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Research paper

Higher circulating leukocytes in women with PCOS is reversed by aerobic exercise

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ABSTRACT

Polycystic ovary syndrome (PCOS) is characterized by insulin resistance, elevated circulating leukocytes, and hypothesized to have higher adipose tissue inflammation. Aerobic exercise reduces circulating leukocytes and improves insulin sensitivity in obese individuals, but the effect of exercise on inflammation in PCOS is not known. We investigated circulating leukocytes, insulin sensitivity by euglycemic-hyperinsulinemic clamp, serum pro- and anti-inflammatory markers (hsCRP, TNF-α, total and high molecular weight adiponectin), and abdominal subcutaneous adipose tissue (SAT) gene expression of proinflammatory markers in 8 PCOS women and 8 obese control females matched for BMI. Additionally, in a prospective study, the 8 women with PCOS underwent a 16-week aerobic exercise regimen with the same measures performed post-intervention. Compared to controls, white blood cell counts (WBC) were 30% higher ($p = 0.04$) and circulating total adiponectin levels were 150% lower ($p = 0.03$) in women with PCOS at baseline/pre-exercise conditions. SAT gene expression of macrophage migration inhibitory factor (MIF, $p < 0.01$) and interleukin-6 (IL-6, $p < 0.05$) were also lower in women with PCOS. In response to 16 weeks of aerobic exercise, insulin sensitivity improved ($p < 0.01$) and WBC counts decreased ($p = 0.02$). The exercise-induced change in WBC and circulating neutrophils correlated inversely with changes in glucose disposal rate ($r = -0.73$, $p = 0.03$; and $r = -0.82$, $p = 0.01$, respectively). Aerobic exercise reduced serum leptin ($p < 0.05$) after 4 weeks, trended to reduce the ratio of leptin-to-high molecular weight adiponectin ($p < 0.1$) by the 8th week, and significantly increased serum dehydroepiandrosterone sulfate (DHEA-S, $p < 0.001$) after 16 weeks. In conclusion, women with PCOS have higher circulating leukocytes compared to controls, which can be reversed by aerobic exercise and is associated with improvements in insulin sensitivity.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder affecting 5–10% of women at reproductive age [1–3]. In addition to menstrual irregularity, hyperandrogenemia, and polycystic ovarian morphology, PCOS is highly associated with abdominal obesity and insulin resistance [4–6]. Chronic, low-grade inflammation, a condition associated with obesity [7] and type 2 diabetes [8,9], is a risk factor for cardiovascular disease [10,11]. Recent evidence suggests that low-grade inflammation may contribute to the metabolic dysregulation associated with PCOS [12].

Although the exact sources of low-grade inflammation associated with insulin resistance and obesity are still debated, adipose tissue inflammation is a likely culprit [13–16]. Adipose tissue from women with PCOS as well as pre-adipocytes treated with testosterone have shown to have reduced lipolytic function [17,18]. Adipose tissue dysfunction, here defined as reduced lipolytic function, is also seen in insulin resistance [19]. Adipose tissue inflammation occurs as a result of monocyte and neutrophil infiltration into

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adipose tissue to “clean up” dysfunctional and dying adipocytes [20–22]. Macrophage accumulation coupled with dysfunctional adipocytes produces pro-inflammatory cytokines and chemokines that spill into the circulation and thus contribute to systemic, chronic, low-grade inflammation [23–25].

Aerobic exercise has been shown to reduce inflammation [26–29]. However, very few studies have focused on how exercise affects adipose tissue inflammation [30]. Prior studies in women with PCOS have shown that there are indeed improvements in insulin resistance with exercise [31,32]. Importantly though, no study to date has examined the role of exercise in adipose specific inflammation in women with PCOS. We have previously shown that menstrual function, insulin resistance, aerobic capacity, and, importantly, adipose tissue lipolytic function were all improved after 16-weeks of moderate intensity aerobic exercise in women with PCOS [33,34]. Improvements in inflammation with aerobic exercise in PCOS women could potentially mediate such improvements in insulin sensitivity. The objectives of this study were: 1) compare markers of systemic and adipose-specific inflammation in 8 women with PCOS and 8 BMI-matched controls; and 2) investigate the effects of 16-weeks of aerobic exercise on adipose and systemic inflammation in the 8 women with PCOS. We hypothesized that women with PCOS would have higher systemic inflammation, which would be attenuated following 16-weeks of exercise.

2. Materials and methods

2.1. Subjects, experimental design, and exercise protocol

As previously reported [33,34], 8 women with PCOS were matched with women without signs of abnormal menses or hyperandrogenemia. The diagnosis of PCOS was assessed by the Rotterdam criteria [35,36]. Women with PCOS had to possess two of the following criteria: confirmation by medical history of menstrual irregularity (oligo- or amenorrhea), presence of more than 10 ovarian follicles 2–9 mm in diameter as assessed by MRI, or either clinical (hirsutism score) or serum measures of androgen excess (elevated free androgen index, FAI). Other causes of oligomenorrhea (hyperprolactinemia, congenital adrenal hyperplasia, Cushing's syndrome, hyperthyroidism) were excluded by medical history. All women in our PCOS group possessed more than 10 ovarian follicles measured 2–9 mm in diameter, and all had irregular menses. Women in the control group were excluded from participation in our study for exercise training, use of contraceptive medications, and menstrual cycle irregularity together with androgen excess. All women in our control group had FAI values below 3.6 as defined as a cut-off value for FAI in the assessment of PCOS according to Hahn et al. [37]. Additionally, all women in our control group reported regular menstruation, thus confirming that they did not have PCOS based on the Rotterdam guidelines. Following an overnight fast, blood and subcutaneous adipose tissue samples were collected, body composition was assessed by dual x-ray absorptiometry (DXA, QDR 4500A; Hologics, Bedford, MA) and insulin sensitivity was determined by a hyperinsulinemic-euglycemic clamp (120-min at 80 mU/min/m^2). Aerobic capacity (VO_2max) was measured during a graded treadmill test (TrueMax 2400; ParvoMedics, Salt Lake City, UT). After baseline testing, women with PCOS underwent 16 weeks of aerobic exercise at the Health and Fitness Center of the Pennington Biomedical Research Center with all exercise sessions performed on a treadmill at 55% VO_2max five times per week under supervision. Details of the exercise protocol are provided elsewhere [33]. Exercise was performed initially to achieve an exercise energy expenditure of 4% of the participants' estimated energy requirement during the first 4 weeks, then incrementally increased to 6% for weeks 5–8, 8% for weeks 9–12, and finally 10% for weeks 13–16. A fasted blood sample was collected every 4 weeks throughout the exercise program. The study was approved by the Institutional Review Board of Pennington Biomedical Research Center and participants provided informed written consent before participating (Clinicaltrials.gov registration NCT01150539).

2.2. Biochemical assays

High sensitive C-reactive protein (hsCRP) and dehydroepiandrosterone sulfate (DHEAS) were determined by chemiluminescent immunoassay (Immulite 2000™, Siemens Healthcare Diagnostics, Deerfield, IL); Tumor necrosis factor-alpha (TNF-α) by immunoassay (Luminex 100™, Luminex Corp. Austin, TX); leptin and adiponectin (total and high molecular weight) by radioimmunoassay (Linco Research Inc., St Charles, MO); serum lipids by an enzymatic assay on a Beckman Coulter DXC 600 (Beckman Coulter, Brea, CA); and complete blood counts using a Beckman Coulter DxH 800 using the Coulter principle (Beckman Coulter, Brea, CA).

2.3. Hyperinsulinemic-euglycemic clamp

Insulin sensitivity was measured as previously described using a hyperinsulinemic euglycemic clamp [33]. After catheter placement, a primed infusion of insulin (80 mU/min/m^2) was initiated for 120 min with plasma glucose levels measured every 5 min; at this insulin infusion level, all splanchnic glucose output was assumed to be blocked. Exogenous 20% glucose was infused at a variable rate to maintain plasma glucose concentration at 90 mg/dL. Glucose disposal rate (GDR) was adjusted for kilograms of fat free mass (FFM) + 17.7, denoted as estimated mean body size (EMBS) [38].

2.4. Subcutaneous adipose tissue biopsy for Real Time PCR for gene expression

Subcutaneous adipose tissue was obtained from the abdomen with a 5-mm needle using the Bergstrom technique after local anesthesia (5 mL 1:1 mixture of 0.5% bupivacaine and 2% lidocaine). Adipose tissue was immediately snap frozen in liquid nitrogen and stored at −80 °C until analysis. Total RNA was extracted from approximately 100 mg of adipose tissue using miRNEasy Kits (Qiagen, Valencia, CA). RNA extracts were converted into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Gene expression was carried out using Real Time-PCR with TaqMan gene expression assays embedded on microfluidic cards on the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Gene expression assays were performed for the following genes: plasminogen activator inhibitor 1 (PAI-1, Hs01126603_m1), macrophage migration inhibitory factor (MIF, Hs00236988_g1), interleukin 6 (IL-6, Hs00985639_m1), cluster of differentiation 68 (CD68, Hs00154355_m1), monocyte chemotactic protein 1 (MCP1, Hs00234140_m1), tumor necrosis factor, alpha (TNFa, Hs00174128_m1), adiponectin (AdipoQ, Hs00605917_m1), and cyclophilin B (PPIB, Hs00168719_m1). Relative gene expression was assessed using ΔΔCt normalized to Cyclophilin B (PPIB) gene expression.

2.5. Statistical analysis

All analyses were performed using GraphPad Prism Software, version 5.0 (GraphPad Software, La Jolla, CA). The Mann–Whitney test was used for cross-sectional comparisons between PCOS and Obese Control women when data was not normally distributed, and an Independent Samples *t*-test was used when the data was normally distributed (Table 1 and Fig. 2). Paired *t*-tests were used