



Exercise differentially affects metabolic functions and white adipose tissue in female letrozole- and dihydrotestosterone-induced mouse models of polycystic ovary syndrome



Rodrigo R. Marcondes ^{a, b}, Manuel Maliqueo ^{a, c}, Romina Fornes ^a, Anna Benrick ^{d, e}, Min Hu ^d, Niklas Ivarsson ^a, Mattias Carlström ^a, Samuel W. Cushman ^f, Karin G. Stenkula ^g, Gustavo A.R. Maciel ^b, Elisabet Stener-Victorin ^{a, *}

^a Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

^b Disciplina de Ginecologia, Laboratório de Ginecologia Estrutural e Molecular (LIM 58), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

^c Endocrinology and Metabolism Laboratory, Department of Medicine, West Division, University of Chile, Santiago, Chile

^d Department of Physiology, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

^e School of Health and Education, University of Skövde, Skövde, Sweden

^f Experimental Diabetes, Metabolism, and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA

^g Department of Experimental Medical Sciences, Lund University, Lund, Sweden

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ABSTRACT

Here we hypothesized that exercise in dihydrotestosterone (DHT) or letrozole (LET)-induced polycystic ovary syndrome mouse models improves impaired insulin and glucose metabolism, adipose tissue morphology, and expression of genes related to adipogenesis, lipid metabolism, Notch pathway and browning in inguinal and mesenteric fat. DHT-exposed mice had increased body weight, increased number of large mesenteric adipocytes. LET-exposed mice displayed increased body weight and fat mass, decreased insulin sensitivity, increased frequency of small adipocytes and increased expression of genes related to lipolysis in mesenteric fat. In both models, exercise decreased fat mass and inguinal and mesenteric adipose tissue expression of Notch pathway genes, and restored altered mesenteric adipocytes morphology. In conclusion, exercise restored mesenteric adipocytes morphology in DHT- and LET-exposed mice, and insulin sensitivity and mesenteric expression of lipolysis-related genes in LET-exposed mice. Benefits could be explained by downregulation of Notch, and modulation of browning and lipolysis pathways in the adipose tissue.

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1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age and is characterized by hyperandrogenism, anovulation and polycystic ovarian morphology (Dumesic et al., 2015). Beyond the reproductive dysfunction, women with PCOS suffers from metabolic dysfunction including insulin resistance, hyperinsulinemia, with an increased risk for type 2 diabetes and cardiovascular diseases (Jayasena and

Franks, 2014). Women with PCOS are more likely to be overweight/obese, specifically abdominal obesity (Lim et al., 2012) with accumulation of white adipose tissue (WAT) (Bartelt and Heeren, 2014). Obesity exacerbates the reproductive and metabolic phenotype in PCOS and increase cardiometabolic risks (Jayasena and Franks, 2014, Moran et al., 2015, Karpe and Pinnick, 2015). Along with abdominal fat accumulation, adipose tissue dysfunction, characterized by increased adipocyte size and altered circulating levels of adipokines, is observed in women with PCOS (Spritzer et al., 2015).

Mice and rats exposed to continuous administration of dihydrotestosterone (DHT) or letrozole from pre-pubertal age develop metabolic and reproductive alterations that resemble features

* Corresponding author. Karolinska Institutet, Department of Physiology and Pharmacology, SE-171 77 Stockholm, Sweden.

E-mail address: elisabet.stener-victorin@ki.se (E. Stener-Victorin).

observed in women with PCOS (van Houten et al., 2012; Kauffman et al., 2015; Maliqueo et al., 2013; Manneras et al., 2007). Previous studies have shown that PCOS mouse model induced by DHT or letrozole presented increased gain of body weight, impaired insulin sensitivity, adipocyte hypertrophy, disrupted estrous cycle, and chronic anovulation (van Houten et al., 2012; Kauffman et al., 2015). However, DHT and letrozole-exposed mice presents different endocrine phenotypes, where DHT-exposed mice presents normal levels of testosterone and high levels of dihydrotestosterone, and letrozole-exposed mice displays high levels of testosterone and unaltered levels of dihydrotestosterone (Caldwell et al., 2014). Both PCOS models are hyperandrogenemic but through different mechanisms; DHT by exogenous administration of androgen, and letrozole by inhibiting the P450 aromatase leading to increased circulating testosterone.

Lifestyle interventions, including diet and exercise, improve PCOS related symptoms (Nybacka et al., 2011; Nybacka et al., 2013). Still, the mechanisms behind the beneficial effects of exercise in women with PCOS are not completely understood. In a PCOS rat model induced by DHT from puberty, exercise decreased adiposity and adipocyte size, improved insulin sensitivity, estrous cyclicity, ovarian morphology, circulating androgens and adipokines levels, and restored the mRNA expression of sympathetic markers in the adipose tissue (Benrick et al., 2013; Manneras et al., 2008; Manneras et al., 2009; Wu et al., 2014).

Exercise can have profound effects on WAT physiology, leading to decreased adipocyte size, and influences the expression of several metabolic proteins, such as glucose transporter 4 (GLUT4) and peroxisome proliferator-activated receptor coactivator 1- α (PGC1- α) (Stanford et al., 2015). Moreover, the effects of exercise in WAT differ depending on adipose tissue depot; subcutaneous versus visceral. Exercise is also able to increase the amount of brown-like adipocytes in the WAT, known as “browning” or “beiging”. These brown-like adipose cells dissipate energy as heat through uncoupling protein-1 (UCP-1) activity (Stanford et al., 2015). The browning of WAT has been related to improvements in metabolic health and is a promising strategy to treat metabolic disorders (Bartelt and Heeren, 2014).

Inhibition of the Notch signaling pathway by knockdown of *Notch1* and *Rbpj* genes promotes browning of WAT, improves insulin sensitivity and glucose tolerance, and increases the expression of genes related to brown-like adipocytes activity, such as *Ucp1*, *Ppargc1a* (PGC1- α), *Prdm16* (PR domain containing 16) and *Cidea* (Cell Death-Inducing DFFA-Like Effector A) (Bi et al., 2014). Pharmacological inhibition of Notch pathway with dibenzazepine increases the expression of *Ucp1*, and improves the insulin sensitivity and glucose tolerance of obese mice models, suggesting that this pathway is a potential target for the treatment of obesity and type 2 diabetes (Bi et al., 2014). To our knowledge, the effects of exercise on Notch signaling pathway in adipose tissue have not previously been analyzed.

The Notch pathway has a strong cross-talk with bone morphogenetic proteins (BMPs) (Kluppel and Wrana, 2005). BMP-4 and BMP-7 induces brown-like phenotype in human adipose stem cells (Elsen et al., 2014), and BMP-4 overexpression *in vivo* induces browning and improves glucose and insulin metabolism (Qian et al., 2013). The role of these molecules in adipose tissue of PCOS is not known.

Herein, we tested the hypotheses that 4–5 weeks of voluntary exercise in either DHT- or letrozole (LET)-induced PCOS mice models would improve impaired insulin sensitivity and glucose metabolism, adipose tissue morphology, and expression of genes related to adipogenesis, lipid metabolism, Notch signaling pathway and browning in subcutaneous and mesenteric WAT. Additionally, we investigated the transcriptional expression of BMP family

growth factors and markers of sympathetic activity in subcutaneous and mesenteric adipose tissue, and liver triglycerides.

2. Materials and methods

2.1. Animals

Forty-seven pre-pubertal, three weeks old female mice (C57BL/6J) were purchased from Janvier Labs (Le Genest Saint-Isle, France). Animals were housed four to five per cage under controlled temperature and 12 h light/12 h dark cycle. Mice were fed with commercial chow (16.5% protein, 4% fat, 58% carbohydrate, 3.5% fiber, 6% vitamins and minerals, and <12% water; Lantmännen Lantbruk, Malmö, Sweden) and tap water *ad libitum*. This study was approved by the Animal Ethical Committee at Karolinska Institutet, protocol number N259-2014.

2.2. Study procedure

At 28 days of age, mice were randomly divided into three groups: control ($n = 9$), DHT ($n = 19$), and letrozole (LET) ($n = 19$), and continuous slow releasing pellets were implanted subcutaneously in the neck region under light anesthesia with isoflurane. The LET group received a 70 days continuous releasing pellet (3.5 mg letrozole; daily dose: 50 μ g; Innovative Research of America, Sarasota, USA) (Kauffman et al., 2015) and the DHT group received a 90 days continuous releasing pellet (2.5 mg DHT; daily dose: 27.5 μ g; Innovative Research of America) (van Houten et al., 2012). Control animals received a pellet lacking the bioactive molecule. Before start of exercise, at 63 days of age and 5 weeks from pellet implantation, when the DHT- and LET-exposed mice developed the PCOS-like phenotype, body composition was measured by Dual-energy X-ray absorptiometry (DEXA) (Lunar PIXImus, GE Medical Systems, Madison, USA) as described previously (Yang et al., 2015). Exercise started after DEXA examination and groups were divided into: control ($n = 9$), DHT ($n = 10$), DHT + exercise (DHT + EX) ($n = 9$), LET ($n = 9$), and LET + exercise (LET + EX) ($n = 10$). At 8 weeks from pellet exposure, after 3 full weeks of exercise, insulin tolerance test (ITT), and 5 days later an oral glucose tolerance test (OGTT), were performed. After 4–5 weeks of exercise, at 10th week from pellet exposure, DEXA measurement was repeated and mice were euthanized after 4 h of fasting (Supplemental Fig. S1). Running wheels were locked 24 h before the euthanasia. Blood was collected by heart puncture and inguinal and mesenteric fat depots were quickly dissected and snap frozen until analyses. Inguinal and mesenteric fat depots were chosen for translational purposes, since these depots are analogous to human fat pads humans (Chusyd et al., 2016).

2.3. Voluntary exercise

Mice in the exercise groups were housed as described previously (Goh and Ladiges, 2015), with free access to a low-profile wireless running wheel for mouse (Med Associates, St Albans, USA). Wheel revolutions were registered.

2.4. Insulin tolerance test (ITT)

After 2 h fasting and locked running wheels, a basal blood sample from the tail was collected to measure glucose with the One Touch Ultra-2 glucometer (LifeScan, Inc., Milpitas, USA). Then animals received insulin dissolved in saline (0.5U/kg) intraperitoneally. Blood glucose was measured at 15, 30 and 45 min after insulin injection.

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