Sprint interval and traditional endurance training increase net intramuscular triglyceride breakdown and expression of perilipin 2 and 5

S. O. Shepherd[1], M. Cocks[1], K. D. Tipton[2], A. M. Ranasinghe[3], T. A. Barker[3], J. G. Burniston[4], A. J. M. Wagenmakers[1] and C. S. Shaw[1]

[1]*School of Sport & Exercise Sciences, University of Birmingham, Birmingham B15 2TT, UK*
[2]*Exercise and Health Sciences Research Group, University of Stirling, Scotland, UK*
[3]*School of Clinical and Experimental Medicine, Cardiovascular and Respiratory Sciences, University of Birmingham, UK*
[4]*Research Institute for Sport & Exercise Science, Liverpool John Moores University, Liverpool, UK*

Key points

- Increases in aerobic capacity and intramuscular triglyceride (IMTG) utilization are well-described adaptations to endurance training (ET) and contribute to improvements in insulin sensitivity.
- Sprint interval training (SIT) also improves aerobic capacity and insulin sensitivity with a lower time commitment than ET.
- This study aimed to determine whether SIT also induces improvements in insulin sensitivity and net IMTG breakdown, and to investigate the underlying mechanisms.
- Six weeks of ET and SIT increased net IMTG breakdown during moderate-intensity cycling, and improved insulin sensitivity. A greater concentration of lipid droplet-associated proteins, perilipin 2 and perilipin 5, was observed following both training modes and contributes to the increases in net IMTG breakdown following training.
- The results suggest a novel mechanism for the training-induced improvements in IMTG breakdown and insulin sensitivity, and clearly demonstrate that SIT is an alternative, time-efficient training strategy that induces similar beneficial metabolic adaptations.

Abstract Intramuscular triglyceride (IMTG) utilization is enhanced by endurance training (ET) and is linked to improved insulin sensitivity. This study first investigated the hypothesis that ET-induced increases in net IMTG breakdown and insulin sensitivity are related to increased expression of perilipin 2 (PLIN2) and perilipin 5 (PLIN5). Second, we hypothesized that sprint interval training (SIT) also promotes increases in IMTG utilization and insulin sensitivity. Sixteen sedentary males performed 6 weeks of either SIT (4–6, 30 s Wingate tests per session, 3 days week^{-1}) or ET (40–60 min moderate-intensity cycling, 5 days week^{-1}). Training increased resting IMTG content (SIT 1.7-fold, ET 2.4-fold; $P < 0.05$), concomitant with parallel increases in PLIN2 (SIT 2.3-fold, ET 2.8-fold; $P < 0.01$) and PLIN5 expression (SIT 2.2-fold, ET 3.1-fold; $P < 0.01$). Pre-training, 60 min cycling at ∼65% pre-training $\dot{V}_{O_2peak}$ decreased IMTG content in type I fibres (SIT 17 ± 10%, ET 15 ± 12%; $P < 0.05$). Following training, a significantly greater breakdown of IMTG in type I fibres occurred during exercise (SIT 27 ± 13%, ET 43 ± 6%; $P < 0.05$), with preferential breakdown of PLIN2- and particularly PLIN5-associated lipid droplets. Training increased the Matsuda insulin sensitivity index (SIT 56 ± 15%, ET 29 ± 12%; main effect $P < 0.05$). No training × group interactions were observed for any variables. In conclusion, SIT and ET both increase net IMTG breakdown during exercise and increase in

PLIN2 and PLIN5 protein expression. The data are consistent with the hypothesis that increases in PLIN2 and PLIN5 are related to the mechanisms that promote increased IMTG utilization during exercise and improve insulin sensitivity following 6 weeks of SIT and ET.

Introduction

High intramuscular triglyceride (IMTG) concentrations are associated with insulin resistance in sedentary obese individuals and type 2 diabetes patients (Phillips *et al.* 1996; Pan *et al.* 1997; Goodpaster *et al.* 2001). However, the athletes' paradox describes a state of elevated IMTG storage alongside high levels of insulin sensitivity in endurance trained (ET) athletes (Goodpaster *et al.* 2001; van Loon *et al.* 2004). ET also increases oxidative capacity and promotes a shift towards greater IMTG utilization during exercise (Schrauwen *et al.* 2002). Therefore, the capacity to oxidize IMTG as a fuel source is believed to be mechanistically important for the preservation of high insulin sensitivity in the face of elevated intramuscular lipid storage (Goodpaster *et al.* 2001; Bruce *et al.* 2003; van Loon & Goodpaster, 2006). The overriding current hypothesis is that high rates of IMTG oxidation during exercise allow the regular turnover of the intramuscular lipid pool and prevent the accumulation of fatty acid metabolites, such as long-chain acyl-CoA, diacylglycerol and ceramides, which are believed to blunt insulin sensitivity (van Loon & Goodpaster, 2006; Moro *et al.* 2008; Shaw *et al.* 2010). Therefore, understanding the mechanisms regulating IMTG lipolysis and IMTG-derived fatty acid oxidation during exercise remain important.

Recent attention has focused on the role of the perilipins (PLINs), a family of lipid droplet (LD) proteins, of which perilipin 2 (PLIN2) and 5 (PLIN5) are expressed in skeletal muscle, but who's precise role is not fully understood. PLIN2 is ubiquitously expressed in the human body (Brasaemle *et al.* 1997) and its content is ~twofold higher in type I muscle fibres compared with type II fibres, which mirrors the fibre type distribution of IMTG (Shaw *et al.* 2009). In non-muscle cells, PLIN2 expression regulates basal lipolytic rates *in vitro* by limiting the interaction of adipose triglyceride lipase (ATGL) with the LD (Listenberger *et al.* 2007; Bell *et al.* 2008). However, in intact rat skeletal muscle in response to lipolytic stimuli (electrically induced contractions and adrenaline) there is an increase in colocalization of hormone-sensitive lipase (HSL) with PLIN2 and of HSL with LDs (Prats

et al. 2006). Furthermore, we recently reported that PLIN2-containing LDs are depleted in human skeletal muscle during moderate-intensity exercise (Shepherd *et al.* 2012). PLIN5, on the other hand, is highly expressed in oxidative tissues (Wolins *et al.* 2006*a*; Yamaguchi *et al.* 2006; Dalen *et al.* 2007). Cell culture studies demonstrate that PLIN5 expression limits basal lipolysis more effectively than PLIN2 (Wang *et al.* 2011*a*). Interestingly, these studies also show that PLIN5 expression (but not PLIN2) recruits ATGL and its coactivator, comparative gene identification-58 (CGI-58), to the LD surface under basal conditions, and it is believed that PLIN5 phosphorylation releases CGI-58 to bind ATGL to stimulate lipolysis in response to protein kinase A (PKA) activation (Wang *et al.* 2011*a*). Skeletal muscle PLIN5 expression is higher in athletes (Amati *et al.* 2011), and increases following training in lean and obese individuals (Peters *et al.* 2012). Collectively, the results from the limited number of studies so far suggest that both PLIN2 and PLIN5 are important in regulating IMTG lipolytic rates, but that PLIN5 may be the key PLIN regulating IMTG lipolysis and net IMTG breakdown during exercise.

Sprint interval training (SIT) promotes skeletal muscle adaptations and improvements in insulin sensitivity comparable to those induced by ET (Burgomaster *et al.* 2008; Gibala *et al.* 2012). In particular, skeletal muscle oxidative capacity is enhanced following SIT (Burgomaster *et al.* 2005, 2007, 2008; Little *et al.* 2010, 2011; Hood *et al.* 2011), and may partly explain the SIT-induced improvements in whole-body insulin sensitivity (Babraj *et al.* 2009; Richards *et al.* 2010; Hood *et al.* 2011; Little *et al.* 2011). However, it has yet to be investigated whether such improvements in insulin sensitivity occur alongside increases in net IMTG breakdown during exercise and enhanced skeletal muscle expression of PLIN2 and PLIN5.

Given the importance of studying IMTG metabolism in a fibre type-specific manner (van Loon, 2004), in the current study we investigated IMTG metabolism and the content and localization of PLIN2 and PLIN5 following different training interventions using immunofluorescence microscopy. We hypothesized that increased

expression of PLIN2 and PLIN5 plays a role in the well-established increase in net IMTG breakdown during exercise following ET. In addition, we investigated the hypothesis that SIT also increases the capacity to breakdown IMTG during moderate-intensity exercise, the protein expression of PLIN2 and PLIN5, and insulin sensitivity.

Methods

Ethical approval

Sixteen healthy, sedentary males volunteered to take part in the study (for characteristics, see Table 1), which was approved by the Black Country NHS Research Ethics Committee (West Midlands, UK) and conformed with the *Declaration of Helsinki*. Written, informed consent was obtained from volunteers following a verbal and written explanation of the nature and risks involved in the experimental procedure. All subjects were healthy (free of any known metabolic or cardiovascular disease) and engaged in less than two 30 min sessions of physical activity per week in the preceding year. Percutaneous muscle biopsies taken in this study before and after each training mode, under basal conditions and after 60 min of moderate-intensity exercise have been used both for the measurements described in this manuscript and for measurements of endothelial eNOS content, eNOS serine[1177] phosphorylation, NOX2 content and capillarization. The latter are reported in a parallel manuscript (Cocks *et al.*, 2012). Measures such as insulin sensitivity and $\dot{V}_{O_2peak}$ also made in the volunteers are relevant for the interpretation of both studies and are presented in both manuscripts.

Pre-experimental procedures

At least 5 days prior to entering the study subjects performed a progressive exercise test to exhaustion on an electronically braked cycle ergometer (Lode BV, Groningen, The Netherlands) in order to determine peak oxygen uptake ($\dot{V}_{O_2peak}$) using an on-line gas collection system (Oxycon Pro, Jaeger, Germany). The test consisted of initially cycling at 95 W, followed by sequential increments of 35 W every 3 min until cadence was reduced to <50 rpm, at which point the test was terminated. $\dot{V}_{O_2peak}$ was taken as the highest value obtained in the last 30 s of the test. During this visit subjects were also allocated to their training intervention. The 16 subjects were divided into pairs, with the best possible match for age, body mass index and $\dot{V}_{O_2peak}$, with one member from each pair randomly assigned to SIT and the other to the ET group. As none of the volunteers was familiar with SIT exercise, the subjects in the SIT group were familiarized to this exercise mode through the performance of one 30 s all out effort (Wingate test; Bar-Or, 1987) on a cycle ergometer against a load equivalent to 0.075 kg (kg body mass)$^{-1}$.

Experimental procedures

Experimental procedures performed before (pre) and after (post) training were identical, and were undertaken across two consecutive days. Post-training experimental procedures were performed >48 h after the final exercise training session in order to minimize any acute effects of the last exercise training bout.

Day 1. All subjects reported to the laboratory after an overnight fast (>10 h), having been instructed to refrain

---

Table 1. Subject characteristics

	SIT		ET	
	Pre-	Post-	Pre-	Post-
Age (years)	22 ± 1		21 ± 1	
Height (m)	1.74 ± 0.02		1.77 ± 0.03	
Body mass (kg)	75.1 ± 3.0	75.2 ± 3.1	70.8 ± 4.4	70.8 ± 4.5
BMI (kg m^{-2})	24.8 ± 0.8	24.8 ± 0.9	22.6 ± 1.6	22.6 ± 1.2
$\dot{V}_{O_2peak}$ (l min^{-1})*	3.13 ± 0.15	3.37 ± 0.18	2.93 ± 0.30	3.40 ± 0.38
$\dot{V}_{O_2peak}$ (l min^{-1} kg^{-1})*	41.9 ± 1.8	45.1 ± 2.3	41.7 ± 4.1	48.2 ± 5.0
W_{max} (W) *	221 ± 11	241 ± 14	218 ± 11	253 ± 16
FFM (kg) *	52.5 ± 1.9	53.6 ± 1.8	50.7 ± 1.5	51.1 ± 2.7
FM (kg)	14.2 ± 1.6	13.6 ± 1.5	12.3 ± 1.5	12.2 ± 1.9
Insulin AUC (μIU ml^{-1} min^{-1})*	8847 ± 1140	5792 ± 688	8434 ± 880	6813 ± 711
Glucose AUC (mg dl^{-1} min^{-1})*	17634 ± 710	14551 ± 398	16835 ± 992	14783 ± 950
ISI-Matsuda*	3.9 ± 0.3	5.8 ± 0.4	3.7 ± 0.5	4.7 ± 0.7

Data provided are means ± SEM ($n = 8$ per group). AUC, area under the curve; BMI, body mass index; ET, endurance training; FM, fat mass; FFM, fat-free mass; ISI, insulin sensitivity index; SIT, sprint interval training; W_{max}, maximum workload. *Indicates main effect for training ($P < 0.05$), such that Pre ≠ Post. No interaction effects (training × group) were observed for any of the variables.

from performing vigorous exercise in the preceding 48 h period. On arrival, a 20G cannula was inserted into the antecubital vein of one arm, and a three-way stop cock attached to allow for multiple blood sampling and flushing of the cannula. After a 20 ml baseline sample was obtained, subjects consumed a beverage containing 75 g glucose (using 82.5 g dextrose monohydrate obtained from Roquette, UK) dissolved in water made up to 300 ml. Further 5 ml blood samples were obtained after 30, 60, 90 and 120 min, and collected into EDTA-containing vacutainers. Isotonic saline (3 ml; B Braun, UK) was used to keep the cannula patent every 15 min during the 2 h test. Plasma samples for each time point were obtained through centrifugation (10 min at 1000 g at 4°C) and stored at −80°C for subsequent analysis. Immediately following the oral glucose tolerance test (OGTT), body composition analysis was performed using dual-energy X-ray absorptiometry. QDR software (Hologic Inc., MA, USA) was used to analyse fat mass and fat-free mass in individual limbs and the trunk region of each subject. Relative fat mass was calculated using absolute fat mass as a proportion of total region mass.

Day 2. On arrival at the laboratory after an overnight fast (>10 h), the thigh of one leg was prepared for muscle biopsy collection. Subjects were randomized as to which leg they received the biopsy from pre-training; the contralateral leg was sampled post-training. Briefly, local anaesthetic (1% lidocaine; B Braun) was administered under the skin and over the fascia of the vastus lateralis before two incisions were made approximately 2 cm apart. A muscle biopsy (∼100 mg) was taken using the Bergström technique (Bergström, 1975) from the distal incision prior to exercise. Skeletal muscle samples were first blotted to remove excess blood, and visible fat and collagen were removed through dissection. A portion of the muscle tissue was then prepared for immunohistochemical analysis by embedding in Tissue-Tek OCT Compound (Sakura Finetek Europe, The Netherlands) and frozen in liquid nitrogen-cooled isopentane. The remaining muscle tissue (∼40 mg) was snap frozen in liquid nitrogen. Subsequently, subjects underwent 60 min of steady state exercise on an electronically braked cycle ergometer set at a workload equivalent to 65% pre-training $\dot{V}_{O_2peak}$. Heart rate and rate of perceived exertion were recorded every 5 min, while gas collection (5 min collection period) was performed at 15 min intervals ($t = 15, 30, 45, 60$ min) using an on-line gas collection system (Oxycon Pro), in order to determine rates of carbohydrate and fat oxidation. A second muscle biopsy was taken immediately post-exercise from the proximal incision.

For all experimental trials, subjects were provided with all food and drinks to be consumed in the preceding 24 h period. The diet was of a standard macronutrient composition (50% carbohydrate, 35% fat, 15% protein), but adjusted for each individual's habitual energy intake, assessed through the completion of a 3 day diet diary. When completing diet diaries, subjects were provided with electronic scales in order to increase the accuracy of portion size determination. All subjects were asked to continue their habitual dietary and physical activity patterns during the training period, again completing a 3 day diet diary post-training in order to assess any differences in energy or macronutrient intake over the training period.

Training interventions

SIT. The SIT training protocol was similar to the protocol used by Burgomaster *et al.* (2008). Subjects performed 30 s 'all out' sprints (Wingate test) on a cycle ergometer against a load equivalent to 0.075 kg per kg body mass and with 4.5 min recovery between each test. During the recovery period subjects cycled against a small load (30 W) maintaining a cadence of below 50 rpm. All participants trained three times a week for 6 weeks, and were excluded from the study if they were absent from more than two sessions. Initially participants performed four Wingate tests per training session, increasing to five tests in weeks 3 and 4, and finishing with six tests per session in weeks 5 and 6.

ET. Subjects in the ET group trained five times a week over the 6 week training period, and were excluded from the study if they were absent from more than three training sessions. All subjects cycled at a workload equivalent to ∼65% $\dot{V}_{O_2peak}$ for 40 min in the first 2 weeks, increasing to 50 min in the following 2 weeks, and 60 min in the final 2 weeks. $\dot{V}_{O_2peak}$ was reassessed after 3 weeks of training and workload adjusted accordingly.

Muscle sample analysis

Immunofluorescence staining. Serial 5 μm sections were cut at −30°C on to ethanol-cleaned glass slides. Sections were fixed in 3.7% formaldehyde for 1 h, rinsed briefly in deionized water, and permeabilized in 0.5% Triton-X 100 for 5 min, followed by 3 × 5 min washes in PBS. Subsequently, slides underwent incubation with appropriate antibodies for 45 min, after which, a further 3 × 5 min PBS wash preceded incubation of sections with appropriately targeted secondary fluorescent conjugated antibodies for 30 min. IMTG (LDs) were visualized using neutral lipid dye oil red O staining (Koopman *et al.* 2001). As such, oil red O solution was applied to washed (3 × 5 min PBS) sections for 30 min following secondary antibody incubation. Slides were then rinsed briefly in deionized water, followed by a 10 min rinse with slow

running tap water, after which coverslips were mounted with a glycerol and mowiol 4–88 solution in 0.2 M Tris buffer (pH 8.5; including 0.1% DABCO anti-fade medium).

Cell border visualization was achieved through incubation of sections with a wheat germ agglutinin Alexa Fluor 350 conjugate (Cat. no. W11263; Invitrogen, Paisley, UK). Muscle fibre type was determined using a mouse anti-myosin antibody for slow twitch fibres (A4.840-c, DSHB, developed by Dr Blau) followed by the application of Alexa Fluor goat anti-mouse IgM 488 or 350. Fibres containing positive staining were classified as type I fibres, while those with no staining were classified as type II fibres. The oil red O working solution was freshly produced for each staining procedure and consisted of 100 mg oil red O in 20 ml 60% triethylphosphate (Sigma–Aldrich, UK). Twelve millilitres of working solution was added to 8 ml deionized water and filtered twice to remove any oil red O crystals. Mitochondria were visualized using a mouse monoclonal anti-OxPhos Complex IV primary antibody (Cat. no. 459600; Invitrogen), followed by an Alexa Fluor goat anti-mouse IgG$_{2a}$ 594 secondary antibody. PLIN2 visualization was achieved using a mouse monoclonal anti-adipophilin primary antibody (Cat. no. 03-610102; American Research Products, MA, USA), followed by an Alexa Fluor goat anti-mouse IgG$_1$ 594 secondary antibody. On occasions when IMTG staining with oil red O was undertaken in parallel with PLIN2, an Alexa Fluor goat-anti mouse IgG$_1$ 488 secondary antibody was used. PLIN5 was visualized using a guinea pig polyclonal anti-OXPAT primary antibody (Cat. no. GP31; Progen Biotechnik, Germany) followed by application of an Alexa Fluor goat-anti guinea pig IgG 488 secondary antibody. All Alexa Fluor secondary antibodies were obtained from Invitrogen. The specificity of the PLIN5 antibody was confirmed in a competition experiment in which a PLIN5 recombinant peptide (Cat. no. NB110–60511PEP; Novus Biologicals, Cambridge, UK) was incubated with the PLIN5 primary antibody, subsequently resulting in removal of the positive fluorescent signal for PLIN5 (Supplementary Fig. S1).

Image capture, processing and data analysis. Images of cross-sectional orientated sections were used for the determination of fibre type differences in the content of IMTG, mitochondria, and PLIN2 and PLIN5. Images were captured using a Nikon E600 microscope with a 40× 0.75 NA objective, coupled to a SPOT RT KE colour 3 shot CCD camera (Diagnostic Instruments Inc., MI, USA). Both DAPI UV (340–380 nm) and FITC (465–495 nm) excitation filters were used to view the Alexa Fluor 350 and 488 fluorophores, respectively. In addition, a Texas red (540–580 nm) excitation filter was used to view sections stained with oil red O or the Alexa Fluor 594 fluoro-

phore. The use of three excitation filters, and one dichroic and one emission filter ('Pinkel' Triple Set; Semrock, Kettering, UK) allowed for semi-automated sequential image capture. Digital images to specifically assess the size and number of IMTG and PLIN2 and PLIN5 objects were obtained using an inverted confocal microscope (Leica DMIRE2, Leica Microsystems) using a 63× 1.4 NA oil immersion objective. An argon laser was used to excite the Alexa Fluor 488 fluorophore, while both the Alexa Fluor 594 fluorophore and oil red O were excited using a helium–neon laser. The same system was used to capture digital images, and to assess IMTG and PLIN2 or PLIN5 colocalization.

Image processing was undertaken using Image-Pro Plus 5.1 software (Media Cybernetics, MD, USA). Fibre type distribution of IMTG, PLIN2 and PLIN5 and mitochondria was assessed using widefield images. To this end, between 6 and 10 widefield images were used per muscle section, resulting in 104 ± 5 fibres analysed per muscle cross-section (42 ± 3 type I fibres, 62 ± 3 type II fibres). Mitochondrial fluorescence staining intensity was used to indicate changes in mitochondrial content after training. An intensity threshold was uniformly selected to represent a positive signal for IMTG, and PLIN2 and PLIN5. The content of IMTG and PLIN2 and PLIN5 were expressed as the positively stained area fraction relative to the total area of each muscle fibre. Images captured using confocal microscopy were used to identify changes in mean IMTG, PLIN2 and PLIN5 size and density. Density was calculated as the number of IMTG objects relative to area. The area of individual IMTG (LDs), PLIN2 or PLIN5 objects was used as a measure of size.

Colocalization analysis was performed separately for PLIN2 or PLIN5 with IMTG. This analysis was restricted to type I fibres, as a previous publication from our laboratory has shown that changes in colocalization with IMTG do not occur in type II fibres in response to the exercise bout used in the present study (Shepherd *et al.* 2012). In this respect, sequential images of either PLIN2 or PLIN5 and IMTG were captured using confocal microscopy (as described) with 8× digital zoom. A positive signal for either PLIN protein and IMTG was obtained through the selection of a uniform intensity threshold. Binary images were created based on the selected threshold and subsequently used for colocalization analysis. A colocalization map was generated, displaying the merged images, with the overlapping area subsequently extracted to create a separate binary image. The total number of objects in this image was expressed relative to the total number of PLIN2 (or PLIN5) as a measure of colocalization (Fig. 4). The extracted objects were also used as a measure of the number of PLIN-associated LDs (PLIN-LDs), and the number of extracted objects was subsequently subtracted from the total number of LDs to quantify the number of LDs not associated with PLIN

(PLIN-null-LDs). Several controls were performed to check for bleed through, non-specific secondary antibody binding and autofluorescence before colocalization analysis was performed. No positive staining was observed in the opposite channel when single staining (IMTG, PLIN2 and PLIN5) was performed, and omission of the primary or secondary antibody, and oil red O abolished all positive signals in the relevant channel discounting the influence of tissue autofluorescence or non-specific secondary antibody binding. In addition, we performed the same colocalization analysis on a series of non-corresponding PLIN2 (or PLIN5) and IMTG images, in order to determine whether the level of random colocalization was statistically different from that obtained for the true corresponding images, as previously described (Lachmanovich *et al.* 2003).

Western blot analysis. Snap frozen muscle tissue was powdered in liquid nitrogen and transferred to Eppendorf tubes containing lysis buffer (1× RIPA buffer, Cell Signalling, including one complete mini protease inhibitor tablet; Roche Diagnostics, Germany). Samples were mixed on ice for 2 h and then homogenized on ice for ∼30 s at slow speed (Polytron), followed by centrifugation for 20 min at 4°C and 10,000 *g*. The supernatant was removed and a small portion used to determine protein concentration (Pierce BCA assay kit). Samples were subsequently diluted to a final concentration of 3 μg μl^{-1}, using homogenization buffer and Laemmli sample buffer (Sigma), and boiled for 5 min. Equal quantities of protein (45 μg) were separated by electrophoresis on 12% SDS–polyacrylamide gels and transferred to nitrocellulose membranes at 25 V for 1.5 h. Ponceau S staining was performed to assess transfer efficacy and subsequently imaged using a ChemiDoc XRS+ system (Bio-Rad, UK). This image was used to quantify the total protein stain of each lane, and this was used as a measure of equal loading when the inter-lane variation was less than 10%. Membranes were subsequently destained (0.1 M NaOH) and blocked for 1 h in 5% non-fat dried milk (NFDM) in PBS with 0.1% Tween (PBST) followed by incubation overnight at room temperature with either the anti-adipophilin (Cat. no. 03-610102; American Research Products) or anti-OXPAT (Cat. no. GP31; Progen Biotechnik) primary antibody diluted in 3% NFDM in PBST. The membranes were then washed three times in PBST, blocked in 5% NFDM in PBST for 1 h, followed by incubation for 1 h at room temperature with appropriate HRP-conjugated IgG secondary antibodies diluted in 3% NFDM in PBST. Antibody binding was detected using enhanced chemiluminescence HRP detection reagent (GE Healthcare, UK), and imaging and band quantification was performed using a ChemiDoc XRS+ system (Bio-Rad).

Blood sample analysis

Plasma glucose concentrations were determined spectrophotometrically using an ILab-600 semi-automatic analyser in combination with the glucose oxidase kit (Instrumentation Laboratory Ltd UK, Warrington, UK). Insulin concentrations during the OGTT were determined using a commercially available direct insulin ELISA kit (Invitrogen). Insulin sensitivity index (Matsuda & DeFronzo, 1999) was calculated based on plasma glucose and insulin values.

Calculations and statistical analysis

Rates of whole-body fat and carbohydrate oxidation (g min^{-1}) were calculated from the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values collected at 15, 30, 45 and 60 min during the steady state cycling exercise. The calculations were made assuming protein oxidation was negligible, and according to previously published equations (Jeukendrup & Wallis, 2005):

$$\text{Carbohydrate oxidation (g min}^{-1}) = 4.21\dot{V}_{CO_2} - 2.962\dot{V}_{O_2}$$

$$\text{Fat oxidation (g min}^{-1}) = 1.695\dot{V}_{O_2} - 1.701\dot{V}_{CO_2}$$

Insulin sensitivity was calculated using the Matsuda index (Matsuda & DeFronzo, 1999), using the following equation:

$$\text{Matsuda (ISI)} = \frac{10,000}{\sqrt{(\text{FPG} \times \text{FPI}) \times (\text{P}\bar{\text{G}} \times \text{P}\bar{\text{I}})}}$$

All data are expressed as means ± SEM. Significance was set at the 0.05 level of confidence. Changes in body composition, exercise capacity, insulin sensitivity and substrate utilization were assessed using a two-factor repeated measures ANOVA, with the between-subject factor '*group*' (SIT *vs.* ET) and the within-subject factor '*training*' (pre- *vs.* post-training). Changes in mitochondrial density and PLIN2 and PLIN5 content were assessed using a mixed three-factor repeated measures ANOVA using the within-subject factors '*fibre*' (type I *vs.* type II fibres) and '*training*', and between-subject (*group*) factor used in the two-factor analysis. To inspect differences in IMTG, we hypothesized *a priori* that IMTG content would be significantly different between type I and type II fibres. Thus, we used a three-factor repeated measures ANOVA with the between-subject factor as '*group*', and the within-subject factors '*training*' and '*time*' (pre- *vs.* post-exercise). The same three-factor repeated measures ANOVA and between-subject and within-subject factors were used when assessing differences in colocalization, following an *a priori* hypothesis that colocalization would be significantly different between actual and randomized images, based on a previous study (Lachmanovich *et al.*

2003). Significant main effects or interactions were assessed using Bonferroni adjustment *post hoc* analysis. Improvements in training performance (SIT group; e.g. mean power) were examined using a paired *t* test analysis. A Pearson's bivariate correlation analysis was used to investigate specific relationships between variables.

Results

Exercise capacity, body composition, substrate utilization and insulin sensitivity

At baseline, no differences between groups were observed for any of the variables mentioned in Table 1. In response to training $\dot{V}_{O_2peak}$ was significantly improved irrespective of training type (SIT $7 \pm 2\%$, ET $15 \pm 3\%$; main effect for condition, $P < 0.001$; Table 1). The difference between the modes of exercise did not reach statistical significance ($P = 0.064$). W_{max} was also increased after training, with no difference between groups (main effect for condition, $P < 0.001$). Fat-free mass increased significantly post-training, with no difference between groups (main training effect, $P = 0.039$). The observed decrease in relative fat mass (Table 1) did not reach statistical significance, with no difference between groups (main training effect, $P = 0.075$). Glucose area under the curve (AUC) was reduced post-training (SIT $17 \pm 2\%$, ET $12 \pm 2\%$; main training effect, $P < 0.001$; Table 1), with no difference between groups. Similarly, training reduced insulin AUC (SIT $33 \pm 3\%$, ET $18 \pm 5\%$; main training effect, $P < 0.001$; Table 1), with no difference between groups. In accordance, training improved insulin sensitivity, calculated using the Matsuda index (Matsuda & DeFronzo, 1999; SIT $56 \pm 15\%$, ET $29 \pm 12\%$; main training effect, $P < 0.01$; Table 1), with no difference

between groups. Carbohydrate (absolute and relative) and fat (relative only) oxidation, as well as respiratory exchange ratio (RER), were all significantly altered compared with pre-training following ET only ($P < 0.05$; Table 2), with no effect apparent following SIT. RER tended to be lower in the SIT group ($P = 0.055$) before training. When considering the effectiveness of SIT, the first and final training sessions were examined. Specifically, mean power output increased during training by $14 \pm 3\%$ ($P = 0.001$), and peak power output increased by $21 \pm 6\%$ ($P = 0.01$).

Analysis of mitochondrial content

Immunofluorescence images demonstrated that COX protein expression (fluorescence intensity), representing mitochondrial density, was greater in type I fibres than type II fibres pre- and post-training (main effect of fibre, $P < 0.001$; Table 3). No group differences were apparent at baseline. A main effect of training was observed, with increases in mitochondrial density in both type I fibres (SIT $39 \pm 4\%$, ET $46 \pm 16\%$) and type II fibres (SIT $39 \pm 3\%$, ET $50 \pm 14\%$; $P < 0.05$; Table 3). No difference between groups was apparent following training.

IMTG analysis

Our *a priori* hypothesis that IMTG content is greater in type I fibres compared with type II fibres meant that we examined IMTG fibre type-specific training effects separately. There were no differences in resting IMTG content between groups at baseline. A significant main effect of training was observed with increased resting IMTG content in type I fibres after both interventions (expressed as percentage fibre stained; SIT

Table 2. Substrate utilization during 60 min cycling at ~65% pre-training $\dot{V}_{O_2peak}$

	SIT		ET	
	Pre-	Post-	Pre-	Post-
Heart rate (beats min^{-1})*	164 ± 3	147 ± 3	165 ± 3	138 ± 4
$\dot{V}_{O_2}$ (l min^{-1})*	2.22 ± 0.11	2.12 ± 0.12	2.19 ± 0.14	2.10 ± 0.13
$\dot{V}_{CO_2}$ (l min^{-1})*	1.94 ± 0.09	2.01 ± 0.13	1.97 ± 0.11	1.81 ± 0.10
CHO oxidation				
(g min^{-1})	1.61 ± 0.07	1.56 ± 0.13	1.80 ± 0.12	1.37 ± 0.07†
(percentage of total oxidation)	62.3 ± 2.0	63.2 ± 4.4	70.9 ± 4.3	56.6 ± 3.1†
Fat oxidation				
(g min^{-1})	0.46 ± 0.04	0.42 ± 0.06	0.36 ± 0.07	0.49 ± 0.05
(percentage of total oxidation)	39.5 ± 1.9	38.6 ± 4.3	31.0 ± 4.3	45.1 ± 3.1†
RER	0.88 ± 0.01	0.88 ± 0.01	0.90 ± 0.01	0.86 ± 0.01†

Values obtained during 60 min of moderate-intensity cycling at ~65% pre-training $\dot{V}_{O_2peak}$. Data provided are means ± SEM ($n = 8$ per group). CHO, carbohydrate; ET, endurance training; RER, respiratory exchange ratio; SIT, sprint interval training. *Main effect for training ($P < 0.05$) such that Pre $\neq$ Post (no significant differences between training interventions). †Interaction effect (training × group; $P < 0.05$), such that the effect of ET was significantly different from the effect of SIT.

Table 3. Effect of 6 weeks of SIT or ET on fibre type-specific mitochondrial content

COX expression (fluorescence intensity)	SIT		ET	
	Pre-	Post-	Pre-	Post-
Type I fibres	54.1 ± 4.6	74.4 ± 4.0†	60.1 ± 7.7	81.5 ± 5.9†
Type II fibres*	41.1 ± 3.0	56.8 ± 3.5†	42.4 ± 4.8	60.4 ± 5.1†

Mitochondrial density, quantified from immunofluorescence images of COX in type I and type II fibres obtained before and after 6 weeks of SIT or ET. Values are presented as means ± SEM ($n = 8$ per group). *Main fibre effect ($P < 0.05$ *vs.* type I fibres). †Main training effect ($P < 0.05$ *vs.* pre-training). ET, endurance training; SIT, sprint interval training.

1.9-fold, ET 2.9-fold; main training effect, $P < 0.05$; Fig. 1*E*). This finding was attributed to a significant rise in IMTG density, expressed as the number of LDs per area, after SIT (pre-training, $0.065 \pm 0.009 \ \mu m^2$; post-training, $0.087 \pm 0.010 \ \mu m^2$) and ET (pre-training, $0.064 \pm 0.009 \ \mu m^2$; post-training, $0.109 \pm 0.012 \ \mu m^2$). A main effect of training was observed for IMTG content in type II fibres after both interventions (SIT 1.4-fold, ET 2.5-fold; main training effect, $P < 0.05$; Fig. 1*F*). This increase in IMTG content was again attributable to an increase in IMTG density. No changes in IMTG size were observed in response to training. There were no interaction effects between training and group in any of the measures of resting IMTG content.

Before training, absolute IMTG content in type I fibres decreased significantly in response to 60 min steady state cycling, with no difference between groups (SIT $17 \pm 9\%$, ET $15 \pm 12\%$; main effect of time, $P < 0.05$; Fig. 1*E*). Following training, net IMTG breakdown during exercise in type I fibres was also significantly increased (SIT $27 \pm 13\%$, ET $43 \pm 5\%$; main effect of time, $P < 0.05$; Fig. 1*E*). In comparison to pre-training, net IMTG breakdown in type I fibres was significantly greater following training (training × time interaction; $P < 0.05$), with no difference in net IMTG breakdown between groups. Both pre- and post-training, the reduction in IMTG content in type I fibres was attributed to decreases in IMTG density after SIT (pre-training, $21 \pm 13\%$; post-training, $38 \pm 7\%$) and ET (pre-training, $20 \pm 17\%$; post-training, $32 \pm 8\%$). No significant breakdown of IMTG occurred during the exercise bout in type II fibres either before or after training (Fig. 1*F*).

PLIN2 and PLIN5 analysis

Immunofluorescence images show that PLIN2 content (expressed as percentage fibre stained) in type I fibres was significantly greater than in type II fibres both before and following training, irrespective of intervention ($P < 0.001$; Fig. 2*C* and *D*). A main effect of training was observed, with an increase in PLIN2 content in both type I (SIT 2.1-fold, ET 3.4-fold;

Fig. 2*C* and *D*) and type II fibres (SIT 2.5-fold, ET 2.2-fold; main training effect, $P < 0.001$). This finding was attributed to an increase in PLIN2 density in both type I (SIT: pre- $0.057 \pm 0.008 \ \mu m^2$, post- $0.092 \pm 0.010 \ \mu m^2$; ET: pre- $0.051 \pm 0.012 \ \mu m^2$, post- $0.100 \pm 0.009 \ \mu m^2$) and type II fibres (SIT: pre- $0.020 \pm 0.004 \ \mu m^2$, post- $0.044 \pm 0.009 \ \mu m^2$; ET: pre- $0.012 \pm 0.002 \ \mu m^2$, post- $0.031 \pm 0.006 \ \mu m^2$; main training effect, $P < 0.001$). No significant condition and group interactions were observed for either PLIN2 content or density. PLIN5 content (expressed as percentage of fibre stained) was significantly greater in type I fibres than type II fibres at all time points, irrespective of intervention ($P < 0.001$; Fig. 3*C* and *D*). A main effect of training was observed for PLIN5 content, with significant increases occurring in both fibre types after training (type I fibres: SIT 2.2-fold, ET 2.8-fold; type II fibres: SIT 2.2-fold, ET 3.4-fold; main training effect, $P < 0.001$; Fig. 3*C* and *D*). This finding was attributed to an increase in PLIN5 density in both type I (SIT: pre- $0.054 \pm 0.005 \ \mu m^2$, post- $0.079 \pm 0.007 \ \mu m^2$; ET: pre- $0.039 \pm 0.003 \ \mu m^2$, post- $0.107 \pm 0.011 \ \mu m^2$) and type II fibres (SIT: pre- $0.009 \pm 0.001 \ \mu m^2$, post- $0.018 \pm 0.004 \ \mu m^2$; ET: pre- $0.005 \pm 0.001 \ \mu m^2$, post- $0.025 \pm 0.005 \ \mu m^2$; main training effect, $P < 0.001$). Again, no significant condition and group interaction effects were observed.

We performed immunoblotting of whole muscle homogenates in order to confirm the finding of an increase in both PLIN2 and PLIN5 content in response to training using immunohistochemical methodology. Immunoblot analysis revealed a main effect of training for PLIN2 content (SIT 2.3-fold, ET 3.3-fold; $P = 0.02$; Fig. 2*E*), with no difference between groups. PLIN5 protein expression was also increased following training (SIT 2.0-fold, ET 2.4-fold; main training effect, $P = 0.01$; Fig. 3*E*), again with no difference between groups.

Colocalization of PLIN2 and PLIN 5 with IMTG

Before training, the fraction of PLIN2 colocalized with IMTG in resting muscle was similar between groups (SIT 0.58 ± 0.04, ET 0.61 ± 0.04; $P = 0.96$). This relationship
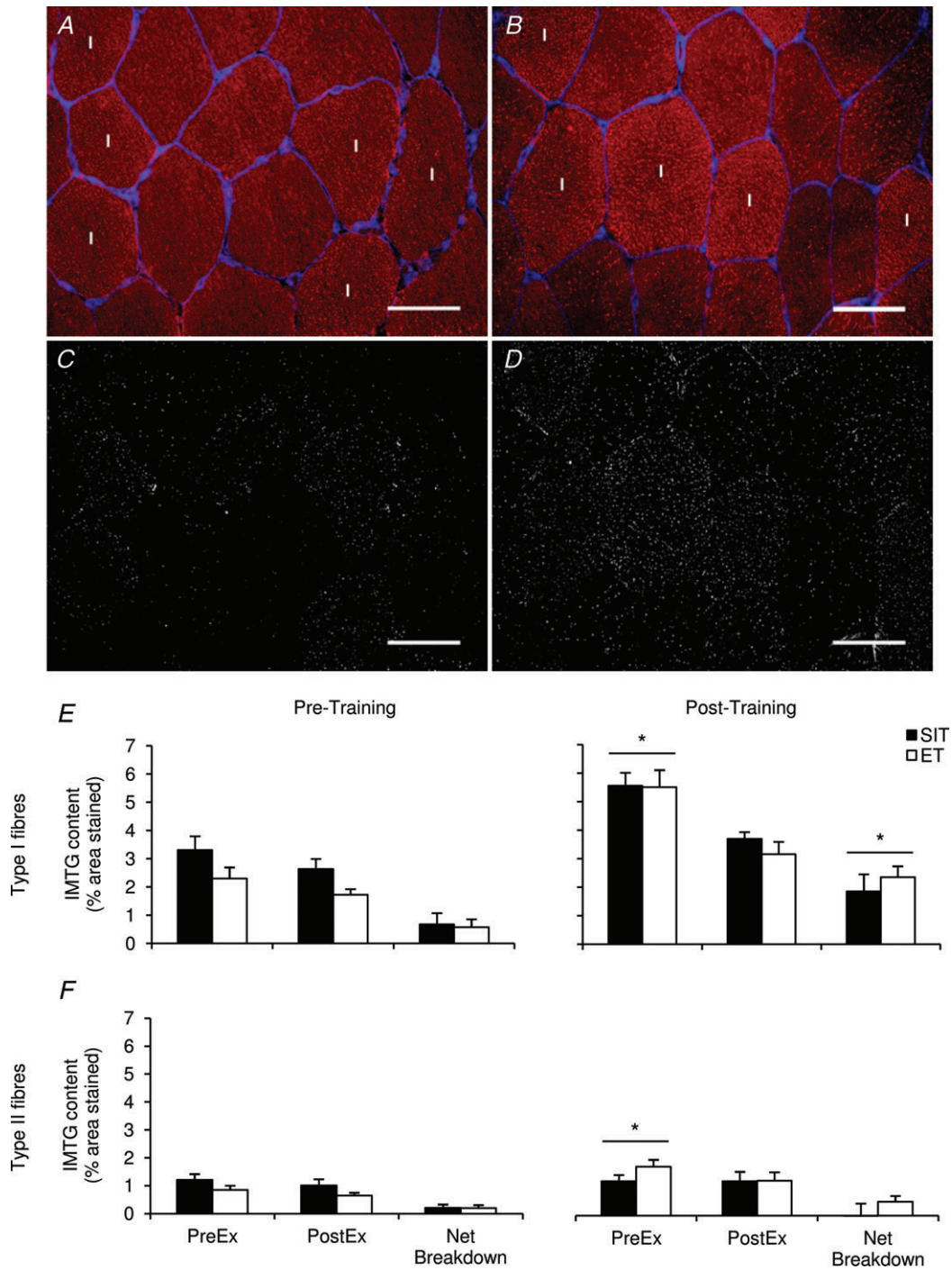
Figure 1. Effect of 6 weeks of sprint interval training (SIT) and endurance training (ET) on fibre type-specific intramuscular triglyceride (IMTG) content and net IMTG breakdown during exercise
Representative immunofluorescence images of IMTG (stained red) in combination with WGA to identify the cell border (stained blue) in skeletal muscle, pre- (*A*) and post- (*B*) 6 weeks of SIT. Type I fibres are indicated with a 'I', all other fibres are assumed type II fibres. White bar, 50 μm. *C* and *D*, the ORO signal obtained after the intensity threshold was applied during quantitation (showing the LDs in white), before and after SIT, respectively. IMTG content, quantified from immunofluorescence images of oil red O-stained muscle sections, before (Pre-training) and after (Post-training) 6 weeks of SIT (filled bars) or ET (open bars). IMTG content was measured before (PreEx) and after (PostEx) 60 min steady state cycling, and net IMTG breakdown calculated (Breakdown) in type I (*E*) and type II fibres (*F*). Values are presented as means ± SEM (*n* = 8 per group). *Main effect of training intervention (*P* < 0.05 *vs.* pre-training).

was unchanged in response to both training interventions. Pre-training, 60 min steady state exercise induced a significant reduction in the fraction of PLIN2 colocalized with IMTG (SIT $30 \pm 6\%$, ET $23 \pm 4\%$; main training effect, $P < 0.01$). This reduction was the result of a decrease in IMTG content and an unchanged PLIN2 content. Post-training, a significantly larger reduction in the fraction of PLIN2 associated with IMTG was observed in response to the exercise bout (SIT $37 \pm 7\%$,

ET $40 \pm 4\%$; $P = 0.01$), with no difference between groups (data not shown). As a change in PLIN2 association with IMTG was observed following steady state exercise both before and after training, we investigated the number of LDs either associated (PLIN2-LD) or not associated (PLIN2-null-LD) with PLIN2 at each time point. Both before and after training, the number of PLIN2-LD was greater than the number of PLIN2-null-LD before exercise. Pre-training, 60 min steady state exercise induced
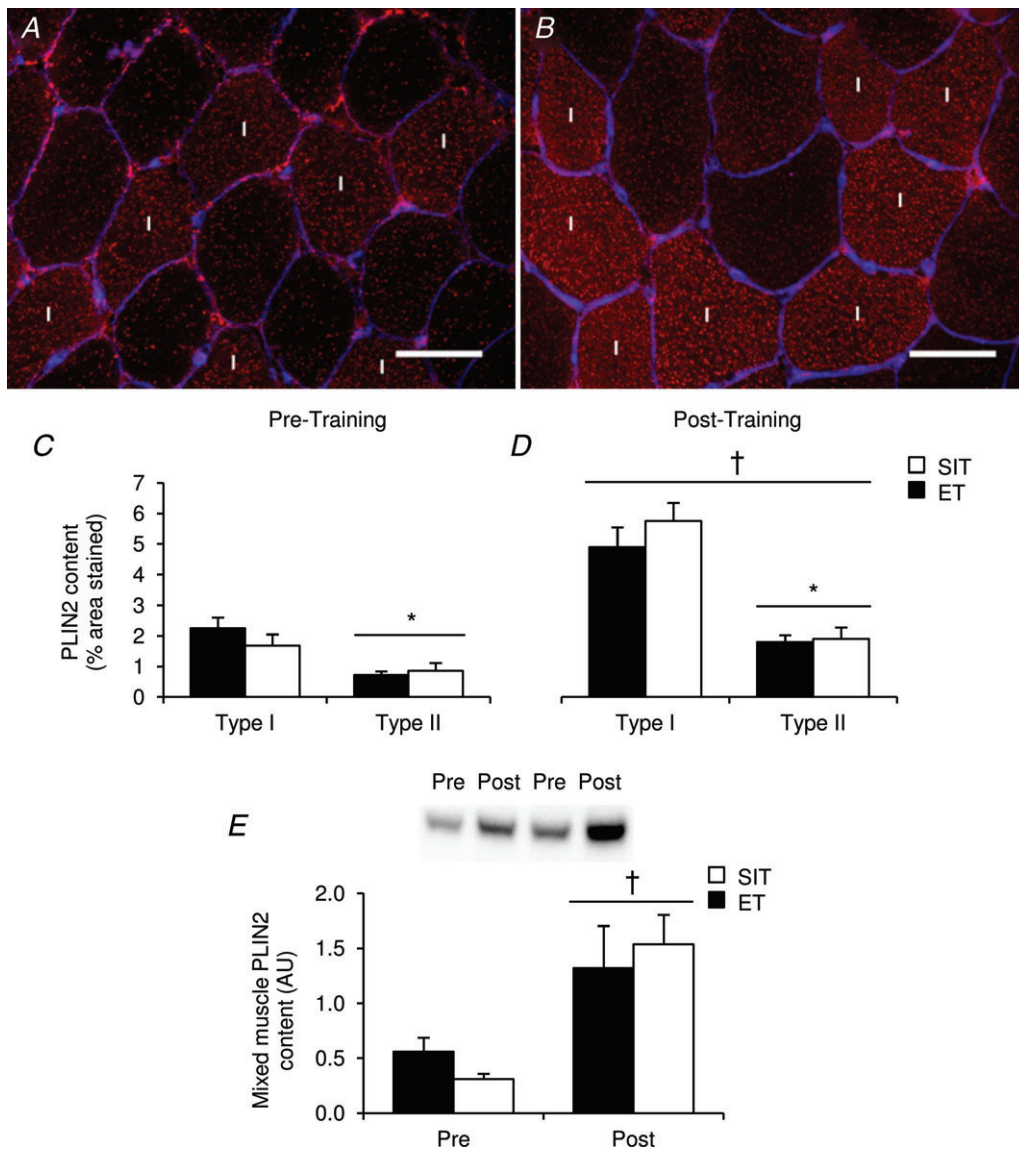

Figure 2. Effect of 6 weeks of sprint interval training (SIT) and endurance training (ET) on fibre type-specific perilipin 2 (PLIN2) expression
Representative immunofluorescence images of PLIN2 (stained red) in combination with WGA to identify the cell border (stained blue) in skeletal muscle, pre- (*A*) and post- (*B*) 6 weeks of SIT. Type I fibres are indicated with a 'I', all other fibres are assumed type II fibres. White bar, 50 μm. PLIN2 expression, quantified from immunofluorescence images of PLIN2, in type I and type II fibres obtained before (*C*) and after (*D*) 6 weeks of SIT (filled bars) or ET (open bars). PLIN2 expression quantified from immunofluorescence images correlated with PLIN2 expression determined following immunoblotting of whole muscle homogenates (*E*). Values are presented as means $\pm$ SEM (*n* = 8 per group). *Main effect of fibre type ($P < 0.05$ *vs.* type I fibres). †Main effect of training intervention ($P < 0.05$ *vs.* pre-training).

a reduction in the number of PLIN2-LD (SIT $22 \pm 10\%$, ET $28 \pm 6\%$; $P = 0.01$; Fig. 4E and F), whereas the number of PLIN2-null-LD was unchanged. In contrast, following training, exercise induced a significant reduction in both the PLIN2-LD (SIT $32 \pm 6\%$, ET $39 \pm 6\%$; $P < 0.001$) and the PLIN2-null-LD (SIT $24 \pm 12\%$, ET $35 \pm 12\%$; $P = 0.01$; Fig. 4E and F). No group interactions were detected with either condition or time for variables of PLIN2 and IMTG association, indicating that observations were statistically similar between groups.

Prior to training, the fraction of PLIN5 colocalized with IMTG was similar between groups (SIT 0.58 ± 0.04, ET 0.50 ± 0.04; $P = 0.20$), and following both training interventions this relationship was unchanged. Pre-training, 60 min steady state exercise significantly reduced the fraction of PLIN5 associated with IMTG (SIT $36 \pm 4\%$, ET $33 \pm 2\%$; main effect of time, $P < 0.01$), which was
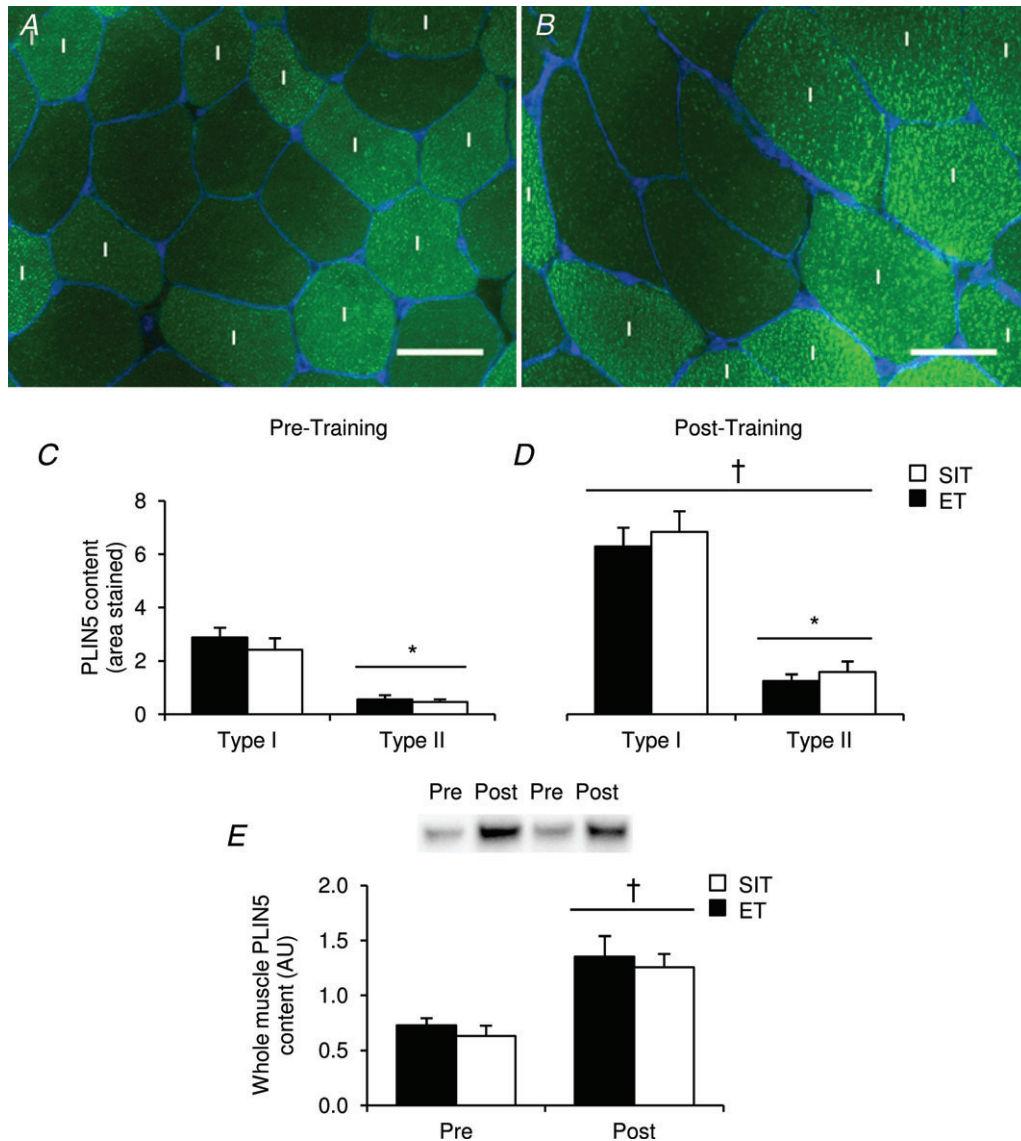

Figure 3. Effect of 6 weeks of sprint interval training (SIT) and endurance training (ET) on fibre type-specific perilipin 5 (PLIN5) expression
Representative immunofluorescence images of PLIN5 (stained green) in combination with WGA to identify the cell border (stained blue) in skeletal muscle, pre- (*A*) and post- (*B*) 6 weeks of SIT. Type I fibres are indicated with a 'I', all other fibres are assumed type II fibres. White bar, 50 μm. PLIN5 expression, quantified from immunofluorescence images of PLIN5, in type I and type II fibres obtained before (*C*) and after (*D*) 6 weeks of SIT (filled bars) or ET (open bars). PLIN5 expression quantified from immunofluorescence images correlated with PLIN5 expression determined following immunoblotting of whole muscle homogenates (*E*). Values are presented as means $\pm$ SEM ($n = 8$ per group). *Main effect of fibre type ($P < 0.05$ *vs*. type I fibres). †Main effect of training intervention ($P < 0.05$ *vs*. pre-training).

the result of a reduction in IMTG with no change in PLIN5 content. Following training, the exercise-induced reduction in the fraction of PLIN5 colocalized with IMTG tended to be larger compared with pre-training, which bordered on statistical significance (SIT $40 \pm 3\%$, ET $41 \pm 3\%$; $P = 0.06$), and with no difference between groups (data not shown). As above, we subsequently investigated the absolute number of LDs either associated

(PLIN5-LD) or not associated (PLIN5-null-LD) with PLIN5 at each time point. Pre-training, the number of PLIN5-LD was larger in the SIT than the ET group ($P < 0.05$), but no difference in the number of PLIN5-null-LD was apparent. Despite this discrepancy, a similar reduction in PLIN5-LD was observed in response to exercise (SIT $32 \pm 4\%$, ET $35 \pm 4\%$; $P < 0.01$; Fig. 4*G* and *H*), with no change in the number of PLIN5-null-LD,
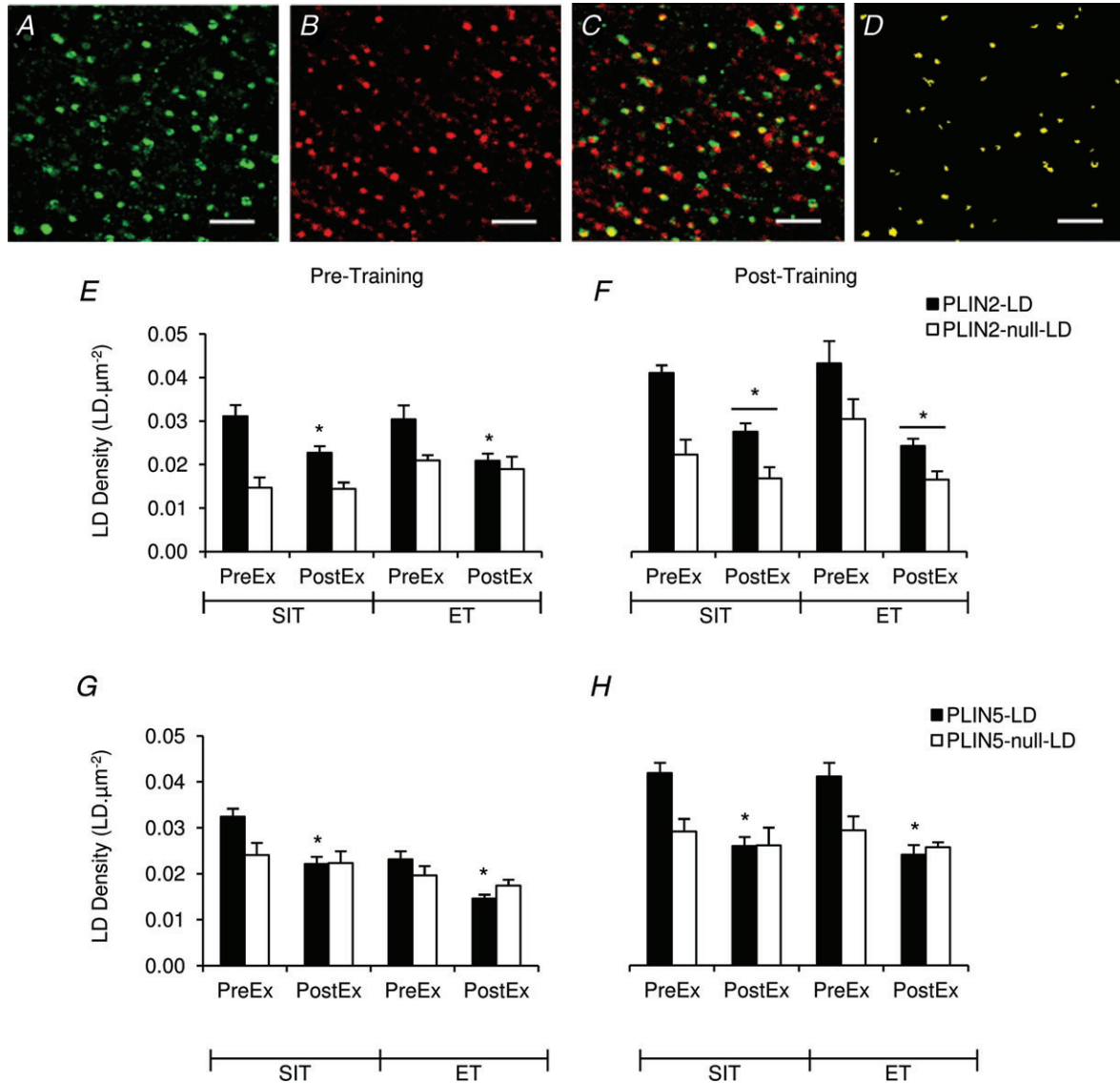

Figure 4. Analysis of the colocalization of perilipin 2 (PLIN2) and perilipin 5 (PLIN5) with lipid droplets (LDs) pre- and post-60 min steady state cycling, before and after 6 weeks of sprint interval training (SIT) or endurance training (ET)
Representative immunofluorescence images of PLIN5 (*A*), IMTG (*B*), and the merged images (*C*) with the yellow areas describing regions of overlap between PLIN5 and IMTG images. The yellow objects were then extracted (to measure the number of PLIN5-LDs; *D*), and the number subtracted from the total number of LDs to quantify the number of PLIN5-null-LD. The same procedure was used to obtain values of colocalization between PLIN2 and IMTG. White bar, 5 μm. The number of PLIN2-LD (filled bars) and PLIN2-null-LD (open bars) were quantified before (PreEx) and after exercise (PostEx), prior to (*E*) and following (*F*) training in both the SIT and ET groups. The same analysis was repeated for PLIN5 for pre- (*G*) and post-training (*H*). *Main effect of exercise bout ($P < 0.05$ *vs.* pre-exercise).

Table 4. Bivariate correlation analysis

	IMTG	Net IMTG breakdown	Insulin sensitivity
PLIN2	0.68‡	0.57‡	0.40*
PLIN5	0.72‡	0.63‡	0.42*
IMTG		0.81‡	0.53†

IMTG, PLIN2 and PLIN5 represent resting content. Net IMTG breakdown refers to absolute change in IMTG concentration from pre- to post-exercise. Values represent Pearson's squared correlation coefficients (*R*-values) for relationships between variables. Due to the lack of group × training interactions in all variables, it was assumed that the exercise training was driving the changes independent of exercise modality. Therefore, the data from both intervention groups were pooled for correlation analysis. Significant correlations: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$. IMTG, intramuscular triglyceride; PLIN 2, perilipin 2; PLIN 5, perilipin 5.

and no difference between groups for either variable. Following training, exercise induced a reduction in the number of PLIN5-LD only (SIT 38 ± 3%, ET 41 ± 5%; $P < 0.01$; Fig. 4*G* and *H*), with no difference between groups.

Correlation analyses

Due to the lack of group × training interactions in all variables, significant training effects were detected independent of exercise modality. Therefore, the data from the SIT and ET interventions (type I and type II fibres, pre- and post-training) were pooled for correlation analysis (Table 4). Both PLIN2 ($R = 0.68$; $P < 0.001$) and PLIN5 content ($R = 0.72$; $P < 0.001$) showed strong positive correlations with IMTG content. A very strong positive correlation was observed between pre-exercise IMTG concentrations and the decrease in IMTG content in response to 60 min steady state exercise ($R = 0.81$, $P < 0.001$). In addition, PLIN2 ($R = 0.57$, $P = 0.001$) and PLIN5 content ($R = 0.63$, $P < 0.001$) were strongly associated with net IMTG breakdown. Insulin sensitivity was modestly associated with PLIN2 ($R = 0.40$; $P = 0.02$) and PLIN5 content ($R = 0.42$; $P = 0.02$), as well as IMTG content ($R = 0.53$; $P = 0.01$).

Discussion

This is the first study to report that greater protein expression of PLIN2 and PLIN5 is associated with larger IMTG stores and enhanced net IMTG breakdown during exercise in type I muscle fibres following a period of traditional ET. This observation is consistent with our first hypothesis. We also provide novel evidence that PLIN5-LDs are utilized during an acute bout of exercise irrespective of training status, whereas after a period of training both PLIN2-LDs and PLIN2-null-LDs are utilized during an acute bout of exercise. In support of our second hypothesis, we demonstrate for the first time that like traditional ET, SIT also enhances IMTG content and net breakdown during 60 min of moderate-intensity exercise and leads to increases in the protein expression of PLIN2 and PLIN5. This common metabolic response following both SIT and ET is also evident in the utilization of PLIN5-LDs, PLIN2-LDs and PLIN2-null-LDs. Taken together, these adaptations may contribute to the mechanisms underlying the observed improvements in insulin sensitivity following SIT and ET.

We report that ET leads to increases in IMTG content in both type I and type II fibres, and is accompanied by increased mitochondrial density, corroborating the findings of previous studies (Schrauwen-Hinderling *et al.* 2003; Pruchnic *et al.* 2004; Tarnopolsky *et al.* 2007; Dubé *et al.* 2011). In addition before training, 60 min moderate-intensity cycling induced a small but significant decrease in IMTG in type I fibres. Importantly, we report that ET leads to increased rates of whole-body fat oxidation during exercise at 65% pre-training $\dot{V}_{O_2peak}$, and that this is accompanied by greater IMTG breakdown primarily in type I fibres. Previous studies combining IMTG content and *A*–*V* balance measurements using [U-^{13}C]palmitate demonstrate that oxidation of plasma fatty acids does not completely account for fat oxidation during exercise, indicating that other fat sources contribute to total fat oxidation (Sacchetti *et al.* 2002; van Hall *et al.* 2002; van Loon *et al.* 2003). Therefore, it is reasonable to assume that fatty acids liberated through the net reduction in IMTG content contribute to total fat oxidation during exercise in lean but sedentary individuals, and the IMTG contribution to total fat oxidation is increased following ET. The latter observation is consistent with a number of recent training studies also examining net IMTG breakdown in a fibre type-specific manner (Van Proeyen *et al.* 2011*a*, *b*).

Our data demonstrate that net IMTG breakdown during exercise is positively correlated with resting IMTG concentration. This corroborates the findings of several other reports in trained individuals (Coggan *et al.* 2000; van Loon *et al.* 2003; Stellingwerff *et al.* 2007), and indicates that the ET-induced increase in IMTG content contributes to the increase in net IMTG breakdown during ET exercise. However, it is worth noting that this is only one of a number of adaptations that probably explain the increase in IMTG breakdown during exercise following ET (van Loon, 2004; Shaw *et al.* 2010). The increase in IMTG content was due to a greater number of LDs rather than an increased volume of pre-existing LDs. This conclusion is in agreement with a previous report using transmission electron microscopy (Tarnopolsky *et al.* 2007). As smaller LDs maintain a higher surface area to volume ratio, they also provide a greater surface area for the interaction of

lipolytic enzymes with the IMTG substrate and regulatory proteins on the LD surface. As a result, the mobilization of fatty acids for oxidation during exercise would be enhanced.

Using immunofluorescence microscopy we previously reported that trained athletes display an ∼twofold greater PLIN2 concentration in type I compared with type II muscle fibres (Shaw *et al.* 2009). The present data extend this finding to sedentary individuals, and further reveal a ∼fourfold difference in PLIN5 protein expression when comparing type I and type II fibres. In addition, we report that ET enhances the intramuscular content of both PLIN2 and PLIN5, assessed through immunoblotting of whole muscle homogenates. The findings are partly in agreement with a recent study showing increased PLIN5, but not PLIN2, protein abundance in the muscle of lean and obese males following 12 weeks of ET (Peters *et al.* 2012), and with a cross-sectional study that reported a ∼sevenfold higher PLIN5 content in older athletes compared with age-matched sedentary controls (Amati *et al.* 2011). However, our study is the first to examine changes in the intramuscular expression of the PLIN proteins in response to training interventions in a fibre type-specific manner using immunofluorescence microscopy. Accordingly, we demonstrate that, like the changes in IMTG, the increase in PLIN2 and PLIN5 protein expression occurs in both type I and type II muscle fibres.

In agreement with data obtained in rodent (Minnaard *et al.* 2009) and human skeletal muscle (Amati *et al.* 2011; Peters *et al.* 2012), both PLIN2 and PLIN5 expression positively correlated with IMTG content, suggesting that the expression of these PLIN proteins is proportional to IMTG content in lean, healthy individuals. Future research will be required to clarify if the increase in PLIN expression is the primary event driving the increase in IMTG content (which is the mechanism that we propose) or the consequence of the increase in IMTG content. Furthermore, the fraction of IMTG associated with either PLIN2 or PLIN5 at rest was similar pre- and post-training in type I fibres (∼0.64 and ∼0.59, respectively). These values are consistent with previous reports in rat (Macpherson *et al.* 2012) and human skeletal muscle (Shaw *et al.* 2009; Shepherd *et al.* 2012). Although the physiological significance of this partial colocalization is currently unknown, this finding is in agreement with a number of observations demonstrating that the protein composition of LDs may vary (Wolins *et al.* 2005, 2006*b*; Ducharme & Bickel, 2008; Fujimoto *et al.* 2008). To further investigate this relationship we assessed the colocalization of these PLINs with LDs before and after 60 min moderate-intensity ET. For each PLIN we describe two pools of LDs: (1) PLIN2-associated LDs (PLIN2-LD) and LDs that do not contain PLIN2 (PLIN2-null-LD); and (2) PLIN5-associated LDs (PLIN5-LD) and LDs that do not contain PLIN5 (PLIN5-null-LD). In the untrained state, 60 min moderate-intensity ET induced a significant decrease in the number of PLIN2-LD and PLIN5-LD, with no change in the number of PLIN2-null-LD and PLIN5-null-LD. These observations confirm a recent report from our laboratory showing a preferential utilization of PLIN2-LD, and not PLIN2-null-LD, in response to moderate-intensity ET in sedentary individuals (Shepherd *et al.* 2012), and provides novel evidence to suggest that a similar relationship exists for PLIN5. After 6 weeks ET, a preferential decrease in the number of PLIN2-LD and PLIN5-LD also occurred in response to 60 min moderate-intensity ET. Interestingly, in the trained state exercise also induced a decrease in the content of PLIN2-null-LD after 60 min moderate-intensity ET, whereas the content of PLIN5-null-LD did not change. This is the first observation to suggest that PLIN5 is more important than PLIN2 in mediating the hydrolysis of intramuscular lipid stores during exercise, and is in line with emerging evidence concerning the precise role of PLIN5 in lipolysis in cultured cells. Indeed, PLIN5 is the only PLIN thus far that has been shown to bind ATGL and its coactivator, CGI-58. In this respect, overexpression of PLIN5 in cultured cells recruits ATGL and CGI-58 under basal conditions, while expression of PLIN2 does not have this effect (Wang *et al.* 2011*a*). Furthermore, PLIN5 is phosphorylated in response to PKA activation, and the expression and PKA-induced activation of PLIN5 stimulates triacylglycerol hydrolysis in Chinese hamster ovary cells, whereas rates of lipolysis are unaffected by PKA activation in cells overexpressing PLIN2 (Wang *et al.* 2011*a*, *b*). It is also important to note that electrical stimulation and adrenaline stimulation of rat skeletal muscle (Prats *et al.* 2006) has been reported to increase the colocalization of HSL with PLIN2. However, it is not known whether this association occurs in man *in vivo* and whether it increases rates of lipolysis. It is also worth acknowledging that we only assessed colocalization of either PLIN2 or PLIN5 with the LDs, and therefore cannot discriminate LDs containing both PLIN2 and PLIN5 from those LDs containing only PLIN2 or PLIN5. Nevertheless, the observation that the number of PLIN5-LD is reduced following exercise, while no reduction occurs in the number of PLIN5-null-LD, appears to underpin the important role of PLIN5 in LD lipolysis and subsequent oxidation in skeletal muscle. In line with this conclusion, observations from various cell culture models and rat skeletal muscle preparations suggest that PLIN5 also recruits mitochondria to the surface of LDs, thus stimulating the formation of functional complexes between mitochondria and LDs (Wang *et al.* 2011*b*; Bosma *et al.* 2012). Accordingly, PKA activation in alpha mouse liver 12 (AML12) cells expressing PLIN5 has recently been shown to promote triacylglycerol hydrolysis and efficient channeling of liberated fatty acids to the mitochondria for

β-oxidation (Wang *et al.* 2011*b*). IMTG and mitochondria in skeletal muscle of endurance athletes are observed in close proximity (Shaw *et al.* 2008), and ET enhances the spatial interaction between IMTG and mitochondria (Tarnopolsky *et al.* 2007). Collectively these observations suggest that increased expression and PKA activation of PLIN5 may also contribute to an increased net IMTG breakdown during moderate-intensity ET by mediating stronger interactions between IMTG and mitochondria.

It is worth noting that there was no net IMTG breakdown in type II fibres during 60 min moderate-intensity exercise pre- or post- 6 weeks of training. This observation is in line with previous studies in well-trained athletes performing 2–3 h moderate-intensity exercise (van Loon *et al.* 2003; De Bock *et al.* 2005; Stellingwerff *et al.* 2007). However, PLIN2 and PLIN5 protein expression was significantly increased in type II fibres, raising the question as to why IMTG breakdown was not enhanced in parallel as seen in the type I fibres. However, as the exercise was performed at a moderate intensity, it is likely that only a small proportion of the type II fibres were recruited, and this therefore may explain why no net IMTG breakdown was observed in type II fibres. In addition, the higher PLIN2 and PLIN5 content after training in both fibre types may also serve other metabolic roles such as to mediate IMTG storage under non-exercise conditions.

Both PLIN2 and PLIN5 also appear to play an important role in limiting rates of lipolysis in the basal state, as expression of either PLIN2 or PLIN5 in cultured cells reduces basal lipolytic rates compared with when PLIN2 is knocked down or PLIN5 is not expressed (Listenberger *et al.* 2007; Bell *et al.* 2008; Wang *et al.* 2011*a*). Specifically, PLIN2 expression reduces the association of ATGL with the LD (Listenberger *et al.* 2007; Bell *et al.* 2008), and PLIN5 binds ATGL under basal conditions thereby reducing its activity (Wang *et al.* 2011*a*). Furthermore, it has recently been shown that PLIN5 expression in AML12 cells reduces basal triacylglycerol hydrolysis and promotes palmitate incorporation into LD triacylglycerol (Wang *et al.* 2011*b*). If such mechanisms exist *in vivo*, these observations suggest that PLIN5 and/or PLIN2 may play a key role in the coupling of TAG hydrolysis to the metabolic demand for fatty acids. As mentioned, ET increases the net breakdown of IMTG during exercise, therefore during an ET intervention IMTG concentrations are reduced on a regular basis. In addition, IMTG synthesis is upregulated in the period following IMTG-depleting exercise (Schenk & Horowitz, 2007). Therefore, undertaking repeated bouts of exercise during an exercise training programme likely results in high rates of IMTG synthesis (Bergman *et al.* 2010). As a result of this combination of training adaptations in the enzymes controlling IMTG metabolism, insulin resistance-inducing fatty acid metabolites have been suggested to be maintained at a lower concentration in skeletal muscle, thereby preserving higher insulin

sensitivity in trained individuals. (Dubé *et al.* 2008, 2011).

The most well-documented adaptations to SIT are an improved $\dot{V}_{\mathrm{O_2peak}}$ and an enhanced skeletal muscle oxidative capacity (reviewed in Gibala *et al.* 2012), both of which are increased by a similar magnitude as ET (Burgomaster *et al.* 2008). We also demonstrate that SIT and ET improve $\dot{V}_{\mathrm{O_2peak}}$ in sedentary individuals as previously described (Burgomaster *et al.* 2008), although in the present study the improvement in $\dot{V}_{\mathrm{O_2peak}}$ tended to be greater in the ET group ($P = 0.064$). Like ET, SIT also increased mitochondrial density, as previously reported (Burgomaster *et al.* 2008), and we provide novel data showing that SIT leads to higher IMTG content in type I and type II muscle fibres. In contrast to ET, no changes in whole-body fat oxidation rates were observed following SIT. This finding is in contrast to the findings of Burgomaster *et al.* (2008), who reported an increase in fat oxidation during exercise following SIT. The SIT group tended to exhibit a lower RER than the ET group pre-training, suggesting that rates of fat oxidation were higher in the SIT group before training, despite the groups being matched for physical activity level, aerobic capacity and fat mass, and diet was controlled in the 48 h prior to the exercise bout. Nevertheless, we observe clear increases in IMTG breakdown in type I fibres during moderate-intensity exercise in response to SIT, suggesting that the source of oxidized fat is shifted from plasma fatty acids towards IMTG. Importantly, the expression of both PLIN2 and PLIN5 correlated with IMTG content, net IMTG breakdown in type I fibres during exercise, and insulin sensitivity. Therefore, the data suggest that the increase in IMTG content and net breakdown during exercise that results from the increased expression of PLIN2 and PLIN5 probably maintains low intramuscular fatty acid metabolite concentrations leading to improvements in insulin sensitivity following both ET and SIT. Accordingly, the present study adds to the growing body of evidence that SIT is a time-efficient exercise intervention to improve insulin sensitivity in healthy individuals and patient populations (Babraj *et al.* 2009; Richards *et al.* 2010; Hood *et al.* 2011; Little *et al.* 2011).

It is important to note that the primary aim of the study was to investigate exercise training-induced increases in net IMTG breakdown during 60 min moderate-intensity exercise, and PLIN2 and PLIN5 protein expression. However, it is likely that the number of subjects in the present study is not sufficiently high to detect between group differences for some of the measured variables. Therefore, although many of the training effects are of a similar magnitude, larger-scale studies are required to determine which exercise mode is superior in increasing the main metabolic outcome measures (IMTG breakdown during exercise, and PLIN2 and PLIN5 expression), and/or

in increasing whole-body insulin sensitivity and aerobic exercise capacity. Studies involving a larger number of participants should be completed before advice can be given about which exercise training mode is the best for the general population and for specific patient populations.

In conclusion, PLIN2 and PLIN5 expression are upregulated in response to ET, and the upregulation of these proteins appears to be important to facilitate the enhanced rates of net IMTG breakdown during exercise. In addition, PLIN2 and PLIN5 expression may contribute to an increase in IMTG content, which probably results in lower fatty acid metabolite concentrations, and in turn may drive the ET-induced improvements in insulin sensitivity. Despite the large differences in duration and energy expenditure between SIT and ET, we provide novel evidence indicating that SIT induces similar improvements in net IMTG breakdown, PLIN2 and PLIN5 protein expression, aerobic exercise capacity and insulin sensitivity as traditional ET. This study, therefore, generates novel information on the mechanism by which net IMTG breakdown during ET increases following both modes of exercise training, and provides evidence that SIT provides a time-efficient exercise alternative to achieve improvements in aerobic fitness and insulin sensitivity.

References

Amati F, Dubé JJ, Carnero EA, Edreira MM, Chomentowski P, Coen PM, Switzer GE, Bickel PE, Stefanovic-Racic M, Toledo FG & Goodpaster BH (2011). Skeletal-muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes* **60**, 2588–2597.

Babraj JA, Vollaard NB, Keast C, Guppy FM, Cottrell G & Timmons JA (2009). Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *BMC Endocr Disord* **9**, 3.

Bar-Or O (1987). The Wingate anaerobic test. An update on methodology, reliability and validity. *Sports Med* **4**, 381–394.

Bell M, Wang H, Chen H, McLenithan JC, Gong DW, Yang RZ, Yu D, Fried SK, Quon MJ, Londos C & Sztalryd C (2008). Consequences of lipid droplet coat protein downregulation in liver cells: abnormal lipid droplet metabolism and induction of insulin resistance. *Diabetes* **57**, 2037–2045.

Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM & Eckel RH (2010). Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes. *J Appl Physiol* **108**, 1134–1141.

Bergström J (1975). Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* **35**, 609–616.

Bosma M, Minnaard R, Sparks LM, Schaart G, Losen M, de Baets MH, Duimel H, Kersten S, Bickel PE, Schrauwen P & Hesselink MK (2012). The lipid droplet coat protein perilipin 5 also localizes to muscle mitochondria. *Histochem Cell Biol* **137**, 205–216.

Brasaemle DL, Barber T, Wolins NE, Serrero G, Blanchette-Mackie EJ & Londos C (1997). Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. *J Lipid Res* **38**, 2249–2263.

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.

Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A & Gibala MJ (2007). Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol* **292**, R1970–R1976.

Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, MacDonald MJ, McGee SL & Gibala MJ (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol* **586**, 151–160.

Burgomaster KA, Hughes SC, Heigenhauser GJF, Bradwell SN & Gibala MJ (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol* **98**, 1985–1990.

Cocks M, Shaw CS, Shepherd SO, Fisher J, Ranasinghe AM, Barker TA, Tipton KD & Wagenmakers AJ. (2013). High intensity interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. *J Physiol* **591**, 641–656.

Coggan AR, Raguso CA, Gastaldelli A, Sidossis LS & Yeckel CW (2000). Fat metabolism during high-intensity exercise in endurance-trained and untrained men. *Metabolism* **49**, 122–128.

Dalen KT, Dahl T, Holter E, Arntsen B, Londos C, Sztalryd C & Nebb HI (2007). LSDP5 is a PAT protein specifically expressed in fatty acid oxidizing tissues. *Biochim Biophys Acta* **1771**, 210–227.

De Bock K, Richter EA, Russell AP, Eijnde BO, Derave W, Ramaekers M, Koninckx E, Leger B, Verhaeghe J & Hespel P (2005). Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *J Physiol* **564**, 649–660.

Dubé JJ, Amati F, Stefanovic-Racic M, Toledo FG, 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.

Dubé JJ, Amati F, Toledo FG, Stefanovic-Racic M, Rossi A, Coen P & Goodpaster BH (2011). Effects of weight loss and exercise on insulin resistance, and intramyocellular triacylglycerol, diacylglycerol and ceramide. *Diabetologia* **54**, 1147–1156.

Ducharme NA & Bickel PE (2008). Lipid droplets in lipogenesis and lipolysis. *Endocrinology* **149**, 942–949.

Fujimoto T, Ohsaki Y, Cheng J, Suzuki M & Shinohara Y (2008). Lipid droplets: a classic organelle with new outfits. *Histochem Cell Biol* **130**, 263–279.

Gibala MJ, Little JP, Macdonald MJ & Hawley JA (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol* **590**, 1077–1084.

Goodpaster BH, He J, Watkins S & Kelley DE (2001). Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* **86**, 5755–5761.

Hood MS, Little JP, Tarnopolsky MA, Myslik F & Gibala MJ (2011). Low-volume interval training improves muscle oxidative capacity in sedentary adults. *Med Sci Sports Exerc* **43**, 1849–1856.

Jeukendrup AE & Wallis GA (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* **26**, S28–S37.

Koopman R, Schaart G & Hesselink MK (2001). Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. *Histochem Cell Biol* **116**, 63–68.

Lachmanovich E, Shvartsman DE, Malka Y, Botvin C, Henis YI & Weiss AM (2003). Co-localization analysis of complex formation among membrane proteins by computerized fluorescence microscopy: application to immunofluorescence co-patching studies. *J Microsc* **212**, 122–131.

Listenberger LL, Ostermeyer-Fay AG, Goldberg EB, Brown WJ & Brown DA (2007). Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover. *J Lipid Res* **48**, 2751–2761.

Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, Jung ME & Gibala MJ (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol* **111**, 1554–1560.

Little JP, Safdar A, Wilkin GP, Tarnopolsky MA & Gibala MJ (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J Physiol* **588**, 1011–1022.

Macpherson RE, Herbst EA, Reynolds EJ, Vandenboom R, Roy BD & Peters SJ (2012). Subcellular localization of skeletal muscle lipid droplets and PLIN family proteins (OXPAT and ADRP) at rest and following contraction (in rat soleus muscle). *Am J Physiol Regul Integr Comp Physiol* **302**, R29–R36.

Matsuda M & DeFronzo RA (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22**, 1462–1470.

Minnaard R, Schrauwen P, Schaart G, Jorgensen JA, Lenaers E, Mensink M & Hesselink MK (2009). Adipocyte differentiation-related protein and OXPAT in rat and human skeletal muscle: involvement in lipid accumulation and type 2 diabetes mellitus. *J Clin Endocrinol Metab* **94**, 4077–4085.

Moro C, Bajpeyi S & Smith SR (2008). Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *Am J Physiol Endocrinol Metab* **294**, E203–E213.

Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB & Storlien LH (1997). Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* **46**, 983–988.

Peters SJ, Samjoo IA, Devries MC, Stevic I, Robertshaw HA & Tarnopolsky MA (2012). Perilipin family (PLIN) proteins in human skeletal muscle: the effect of sex, obesity, and endurance training. *Appl Physiol Nutr Metab* **37**, 724–735.

Phillips DI, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC & Taylor R (1996). Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* **45**, 947–950.

Prats C, Donsmark M, Qvortrup K, Londos C, Sztalryd C, Holm C, Galbo H & Ploug T (2006). Decrease in intramuscular lipid droplets and translocation of HSL in response to muscle contraction and epinephrine. *J Lipid Res* **47**, 2392–2399.

Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C & Goodpaster BH (2004). Exercise training increases intramyocellular lipid and oxidative capacity in older adults. *Am J Physiol Endocrinol Metab* **287**, E857–E862.

Richards JC, Johnson TK, Kuzma JN, Lonac MC, Schweder MM, Voyles WF & Bell C (2010). Short-term sprint interval training increases insulin sensitivity in healthy adults but does not affect the thermogenic response to beta-adrenergic stimulation. *J Physiol* **588**, 2961–2972.

Sacchetti M, Saltin B, Osada T & van Hall G (2002). Intramuscular fatty acid metabolism in contracting and non-contracting human skeletal muscle. *J Physiol* **540**, 387–395.

Schenk S & Horowitz JF (2007). Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. *J Clin Invest* **117**, 1690–1698.

Schrauwen P, van Aggel-Leijssen DP, Hul G, Wagenmakers AJ, Vidal H, Saris WH & van Baak MA (2002). The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-CoA carboxylase-2 expression. *Diabetes* **51**, 2220–2226.

Schrauwen-Hinderling VB, Schrauwen P, Hesselink MK, van Engelshoven JM, Nicolay K, Saris WH, Kessels AG & Kooi ME (2003). The increase in intramyocellular lipid content is a very early response to training. *J Clin Endocrinol Metab* **88**, 1610–1616.

Shaw CS, Clark J & Wagenmakers AJ (2010). The effect of exercise and nutrition on intramuscular fat metabolism and insulin sensitivity. *Annu Rev Nutr* **30**, 13–34.

Shaw CS, Jones DA & Wagenmakers AJ (2008). Network distribution of mitochondria and lipid droplets in human muscle fibres. *Histochem Cell Biol* **129**, 65–72.

Shaw CS, Sherlock M, Stewart PM & Wagenmakers AJ (2009). Adipophilin distribution and colocalization with lipid droplets in skeletal muscle. *Histochem Cell Biol* **131**, 575–581.

Shepherd SO, Cocks M, Tipton KD, Ranasinghe AM, Barker TA, Burniston JG, Wagenmakers AJ & Shaw CS (2012). Preferential utilization of perilipin 2-associated intramuscular triglycerides during 1 h of moderate-intensity endurance-type exercise. *Exp Physiol* **97**, 970–980.

Stellingwerff T, Boon H, Jonkers RA, Senden JM, Spriet LL, Koopman R & van Loon LJ (2007). Significant intramyocellular lipid use during prolonged cycling in endurance-trained males as assessed by three different methodologies. *Am J Physiol Endocrinol Metab* **292**, E1715–E1723.

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.

van Hall G, Sacchetti M, Radegran G & Saltin B (2002). Human skeletal muscle fatty acid and glycerol metabolism during rest, exercise and recovery. *J Physiol* **543**, 1047–1058.

van Loon LJ (2004). Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *J Appl Physiol* **97**, 1170–1187.

van Loon LJ & Goodpaster BH (2006). Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Pflugers Arch* **451**, 606–616.

van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA & Saris WH (2003). Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *J Physiol* **553**, 611–625.

van Loon LJC, Koopman R, Manders R, van der Weegen W, van Kranenburg GP & Keizer HA (2004). Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. *Am J Physiol Endocrinol Metab* **287**, E558–E565.

Van Proeyen K, Szlufcik K, Nielens H, Deldicque L, Van Dyck R, Ramaekers M & Hespel P (2011*a*). High-fat diet overrules the effects of training on fiber-specific intramyocellular lipid utilization during exercise. *J Appl Physiol* **111**, 108–116.

Van Proeyen K, Szlufcik K, Nielens H, Ramaekers M & Hespel P (2011*b*). Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *J Appl Physiol* **110**, 236–245.

Wang H, Bell M, Sreenevasan U, Hu H, Liu J, Dalen K, Londos C, Yamaguchi T, Rizzo MA, Coleman R, Gong D, Brasaemle D & Sztalryd C (2011*a*). Unique regulation of adipose triglyceride lipase (ATGL) by perilipin 5, a lipid droplet-associated protein. *J Biol Chem* **286**, 15707–15715.

Wang H, Sreenevasan U, Hu H, Saladino A, Polster BM, Lund LM, Gong DW, Stanley WC & Sztalryd C (2011*b*). Perilipin 5, lipid droplet associated protein provides physical and metabolic linkage to mitochondria. *J Lipid Res* **52**, 2159–2168.

Wolins NE, Brasaemle DL & Bickel PE (2006*a*). A proposed model of fat packaging by exchangeable lipid droplet proteins. *FEBS Lett* **580**, 5484–5491.

Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A & Bickel PE (2005). S3–12, Adipophilin, and TIP47 package lipid in adipocytes. *J Biol Chem* **280**, 19146–19155.

Wolins NE, Quaynor BK, Skinner JR, Tzekov A, Croce MA, Gropler MC, Varma V, Yao-Borengasser A, Rasouli N, Kern PA, Finck BN & Bickel PE (2006*b*). OXPAT/PAT-1 is a PPAR-induced lipid droplet protein that promotes fatty acid utilization. *Diabetes* **55**, 3418–3428.

Yamaguchi T, Matsushita S, Motojima K, Hirose F & Osumi T (2006). MLDP, a novel PAT family protein localized to lipid droplets and enriched in the heart, is regulated by peroxisome proliferator-activated receptor alpha. *J Biol Chem* **281**, 14232–14240.

Authors' current addresses

S. O. Shepherd, M. Cocks and A. J. M. Wagenmakers: Research Institute for Sport & Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom Street Campus, Liverpool L3 3AF, UK.

C. S. Shaw: Institute of Sport, Exercise & Active Living, Victoria University, Melbourne, Australia.

Author contributions

S.O.S.: design of the experiments; collection, analysis and interpretation of the data; drafting and final revisions of the manuscript. M.C.: design of the experiments; collection, analysis and interpretation of the data. K.D.T.: design of the experiments; collection of the data; critical revision of the manuscript for intellectual content. A.M.R.: collection of the data; critical revision of the manuscript for intellectual content. T.A.B.: collection of the data; critical revision of the manuscript for intellectual content. J.G.B.: provided technical expertise and access to his laboratory for Western blotting analysis; and critical revision of the manuscript for intellectual content. A.J.M.W.: design of the experiments; analysis and interpretation of the data; drafting a final revision of the manuscript. C.S.S.: design of the experiments; analysis and interpretation of the data; drafting a final revision of the manuscript. All authors have approved the final version of the manuscript.

Translational perspective

It is well established that individuals who perform 3–5 h of continuous moderate-intensity exercise per week maintain a higher physical fitness level and have a reduced risk of developing metabolic and cardiovascular diseases. However, these current physical activity guidelines are not met by the majority of the UK population, with 'a lack of time' cited as the primary reason for failure to engage in regular exercise. Sprint interval training (SIT) may provide an effective alternative to traditional endurance-type exercise training (ET), with a substantial reduction in time commitment. Indeed, SIT is reported to induce similar increases in $\dot{V}_{O_2\,max}$, mitochondrial enzyme abundance and whole-body insulin sensitivity. Shepherd and colleagues show that like ET, 6 weeks of SIT increases the capacity to oxidize intramuscular triglyceride (IMTG) during exercise. Furthermore, they show that this adaptation may be mediated by an upregulation of PLIN2 and PLIN5, proteins known to be involved in regulating IMTG breakdown. The depletion and subsequent replenishment of the IMTG pool during exercise and recovery periods is postulated to prevent the accumulation of insulin resistance-inducing fatty acid metabolites. Therefore, increasing IMTG oxidation is an important training adaptation contributing to the improvement of insulin sensitivity following both ET and SIT. These findings add to the evidence that SIT could provide an effective exercise training mode to improve fitness and metabolic health in obese individuals, and elderly individuals with sarcopaenia, type II diabetes and cardiovascular disease. Long-term, large-scale studies investigating the clinical benefits of SIT in such populations are now warranted.