

Short-term endurance training does not alter the oxidative capacity of human subcutaneous adipose tissue

Donny M. Camera · Mitchell J. Anderson ·
John A. Hawley · Andrew L. Carey

Accepted: 5 January 2010 / Published online: 19 January 2010
© Springer-Verlag 2010

Abstract Endurance training results in adaptations that enhance regulation of energy storage and expenditure at rest and during exercise. While processes involved in skeletal muscle oxidative remodelling are well described, it is unknown whether oxidative capacity of human subcutaneous white adipose tissue (WAT) is modified by endurance training. Since human WAT retains rudimentary characteristics required for upregulation of oxidative function, we hypothesised that 10 days of intense endurance training would promote changes in WAT that favour an increase in oxidative capacity. Eleven untrained males (age 22 ± 1 years, body mass 81 ± 5 kg, peak oxygen uptake ($\text{VO}_{2\text{peak}}$) 3.7 ± 0.2 l/min) undertook a 10-day endurance training protocol. Subcutaneous adipose tissue biopsies were taken from the abdomen prior to and 1 day after completion of training and analysed for fatty acid oxidative capacity, citrate synthase activity, and mitochondrial content via electron microscopy and gene expression analyses. There was a reduction in whole-body rates of carbohydrate oxidation, and concomitant increases in fat oxidation rate measured during 20-min of submaximal cycling (70% of pre-training $\text{VO}_{2\text{peak}}$) and an increase in basal GLUT4 protein in skeletal muscle. Despite these training-induced adaptations, there were no changes in WAT of ex-vivo fat oxidation rate,

maximal citrate synthase activity, mitochondrial volume or in selected genes involved in adipose tissue oxidative capacity. We conclude that 10 days training in previously untrained subjects results in adaptations in skeletal muscle but does not increase the oxidative capacity of WAT.

Keywords White adipose · Brown adipose · WAT · BAT · Mitochondrial · Obesity · Adrenergic · UCP1

Introduction

White adipose tissue (WAT) is necessary for maintenance of energy homeostasis via regulation of fuel storage and essential endocrine functions (Hotamisligil 2006). In addition, WAT retains the capacity to up- or down-regulate oxidative capacity. For example, treatment with anti-diabetic thiazolidinedione drugs, while increasing white adipogenesis (Lehmann et al. 1995), up-regulates adipocyte mitochondrial content (Wilson-Fritch et al. 2003), whereas obesity and the inflammatory cytokine $\text{TNF}\alpha$ reduce WAT mitochondrial content (Valerio et al. 2006). Taken collectively, these observations suggest that reductions in adipose oxidative capacity may contribute to the progression of metabolic disease, and that adipose tissue oxidative capacity is labile. While it has been previously argued that the oxidative capacity of WAT is so low as to be negligible with respect to whole-body energy expenditure (Frayn et al. 2008), a small increase in adipose fuel turnover may become physiologically meaningful over an extended time period (Maassen et al. 2007).

Until recently it was thought that humans contain less or no functional brown adipose tissue (BAT), however recent studies (Cypess et al. 2009; Nedergaard et al. 2007; Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen

Communicated by Jacques Poortmans.

D. M. Camera · M. J. Anderson · J. A. Hawley · A. L. Carey
Exercise Metabolism Group, School of Medical Sciences,
RMIT University, Bundoora, VIC, Australia

A. L. Carey (✉)
Metabolic and Vascular Physiology Laboratory,
Baker IDI Heart and Diabetes Institute,
75 Commercial Road, Melbourne, VIC 3004, Australia
e-mail: andrew.carey@bakeridi.edu.au

et al. 2009) corroborate those from the past (Huttunen et al. 1981; Ricquier et al. 1982) and suggest that humans do contain BAT, and preliminarily demonstrate that it is activated by acute cold exposure. The primary means by which BAT proliferation and activation are promoted is through adrenergic stimulation, primarily, but not exclusively, via the β 3-adrenergic receptor (β 3-AR) which is expressed only minimally on other tissues (Collins and Surwit 2001). Interestingly, low levels of β 3-AR expression have been detected in human WAT cells (De Matteis et al. 2002; Krief et al. 1993).

Regular endurance exercise training modulates numerous aspects of cellular biochemistry in multiple tissues, with rapid and marked increases in the oxidative capacity of skeletal muscle (Gibala et al. 2006; Youngren et al. 2001). Further, in rats prolonged endurance training results in increased oxidative capacity of the epididymal fat pad (Stallknecht et al. 1991) and prevents the obesity-induced reduction in omental adipose mitochondrial content (Laye et al. 2009). A well-described endocrine response and primary stimulus for these adaptive responses to training is the increase in circulating catecholamines that occurs during exercise (Peronnet et al. 1981), and this has been shown to contribute to exercise-induced upregulation of PGC-1 α expression in rodent epididymal and retroperitoneal fat pads (Sutherland et al. 2009). However, it is currently unknown whether exercise training coincides with increased oxidative capacity of WAT in response to short-term endurance training in humans, as is the case for skeletal muscle, therefore the purpose of this study was to directly examine this concept in previously untrained male subjects. Since human WAT expresses the β 3-AR, endurance training results in significant adrenergic stimulation and WAT has the capability to increase its oxidative capacity, we hypothesised that a period of short-term intense endurance exercise training that is known to increase skeletal muscle oxidative capacity would result in detectable changes in markers of oxidative function and gene expression in human WAT. Such findings would be important since they may explain some of the beneficial health-related adaptations to this type of physical training, and most importantly, provide evidence as to whether human WAT oxidative capacity can be altered via a physiological stimulus other than prolonged cold exposure, in favour of more BAT-like (increased oxidative capacity) functionality.

Methods

Subjects

Eleven healthy non-endurance trained, non-smoking male subjects volunteered to participate in this study that was

approved by the RMIT University Human Research Ethics Committee. The baseline physical characteristics (standing height, body weight, and body composition) of all participants are presented in Table 1. Body composition was determined using dual-energy X-ray absorptiometry (DEXA) (Lunar DPX densitometer, Lunar Radiation Corp., Madison, WI, USA). A total body scan was conducted with appropriate software to provide estimates of fat and lean mass for the entire body. None of the subjects were on medications before or during the study and were asked to abstain from alcohol consumption. The subjects were given both oral and written information about the purpose, nature, and potential risks of the study, and informed written consent was obtained prior to participation.

Preliminary testing

Approximately 2 weeks prior to beginning experiments, subjects performed an incremental test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands) to measure peak oxygen consumption (VO_{2peak}). This test protocol, described in detail previously (Hawley and Noakes 1992), was modified slightly: after a 5 min (min) warm up at 100 Watts (W), the workload was increased by 25 W every 2.5 min until volitional fatigue or until a cadence of 60 revolutions per minute could no longer be maintained. Throughout the maximal test and during portions of the subsequently described exercise training program, subjects inspired air through a mouthpiece attached to an open-circuit metabolic cart (Parvo-medics, Salt Lake City, UT, USA). Expired gas was passed through a flowmeter, an O_2 analyser, and a CO_2 analyser. The flowmeter and gas analysers were connected to an IBM personal computer that calculated the instantaneous rates of O_2 uptake (VO_2), CO_2 production (VCO_2), minute ventilation (VE_{STPD}), and the respiratory exchange ratio (RER) every 15 s from conventional equations. Before

Table 1 Physical characteristics of the study subjects ($n = 11$)

Age (years)	21.7 \pm 0.7
Height (cm)	182 \pm 1.7
Body composition	
Body mass (kg)	81.0 \pm 4.5
BMI (kg/m ²)	24.3 \pm 1.1
DEXA total body fat mass (%)	15.2 \pm 2.4
DEXA total body lean mass (%)	84.1 \pm 3.5
Exercise capacity	
VO_{2peak} (l/min STPD)	3.7 \pm 0.2
Peak power output (W)	254 \pm 14

Values presented as mean \pm SEM

BMI body mass index, DEXA dual-energy X-ray absorptiometry, VO_{2peak} peak oxygen consumption

each maximal test and all experimental trials, the analysers were calibrated using a 3 l Hans-Rudolph syringe with commercially available gases of known O₂ and CO₂ concentration (4.00% CO₂ and 16.00% O₂). The value for VO_{2peak} (l/min) corresponded to an average of the two highest 15 s values achieved during the collection period. The results of the VO_{2peak} test were used to determine the power output (W) that corresponded to 70% and 90% of each subject's VO_{2peak} to be used in the subsequently described exercise training program.

Exercise training program

The exercise training program was designed according to previous study protocols that have been shown to improve whole-body and skeletal muscle energy metabolism in untrained subjects (McConnell et al. 2005). Subjects reported to the laboratory everyday of the study, and completed all training sessions under supervision for the prescribed durations and intensities. Subjects rode on Lode ergometers for 10 consecutive days, alternating between continuous moderate intensity and shorter high-intensity interval training (HIT) sessions. Moderate intensity bouts involved cycling 60–90 min/day at 70% of VO_{2peak}, while HIT bouts involved six 5 min repetitions at 90% VO_{2peak}, with 3 min active recovery at 40% VO_{2peak} between each repetition. Subjects were allowed to consume water and sports drink ad libitum during training sessions.

Rates of carbohydrate and fat oxidation

On the first and last days of the exercise training program, pulmonary gas samples were measured for a 20 min period (between the 20th and 40th min of each session) as described above. On the evenings prior to the collection of the pulmonary gas samples and the tissue biopsies (described below), subjects were provided with a standard pre-exercise carbohydrate meal (3 g/kg carbohydrate), to be consumed a minimum of 10 h before analysis and without any other food or drink aside from water until after sampling. Whole-body rates of carbohydrate (g/min) and fat oxidation (g/min) were calculated from VCO₂ and VO₂ values (l/min) using non-protein RER values, according to the following equations (Jeukendrup et al. 1995; Peronnet and Massicotte 1991):

$$\text{Carbohydrate oxidation} = (4.585 \times \text{VCO}_2) - (3.226 \times \text{VO}_2)$$

$$\text{Fat oxidation} = (1.695 \times \text{VO}_2) - (1.701 \times \text{VCO}_2)$$

Rates of substrate oxidation (μmol/kg/min) were determined by converting the gram-per-minute rate of triglyceride oxidation to its molar (mol) equivalent,

assuming the average weight of human triglyceride to be 855.26 g/mol, and glucose to be 180 g/mol, and then corrected for total body mass (Jeukendrup et al. 1995; Peronnet and Massicotte 1991).

Skeletal muscle and adipose tissue biopsy

Skeletal muscle and subcutaneous adipose tissue biopsies were undertaken on the mornings of the first and 24–26 h after the final training bout. Subjects reported to the laboratory in the morning after a 10–12 h overnight fast and having abstained from exercise, alcohol, and caffeine for 24 h. Subjects reclined on a bed and while under local anaesthesia (2–3 ml of 1% Xylocain (lignocaine)), *vastus lateralis* muscle and abdominal subcutaneous adipose tissue samples were obtained under sterile conditions using a 5- or 6-mm Bergstrom needle modified with suction. Approximately 120–150 mg of adipose and muscle tissue was obtained and cleaned of visible connective tissue and blood vessels. Approximately 40 mg of adipose sample was rinsed in PBS containing 0.1% BSA and used for fatty acid oxidation analysis (described in Sect. [Palmitate oxidation](#)). The remainder of the samples were allocated towards other assays described subsequently.

Palmitate oxidation

Adipose tissue fatty acid oxidation rate, evaluated before and after the exercise training intervention from adipose tissue biopsies obtained during resting, fasted conditions, was determined from ¹⁴C₂ production and ¹⁴C-labelled acid-soluble metabolites within a sealed system. A volume of 2.0 ml modified Krebs–Henseleit buffer, as previously described (Crowe et al. 2008), was pre-gassed for 40 min with 95% O₂/5% CO₂, added to a 20-ml glass scintillation vial, and placed in a shaking water bath at 30°C. The 20-ml glass scintillation vial contained a 1.7 ml plastic centrifuge tube and a smaller 0.5 ml microfuge tube that contained 400 μl of 1 M benzethonium hydroxide to capture ¹⁴C₂ produced during the oxidation reaction. Before all experiments the buffer was supplemented with 3.5% fatty acid-free BSA, 0.5 mM unlabelled palmitate, 5 mM glucose, and 0.8 μCi/ml ¹⁴C-palmitate (GE Healthcare Life Sciences, Buckinghamshire, UK). Approximately 30–40 mg of adipose tissue from biopsy samples was quickly added to the buffer medium, gassed with 95% O₂/5% CO₂ for 10 s and incubated for 5 h in a shaking water bath at 30°C. Post incubation, the adipose tissue was removed from the buffer medium and immediately snap-frozen in liquid nitrogen and stored at –80°C for later quantification of acid soluble metabolites (ASMs). Gaseous CO₂ produced from the oxidation of ¹⁴C-labelled palmitate was measured by acidifying the remaining buffer medium in the 20-ml

glass scintillation vial with 2 ml of 1 M acetic acid, and the buffer was incubated for a further 2 h in a shaking water bath at 30°C. Post incubation, the inner 0.5-ml tube was carefully removed and placed into a scintillation vial with 5 ml of cocktail for quantification by liquid scintillation. To quantify ASMs, the frozen tissue sample was transferred to a 10-ml plastic centrifuge tube and homogenised in 5.0 ml of ice-cold 2:1 chloroform–methanol mixture (vol/vol). After adding 2.0 ml of distilled H₂O and shaking intermittently for 10 min, the samples were centrifuged at 3,000g for 10 min at 4°C. A 3 ml aliquot of the top aqueous phase was removed, placed in a scintillation vial with 10 ml of cocktail, and quantified by liquid scintillation.

Citrate synthase activity

Previously snap-frozen adipose (~10–20 mg) tissue samples were homogenised in 100 mM potassium phosphate buffer (pH 7.3, 1:20 dilution), and citrate synthase was assayed spectrophotometrically at 25°C by the reduction of DNTB, as published previously with slight modifications (Bruce et al. 2003).

Gene expression analysis

Selected genes that, when highly expressed, are representative of either WAT (HOXC9, RB1, TCF21, and Resistin) or an oxidative/BAT phenotype (PGC-1 α , COX IV, UCP1, UCP2, UCP3, HOXC4, ADRB3, and PRMD16) (Gesta et al. 2007; Nedergaard et al. 2007; Seale et al. 2007; Timmons et al. 2007; Tseng et al. 2008) were measured to determine whether the training program was associated with changes in mRNA expression. All reagents used in this and subsequent procedures were obtained from Sigma–Aldrich (St Louis, MO, USA) unless specified. Adipose tissue RNA extraction was performed on previously snap-frozen samples with TRIzol according to the manufacturer's directions. Briefly, ~50 mg of adipose tissue was placed in 10 times volume of TRIzol and homogenised with a handheld, motorised Teflon pestle for approximately 30 s. Samples were allowed to stand for a few minutes, after which chloroform (1/5 volume TRIzol) was added and the sample vigorously shaken. Samples were allowed to stand for 5 min and spun at 12,000g for 15 min at 4°C after which the upper aqueous phase was transferred to a new tube. The aqueous phase was mixed with 2 μ l of 20 mg/ml glycogen and then precipitated by mixing with isopropanol alcohol (1/2 volume TRIzol). Samples were incubated at room temperature for 10 min and then centrifuged at 12,000g for 15 min at 4°C. The supernatant was removed and the resulting pellet was washed with 1 ml of 75% ethanol in diethylpyrocarbonate (DEPC)-treated

water. After centrifugation at 7,500g for 10 min at 4°C, the ethanol supernatant was removed and the RNA pellets were left to air-dry for 5–10 min before being re-dissolved in 15 μ l of DEPC-treated water. The quality and quantity of the RNA were determined on a NanoDrop 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) by measuring absorbance at 260 nm and 280 nm with a 260/280 ratio between 1.76 and 1.96 recorded for all samples. The RNA samples were diluted as appropriate to equalise concentrations, and stored at –80°C for subsequent reverse transcription.

First-strand complementary DNA (cDNA) synthesis was performed using commercially available TaqMan Reverse Transcription kit reagents (Applied Biosystems, Melbourne, Australia) at a final concentration of 10 ng/ μ l. All RNA samples were reverse transcribed to cDNA in a single run from the same reverse transcription master mix. Negative control samples (no RNA or no reverse transcriptase enzyme) were run simultaneously with test samples to control for and verify that amplification did not proceed from genomic DNA and other potential contaminants.

As much as 10 ng of cDNA was aliquoted into 96-well PCR plates and subjected to PCR analysis using a BioRad iCycler RT-qPCR system (BioRad, Gladesville, Australia). PCR with reactions contained 4 μ l of cDNA template (2.5 ng/ μ l), 10 μ l of 2 \times BioRad iQTM Supermix (BioRad), 1 μ l of 20 \times Taqman FAM-labelled assay-on-demand gene expression reagents, 0.5 μ l of 20 \times 18S ribosomal RNA (rRNA) control reagents (Applied Biosystems), and DEPC H₂O added to a final volume of 20 μ l. Multiplex PCR conditions involved 50 cycles of 95°C for 15 s and 58.5°C for 60 s, using VIC-labelled 18S rRNA as a housekeeping gene to normalise threshold cycle (C_T) values. A C_T value reflects the cycle number at which the DNA amplification is first detected. The relative amounts of mRNAs were calculated using the comparative C_T method. For each sample, a ΔC_T value was obtained by subtracting 18S C_T values from the C_T of the gene of interest. Therefore, a higher ΔC_T value indicates a lower relative expression. All samples were run in duplicate simultaneously with RNA- and RT-negative controls.

Western blotting

Vastus lateralis muscle lysates were solubilised in Laemmli buffer, separated by SDS–PAGE, and transferred to polyvinylidene fluoride membranes. The membranes were blocked and then incubated overnight at 4°C with antibodies specific for GLUT4 (1:1,000, Biogenesis Ltd, Poole, UK) and β -actin (1:1,000, Cell Signaling, Danvers, MA, USA). The immunoreactive proteins were detected via

chemiluminescence (Pierce, Rockford, IL, USA) and quantified by densitometry (BioRad, Gladesville, NSW, Australia). Of note, insufficient quantities of adipose tissue remained from biopsy samples to allow protein analyses.

Electron microscopy

Subcutaneous WAT was analysed by transmission electron microscopy for mitochondrial content before and after exercise training from three subjects. Approximately 3–5 mg of adipose tissue was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 2 h, and then stored at 4°C in cacodylate buffer. Samples were next post-fixed in 2% osmium tetroxide solution for 1 h. After dehydrating in graded acetone, the tissue was embedded in araldite/epon resin. Sections (0.5 µm) were cut using an Ultracut S ultramicrotome mounted on copper/palladium 200 mesh grids and then stained with 3% aqueous solution of uranyl acetate and lead citrate. Grids were examined in a Siemens Elmiskop 102 electron microscope at 60 K after magnification at 8–10,000×. Mitochondrial numbers were subsequently quantified by counting visible mitochondria from three separate images of white adipocyte cytoplasm from each sample (Crowe et al. 2008).

Statistical analysis

All data are expressed as mean ± SEM. Data were evaluated using paired *t* tests. Statistical significance was considered to be $P < 0.05$.

Results

Respiratory exchange ratio (Fig. 1a) and whole-body carbohydrate oxidation rate (Fig. 1b) decreased, and whole-body fat oxidation rate (Fig. 1c) increased during submaximal cycling (70% of pre-training $\text{VO}_{2\text{peak}}$) after 10 days of endurance training ($P < 0.05$). There was also a training-induced increase in skeletal muscle GLUT4 protein (Fig. 1d; $P < 0.05$).

WAT oxidative capacity

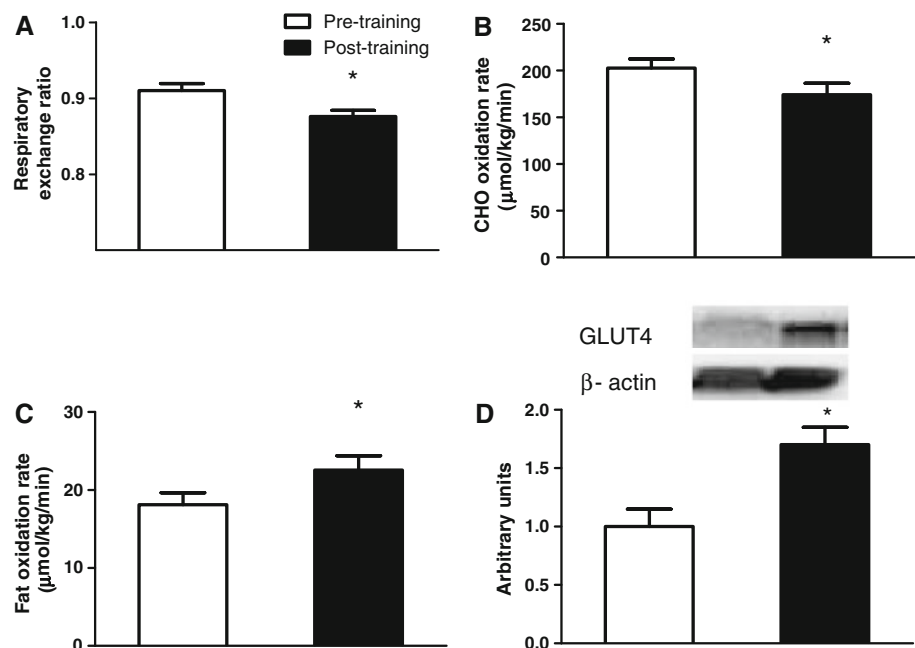
There was no change in complete ($^{14}\text{CO}_2$ production) or incomplete ($^{14}\text{CO}_2$ -labelled ASMs) palmitate oxidation after the training intervention (Fig. 2a), or in the ratio of complete-to-incomplete oxidation (Fig. 2b). While there appeared to be a modest increase in the ratio of complete-to-incomplete fatty acid oxidation (Fig. 2b) this failed to reach statistical significance ($P = 0.15$). Maximal citrate synthase activity was unchanged after the training intervention (Fig. 2c).

White adipose tissue sections magnified 8–10,000× via electron microscopy revealed a small, characteristic cytosolic compartment in each cell (Fig. 2d). Very few mitochondria were seen, and the number of mitochondria was unchanged in response to the training intervention.

Gene expression analysis

Training had no measurable effect on any of the genes under investigation (Fig. 3a). Of note, of 10 subjects in

Fig. 1 Non-protein respiratory exchange ratio (RER) (a), carbohydrate (CHO) (b), and fat (c) oxidation rates ($\mu\text{mol}/\text{kg}/\text{min}$) during cycling at 70% of pre-training $\text{VO}_{2\text{peak}}$ before and after 10 days of intense cycle exercise training. **d** Skeletal muscle GLUT4 content before and after 10 days of intense cycle exercise training. Values are expressed as mean ± SEM ($n = 11$). * $P < 0.05$



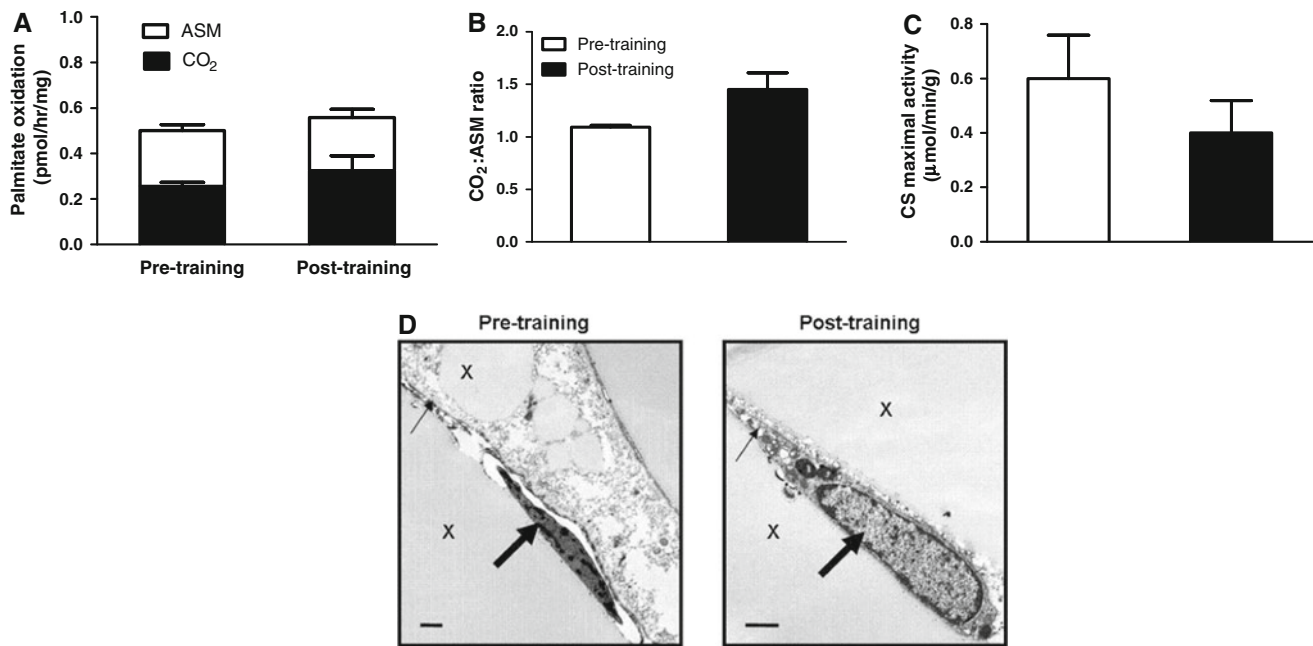


Fig. 2 **a** Palmitate oxidation rate (pmol/h/mg) in ex-vivo subcutaneous adipose tissue before and after the exercise training intervention. **b** Ratio of complete (CO₂) to incomplete (ASM) fatty acid oxidation rate. ($n = 9$). **c** Maximal activity of citrate synthase (CS, $\mu\text{mol}/\text{min}/\text{g}$) before and after training in subcutaneous adipose tissue. **d** Electron

microscopic images of adipocytes from subcutaneous adipose tissue before and after 10 days of intense endurance exercise training. *Large arrows* indicate nuclei, *small arrows* indicate mitochondria, *crosses* indicate intracellular lipid droplets. *Bars* represent 1 μm . Values are expressed as mean \pm SEM ($n = 9$)

which gene expression was measured ADRB3 (the β_3 -AR) was detectable in eight subjects before and after training, and UCPI in seven before training and these plus an additional two after training. Resistin mRNA was detected in samples from four subjects before training, whereas it was found in these same four plus an additional three subjects after training. Figure 3b shows individual gene expression patterns for a number of these genes that are important in mitochondrial production and function, as well as brown and white adipocyte determination.

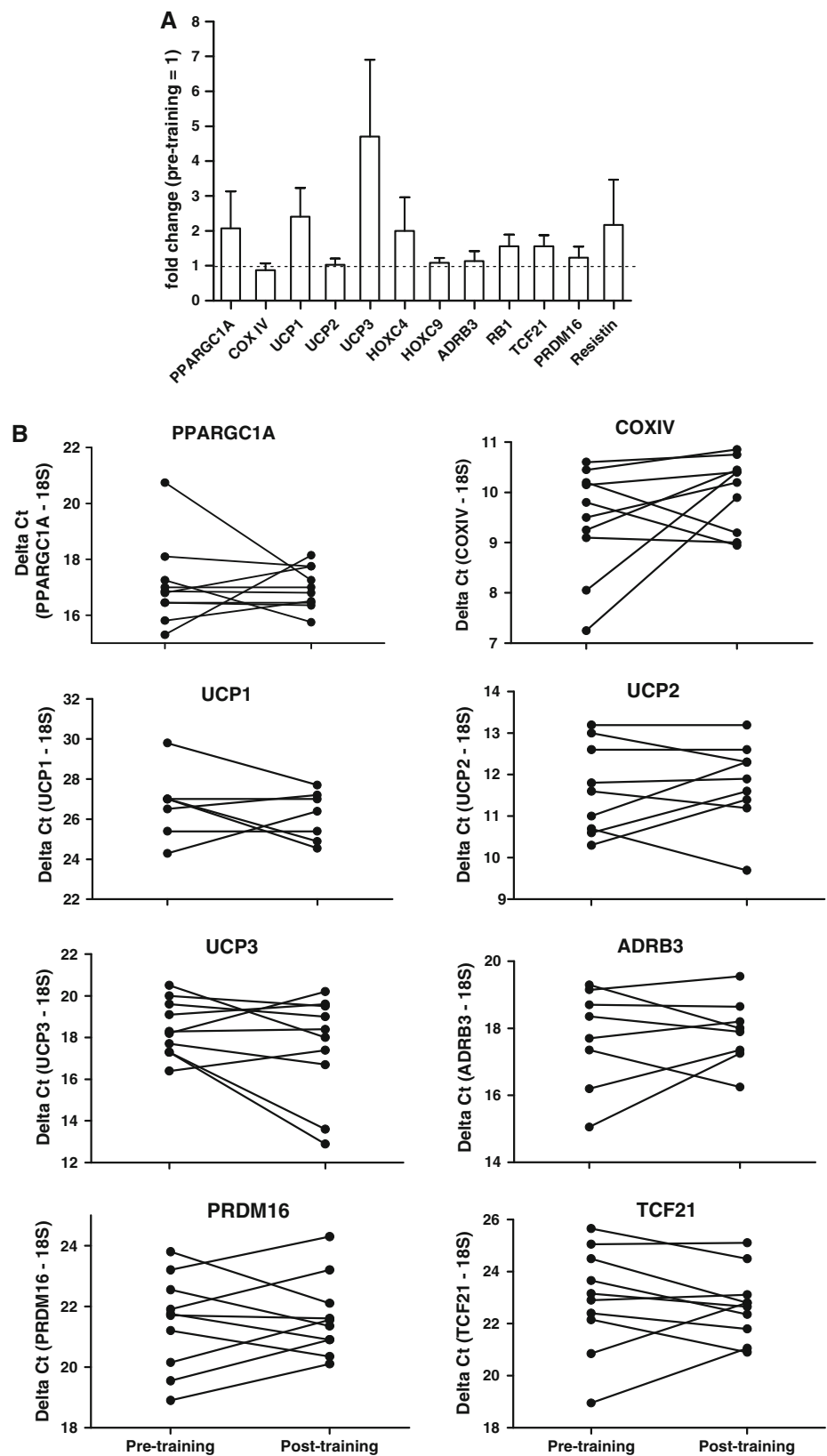
Discussion

We hypothesised that short-term endurance exercise training would promote a transition in white adipocyte phenotype from primarily storage towards a more oxidative phenotype. In contrast to our original hypothesis, and despite an intervention that resulted in significant whole-body and skeletal muscle adaptations, we were unable to detect any measurable changes in the oxidative capacity of human adipose tissue after 10 days of intense endurance exercise training.

The question of whether human WAT oxidative capacity can be increased is important because the ability to dissipate energy through uncoupled respiration and thermogenesis offers the possibility of increasing energy expenditure without necessarily causing dysfunction in

other tissues, thus providing a suitable strategy to attenuate obesity (Feldmann et al. 2009). Since prolonged exercise bouts induce a systemic adrenergic response (Peronnet et al. 1981), human WAT expresses a small quantity of the β_3 -AR (De Matteis et al. 2002; Krief et al. 1993), and that rodents increase WAT oxidative capacity in response to training (Stallknecht et al. 1991) we postulated that exercise training would provide a potent physiological stimulus to alter the functionality of WAT. Accordingly, in addition to functional (ex-vivo fatty acid oxidation rate and citrate synthase activity) and morphological (electron microscopic imaging of mitochondrial content) measures, we quantified the expression of a number of genes that were selected a priori as markers of adipocyte phenotype. Based on the similar mRNA abundances before and after the training intervention, it would appear that a change in phenotype towards a greater oxidative capacity did not take place. Furthermore, this highlights the marked variability with respect to both individual differences, as well as the response to training in this tissue. It is possible that the genes selected might be too small a cohort with respect to detecting useful changes in cell function, and perhaps conducting a gene array analysis would provide a clearer indication as to whether a global change in tissue function had occurred. However, the genes selected here have been previously shown to be strong markers of both brown and WAT function (Gesta et al. 2007; Nedergaard et al. 2007;

Fig. 3 a Gene expression represented as fold change compared with pre-exercise level (pre exercise = 1, *dashed line*) after 10 days intense cycle exercise training. Values are expressed as mean \pm SEM. **b** Gene expression values representing genes that are important in WAT/BAT oxidative function and differentiation for individual subjects before and after 10 days of intense cycle exercise training. Values are presented as Delta-Ct: the value obtained after subtraction of the threshold cycle for the housekeeping gene 18S from the gene of interest ($n = 7-10$)



Puigserver 2005; Seale 2007, 2008), and their expression reflects a lack of change in functional measures, therefore we suggest that these analyses are an accurate representation of the tissue phenotype.

It is possible that the transition of human WAT away from storage to an oxidative phenotype might require a much more prolonged stimulus (i.e., months to years), as has been demonstrated in rats (Stallknecht et al. 1991). In this regard, although significant increases in skeletal muscle oxidative capacity are evident after just 1–2 weeks of training (Gibala et al. 2006; Youngren et al. 2001), larger changes are evident after longer intervention periods (Green et al. 1995; Green et al. 1991; Phillips et al. 1996). Second, white adipocytes in humans are replaced at a rate of only $\sim 10\%$ per year (Spalding et al. 2008), therefore a stimulus that has potential to modify function may need to be present for a much longer duration than several weeks, as appears to be the case in rodents (Laye et al. 2009). In this light, the development of obesity and dysregulation of adipose tissue takes many months/years, and in this instance, akin to the opposite of chronic exercise training, BAT-like features in human WAT are reduced (Krief et al. 1993; Oberkofler et al. 1997; Yang et al. 2003). Additionally, even though adipose tissue blood flow is increased during intense exercise (Bulow and Madsen 1978), using exercise as a mode to induce an adrenergic stimulus is transient, and subcutaneous WAT $\beta 3$ -AR content too low. For instance, the adrenergic stimulus might need to be continuous, as is the case in patients with pheochromocytoma (Ricquier et al. 1982), or during cold exposure (Smith et al. 1990). Further, despite training bouts similar to those in the current study resulting in a significant adrenergic response, unlike rodents (Sutherland et al. 2009) the relatively low number of $\beta 3$ -AR within the subcutaneous depot may not be sufficient to activate the subsequent intracellular signalling pathways necessary for a transition in cell phenotype to materialise.

In regard to the capacity for WAT to diverge with respect to oxidative capacity, and the ability to detect such changes, the site of adipose extraction may be critical. In the current study it was possible only to extract samples from the subcutaneous depot, however studies that have compared subcutaneous and visceral fat depots in humans and rodents have shown differences in tissue phenotype (Lefebvre et al. 1998). In particular, immunohistochemical (De Matteis et al. 2002) and gene expression (Krief et al. 1993) studies have shown a greater presence of the $\beta 3$ -AR in human omental adipose depot when compared with subcutaneous WAT. The omental adipose depot has also been shown to have much greater metabolic activity compared to subcutaneous adipose tissue (Lefebvre et al. 1998), with threefold higher rates of lipolysis (Hamosh et al. 1963) and lipid turn-over (Frayn 1998), and a greater response to noradrenaline (Ostman et al. 1979). Likewise,

similar rodent studies that have identified increased oxidative capacity in WAT depots have examined epididymal, omental, and retroperitoneal fat pads, all of which are in the visceral compartment (Laye et al. 2009; Stallknecht et al. 1991; Sutherland et al. 2009). In addition, different subcutaneous WAT depots may vary with respect to oxidative capacity, and therefore might respond differently to training. Lipolytic rates of subcutaneous WAT vary when comparing the upper and lower body of lean humans (Horowitz and Klein 2000), and adipose tissue in close proximity the thigh muscle of obesity resistant mice has been shown to contain brown adipocytes (Almind et al. 2007). This raises the possibility that the hypothesised transition may occur in response to training in adipocytes from the omental depot, or from subcutaneous adipose tissue surrounding the lower body musculature, but remained undetected by the methods employed here. In the present study, obtaining a tissue sample from the omental and leg/gluteal adipose depots would have presented numerous difficulties with respect to practical and ethical constraints and was therefore not possible.

The results presented here offer the first direct quantitation of the absolute fatty acid oxidation rate by human subcutaneous adipose tissue. Oxidation rates varied between ~ 0.3 and ~ 0.9 pmol/h/mg. Other researchers (Maassen et al. 2007) have suggested that hyperlipidemia observed in the metabolic syndrome, resulting from liberation of fatty acids during WAT lipolysis, could be reduced by increasing the oxidative capacity of WAT, thereby preventing release of liberated fatty acids into circulation. Levels of palmitate oxidation reported here (<1 pmol/h/mg) are in the order of <100 mg of lipid oxidation per day in the WAT of a healthy human. On the surface this indicates that WAT lipid oxidation is an extremely small contributor to fuel oxidation, and any attempt to elevate its oxidative capacity would probably result in a minimal impact upon whole-body fat oxidation rate, and hyperlipidemia. This does not, however, account for the issues described in the previous paragraph; i.e., that other depots might contribute a much greater quantity of lipid oxidation. Further our assays do not account for (a) other substrates which WAT may preferentially oxidise; (b) that stimulated (e.g., adrenergic) oxidation rate, as distinct from the basal rate that was calculated here, might be dramatically greater; or c) that continuous circulation rather than a closed system might allow for better perfusion therefore higher oxidation rates. This is evidenced by studies in human ex-vivo WAT tissue slices that reported oxygen consumption rates in a closed continuously gas-perfused system that equate to substrate oxidation rates approximately one order of magnitude higher than our data (Hallgren et al. 1986). Finally, since BAT oxidative capacity is far in excess of WAT, this has no bearing on the

concept that conversion of WAT to BAT might provide a meaningful increase in whole-body energy expenditure (Cinti 2009).

It is important to consider that a true functional adipocyte transition may not be possible, at least within the context of exercise training. In rodents it has been shown acute exercise is able to stimulate PGC-1 α mRNA expression in muscle (Sutherland et al. 2009), while cultured human primary white adipocytes can upregulate oxidative capacity after induction of PGC-1 α expression (Tiraby et al. 2003), and certain signalling cues can promote the development of immature brown fat cells into mature cells (Hull and Segall 1965; Petrovic et al. 2008; Seale et al. 2007; Tseng et al. 2008), prior evidence suggest that BAT activity is reduced in exercise trained rodents (Larue-Achagiotis et al. 1994). Since upregulation of oxidative capacity in BAT is primarily for the purpose of adaptive thermogenesis (e.g., during cold exposure), and training provides a regular source of non-adaptive thermogenic heat production, WAT may respond in a similar manner to BAT in that its oxidative capacity might be reduced by regular training.

In conclusion, a short-term (10 days) endurance exercise training regimen resulted in major shifts in whole-body metabolism from carbohydrate to fat oxidation during submaximal exercise. Yet despite this robust adaptation, our training program had less effect on a number of metabolic and oxidative markers in subcutaneous WAT. Furthermore, the training intervention failed to alter the expression of a number of genes with putative roles in the function and differentiation of WAT and BAT. Future studies should determine whether long-term (3–12 month) training interventions influence human adipose tissue oxidative capacity from regional-specific adipose depots.

Acknowledgments This study was supported by an Early Career Researcher grant from RMIT University. ALC was supported by a Peter Doherty Fellowship from the National Health and Medical Research Council of Australia. The authors would like to thank the research participants for volunteering their time and effort, and Dr Matthew Watt, Monica Amezcua and Kate Pattison for technical assistance. No conflict of interest exists.

References

- Almind K, Manieri M, Sivitz WI, Cinti S, Kahn CR (2007) Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proc Natl Acad Sci USA* 104:2366–2371
- Bruce CR, Anderson MJ, Carey AL, Newman DG, Bonen A, Kriketos AD, Cooney GJ, Hawley JA (2003) Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. *J Clin Endocrinol Metab* 88:5444–5451
- Bulow J, Madsen J (1978) Human adipose tissue blood flow during prolonged exercise II. *Pflugers Arch* 376:41–45
- Cinti S (2009) Transdifferentiation properties of adipocytes in the adipose organ. *Am J Physiol Endocrinol Metab* 297:E977–E986
- Collins S, Surwit RS (2001) The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog Horm Res* 56:309–328
- Crowe S, Turpin SM, Ke F, Kemp BE, Watt MJ (2008) Metabolic remodeling in adipocytes promotes ciliary neurotrophic factor-mediated fat loss in obesity. *Endocrinology* 149:2546–2556
- Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 360:1509–1517
- De Matteis R, Arch JR, Petroni ML, Ferrari D, Cinti S, Stock MJ (2002) Immunohistochemical identification of the beta(3)-adrenoceptor in intact human adipocytes and ventricular myocardium: effect of obesity and treatment with ephedrine and caffeine. *Int J Obes Relat Metab Disord* 26:1442–1450
- Feldmann HM, Golozoubova V, Cannon B, Nedergaard J (2009) UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab* 9:203–209
- Frayn KN (1998) Regulation of fatty acid delivery in vivo. *Adv Exp Med Biol* 441:171–179
- Frayn KN, Langin D, Karpe F (2008) Fatty acid-induced mitochondrial uncoupling in adipocytes is not a promising target for treatment of insulin resistance unless adipocyte oxidative capacity is increased. *Diabetologia* 51:394–397
- Gesta S, Tseng Y, Kahn R (2007) Developmental origin of fat: tracking obesity to its source. *Cell* 131:242–256
- Gibala MJ, Little JP, van Essen M, Wilkin GP, Burgomaster KA, Safdar A, Raha S, Tarnopolsky MA (2006) Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* 575:901–911
- Green HJ, Jones S, Ball-Burnett ME, Smith D, Livesey J, Farrance BW (1991) Early muscular and metabolic adaptations to prolonged exercise training in humans. *J Appl Physiol* 70:2032–2038
- Green HJ, Jones S, Ball-Burnett M, Farrance B, Ranney D (1995) Adaptations in muscle metabolism to prolonged voluntary exercise and training. *J Appl Physiol* 78:138–145
- Hallgren P, Korsback S, Sjostrom L (1986) Measurements of adipose tissue respiration in a closed chamber using an oxygen sensor: methodological considerations. *J Lipid Res* 27:996–1005
- Hamosh M, Hamosh P, Bar-Maor JA, Cohen H (1963) Fatty-acid metabolism by human adipose tissues. *J Clin Invest* 42:1648–1652
- Hawley JA, Noakes TD (1992) Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. *Eur J Appl Physiol Occup Physiol* 65:79–83
- Horowitz JF, Klein S (2000) Whole body and abdominal lipolytic sensitivity to epinephrine is suppressed in upper body obese women. *Am J Physiol Endocrinol Metab* 278:E1144–E1152
- Hotamisligil G (2006) Inflammation and metabolic disorders. *Nature* 444:860–867
- Hull D, Segall MM (1965) Sympathetic nervous control of brown adipose tissue and heat production in the new-born rabbit. *J Physiol* 181:458–467
- Huttunen P, Hirvonen J, Kinnula V (1981) The occurrence of brown adipose tissue in outdoor workers. *Eur J Appl Physiol Occup Physiol* 46:339–345
- Jeukendrup A, Saris W, Schrauwen P, Brouns F, Wagenmakers A (1995) Metabolic availability of medium chain triglycerides co-ingested with carbohydrates during prolonged exercise. *J Appl Physiol* 79:756–762

- Krief S, Lonnqvist F, Raimbault S, Baude B, Van Spronsen A, Arner P, Strosberg AD, Ricquier D, Emorine LJ (1993) Tissue distribution of beta 3-adrenergic receptor mRNA in man. *J Clin Invest* 91:344–349
- Larue-Achagiotis C, Goubern M, Laury MC, Louis-Sylvestre J (1994) Energy balance in an inbred strain of rats: comparison with the Wistar strain. *Physiol Behav* 55:483–487
- Laye MJ, Rector RS, Warner SO, Naples SP, Perretta AL, Uptergrove GM, Laughlin MH, Thyfault JP, Booth FW, Ibdah JA (2009) Changes in visceral adipose tissue mitochondrial content with type 2 diabetes and daily voluntary wheel running in OLETF rats. *J Physiol* 587:3729–3739
- Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, Vidal H (1998) Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* 47:98–103
- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem* 270:12953–12956
- Maassen JA, Romijn JA, Heine RJ (2007) Fatty acid-induced mitochondrial uncoupling in adipocytes as a key protective factor against insulin resistance and beta cell dysfunction: a new concept in the pathogenesis of obesity-associated type 2 diabetes mellitus. *Diabetologia* 50:2036–2041
- McConnell GK, Lee-Young RS, Chen ZP, Stepto NK, Huynh NN, Stephens TJ, Canny BJ, Kemp BE (2005) Short-term exercise training in humans reduces AMPK signalling during prolonged exercise independent of muscle glycogen. *J Physiol* 568:665–676
- Nedergaard J, Bengtsson T, Cannon B (2007) Active brown adipose tissue and adult humans. *Am J Physiol Endocrinol Metab* 293:444–452
- Oberkofler H, Dallinger G, Liu YM, Hell E, Krempler F, Patsch W (1997) Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. *J Lipid Res* 38:2125–2133
- Ostman J, Arner P, Engfeldt P, Kager L (1979) Regional differences in the control of lipolysis in human adipose tissue. *Metabolism* 28:1198–1205
- Peronnet F, Massicotte D (1991) Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* 16:23–29
- Peronnet F, Cleroux J, Perrault H, Cousineau D, De Champlain J, Nadeau R (1981) Plasma norepinephrine response to exercise before and after training in humans. *J Appl Physiol* 51:812–815
- Petrovic N, Shabalina IG, Timmons JA, Cannon B, Nedergaard J (2008) Thermogenically competent nonadrenergic recruitment in brown preadipocytes by a PPARgamma agonist. *Am J Physiol Endocrinol Metab* 295:E287–E296
- Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJ, Grant SM (1996) Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *Am J Physiol* 270:E265–E272
- Puigserver P (2005) Tissue-specific regulation of metabolic pathways through the transcriptional coactivator PGC1-alpha. *Int J Obes (Lond)* 29:S5–S9
- Ricquier D, Nechad M, Mory G (1982) Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma. *Clin Endocrinol Metab* 54:803–807
- Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58:1526–1531
- Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM (2007) Transcriptional control of brown fat determination by PRDM16. *Cell Metab* 6:38–54
- Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe H, Erdjument-Bromage H, Tempst P, Rudnick M, Beier D, Spiegelman B (2008) PRMD16 controls a brown fat/skeletal muscle switch. *Nature* 454:21
- Smith DJ, Deuster PA, Ryan CJ, Doubt TJ (1990) Prolonged whole body immersion in cold water: hormonal and metabolic changes. *Undersea Biomed Res* 17:139–147
- Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P (2008) Dynamics of fat cell turnover in humans. *Nature* 453:783–787
- Stallknecht B, Vinten J, Ploug T, Galbo H (1991) Increased activities of mitochondrial enzymes in white adipose tissue in trained rats. *Am J Physiol* 261:E410–E414
- Sutherland LN, Bomhof MR, Capozzi LC, Basaraba SA, Wright DC (2009) Exercise and adrenaline increase PGC-1{alpha} mRNA expression in rat adipose tissue. *J Physiol* 587:1607–1617
- Timmons J, Wennmalm K, Larsson O, Walden T, Lassman T, Petrovic N, Hamilton D, Gimeno R, Wahlestedt C, Baar K, Nedergaard J, Cannon B (2007) Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci USA* 104:4401–4406
- Tiraby C, Tavernier G, Lefort C, Larrouy D, Bouillaud F, Ricquier D, Langin D (2003) Acquisition of brown fat cell features by human white adipocytes. *J Biol Chem* 278:33370–33376
- Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN, Kahn CR (2008b) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454:1000–1004
- Valerio A, Cardile A, Cozzi V, Bracale R, Tedesco L, Pisconti A, Palomba L, Cantoni O, Clementi E, Moncada S, Carruba MO, Nisoli E (2006) TNF-alpha downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents. *J Clin Invest* 116:2791–2798
- van Marken Lichtenbelt WD, Vanhomerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. *N Engl J Med* 360:1500–1508
- Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. *N Engl J Med* 360:1518–1525
- Wilson-Fritch L, Burkart A, Bell G, Mendelson K, Leszyk J, Nicoloro S, Czech M, Corvera S (2003) Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. *Mol Cell Biol* 23:1085–1094
- Yang X, Enerback S, Smith U (2003) Reduced expression of FOXC2 and brown adipogenic genes in human subjects with insulin resistance. *Obes Res* 11:1182–1191
- Youngren JF, Keen S, Kulp JL, Tanner CJ, Houmard JA, Goldfine ID (2001) Enhanced muscle insulin receptor autophosphorylation with short-term aerobic exercise training. *Am J Physiol Endocrinol Metab* 280:E528–E533