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Increased androgen levels in rats impair glucose-stimulated insulin secretion through disruption of pancreatic beta cell mitochondrial function

Hongdong Wang[a,b], Xiaping Wang[b], Yunxia Zhu[b], Fang Chen[b], Yujie Sun[b], Xiao Han[a,b,*]

[a] State Key Laboratory of Reproductive Medicine, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, China
[b] Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, China

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ABSTRACT

Although insulin resistance is recognized to contribute to the reproductive and metabolic phenotypes of polycystic ovary syndrome (PCOS), pancreatic beta cell dysfunction plays an essential role in the progression from PCOS to the development of type 2 diabetes. However, the role of insulin secretory abnormalities in PCOS has received little attention. In addition, the precise changes in beta cells and the underlying mechanisms remain unclear. In this study, we therefore attempted to elucidate potential mechanisms involved in beta cell alterations in a rat model of PCOS. Glucose-induced insulin secretion was measured in islets isolated from DHT-treated and control rats. Oxygen consumption rate (OCR), ATP production, and mitochondrial copy number were assayed to evaluate mitochondrial function. Glucose-stimulated insulin secretion is significantly decreased in islets from DHT-treated rats. On the other hand, significant reductions are observed in the expression levels of several key genes involved in mitochondrial biogenesis and in mitochondrial OCR and ATP production in DHT-treated rat islets. Meanwhile, we found that androgens can directly impair beta cell function by inducing mitochondrial dysfunction in vitro in an androgen receptor dependent manner. For the first time, our study demonstrates that increased androgens in female rats can impair glucose-stimulated insulin secretion partly through disruption of pancreatic beta cell mitochondrial function. This work has significance for hyperandrogenic women with PCOS: excess activation of the androgen receptor by androgens may provoke beta cell dysfunction via mitochondrial dysfunction.

1. Introduction

Polycystic ovary syndrome (PCOS) is found in 5–10% of women of reproductive age, making it the most common endocrine disorder in women [1]. Insulin resistance, as a central component of PCOS, characteristically occurs in 30–40% of women with PCOS, whereas the prevalence of type 2 diabetes has been placed at 5–10% [1]. PCOS is a heterogeneous condition with unknown etiology that is characterized by hyperandrogenism and anovulatory infertility. In addition, PCOS frequently occurs with insulin resistance, which, if severe, results in compensatory hyperinsulinemia that stimulates ovarian androgen production [2].

Studies have shown that insulin resistance precedes the onset of type 2 diabetes [3]; indeed, insulin resistance has been postulated as the primary factor in the pathogenesis of this disease. The high prevalence of type 2 diabetes in women with PCOS is consistent with the increased risk observed in other populations where insulin resistance is common [4,5]. However, a pathophysiologic model involving insulin resistance as the primary risk factor is not sufficient to explain the finding that only a subset of insulin-resistant women with PCOS develops type 2 diabetes. Therefore, defects in insulin secretion as well as familial and environmental factors also need to be considered to obtain a better understanding of why women with PCOS are predisposed to type 2 diabetes. Insulin resistance has been well documented as a factor that contributes to the reproductive and metabolic

Abbreviations: GSIS, glucose-stimulated insulin secretion; T, testosterone; DHT, 5α-dihydrotestosterone; AR, androgen receptor; Tfam, mitochondrial transcription factor A; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; NRF1, nuclear respiratory factor 1; ND1, NADH dehydrogenase subunit 1; ND3, NADH dehydrogenase subunit 3; mtDNA, mitochondrial DNA; mtCopII, mitochondrial respiratory chain complex II; mtCop IV, mitochondrial respiratory chain complex IV; AS-β, ATP synthase subunit β; siRNA, small interfering RNA; Ins, insulin; h, hour; min, minute; mtDNA, mitochondrial DNA.

* Corresponding author at: State Key Laboratory of Reproductive Medicine, Key Laboratory of Human Functional Genomics of Jiangsu Province, Nanjing Medical University, 140 Hanzhong Road, Nanjing, 210029, China. Fax: +86 25 86862731.
E-mail address: hanxiao@njmu.edu.cn (X. Han).

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phenotypes of PCOS, but the role of abnormal insulin secretion has received little attention.

Dysfunction of pancreatic beta cells – which are responsible for the secretion of insulin – is a characteristic of both type 1 and type 2 diabetes [6,7]. Of the limited number of studies that have investigated pancreatic beta cell function in PCOS, some have described increased insulin secretion, while others suggest decreased insulin secretion. For example, beta cell dysfunction may be a key pathogenic determinant in PCOS [8]. Women with functional ovarian hyperandrogenism have also shown significantly higher basal insulin secretion rates and attenuated secretion in response to meals [9]. In addition, a progressive decline in beta cell function over time has been reported [10]. However, Holte et al. reported an increase in the early insulin response to glucose in women with PCOS; this was closely associated with increased androgenicity that was not accounted for by insulin resistance [11]. Another study concluded that beta cell function was increased in both lean and obese PCOS patients, based on the finding of greater early-phase insulin secretion [12]. The few studies describing beta cell function in PCOS have ascribed the dysfunction of pancreatic beta cells to insulin resistance [13]. Recently, a role for oxidative stress and inflammation induced in mononuclear cells has been confirmed in beta cell dysfunction in PCOS [14]; however, the underlying mechanisms remain unclear. In particular, the relationship between increased androgens and impaired pancreatic beta cell function in PCOS has not been clearly established.

The aim of the present study was to elucidate potential mechanisms involved in beta cell alterations in a previously described rat model of PCOS that exhibits metabolic as well as reproductive features of PCOS [15]. We used this PCOS rat model to investigate changes in beta cell function and the potential mechanisms underlying these changes.

2. Materials and methods

2.1. Reagents

Testosterone (T) was purchased from Acros Organics (Geel, Belgium), 5α-dihydrotestosterone (DHT) from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and the androgen receptor (AR)-specific antagonist flutamide from Sigma-Aldrich (St Louis, MO, USA). Silastic® Laboratory Tubing (for implantation) was obtained from Dow Corning Corporation (Midland, MI, USA). The following antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA): mitochondrial transcription factor A (sc-23588) and nuclear respiratory factor 1 (NRF1) (sc-33771). The AR antibody (ab9474) was purchased from Abcam (Cambridge, MA, USA).

2.2. Vaginal smears

The estrous cycle stage of each rat was determined by microscopic analysis to identify the predominant cell type in vaginal smears obtained daily from Day 90 after tubing implantation [16].

2.3. Animal studies

All experiments were performed with female Sprague-Dawley rats. Littermate controls were used throughout this study. Female rats (21 days old) were purchased from Nanjing Medical University Laboratory Animal Center and housed in groups of five per cage under controlled environmental conditions at the same animal center. Rats were fed a standard rodent chow diet (Shanghai Slac Laboratory Animal Co., Ltd., Shanghai, China) and tap water ad libitum. Normal adult female rats (200–250 g) used for islet isolation were purchased and housed as described above. Rats (21 days of age) were randomly divided into two experimental groups: control ($n = 15$) and DHT-treated ($n = 15$), and allowed to acclimate for 12 h before surgical procedures. After anesthesia with pentobarbital sodium, DHT-treated rats were implanted subcutaneously in the cervical region with 90-day continuous-release silastic tubing (1.47 mm inner diameter, 1.95 mm outer diameter) containing 7.5 mg DHT [rather than using DHT continuous-release pellets [15,17]. Littermate control rats were implanted with empty tubing. The body weights of all rats were measured weekly. The stage of the estrous cycle was determined from daily vaginal smears taken from DHT-treated and control rats (5 per group) for 12 days at the end of the 90-day treatment period. At the end of the treatment period, prior to islet isolation, blood samples were collected from hearts of DHT-treated and control rats ($n = 6$–8 per group). Immediately after islet isolation, ovaries of DHT-treated and control rats were removed and fixed overnight in 4% (g/vol) paraformaldehyde. The numbers of rats used for each experimental analysis are specified in the. Total serum testosterone and 17β-estradiol concentrations were determined for each rat using commercial radioimmunoassay (RIA) kits (Beckman Coulter, CA, USA). Total serum DHT concentrations were determined for each rat using commercial radioimmunoassay (RIA) kits (DIAsource ImmunoAssays, Belgium). Total serum dehydroepiandrosterone and androstenedione concentrations were determined for each rat using rat dehydroepiandrosterone/androstenedione ELISA kits (Xinqidi Biological Technology, Wuhan, China). All animal studies were performed according to guidelines established by the Research Animal Care Committee of Nanjing Medical University, China.

2.4. Measurement of metabolic factors and intraperitoneal glucose tolerance test

Fasting glucose and serum lipid levels were determined from blood samples collected at the end of the 90-day treatment period. Blood glucose levels were measured using a freestyle mini glucometer (Abbot, Alameda, CA, USA). The circulating cholesterol, triglycerides, and free fatty acids levels were assessed by an enzymatic colorimetric method (Wako Pure Chemical Industries, Osaka, Japan). DHT-treated and control rats were fasted overnight prior to conducting intraperitoneal glucose tolerance tests (IPGTTs). In brief, after measurement of basal blood glucose levels, rats received an intraperitoneal (i.p.) injection of glucose (2 g of glucose per kg body mass as a 20% glucose solution), and tail vein blood samples were taken at 5, 15, 30, 60, and 120 min. In addition, blood samples were collected for insulin measurements and rats that underwent IPGTTs were sacrificed via euthanasia at the end of the assay. Insulin levels were measured using rat insulin ELISA kits (Millipore, Billerica, MA, USA).

2.5. Islet isolation and culture

DHT-treated rats and their controls, or normal adult rats at diestrus, were fasted overnight before islet isolation and culture, performed as previously described [18]. Freshly isolated islets were transferred to sterile 6-well plates and cultured in RPMI 1640 containing 5.5 mmol l^{-1} glucose supplemented with 10% fetal bovine serum (FBS), 100 units ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin. Islets were allowed to equilibrate overnight before further treatment.

2.6. Glucose-stimulated insulin secretion assays and insulin content extraction

Islets (seven islets of similar size per well, six wells per group) isolated from normal adult female rats were cultured in 48-well