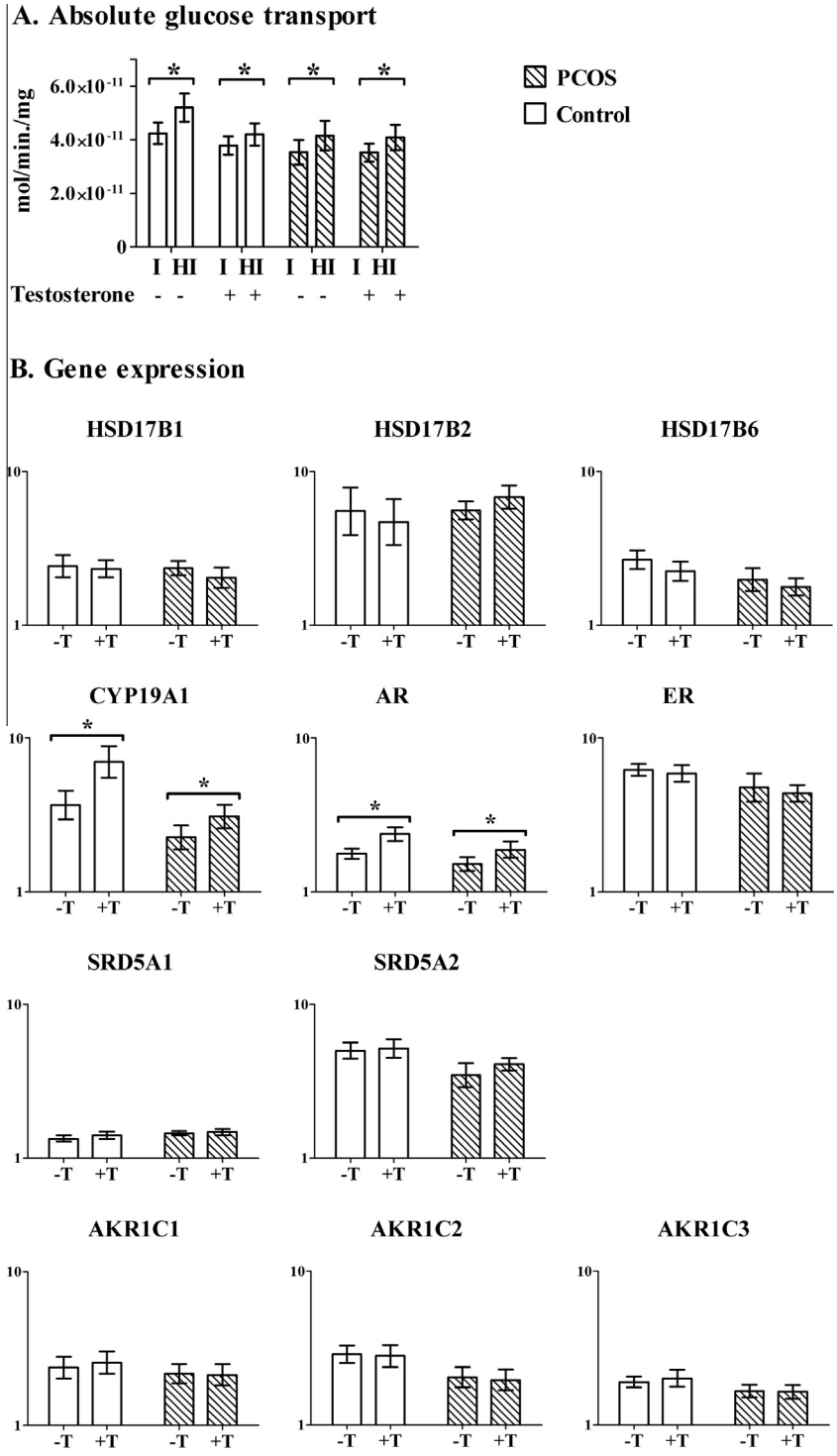

Fig. 1. Effect of *in vitro* testosterone treatment on myotubes established from patients with PCOS and healthy controls. (A) Absolute glucose transport measured in testosterone treated or untreated myotubes established from patients with PCOS and controls during either baseline insulin (25 pmol/l) or high insulin stimulation (1 μmol/l). $^{*}p < 0.05$ – baseline insulin vs. high insulin stimulation. (B) Relative gene expression of hormone receptors and enzymes involved in the synthesis and conversion of testosterone. Within each gene the expression levels were relative to the lowest expression level, which was set to 1. $^{*}p < 0.05$ testosterone treatment vs. no treatment. Results are presented as means of absolute of glucose transport ± s.e.m. I: baseline insulin (25 pmol/l); HI: high insulin (1 μmol/l). $n = 10$ patients with PCOS and $n = 10$ controls.

abolished the effect of testosterone on glucose transport and palmitate oxidation [8,27]. Testosterone furthermore induced a non-significant increase in androgen receptor gene expression levels in female myotubes [27]. These results support the idea that the effect of testosterone on the glucose metabolism is mediated through the androgen receptor [8,27]. Our findings suggest that testosterone affects androgen receptor gene expression levels in myotubes without inducing insulin resistance. These *in vitro* findings may suggest that testosterone is unlikely to be primary responsible for the muscular *in vivo* insulin resistance in patients with PCOS. It may be speculated if testosterone indirectly affects insulin resistance in muscle, rather than exerting a direct effect.

Hence, our study provides no evidence that treatment with testosterone, at a concentration that induced insulin resistance in

other cell types than myotubes [8,9], induced insulin resistance in myotubes established from patients with PCOS or controls.

Considering the complex pathogenesis of PCOS, the absence of insulin resistance in myotubes from PCOS *in vitro* [5] and insufficiency of testosterone to induce insulin resistance on myotubes, the triggering factor for insulin resistance in skeletal muscle may be dependent on the presence of other cell types. A novel study by Ciaraldi et al. suggest that PCOS may represent a state of increased sensitivity of inflammatory cells to cytokines and chemokines, which through increased neutrophil and macrophage infiltration into skeletal muscle could contribute to insulin resistance [28]. It is possible that testosterone has a tissue-specific impact on this sensitivity of inflammatory cells.

Corbould et al. found no differences in the production of IL-6 and TNF-α in preadipocytes, however the study was underpowered to detect a difference in TNF-α, and the study did not investigate the effect of testosterone on cytokine production [29]. We found that testosterone treatment increased aromatase and androgen receptor gene expression levels, but did not induce insulin resistance in myotubes established from either patients with PCOS or healthy, matched controls. This observation suggests that testosterone may not be solely implicated in skeletal muscular insulin resistance in patients with PCOS and if implicated, it may be as a result of an indirect mechanism.

Conflict of interests

The authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.08.033.

References

[1] E.A.-S.P.C.W.G. Rotterdam, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, Fertil. Steril. 81 (2004) 19–25.
[2] R. Azziz, K.S. Woods, R. Reyna, T.J. Key, E.S. Knochenhauer, B.O. Yildiz, The prevalence and features of the polycystic ovary syndrome in an unselected population, J. Clin. Endocrinol. Metab. 89 (2004) 2745–2749.
[3] E.S. Knochenhauer, T.J. Key, M. Kahsar-Miller, W. Waggoner, L.R. Boots, R. Azziz, Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study, J. Clin. Endocrinol. Metab. 83 (1998) 3078–3082.
[4] D. Glintborg, J.E. Henriksen, M. Andersen, C. Hagen, J. Hangaard, P.E. Rasmussen, K. Schousboe, A.P. Hermann, Prevalence of endocrine diseases and abnormal glucose tolerance tests in 340 Caucasian premenopausal women with hirsutism as the referral diagnosis, Fertil. Steril. 82 (2004) 1570–1579.
[5] M. Eriksen, A.D. Porneki, V. Skov, J.S. Burns, H. Beck-Nielsen, D. Glintborg, M. Gaster, Insulin resistance is not conserved in myotubes established from women with PCOS, PLoS One 5 (2010) e14469.
[6] D. Glintborg, K. Hojlund, N.R. Andersen, B.F. Hansen, H. Beck-Nielsen, J.F. Wojtaszewski, Impaired insulin activation and dephosphorylation of glycogen synthase in skeletal muscle of women with polycystic ovary syndrome is reversed by pioglitazone treatment, J. Clin. Endocrinol. Metab. 93 (2008) 3618–3626.
[7] R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H.F. Escobar-Morreale, W. Futterweit, O.E. Janssen, R.S. Legro, R.J. Norman, A.E. Taylor, S.F. Witchel, E. Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen, P. Society, The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report, Fertil. Steril. 91 (2009) 456–488.
[8] A. Corbould, Chronic testosterone treatment induces selective insulin resistance in subcutaneous adipocytes of women, J. Endocrinol. 192 (2007) 585–594.
[9] P. Ormazabal, C. Romero, A.F. Quest, M. Vega, Testosterone modulates the expression of molecules linked to insulin action and glucose uptake in endometrial cells, Horm. Metab. Res. 45 (2013) 640–645.
[10] L. Wang, S. Li, A. Zhao, T. Tao, X. Mao, P. Zhang, W. Liu, The expression of sex steroid synthesis and inactivation enzymes in subcutaneous adipose tissue of PCOS patients, J. Steroid Biochem. Mol. Biol. 132 (2012) 120–126.
[11] K. Aizawa, M. Iemitsu, S. Maeda, S. Jesmin, T. Otsuki, C.N. Mowa, T. Miyauchi, N. Mesaki, Expression of steroidogenic enzymes and synthesis of sex steroid hormones from DHEA in skeletal muscle of rats, Am. J. Physiol. Endocrinol. Metab. 292 (2007) E577–E584.
[12] K. Sato, M. Iemitsu, K. Aizawa, R. Ajisaka, Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E961–E968.
[13] M. Gaster, I. Petersen, K. Hojlund, P. Poulsen, H. Beck-Nielsen, The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity, Diabetes 51 (2002) 921–927.
[14] K. Hojlund, D. Glintborg, N.R. Andersen, J.B. Birk, J.T. Treebak, C. Frosig, H. Beck-Nielsen, J.F. Wojtaszewski, Impaired insulin-stimulated phosphorylation of Akt and AS160 in skeletal muscle of women with polycystic ovary syndrome is reversed by pioglitazone treatment, Diabetes 57 (2008) 357–366.
[15] D. Glintborg, A.P. Hermann, M. Andersen, C. Hagen, H. Beck-Nielsen, J.D. Veldhuis, J.E. Henriksen, Effect of pioglitazone on glucose metabolism and luteinizing hormone secretion in women with polycystic ovary syndrome, Fertil. Steril. 86 (2006) 385–397.
[16] D. Glintborg, K. Hojlund, M. Andersen, J.E. Henriksen, H. Beck-Nielsen, A. Handberg, Soluble CD36 and risk markers of insulin resistance and atherosclerosis are elevated in polycystic ovary syndrome and significantly reduced during pioglitazone treatment, Diabetes Care 31 (2008) 328–334.
[17] M.B. Eriksen, A.D. Minet, D. Glintborg, M. Gaster, Intact primary mitochondrial function in myotubes established from women with PCOS, J. Clin. Endocrinol. Metab. 96 (2011) E1298–E1302.
[18] D. Glintborg, H. Mumm, D. Hougaard, P. Ravn, M. Andersen, Ethnic differences in Rotterdam criteria and metabolic risk factors in a multiethnic group of women with PCOS studied in Denmark, Clin. Endocrinol. (Oxf.) 73 (2010) 732–738.
[19] A. Vermeulen, L. Verdonck, J.M. Kaufman, A critical evaluation of simple methods for the estimation of free testosterone in serum, J. Clin. Endocrinol. Metab. 84 (1999) 3666–3672.
[20] G. Lykkesfeldt, P. Bennett, A.E. Lykkesfeldt, S. Micic, S. Moller, B. Svenstrup, Abnormal androgen and oestrogen metabolism in men with steroid sulphatase deficiency and recessive X-linked ichthyosis, Clin. Endocrinol. (Oxf.) 23 (1985) 385–393.
[21] M. Gaster, S.R. Kristensen, H. Beck-Nielsen, H.D. Schroder, A cellular model system of differentiated human myotubes, APMIS 109 (2001) 735–744.
[22] M. Gaster, H. Beck-Nielsen, H.D. Schroder, Proliferation conditions for human satellite cells. The fractional content of satellite cells, APMIS 109 (2001) 726–734.
[23] M. Gaster, H. Beck-Nielsen, The reduced insulin-mediated glucose oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin – evidence from cultured myotubes, Biochim. Biophys. Acta 1690 (2004) 85–91.
[24] T.R.P.f.S. Computing, Free software package R, 2014.
[25] T.P. Ciaraldi, V. Aroda, S. Mudaliar, R.J. Chang, R.R. Henry, Polycystic ovary syndrome is associated with tissue-specific differences in insulin resistance, J. Clin. Endocrinol. Metab. 94 (2009) 157–163.
[26] A. Corbould, Y.B. Kim, J.F. Youngren, C. Pender, B.B. Kahn, A. Lee, A. Dunaif, Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E1047–E1054.
[27] F. Salehzadeh, A. Rune, M. Osler, L. Al-Khalili, Testosterone or 17{beta}-estradiol exposure reveals sex-specific effects on glucose and lipid metabolism in human myotubes, J. Endocrinol. 210 (2011) 219–229.
[28] T.P. Ciaraldi, V. Aroda, S.R. Mudaliar, R.R. Henry, Inflammatory cytokines and chemokines, skeletal muscle and polycystic ovary syndrome: effects of pioglitazone and metformin treatment, Metabolism 62 (2013) 1587–1596.
[29] A. Corbould, A. Dunaif, The adipose cell lineage is not intrinsically insulin resistant in polycystic ovary syndrome, Metabolism 56 (2007) 716–722.