Diabetologia (2012) 55:1424–1434
DOI 10.1007/s00125-011-2442-8

ARTICLE

Effect of exercise training on insulin sensitivity, mitochondria and computed tomography muscle attenuation in overweight women with and without polycystic ovary syndrome

S. K. Hutchison · H. J. Teede · D. Rachoń · C. L. Harrison · B. J. Strauss · N. K. Stepto

Received: 25 August 2011 / Accepted: 9 December 2011 / Published online: 13 January 2012

Abstract
Aims/hypothesis Polycystic ovary syndrome (PCOS) is an insulin resistant (IR) state. Increased skeletal muscle lipid content and impaired mitochondrial biogenesis have been implicated in the pathogenesis of IR. We investigated whether differences in these variables explain the IR of women affected by PCOS and whether improvements in IR with exercise are reflected by changes in these variables.
Methods Sixteen PCOS and 13 non-PCOS overweight women were assessed, and eight PCOS and seven non-PCOS women were reassessed after 12 weeks of moderate and vigorous exercise training. Outcomes included insulin sensitivity (glucose infusion rate [GIR]), skeletal muscle gene expression and protein abundance, enzyme activity of selected mitochondrial components, and computed tomography (CT) attenuation-estimated muscle lipid.
Results GIR was lower in women with PCOS versus those without (p=0.01) and increased with exercise in both groups. Baseline CT muscle attenuation suggested a trend to less muscle lipid in PCOS, which increased with exercise training, with a difference in the change in muscle lipid (p=0.01, age-corrected), compared with non-PCOS women. GIR correlated with *PGC1A* gene expression across the whole group; skeletal muscle expression of mitochondrial biogenesis markers was not different between groups at baseline, or after training. Neither lipid changes nor mitochondrial changes correlated with changes in GIR.
Conclusions/interpretation Differences in IR in women with and without PCOS were not explained by differences in skeletal muscle lipid or mitochondrial parameters. Improvements in IR with exercise were dissociated from mitochondrial parameters. CT muscle attenuation suggested a differential capacity of PCOS muscle to store lipid compared with non-PCOS.

Trial registration: Clinicaltrials.gov ISRCTN84763265
Funding: National Health & Medical Research Council (Grant number 606553), Monash University and The Jean Hailes Foundation.

Keywords Insulin resistance · Mitochondrial function · Muscle lipid content · Polycystic ovary syndrome

Electronic supplementary material The online version of this article (doi:10.1007/s00125-011-2442-8) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

S. K. Hutchison · H. J. Teede · D. Rachoń · C. L. Harrison · N. K. Stepto
Jean Hailes Foundation Research, School of Public Health and Preventative Medicine, Monash University,
Melbourne, VIC, Australia

S. K. Hutchison · H. J. Teede
Diabetes Unit, Southern Health,
Melbourne, VIC, Australia

D. Rachoń
Department of Clinical and Experimental Endocrinology,
Medical University of Gdańsk,
Gdańsk, Poland

C. L. Harrison
Department of Physiology, Monash University,
Melbourne, VIC, Australia

B. J. Strauss
Department of Medicine, Monash University,
Melbourne, VIC, Australia

N. K. Stepto
Institute of Sport Exercise and Active Living, Victoria University,
Melbourne, VIC, Australia

N. K. Stepto (✉)
School of Sport and Exercise Science, Victoria University,
PO Box 14428, Melbourne, VIC 8001, Australia
e-mail: Nigel.Stepto@vu.edu.au

Abbreviations

β-HAD	β-Hydroxyacyl-CoA dehydrogenase
CS	Citrate synthase
CT	Computed tomography
C_t	Cycle threshold
DGAT1	Diacylglycerol acyltransferase
GIR	Glucose infusion rate
HU	Hounsfield unit
IMCL	Intramyocellular lipid
IR	Insulin resistance
NRF1	Nuclear respiratory factor-1
OXPHOS	Oxidative phosphorylation
PCOS	Polycystic ovary syndrome
PGC1α	Peroxisome-proliferator activated receptor γ coactivator 1α
SHBG	Sex hormone-binding globulin
TFAM	Mitochondrial transcription factor A
$\dot{V}O_{2max}$	Maximal oxygen uptake

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of reproductive-age women, affecting 8–18% [1]. Women with PCOS (PCOS women) have both increased intrinsic insulin resistance (IR) compared with body composition of matched women without PCOS (non-PCOS women) [2–4] and obesity-related extrinsic IR. IR in PCOS underpins reproductive and metabolic features [2] including increased risk of prediabetes and type 2 diabetes [3, 5]. The mechanism of IR in PCOS remains unclear [3].

Muscle lipid content has been proposed to play a role in IR, with various measures correlating with IR (reviewed by Lara-Castro and Garvey [6]) including computed tomography (CT) muscle attenuation [7]. Lipid is stored both around and within muscle cells. Elevated intramyocellular lipid (IMCL) is hypothesised to mediate IR. IMCL increase itself may be a consequence of impaired mitochondrial function [8]. CT muscle attenuation, although unable to distinguish intra- from extra-myocellular lipid, is a non-invasive assessment of muscle lipid content that correlates with IMCL assessed using biopsy tissue and magnetic resonance spectroscopy [9, 10]. The role of muscle lipid content in IR is not clear in PCOS.

Reduced skeletal muscle mitochondrial function has been associated with IR in patients with type 2 diabetes [11], those at risk of diabetes [12], and the elderly [13]. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) is a key nuclear-encoded regulator of mitochondrial biogenesis and energy metabolism [14, 15]. *PGC1A* (also known as *PPARGC1A*) gene expression is lower in patients with type 2 diabetes than controls [16] and correlates with downregulation of genes encoding enzymes involved with oxidative phosphorylation (OXPHOS) [17]. Similar findings were obtained in a cross-sectional study of IR PCOS women [18]; however, confounding factors were not documented. Protein abundance of OXPHOS components and enzyme activity has not been studied in PCOS to date.

Some interventions in obesity and type 2 diabetes improve IR and mitochondrial function in parallel; however, results can be discordant (reviewed by Turner and Heilbronn [19]). The only interventional study in PCOS to investigate mitochondrial function showed that the insulin sensitiser, pioglitazone, improved both IR and mitochondrial function [20]. Improved mitochondrial function has been demonstrated with exercise [21]. The response to exercise is not as clear in obese and diabetic patient groups. Exercise combined with energy restriction improves mitochondrial function in obese people [22], but exercise alone failed to improve mitochondrial function in those whose IR improved [23]. IMCL has been shown to decrease with diet-induced weight loss in type 2 diabetes, but did not change with a combination of diet and exercise [24]. Despite the relationship between increased IMCL and IR, exercise may increase IMCL content while improving IR, the so-called 'athlete's paradox' [25]. To our knowledge, the effect of exercise training on mitochondrial function and muscle lipid content has not been studied in PCOS.

PCOS is a condition characterised by IR greater than expected for body weight. We hypothesise that high muscle lipid content and/or low mitochondrial content contribute to this IR. Furthermore we expected exercise-induced improvements in IR would be accompanied by reduced muscle lipid content and increased mitochondrial biogenesis.

Methods

Participants Overweight and obese (BMI >27 kg/m^2) sedentary premenopausal women with (n=16) and without (n=13) PCOS were recruited from community advertisements. PCOS was diagnosed by an endocrinologist (S.K. Hutchison) after clinical exclusion of other causes of hyperandrogenism based on the 1990 National Institutes of Health criteria as previously reported [26]. All non-PCOS women had regular menses and no evidence of clinical or biochemical hyperandrogenism. Exclusion criteria included type 2 diabetes, regular physical activity and pregnancy [26]. The Southern Health Research Advisory and Ethics Committee approved the study, and participants gave written informed consent.

Study design At screening 3 months before baseline, standard diet and lifestyle advice was delivered (Heart Foundation recommendations [www.heartfoundation.org.au]). Medications affecting end points, including insulin sensitisers, anti-

androgens and hormonal contraceptives, were ceased. Data were collected after 3 months (baseline) and after 12 weeks of exercise training (study completion) in the follicular phase of the menstrual cycle wherever feasible.

Exercise intervention Participants undertook 12 weeks of supervised, progressive, moderate and vigorous exercise training on a motorised treadmill as described previously. Briefly, participants attended three 1 h sessions each week, which sequentially alternated between moderate-intensity (walking or jogging at 70% of maximal oxygen uptake [$\dot{V}O_{2max}$]) and high-intensity (six 5 min intervals with a 2 min recovery period at ~95–100% of $\dot{V}O_{2max}$) interval training. Participants' exercise was progressively increased over the study [27]. $\dot{V}O_{2max}$ tests were repeated at 6 and 12 weeks to assess changes in fitness and maximal heart rate. Heart rate monitors were used in all sessions (Polar Electro Oy, Kempele, Finland).

Clinical and biochemical measurements Participants' body weight, height, BMI, waist circumference and percentage body fat were measured by body composition technicians in Monash Medical Centre Body Composition Laboratory.

Mean thigh muscle attenuation on CT scan was used to assess muscle lipid content. Participants were placed in a supine position, and a cross-sectional scan of both legs was obtained at the mid-thigh (defined as the mid-point between the anterior iliac crest and the patella). All scans were performed using a General Electric Lightspeed CT scanner (GE Medical Systems, Milwaukee, WI, USA) and saved as DICOM images for analysis. Standard CT procedures of 120 kV, 5 mm thickness and a 512×512 matrix were used for all participants, and images were analysed using Slice-O-Matic version 4.3 software (Tomovision, Magog, QC, Canada). Attenuation levels for delineating fat (less than –30 Hounsfield units [HU]) and muscle (−29 to 150 HU) and manual demarcation of muscle from bone and subcutaneous and intermuscular fat were used as previously described [28]. Mean muscle attenuation was determined by averaging all pixels within the range −29 to 150 HU. The higher the attenuation, the less lipid is present in the muscle [7].

$\dot{V}O_{2max}$ and maximum heart rate were assessed using the MOXUS modular system (AEI Technologies, Pittsburgh, PA, USA) while participants exercised on a treadmill (Biodex RTM 500, New York, USA) until volitional fatigue [27].

Insulin sensitivity was assessed by the euglycaemic–hyperinsulinaemic clamp technique as previously described [26]. Clamp timing was standardised to 48 h after exercise, and included a standardised high-carbohydrate diet before an overnight fast. Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at 40 mU m^{-2} min^{-1} for 120 min, with plasma glucose maintained at ~5 mmol/l using variable infusion rates of 25% glucose. Glucose infusion rates (GIRs) were calculated during steady state, achieved in the last 30 min of the clamp and expressed as glucose (mg) per body surface area (m^2) per min [26].

Blood sampling and analysis were performed as previously described [26]. HbA_{1c} was determined using high-performance liquid chromatography using the Glycohemoglobin Analyzer model HLC-723 GHbV A1c2.2 (Tosoh Corporation, Tokyo, Japan). The free androgen index was calculated as testosterone/sex hormone-binding globulin (SHBG)×100.

Muscle samples Thigh vastus lateralis muscle was obtained by percutaneous biopsy under local anaesthesia immediately before the insulin clamp [29]. Muscle biopsy samples were blotted and dissected free of any connective and fat tissue, immediately frozen in liquid nitrogen, and then stored at −80°C for later analysis.

Muscle total RNA isolation Total RNA was isolated from the muscle (15–20 mg) using the RNeasy Total RNA Kit (Qiagen, Hilden, Germany) as previously described [29]. The total RNA content and purity were established by measuring absorbance at 260 and 280 nm (NanoDrop; Eppendorf South Pacific, North Ryde, NSW, Australia). Afterwards, each sample was diluted with RNase-free water to a concentration of 10 ng/μl and stored at −80°C for subsequent analysis.

Reverse transcription and real-time PCR RNA samples were reverse transcribed in a thermal cycler (Perkin Elmer GeneAmp PCR 2400 thermal cycler; Perkin Elmer, Rowville, VIC, Australia) using Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA, USA) in 10 μl reaction mixtures containing 1× Taqman RT buffer, 5.5 mmol/l $MgCl_2$, 500 μmol/l 2′-deoxynucleoside 5′-triphosphate, 2.5 μmol/l random hexamers, 0.4 U/μl RNase inhibitor and 1.25 U/μl multiscribe reverse transcriptase. The reaction conditions were as follows: 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min.

Relative gene expression was quantified by real-time PCR. All reactions were performed according to the multiplex cycle threshold (C_t) method using the reference gene (ribosomal 18S) and the gene of interest in the same well. The reference gene did not change with exercise. PCRs were performed on a BioRad i-CYCLER iQ real-time PCR detection system in 25 μl reaction volume of BioRad iQ Supermix PCR mix (BioRad Laboratories, Gladesville, NSW, Australia), Applied Biosystems pre-developed assay reagent for 18S, the forward and reverse primers and probes of the genes of interest (electronic supplementary material [ESM] Table 1) and sterile water. Probes and primers were designed (Primer Express version 1.0; Applied Biosystems) from the human gene sequence accessed from GenBank/EMBL [30].

Comparative C_t calculations for the expression of the studied genes were performed subtracting the 18S C_t values from C_t values of the gene of interest to derive a ΔC_t value. The expression of the studied genes was then calculated according to the formula: $2^{-\Delta C_t}$[31].

Protein extraction and analyses (western blots) Muscle tissue (15–20 mg) was homogenised (Polytron; Brinkman Instruments, New York, NY, USA) in ice-cold buffer containing 50 mmol/l HEPES, 150 mmol/l NaCl, 10 mmol/l NaF, 1 mmol/l Na_3VO_4, 5 mmol/l EDTA, 0.5% Triton X-100, 10% glycerol (vol./vol.), 2 μg/ml leupeptin, 100 μg/ml phenylmethanesulfonyl fluoride and 2 μg/ml aprotinin. All chemicals were from Sigma-Aldrich (North Ryde, NSW, Australia). Homogenates were then centrifuged (16,000×*g* for 60 min at 4°C), and the supernatant fractions were removed and rapidly frozen in liquid nitrogen. Protein concentrations of the muscle lysates were determined using the BCA assay kit (Pierce, Rockford, IL, USA). For analysis of protein abundance , equal quantities of protein (35 μg) were resolved by SDS-PAGE on 10% polyacrylamide gels, transferred to a nitrocellulose membrane, blocked with 5% BSA, and immunoblotted overnight with the antibodies (diluted 1:1000) directed against: complex I subunit NDUFB8 (MS105); complex II–30 kDa (MS203); complex III–core protein 2 (MS304); complex IV subunit II (MS405); complex V α subunit (MS507; MitoProfile Total OXPHOS Complexes Detection Kit, Eugene, OR, USA); and PGC1α (1:1,000; Chemicon International, Boronia, VIC, Australia). After incubation with horseradish peroxidase-conjugated secondary antibody (1:2,000; Amersham Biosciences, Castle Hill, NSW, Australia), the immunoreactive proteins were detected with enhanced chemiluminescence (Perkin Elmer) and quantified by densitometry.

Analysis of muscle enzyme activity The remaining muscle biopsy fragments (5–10 mg) were homogenised in 1:50 dilution (wt/vol.) of a 175 mmol/l potassium buffer solution. Citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities were analysed by measuring the disappearance of NADH spectrophotometrically at a constant temperature of 25°C [32].

Statistical analysis All data are presented as mean±SE. Data were assessed for normality using Kolmogorov–Smirnov tests and log-transformed where appropriate (insulin). Results are presented for 29 participants at baseline (16 PCOS and 13 non-PCOS women) except for GIR (n=28, PCOS n=16, non-PCOS n=12). At completion, results are presented for n=15 (PCOS n=8, non-PCOS n=7) except for GIR (n=14, PCOS n=7, non-PCOS n=7) and CT data (n=14, PCOS n=8, non-PCOS n=6). Two-tailed statistical analysis was performed using SPSS for Windows 17.0 software (SPSS, Chicago, IL, USA), with statistical significance set at α level of $p<0.05$. Data were assessed using Student's t test with general linear modelling to correct for age. The χ^2 test was used for difference in proportions. Relationships between variables were examined using bivariate (Spearman) correlations. The effect of exercise training was examined using repeated-measures ANOVA (PCOS status×time) with correction for age and BMI. Change in variable was defined as ratio of pretreatment to post-treatment value.

Results

Participants comprised a subset from a previous study [26] and were included if adequate muscle biopsy tissue was available. In total, 16 PCOS and 13 non-PCOS women completed the 3-month run-in with stable diet and withdrawal of relevant medications. Eight PCOS and seven non-PCOS women completed 12 weeks of training.

PCOS vs non-PCOS women: baseline characteristics (Table 1) PCOS women were younger than non-PCOS women (30.7±1.4 vs 34.5±1.1 years, p=0.04) and had higher androgen concentrations and lower SHBG and HDL concentrations. PCOS women had ~36% lower GIR (p=0.01) than non-PCOS women despite similar BMI and body fat percentage, fitness measured by $\dot{V}O_{2max}$, and frequency of family history of type 2 diabetes. There was a trend to greater CT thigh muscle attenuation in the PCOS women (49.5±0.67 vs 47.5±0.93 HU, p=0.08), reflecting lower muscle lipid content. Correction for age did not alter the findings (data not shown).

PCOS vs non-PCOS women: markers of mitochondrial biogenesis and function There were no differences between PCOS and non-PCOS women in *PGC1A*, *TFAM* and *NRF1* gene expression (Fig. 1a) and no differences in protein abundance or gene expression of OXPHOS enzymes (Fig. 1a,c). However, there were trends to lower *PGC1A* gene expression (p=0.16) and higher PGC1α protein abundance (p=0.11) in the PCOS group with an inverse correlation between protein and mRNA levels (r=−0.37, p=0.05). There was no difference in CS and β-HAD activity between PCOS and non-PCOS women (Fig. 1b).

There was a correlation between GIR and *PGC1A* gene expression (r=0.44, p=0.02) (Fig. 2a) irrespective of PCOS status with a trend to a negative correlation with PGC1α protein abundance (r=−0.34, p=0.09). None of the other mitochondrial markers correlated with GIR. Triacylglycerol was associated with *PGC1A* gene expression (r=−0.68, $p<0.01$) but not PGC1α protein abundance (Fig. 2b). There was no correlation between the mitochondrial measurements and $\dot{V}O_{2max}$, BMI, weight or age. Thigh muscle attenuation

Table 1 Baseline characteristics of participants

Characteristic	Non-PCOS (n=13)	PCOS (n=16)	p value
Age (years)	34.5±1.1	30.7±1.4	0.04
Waist (cm)	103.1±2.8	106.3±3.2	0.47
BMI (kg/m²)	36.0±1.3	37.0±1.7	0.64
Body fat (%)	49.7±1.4	48.1±1.0	0.33
Thigh muscle attenuation (HU)	47.5±0.93	49.5±0.67	0.08
GIR (mg m^{-2} min^{-1})	257.6±18.6	170±24.1	0.01
Fasting glucose (mmol/l)	4.8±0.1	4.9±0.1	0.36
Fasting insulin (pmol/l)	84.2 (68.3–134.1)	169.2 (114.0–212.7)	0.03
Testosterone (nmol/l)	1.6±0.2	2.7±0.2	<0.01
SHBG (nmol/l)	45.3±8.2	28.3±2.0	0.04
FAI	4.6 ±1.0	10.4±1.1	<0.01
Cholesterol (mmol/l)	4.8±0.2	5.0±0.3	0.68
Triacylglycerol (mmol/l)	1.2±0.2	1.5±0.2	0.22
HDL-cholesterol (mmol/l)	1.3±0.1	1.0±0.1	0.02
LDL-cholesterol (mmol/l)	3.0±0.2	3.3±0.2	0.43
$\dot{V}O_{2max}$ (ml kg^{-1} min^{-1})	25.0±0.9	23.7±1.3	0.43

Data are means±SE except for insulin (median [interquartile range]; p values from log-transformed data)

FAI, free androgen index

correlated with β-HAD (r=0.38, p=0.04) and was inversely correlated with HDL (r=−0.38, p=0.04).

PCOS vs non-PCOS women: effect of exercise training (Table 2) Exercise attendance was similar for both groups (97% PCOS, 92% non-PCOS, p=0.19). $\dot{V}O_{2max}$ improved with exercise training (p<0.01) within each group (Table 2). Exercise training resulted in decreased BMI and weight across the whole group, and there was a significant between-group difference in change in weight (p=0.03) and waist circumference (p=0.02), both decreasing more in the non-PCOS than in the PCOS women (Table 2). GIR increased with training, with a significant within-group improvement in the PCOS group (p=0.01) and a trend to improvement in the non-PCOS group (p=0.07), with no between-group difference.

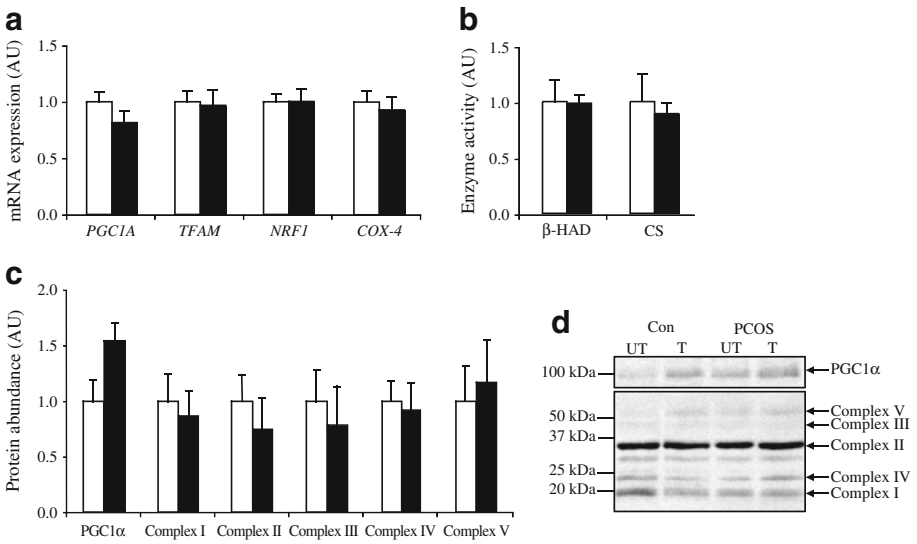

Fig. 1 mRNA expression, protein production and enzyme activity of mitochondrial biogenesis genes. **a** mRNA expression of *PGC1A*, mitochondrial transcription factor A (*TFAM*), nuclear respiratory factor-1 (*NRF1*) and cytochrome oxidase subunit 4 (*COX4*) genes determined by real time quantitative PCR. **b** β-HAD and CS enzyme activity was determined by measuring the disappearance of NADH spectrophotometrically. **c** Protein production of PGC1α, complex I (subunit NDUFB8), complex II (30 kDa subunit), complex III (core protein 2), complex IV (subunit II), complex V (α subunit) measured by western blotting. **d** Representative immunoblots of PGC1α and the mitochondrial complex proteins for a control (Con) and a PCOS woman (PCOS), in both untrained (UT) and trained (T) state. Data represent means ± SE from 16 PCOS (black bars) expressed relative to 13 non-PCOS (white bars) women (AU, arbitrary units; control =1)

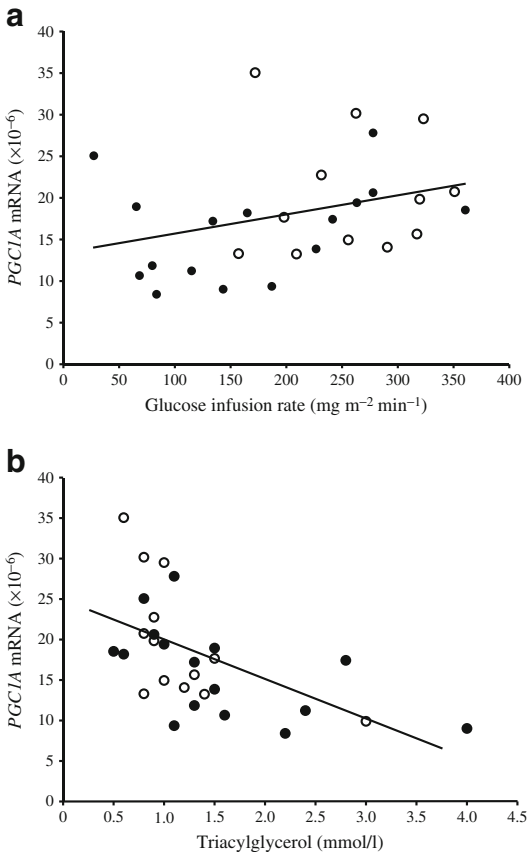

Fig. 2 Scatterplot of *PGC1A* gene expression versus (**a**) GIR (trend line $PGC1A = 1.32\times10^{-6} + 2.34\times GIR\times10^{-6}$) and (**b**) triacylglycerol (trend line $PGC1A = 2.43\times10^{-6} - 4.75\times TG\times10^{-7}$, where TG is triacylglycerol). Black circles, PCOS; white circles, non-PCOS

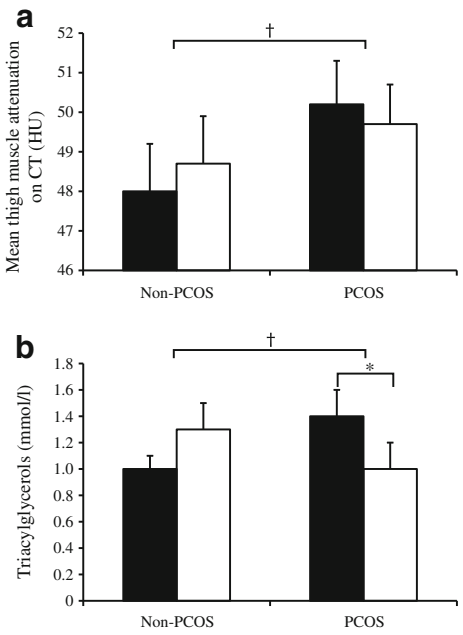

Fig. 3 Change in CT thigh muscle attenuation and triacylglycerol with exercise training. Data represent means±SE from eight PCOS and seven non-PCOS women before and after exercise (black and white bars, respectively). Significant within-group change with exercise (*$p<0.05$). Significant between-group differences in change in CT attenuation and triacylglycerol with exercise training (†$p<0.05$ adjusted for age)

As previously reported, there was a between-group difference in the change in triacylglycerol ($p=0.01$), with PCOS women showing a reduction in triacylglycerol ($p=0.02$), and no change in non-PCOS women ($p=0.09$; Fig. 3) [26]. Fasting insulin decreased within the PCOS group ($p=0.04$). There was a between-group difference in the change in CT muscle attenuation ($p=0.05$, $p=0.01$ corrected for age) with trends to decreased attenuation in the PCOS women ($p=0.19$), reflecting increased muscle lipid content, compared with the increased attenuation in non-PCOS women ($p=0.18$), reflecting the opposite (Fig. 3). The change in triacylglycerol correlated with change in thigh muscle attenuation ($r=0.54$, $p=0.04$).

There were no changes in any of the mitochondrial markers with exercise training within the whole group or between the two groups (Table 3). Within-group analyses revealed that electron-transport chain complex V α subunit and core 2 protein (complex III) increased in the non-PCOS group ($p=0.02$ and $p=0.04$, respectively), and expression of the *COX4* (also known as *COX4I1*) gene increased in the PCOS group ($p=0.02$; Table 3).

Discussion

The results of this study show that overweight women with PCOS had lower insulin sensitivity than a weight- and fitness-matched comparison group. Contrary to our prediction, however, there was no evidence that this difference in insulin sensitivity could be explained by a corresponding reduction in muscle mitochondrial content or functional markers. A novel finding was a trend to higher muscle attenuation (lower muscle lipid) in the PCOS women at baseline and a differential between-group effect of exercise training on thigh muscle attenuation. We have previously reported a similar between-group effect on serum triacylglycerol [26], with levels decreasing in the PCOS women with training. CT thigh muscle attenuation tended to decrease in the PCOS women, reflecting higher muscle lipid content after exercise training, whereas there was a trend to increased thigh muscle attenuation in the non-PCOS women. No direct correlation was found between these measures and GIR, but it does suggest an unexpected differential capacity for lipid storage in PCOS women that may contribute to the metabolic phenotype. There were no differences in a broad range of genes, proteins and enzyme activities reflecting mitochondrial biogenesis and function when compared with non-PCOS women of similar weight. Furthermore, mitochondrial markers did not change with exercise training-

Table 2 Effects of exercise training on weight, hormonal and metabolic variables

Variable	Non-PCOS (n=7)		PCOS (n=8)		p value for effect of exercise training	p value for change over study, PCOS vs non-PCOS
	Pre	Post	Pre	Post		
BMI (kg/m²)	37.6±2.3	36.4±2.0[a]	35.6±2.2	35.5±2.3	0.02	0.06
Weight (kg)	100.4±5.6	97.6±5.0[a]	95.2±6.6	94.6±6.8	<0.01	0.03
Waist (cm)	105.6±3.7	100.6±4.7[a]	104.6±5.1	105.6±4.9	0.09	0.02
Thigh muscle attenuation (HU)	48.0±1.2	48.7±1.2	50.2±1.1	49.7±1.0	0.82	0.05[b]
Fasting glucose (mmol/l)	4.7±0.1	4.8±0.1	5.1±0.2	5±0.1	0.97	0.58
Fasting insulin (pmol/l)	115.8 (76.0–149.0)	127.2 (79.1–158.0)	169.2 (136.1–285.1)	120.6 (78.7–286.3)[a]	0.73	0.09
GIR (mg m^{-2} min^{-1})	240.4±20.0	297.5±34.7	142.1±46.2	178.4±41.5[a]	0.01	0.46
SHBG (nmol/l)	56±13.7	58.6±11.2	26.7±2.9	30.5±3.5	0.31	0.85
Testosterone (nmol/l)	1.5±0.2	1.9±0.4	2.7±0.2	2.7±0.3	0.21	0.21
FAI	3.4±0.8	4.0±1.3	10.7±1.5	9.5±1.5	0.67	0.15
Cholesterol (mmol/l)	4.7±0.4	5.0±0.4	4.6±0.4	4.5±0.3	0.74	0.29
Triacylglycerol (mmol/l)	1.0±0.1	1.3±0.2	1.4±0.2	1.0 ±0.2[a]	0.43	<0.01
HDL-cholesterol (mmol/l)	1.2±0.1	1.3±0.1	0.9±0.1	1.0±0.1	0.17	0.87
LDL-cholesterol (mmol/l)	3.0±0.3	3.2±0.4	3.1±0.4	3.0±0.3	0.80	0.6
$\dot{V}O_{2max}$ (ml kg^{-1} min^{-1})	25.8±1.4	30.5±1.5[a]	25.9±3.3	33.3±3.5[a]	<0.01	0.19

Data are means±SE except for insulin (median [interquartile range]; p values from log-transformed data)

[a] Significant within-group change with exercise (p<0.05)

[b] Significant between-group change with exercise when corrected for age (p=0.01)

FAI, free androgen index

induced increase in insulin sensitivity in either group. This suggests that previously observed relationships between IR states and mitochondrial dysfunction are not applicable to the intrinsic IR of PCOS.

Previous data have linked high levels of IMCL, measured directly and with imaging techniques, with IR (reviewed by Lara-Castro and Garvey [6]). Using CT thigh muscle attenuation, an estimate of muscle lipid content that correlates with IMCL [9], we found a trend to lower baseline muscle lipid content in the more IR PCOS versus non-PCOS women. In support of this finding, an earlier study found that the relationship between IR and IMCL was present only in lean men [33]. Obese men in the same study had surprisingly low levels of IMCL. In the present study, there was an unexpected differential response of muscle lipid content to exercise training, with PCOS women increasing and non-PCOS women decreasing lipid, while IR decreased in both groups. Another study in overweight and obese adults found that exercise-induced improvements in insulin sensitivity were accompanied by increases in IMCL [34, 35]. Meex et al. [35] demonstrated a trend to increased IMCL with exercise in male patients with type 2 diabetes, whose IR improved, but, in contrast with the present study, mitochondrial function also improved. It was postulated that the increased IMCL may represent recruitment of non-oxidative type 2 fibres or improved lipid partitioning through the enzyme diacylglycerol acyltransferase (DGAT1) [35]. DGAT1 is critical for triacylglycerol synthesis, and overexpression in rodent skeletal muscle leads to muscle triacylglycerol accumulation with paradoxically decreased IR [36]. This partitioning of lipids may reduce build-up of triacylglycerol-derived metabolites, such as diacylglycerol and ceramides, that interfere with insulin signalling [37].

Our data suggest a difference in the capacity of sedentary PCOS women to store lipid in skeletal muscle compared with non-PCOS women. These findings parallel differences between men and non-PCOS women. Men have lower IMCL than women despite being more IR [38]. With endurance exercise, men exhibit lower lipid oxidation than women [39], and an acute exercise bout leads to muscle triacylglycerol breakdown in women but not in men [40]. Furthermore, PCOS women have more visceral fat than non-PCOS women, which decreases with exercise training in PCOS women only [26], again mimicking the response of visceral fat to exercise that occurs in men when compared with non-PCOS women [41]. The influence of hyperandrogenism on the metabolic phenotype of PCOS is not clear. These findings suggest a possible 'androgenic' pattern of lipid storage and its response to exercise training in PCOS. Androgens did not correlate with any of these lipid measures. This warrants further direct assessment of IMCL in PCOS including its cellular distribution, the presence of ceramides and diacylglycerol, and the activity of lipolytic and liposynthetic pathways such as DGAT1.

Interaction between mitochondrial function and IMCL accumulation may be the important factor for determining insulin sensitivity [42]. The literature supports an association

Table 3 Effect of exercise on protein abundance and gene expression and enzyme function

Characteristic	PCOS (n=8)		Non-PCOS (n=7)		p value for effect of exercise training	p value for change over study, PCOS vs non-PCOS
	Pre	Post	Pre	Post		
Protein abundance						
PGC1α	2.0±0.6	1.7±0.4	1.0±0.3	1.5±0.4	0.83	0.46
Complex I	0.9±0.2	0.8±0.2	1.0±0.3	1.3±0.3	0.48	0.26
Complex II	0.8±0.2	0.7±0.2	1.0±0.2	1.5±0.4	0.36	0.10
Complex III	1.0±0.4	1.0±0.3	1.0±0.3	1.4±0.4[a]	0.18	0.15
Complex IV	1.1±0.3	1.0±0.2	1.0±0.2	1.3±0.2	0.24	0.13
Complex V	1.5±0.5	1.4±0.4	1.0±0.3	1.5±0.4[a]	0.19	0.08
Enzyme activity						
β-HAD	0.9±0.1	1.0±0.1	1.0±0.0	1.1±0.1	0.16	0.97
CS	0.9±0.1	0.9±0.1	1.0±0.1	1.0±0.1	0.84	1.00
Gene expression						
PGC1A	0.8±0.1	0.8±0.1	1.0±0.1	0.8±0.1	0.24	0.14
TFAM	0.8±0.1	0.9±0.1	1.0±0.2	0.8±0.0	0.57	0.12
NRF1	0.8±0.1	0.7±0.1	1.0±0.1	0.9±0.1	0.38	0.94
COX4	0.8±0.1	1.0±0.1[a]	1.0±0.1	1.0±0.1	0.20	0.20

Data are means±SE

Arbitrary units expressed relative to non-PCOS women at baseline (1.0)

[a] Significant within-group change with exercise (p<0.05)

between skeletal muscle mitochondrial dysfunction, high adiposity and IR in people with obesity and type 2 diabetes and IR in first-degree relatives of those with type 2 diabetes, but controversy remains [19]. The present study found a modest correlation between IR and expression of *PGC1A*, but no difference in any mitochondrial markers between PCOS women and non-PCOS women. Some studies have reported a similar dissociation between IR and mitochondrial function [19, 43], which was highlighted by Nair et al [44] when comparing mitochondrial function of Asian–Indians with northern Europeans. In contrast with the present study, most studies of mitochondrial function and IR do not adequately control for physical activity, family history of type 2 diabetes, and body composition. However, as with our data, when patients with diabetes are well matched with normoglycaemic controls for body composition and physical activity, the two groups have similar mitochondrial function [45].

In PCOS, one previous study on the role of mitochondria [18] used a microarray approach and found reduced OXPHOS gene expression in skeletal muscle of PCOS women compared with weight-matched controls. The authors linked this to reduced *PGC1A* expression in PCOS, previously shown in type 2 diabetes [16, 17]. In contrast, our study found no difference in either OXPHOS gene expression and protein abundance or *PGC1A* gene expression. The reasons for disparities between this and other studies of IR and mitochondrial function are not clear [19]. Skov et al [18] selected PCOS women on the basis of IR severity, perhaps amplifying differences found, and family history of type 2 diabetes, fitness and body composition were not documented. In the present study, in which the PCOS group was not selected on the basis of IR and potential confounders were addressed, mitochondrial dysfunction does not appear to contribute to intrinsic PCOS-related IR.

PGC1α, through its effects on mitochondrial biogenesis and energy metabolism, has been implicated in the pathogenesis of IR [15]. A correlation between GIR and *PGC1A* was found for the whole group, supporting a relationship between *PGC1A* and IR but not specific to women with PCOS. However, animal studies using gene knockout and transgenic overexpression strategies have been conflicting but, overall, not supportive of the hypothesis that skeletal muscle PGC1α is causally related to IR (reviewed by Patti and Corvera [46]). Apart from the relationship with *PGC1A*, the present study found no relationship between GIR and downstream factors, including PGC1α protein production, nuclear respiratory factor-1 (NRF1), mitochondrial transcription factor A (TFAM) or mitochondrial genes and proteins. Post-transcriptional regulation of PGC1α, such as acetylation [47], may in part account for the dissociation between gene expression and protein abundance and the expected downstream effects.

A number of interventions that improve IR, including physical activity, weight loss and insulin sensitisers, also improve mitochondrial function (reviewed by Turner and Heilbronn [19]). However, other studies have demonstrated improved IR without improved mitochondrial function [19, 24, 48, 49]. Exercise training has long been shown to improve mitochondrial function [21]. In the present study, although exercise improved fitness and IR in both groups, mitochondrial variables did not change. In support of our findings, Heilbronn et al [23] demonstrated improvement in IR in obese men with exercise training without change in mitochondrial enzyme activity or mitochondrial biogenesis. Absence of responses to exercise may reflect the type and length of exercise training, site of muscle sampled, or resistance of muscle to increases in mitochondrial biogenesis and function. Taken together these data suggest that obese women with and without PCOS respond to exercise differently and warrant further exploration with inclusion of lean control groups.

Limitations of this study include small sample size, albeit larger than similar studies investigating differences in mitochondrial function between groups [11]. Our groups were not age-matched, but correcting for age did not affect, and age did not correlate with, any mitochondrial markers (not shown). This study did assess a number of different markers of mitochondrial biogenesis, but did not assess mitochondrial function, size or number. CT was used to measure muscle lipid content, but cannot distinguish intra- from extra-myocellular lipid. However, CT muscle attenuation correlated more closely with IMCL than with extramyocellular lipid [9, 10]. Further study of muscle lipid content in PCOS by more direct techniques is warranted. Despite these limitations, CT does sample large areas of muscle not possible with biopsy techniques, and would be more amenable to performing larger scale clinical studies in both lean and obese PCOS and non-PCOS women.

Conclusions In summary, there were differential effects of exercise training on circulating and muscle lipids between groups. PCOS women had significantly higher serum triacylglycerol at baseline and a trend to higher CT muscle attenuation, or less muscle lipid. Exercise led to a decrease in serum triacylglycerol and CT muscle attenuation relative to non-PCOS women. This suggests that PCOS women may store less lipid in skeletal muscle than non-PCOS women and that exercise may increase muscle lipid storage in PCOS women relative to non-PCOS women. No differences were observed in markers of mitochondrial function between overweight PCOS and non-PCOS women of comparable weight, despite a clear difference in IR. No major changes in mitochondrial markers were seen with 12 weeks of exercise training in either group. Therefore muscle lipid storage, but not skeletal muscle mitochondrial function, may contribute to

IR in women affected by PCOS and its amelioration with exercise. Further investigations on other potential mediators of IR in PCOS and the effects of exercise are warranted.

Acknowledgements Pathology was completed at Southern Health Laboratories. Tissue analysis of mitochondrial genes, proteins and enzyme activities was completed at the Baker Research Institute, Monash University, Melbourne, VIC, Australia, under the supervision of M. Febbraio and C. Bruce. Reagents were provided by the Baker Research Laboratory. E. Paul assisted with statistical analysis. Muscle biopsies were performed by B. Canny. An abstract was presented at the 7th Annual meeting of the Androgen Excess-PCOS Society, 2009.

Funding This investigator-initiated trial was supported by grants from the National Health & Medical Research Council (NH&MRC) Grant number 606553 (to H.J. Teede, B.J. Strauss, N.K. Stepto and S.K. Hutchison) as well as Monash University (N.K. Stepto and H.J. Teede) and The Jean Hailes Foundation. H.J. Teede is an NH&MRC Research Fellow. S.K. Hutchison and C.L. Harrison are NH&MRC PhD Scholars.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement SKH analysed and interpreted the data, and drafted the manuscript. HJT and NKS were responsible for conception and design and critically revised the manuscript for important intellectual content. DR, CLH and BJS analysed the data, and critically revised the manuscript for important intellectual content. All authors approved the final version for publication.

References

1. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ (2010) The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 25:544–551
2. Meyer C, McGrath BP, Teede HJ (2005) Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. J Clin Endocrinol Metab 90:5711–5716
3. Teede HJ, Hutchison SK, Zoungas S (2007) The management of insulin resistance in polycystic ovary syndrome. Trends Endocrinol Metab 18:273–279
4. Corbould A, Kim Y-B, Youngren JF et al (2005) Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. Am J Physiol Endocrinol Metab 288:E1047–E1054
5. Moran LJ, Misso ML, Wild RA, Norman RJ (2010) Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Updat 16:347–363
6. Lara-Castro C, Garvey WT (2008) Intracellular lipid accumulation in liver and muscle and the insulin resistance syndrome. Endocrinol Metab Clin North Am 37:841–856
7. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE (1997) Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes 46:1579–1585
8. Lowell BB, Shulman GI (2005) Mitochondrial dysfunction and type 2 diabetes. Science 307:384–387
9. Goodpaster BH, Kelley DE, Thaete FL, He J, Ross R (2000) Skeletal muscle attenuation determined by computed tomography is associated with skeletal muscle lipid content. J Appl Physiol 89:104–110
10. Larson-Meyer DE, Smith SR, Heilbronn LK, Kelley DE, Ravussin E, Newcomer BR (2006) Muscle-associated triglyceride measured by computed tomography and magnetic resonance spectroscopy. Obesity (Silver Spring) 14:73–87
11. Kelley DE, He J, Menshikova EV, Ritov VB (2002) Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 51:2944–2950
12. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 350:664–671
13. Petersen KF, Befroy D, Dufour S et al (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300:1140–1142
14. Wu Z, Puigserver P, Andersson U et al (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115–124
15. Finck BN, Kelly DP (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest 116:615–622
16. Patti ME, Butte AJ, Crunkhorn S et al (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A 100:8466–8471
17. Mootha VK, Lindgren CM, Eriksson K-F et al (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34:267–273
18. Skov V, Glintborg D, Knudsen S et al (2007) Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. Diabetes 56:2349–2355
19. Turner N, Heilbronn LK (2008) Is mitochondrial dysfunction a cause of insulin resistance? Trends Endocrinol Metab 19:324–330
20. Skov V, Beck-Nielsen H, Kruse T et al (2008) Pioglitazone enhances mitochondrial biogenesis and ribosomal protein biosynthesis in skeletal muscle in polycystic ovary syndrome. PLoS One 3:e2466
21. Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831–838
22. Menshikova EV, Ritov VB, Toledo FGS, Ferrell RE, Goodpaster BH, Kelley DE (2005) Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. Am J Physiol Endocrinol Metab 288:E818–E825
23. Heilbronn LK, Gan SK, Turner N, Campbell LV, Chisholm DJ (2007) Markers of mitochondrial biogenesis and metabolism are lower in overweight and obese insulin-resistant subjects. J Clin Endocrinol Metab 92:1467–1473
24. Toledo FGS, Menshikova EV, Azuma K et al (2008) Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. Diabetes 57:987–994
25. Dube JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH (2008) Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited. Am J Physiol Endocrinol Metab 294:E882–E888
26. Hutchison SK, Stepto NK, Harrison CL, Moran LJ, Strauss BJ, Teede HJ (2011) Effects of exercise on insulin resistance and body composition in overweight and obese women with and without polycystic ovary syndrome. J Clin Endocrinol Metab 96:E48–E56

27. Harrison CL, Stepto NK, Hutchison SK, Teede HJ (2011) The impact of intensified exercise training on insulin resistance and fitness in overweight and obese women with and without polycystic ovary syndrome. Clin Endocrinol (Oxf). doi:10.1111/j.1365-2265.2011.04160.x
28. Mitsiopoulos N, Baumgartner RN, Heymsfield SB, Lyons W, Gallagher D, Ross R (1998) Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. J Appl Physiol 85:115–122
29. Stepto NK, Coffey VG, Carey AL et al (2009) Global gene expression in skeletal muscle from well-trained strength and endurance athletes. Med Sci Sports Exerc 41:546–565
30. Watt MJ, Southgate RJ, Holmes AG, Febbraio MA (2004) Suppression of plasma free fatty acids upregulates peroxisome proliferator-activated receptor (PPAR) alpha and delta and PPAR coactivator 1alpha in human skeletal muscle, but not lipid regulatory genes. J Mol Endocrinol 33:533–544
31. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3:1101–1108
32. Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis. Academy Press, New York
33. Gan SK, Kriketos AD, Poynten AM et al (2003) Insulin action, regional fat, and myocyte lipid: altered relationships with increased adiposity. Obes Res 11:1295–1305
34. Dube JJ, Amati F, Toledo FG et al (2011) Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. Diabetologia 54:1147–1156
35. Meex RCR, Schrauwen-Hinderling VB, Moonen-Kornips E et al (2010) Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 59:572–579
36. Liu L, Zhang Y, Chen N, Shi X, Tsang B, Yu YH (2007) Upregulation of myocellular DGAT1 augments triglyceride synthesis in skeletal muscle and protects against fat-induced insulin resistance. J Clin Invest 117:1679–1689
37. Watt MJ (2009) Storing up trouble: does accumulation of intramyocellular triglyceride protect skeletal muscle from insulin resistance? Clin Exp Pharmacol Physiol 36:5–11
38. Hoeg L, Roepstorff C, Thiele M, Richter EA, Wojtaszewski JF, Kiens B (2009) Higher intramuscular triacylglycerol in women does not impair insulin sensitivity and proximal insulin signaling. J Appl Physiol 107:824–831
39. Tarnopolsky MA, Rennie CD, Robertshaw HA, Fedak-Tarnopolsky SN, Devries MC, Hamadeh MJ (2007) Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. Am J Physiol Regul Integr Comp Physiol 292: R1271–R1278
40. Steffensen CH, Roepstorff C, Madsen M, Kiens B (2002) Myocellular triacylglycerol breakdown in females but not in males during exercise. Am J Physiol Endocrinol Metab 282:E634–E642
41. Kuk JL, Ross R (2009) Influence of sex on total and regional fat loss in overweight and obese men and women. Int J Obes 33:629–634
42. van Loon LJ, Goodpaster BH (2006) Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. Pflugers Arch 451:606–616
43. Karakelides H, Irving BA, Short KR, O'Brien P, Nair KS (2010) Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. Diabetes 59:89–97
44. Nair KS, Bigelow ML, Asmann YW et al (2008) Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. Diabetes 57:1166–1175
45. De Feyter HM, van den Broek NMA, Praet SFE, Nicolay K, van Loon LJC, Prompers JJ (2008) Early or advanced stage type 2 diabetes is not accompanied by in vivo skeletal muscle mitochondrial dysfunction. Eur J Endocrinol 158:643–653
46. Patti ME, Corvera S (2010) The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev 31:364–395
47. Cheng Z, Guo S, Copps K et al (2009) Foxo1 integrates insulin signaling with mitochondrial function in the liver. Nat Med 15:1307–1311
48. Schrauwen-Hinderling VB, Mensink M, Hesselink MKC, Sels J-P, Kooi ME, Schrauwen P (2008) The insulin-sensitizing effect of rosiglitazone in type 2 diabetes mellitus patients does not require improved in vivo muscle mitochondrial function. J Clin Endocrinol Metab 93:2917–2921
49. Irving BA, Short KR, Nair KS, Stump CS (2011) Nine days of intensive exercise training improves mitochondrial function but not insulin action in adult offspring of mothers with type 2 diabetes. J Clin Endocrinol Metab 96:E1137–E1141