Short-term sprint interval *versus* traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance

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Brief, intense exercise training may induce metabolic and performance adaptations comparable to traditional endurance training. However, no study has directly compared these diverse training strategies in a standardized manner. We therefore examined changes in exercise capacity and molecular and cellular adaptations in skeletal muscle after low volume sprint-interval training (SIT) and high volume endurance training (ET). Sixteen active men (21 ± 1 years, $\dot{V}_{O_2peak} = 4.0 \pm 0.21 \min^{-1}$) were assigned to a SIT or ET group ($n = 8$ each) and performed six training sessions over 14 days. Each session consisted of either four to six repeats of 30 s 'all out' cycling at ~250% $\dot{V}_{O_2peak}$ with 4 min recovery (SIT) or 90–120 min continuous cycling at ~65% $\dot{V}_{O_2peak}$ (ET). Training time commitment over 2 weeks was ~2.5 h for SIT and ~10.5 h for ET, and total training volume was ~90% lower for SIT *versus* ET (~630 *versus* ~6500 kJ). Training decreased the time required to complete 50 and 750 kJ cycling time trials, with no difference between groups (main effects, $P \le 0.05$). Biopsy samples obtained before and after training revealed similar increases in muscle oxidative capacity, as reflected by the maximal activity of cytochrome c oxidase (COX) and COX subunits II and IV protein content (main effects, $P \le 0.05$), but COX II and IV mRNAs were unchanged. Training-induced increases in muscle buffering capacity and glycogen content were also similar between groups (main effects, $P \le 0.05$). Given the large difference in training volume, these data demonstrate that SIT is a time-efficient strategy to induce rapid adaptations in skeletal muscle and exercise performance that are comparable to ET in young active men.

Regular endurance training induces numerous physiological adaptations that facilitate improved exercise capacity, i.e. the ability to sustain a given submaximal workload for a longer period of time or achieve a higher average power output over a fixed distance or time (Coyle, 1995; Hawley, 2002). One of the most prominent adaptations to training is a change in skeletal muscle substrate metabolism (Holloszy & Coyle, 1984). For example, even a short period of endurance training (5–7 days) increases glycogen availability but reduces the rate of glycogen catabolism during matched-work exercise (Green *et al.* 1992; Chesley *et al.* 1996), resulting in improved endurance capacity (Green *et al.* 1995). Training-induced shifts in substrate utilization are classically attributed to the improved respiratory control sensitivity that results from an increase in mitochondrial density, as reflected by changes in the maximal activity or protein content of enzymes in the tricarboxylic acid cycle and electron transport chain (Saltin & Gollnick, 1983; Holloszy & Coyle, 1984). However, other factors must also contribute to the training response, as shown by studies that show metabolic (Green *et al.* 1992; Clark *et al.* 2004) or performance adaptations (Coyle *et al.* 1988; Weston *et al.* 1997) despite no change in muscle oxidative capacity.

In contrast to traditional endurance training (ET), high-intensity sprint interval training (SIT) is generally thought to have less of an effect on muscle oxidative capacity, substrate utilization and endurance performance (Gleeson, 2000; Kubukeli *et al.* 2002). However, SIT increases the maximal activities of mitochondrial enzymes (Henriksson & Reitman, 1976; Saltin *et al.* 1976;

Burgomaster *et al.* 2005), reduces glycogen utilization and lactate accumulation during matched-work exercise (Harmer *et al.* 2000; Clark *et al.* 2004; Burgomaster *et al.* 2006) and improves performance during tasks that primarily rely on aerobic metabolism (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006; Eddy *et al.* 1977). SIT may also be more effective than ET for improving other important determinants of endurance performance, such as muscle buffering capacity (Weston *et al.* 1997; Edge *et al.* 2006). Low volume SIT may therefore represent a time-efficient strategy to induce muscle and performance adaptations similar to high volume ET (Coyle, 2005). However, no study has directly compared these diverse training approaches in a standardized manner.

The unique purpose of the present study was to compare changes in exercise capacity and molecular and cellular adaptations in skeletal muscle after low volume SIT and high volume ET. The SIT protocol was based on recent work from our laboratory (Burgomaster *et al.* 2005, 2006) and consisted of six sessions of brief, repeated 'all out' 30 s cycling efforts, interspersed with a short recovery, over 14 days. The ET protocol was modelled after work by others (Green *et al.* 1992; Spina *et al.* 1996) and consisted of six sessions of 90–120 min of moderate intensity cycling exercise, with 1–2 days of recovery interspersed between training sessions. As a result, subjects in both groups performed the same number of training sessions on the same days with the same number of recovery days; however, total training volume was ∼90% lower in the SIT group. Needle biopsy samples were obtained before and after training to examine changes in muscle factors related to exercise tolerance, including muscle oxidative capacity and buffering capacity (Hawley, 2002). Performance tests included 50 kJ and 750 kJ cycling time trials, which required ∼2 min and ∼1 h to complete, respectively, and thus differed considerably in the relative energy contribution from oxidative and non-oxidative metabolism. We hypothesized that both SIT and ET would increase muscle oxidative capacity and 750 kJ time trial performance, given the major contribution from aerobic metabolism during this task. In contrast, we hypothesized that SIT but not ET would increase muscle buffering capacity and 50 kJ time trial performance given the large contribution from non-oxidative metabolism during this task.

Methods

Subjects

Sixteen healthy men were recruited to take part in the experiment (Table 1). All subjects were physically active students at McMaster University who took part in some form of recreational exercise two to three times per week (jogging, cycling, etc.). None of the subjects were engaged in regular training for a particular sporting event. Eight

Table 1. Subject characteristics

Variable	SIT group	ET group
Age (years)	22 ± 1	21 ± 1
Weight (kg)	78 ± 2	81 ± 4
Height (cm)	183 ± 2	184 ± 2
Body mass index	23.3 ± 0.5	24.0 ± 1.0
$\dot{V}_{O_2peak}$ (l min^{-1})	4.1 ± 0.2	4.0 ± 0.3

Values are means $\pm$ s.e.m., $n = 8$ per group. SIT, sprint interval training: ET, endurance training: $\dot{V}_{O_2peak}$, peak oxygen uptake. There were no differences between groups in any descriptive characteristic.

subjects were randomly assigned to a SIT group and the other eight were assigned to an ET group. Following routine medical screening, subjects were advised of the purpose of the study and associated risks, and all provided written informed consent. The experimental protocol was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board and conformed to the Declaration of Helsinki.

Pre-experimental procedures

Prior to baseline measurements, subjects made several familiarization visits to the laboratory in order to become orientated with the testing procedures and training devices. During one of these visits, subjects performed an incremental test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport V2.0, Groningen, the Netherlands) to determine $\dot{V}_{O_2peak}$ using an online gas collection system (Moxus modular oxygen uptake system, AEI technologies, Pittsburgh, PA, USA). Following a 5 min warm-up at 50 W, the test began with the workload increasing by 1 W every 2 s until volitional exhaustion. The value used for $\dot{V}_{O_2peak}$ corresponded to the highest value achieved over a 30 s collection period. All subjects also performed a 50 kJ time trial and 750 kJ time trial in order to become familiarized with the exercise tests employed during the main experimental trials. Subjects in the SIT group also performed a familiarization Wingate test, and subjects in the ET group performed a submaximal exercise test to determine the workload that elicited ∼65% $\dot{V}_{O_2peak}$. All exercise tests performed during the familiarization period were performed on separated days, separated by at least 24 h, and at least 3 days prior to baseline testing.

Details of exercise performance tests

Time trials. Subjects were instructed to complete 50 and 750 kJ self-paced laboratory time trials on an electronically braked cycle ergometer (Lode) as quickly as possible with no temporal, verbal or physiological feedback. The only feedback provided during the time trials was work completed, which was presented as

Table 2. Training protocols

Parameter	SIT group	ET group
Work intensity	'All out' supramaximal ($\sim$700 w)	65% $\dot{V}_{O_2peak}$ ($\sim$175 w)
Exercise protocol (per session)	30 s $\times$ 4–6 repeats, 4 min recovery	90–120 min of continuous exercise
Total exercise/training time commitment per session	2–3 min (intervals only) 18–27 min (incl. recovery)	90–120 min
Total exercise/training time commitment over 2 weeks	15 min (intervals only) 135 min (incl. recovery)	630 min
Total exercise volume[1] over 2 weeks	$\sim$630 kJ (intervals only) $\sim$950 kJ (incl. recovery)[2]	$\sim$6500 kJ

SIT, sprint interval training; ET, endurance training; $\dot{V}_{O_2peak}$, peak oxygen uptake. [1]Based on average workloads sustained during training and [2]assuming subjects cycled at the highest workload permitted during recovery (30 W) for the maximum duration (4 min) after every interval performed during training (total of 30 intervals over 2 weeks).

'distance covered' on a computer monitor (i.e. 50 kJ was equated to 2 km, and 750 kJ was equated to 30 km, such that visual feedback at any point during the time trial was presented in units of distance rather than work completed). Exercise duration and average power were recorded upon completion of each test.

Wingate test. Subjects in the SIT group completed a 30 s maximal effort on an electronically braked cycle ergometer (Lode) at a resistance equivalent to 7.5% of their body mass. The ergometer was interfaced with a computer loaded with software (Wingate Software Version 1.11, Lode BV) that applied the appropriate load for each subject. Subjects were instructed to begin pedalling as fast as possible $\sim$2 s before the computer applied the load and received extensive verbal encouragement throughout the test. Peak power, mean power and fatigue index were calculated and recorded by an online data acquisition system.

Experimental protocol

The experimental protocol consisted of (i) baseline testing (i.e. following familiarization); (ii) a 2 week training intervention, and (iii) post-training procedures.

Baseline testing. Prior to training, all subjects underwent a resting needle muscle biopsy procedure. The lateral portion of one thigh was anaesthetized (1% xylocaine) and a small incision made through the skin and underlying fascia in order to obtain a tissue sample from the vastus lateralis muscle. The muscle sample was immediately frozen in liquid nitrogen after removal from the leg. Subjects also performed two baseline performance tests and the timing of the tests was standardized for all subjects. Subjects performed a 50 kJ cycling time trial 1 h

after the biopsy procedure, followed 48 h later by a 750 kJ cycling time trial.

Training. The training protocol commenced $\sim$48 h after the 750 kJ time trial and consisted of six sessions spread over 14 days, with 1–2 days recovery between training sessions (Table 2). Both groups performed training on Mondays, Wednesdays and Fridays for 2 weeks. For the SIT group, training consisted of repeated 30 s maximal cycling efforts, interspersed with 4 min of recovery (rest or light cycling at 30 W). Training progression was implemented by increasing the number of repeats from four repetitions during sessions 1 and 2, to five repetitions during sessions 3 and 4, and finally to six repetitions during sessions 5 and 6. For the ET group, training consisted of 90–120 min of continuous cycling at an intensity corresponding to 65% of $\dot{V}_{O_2peak}$. Training progression in the ET group was implemented by increasing the duration of exercise from 90 min during sessions 1 and 2, to 105 min during sessions 3 and 4, and finally to 120 min during sessions 5 and 6. All training sessions for both groups were directly supervised by one of the study investigators.

Post-training procedures. The nature and timing of the post-training tests was identical in all respects to the pre-training procedures. A resting needle muscle biopsy sample was obtained $\sim$72 h after the final training session, using the same leg as for the pre-training sample but separated by a minimum of 5 cm from the first incision. A 50 kJ time trial was performed 1 h after the biopsy procedure, followed 48 h later by the 750 kJ time trial.

Physical activity and nutritional controls

Subjects were instructed to continue their normal dietary and physical activity practices throughout the experiment.

Subjects were also instructed to refrain from any exercise aside from activities of daily living for 2 days prior to the biopsy procedures and exercise performance tests. In order to minimize diet-induced variability in muscle metabolism, subjects were also instructed to consume the same types and quantities of food for 2 days prior to the resting muscle biopsy and the time trials. Subjects were required to complete food diaries prior to the baseline biopsy and performance tests. The food diaries were collected, photocopied and returned to the subjects before the post-training tests so that subjects could replicate their individual pattern of food intake.

Muscle analyses

Frozen wet muscle samples were initially sectioned into several pieces under liquid nitrogen and stored at $-80°C$. One piece of frozen muscle was freeze dried, powdered and dissected free of all non-muscle elements and stored at $-80°C$.

Enzyme activity. One piece of frozen wet muscle was homogenized using a glass tissue grinder in a buffer containing 50 mм K_2HPO_4, 1 mм EDTA and 1 mм DTT (pH 7.4). The maximal activity of cytochrome c oxidase (COX) was determined on a spectrophotometer (Cary BIO 300, Varian Cary Instruments, Toronto, ON, Canada) using previously described methods (Carter *et al.* 2001). Reactions were carried out over a 3 min period and all assays were performed in duplicate. Protein content of the muscle homogenate was determined by the method of Lowry *et al.* (1951) and enzyme activity was calculated in mol (kg protein)$^{-1}$ h^{-1}.

Western blotting. The amounts of COX subunit II (mitochondrial encoded) and COX subunit IV (nuclear encoded) were quantified using Western blotting as previously described (Raha *et al.* 2002). One piece of frozen muscle was homogenized and proteins were separated using 12.5% Tris–glycine polyacrylamide gel electrophoresis for 1.5 h at 110 V constant voltage (Bio-Rad Protean III, Bio-Rad Laboratories Ltd, Mississauga, ON, Canada). The electrophoretogram was transferred to nitrocellulose using 100 V for 1 h in 1 × transfer buffer (25 mм Tris base, 192 mм glycine, 24% v/v methanol and 0.1% SDS). The nitrocellulose was blocked with 5% skim milk in Tris-buffered saline (TBST: 137 mм NaCl, 2.7 mм KCl, 25 mм Tris-Cl, pH 8.0, 0.1% Tween-20) for 2 h at room temperature. The membrane was incubated with monoclonal antibodies against COX II and COX IV (Mitosciences, Eugene, OR, Canada) using 1 : 2500 and 1 : 1000 dilutions, respectively, and incubated in TBST supplemented with 3% skim milk. The two antibodies were used simultaneously to probe the Western blot in a volume of 10 ml at 4°C for 16 h. The blots

Table 3. Primer sequences

Gene	Forward primer	Reverse primer
β2M	ggctatccagcgtactccaa	gatgaaacccagacacatagca
COX II	cgactacggcggactaatct	tcgattgtcaacgtcaagga
COX IV	cgagcaatttccacctctgt	ggtcacgccgatccatataa

β2M, beta2-microglobulin; COX, cyctochrome c oxidase.

were developed using horseradish peroxidase conjugated secondary antibody targeted to mouse and/or rabbit in conjunction with an enhanced chemiluninescence kit (ECL Plus, Amersham Biosciences, Laval, PQ, Canada) and analysed using a software package (Image J, NIH, Bethesda, MD, USA).

RNA extraction, reverse transcription and RT-PCR. Total RNA was extracted from wet muscle using a commercially available TRIzol Reagent (Invitrogen Canada, Burlington, ON, Canada) following the manufacturer's instructions. The concentration and purity of the RNA was determined spectrophotometrically by measuring the absorbance at 260 (OD_{260}) and 280 (OD_{280}) with an OD_{260}/OD_{280} ratio of 1.5–1.6. The integrity of RNA samples were assessed by RNA agarose–formaldehyde gel electrophoresis and evaluating the ratio of 28S to 18S rRNA bands.

One microgram of RNA, DNase treated (DNA-free, Ambion Inc., Austin, TX, USA), was converted to cDNA using a commercially available kit (1st Strand cDNA Synthesis Kit for RT-PCR (AMV), Roche Applied Science, Laval, PQ, Canada). Briefly, the reaction volume of 20 μl for RT contained: 1 × reaction buffer, 5 mм $MgCl_2$, 1 mм dNTP mixture, 3.2 μg of random primer p(dN)$_6$, 50 U RNase inhibitor, 0.8 μl avian myeloblastosis virus (AMV) reverse transcriptase, and 1 μg DNA-free RNA. Reverse transcription was performed in a thermal cycler (Applied Biosystems, Foster City, CA, USA): 25°C for 10 min, 42°C for 60 min, 99°C for 5 min, and 4°C for 5 min. All RNA samples were converted to cDNA together to prevent technical variation. Negative controls (no RNA or no reverse transcriptase enzyme) were run simultaneously with samples to control for RNA and genomic DNA contamination.

The primers for real-time PCR were designed using Primer3 software (Whitehead Institute for Biomedical Research) and their thermodynamic specificity was determined using BLAST sequence alignments (NCBI) and Oligo analyser software (Integrated DNA Technologies, Coralville, IA, USA). Real-time PCR was performed using an iCycler real-time PCR system (Bio-Rad Laboratories, CA) using SYBR Green 1 chemistry (iQ SYBR Green Supermix) according to the manufacturer's instructions. Briefly, the reaction volume of 25 μl for PCR contained: 1 × iQ SYBR Green Supermix, forward and reverse primers (Table 3) and 10 ng cDNA template. The application was performed for 1 cycle (50°C for 2 min, 95°C for 10 min) followed by 40 cycles

(95°C for 15 s, 60°C for 60 s) and the beta2-microglobulin (β2M) signal was used as a housekeeping gene to normalize threshold cycle (C_T) values. All samples were run in duplicate simultaneously with RNA- and RT-negative controls. In addition, the melting point dissociation curve generated by the instrument was also used to confirm the specificity of the amplified product.

Muscle buffering capacity. The *in vitro* method of Marlin & Harris (1991) was employed, except that NaF as opposed to iodoacetic acid was used to inhibit glycolysis because it is acid–base neutral and would not affect the initial pH measurement (Mannion *et al.* 1993). Briefly, ~3 mg of freeze-dried muscle was homogenized to a dilution of 5.0 mg ml^{-1} in a solution of 145 mM KCl, 10 mM NaCl and 5 mM NaF. After homogenization, the muscle sample was incubated for 5 min in a hot water bath at 37°C and an initial pH measurement was obtained using a microelectrode (MI-415, Microelectrodes, Inc., Bedford, NH, USA) connected to a pH meter (Denver Instruments, Denver, CO, USA). If the initial pH was below 7.10, it was adjusted to 7.10 with 10 mM NaOH (Marlin & Harris, 1991; Mannion *et al.* 1993). The sample was then titrated across a physiological pH range from 7.10 to 6.50 by the addition of 2.5 μl aliquots of 10 mM HCl. Muscle buffering capacity was subsequently calculated in μmol H$^+$ (g dry muscle)$^{-1}$ pH unit^{-1}.

Muscle glycogen. Briefly, ~2 mg of freeze-dried muscle was incubated in 2.0 N HCl and heated for 2 h at 100°C to hydrolyse the glycogen to glucosyl units. The solution was subsequently neutralized with an equal volume of 2.0 N NaOH and analysed for glucose using an enzymatic assay adapted for fluorometry (Passoneau & Lowry, 1993).

Statistical analyses

All data were analysed using a 2-factor analysis of variance, with one between factor (group; SIT *versus* ET) and one within factor (time; pre-training *versus* post-training). Analyses of mRNA data were performed by comparing the difference between the target and reference C_T values (delta C_T) as previously described (Livak & Schmittgen, 2001; Mahoney *et al.* 2004). The differences in the C_T values are expressed numerically using the equation mRNA = $2^{-\text{delta}C_T}$. The level of significance for all analyses was set at $P = 0.05$. All data are presented as means ± s.e.m. based on $n = 8$ subjects per group, except for glycogen where $n = 7$ for the SIT group only.

Results

Exercise performance

The time required to complete the 750 kJ cycling test decreased after training by 10.1% and 7.5% in the SIT and ET groups, respectively, with no difference between groups (main effect for time, $P < 0.001$) (Fig. 1). Following training, there was a corresponding increase in mean power output during the 750 kJ time trial from 212 ± 17 to 234 ± 16 W in the SIT group and from 199 ± 13 to 212 ± 12 W in the ET group (main effect for time, $P < 0.001$). Although the magnitude of improvement was smaller, the time required to complete the 50 kJ test also decreased after training by 4.1% in the SIT group (Post: 113 ± 6 *versus* Pre: 117 ± 6 s) and 3.5% in the ET group (Post: 122 ± 10 *versus* Pre: 115 ± 9 s) (main effect for time, $P = 0.02$). Following training, mean power output during the 50 kJ time trial increased from 435 ± 23 to 453 ± 25 W in the SIT group and 416 ± 39 to 433 ± 40 W in the ET group (main effect for time, $P = 0.02$).

Muscle oxidative capacity

The maximal activity of COX increased after training (main effect for time, $P = 0.04$), but there was no difference between groups (Fig. 2). Similarly, there were training-induced increases in COX II and COX IV protein contents (main effects for time, $P = 0.03$ and 0.04, respectively), but no difference between groups (Fig. 3). COX II and COX IV mRNAs were unchanged ($P > 0.05$) after training in both groups (Table 4).

Muscle buffering capacity

Muscle buffering capacity increased after training by 7.6 and 4.2% for the SIT and ET groups, respectively, with no difference between groups (main effect for time, $P = 0.03$) (Fig. 4).

Muscle glycogen content

Resting muscle glycogen content increased after training by 28 and 17% for the SIT and ET groups, respectively, with no difference between groups (main effect for time, $P = 0.006$) (Fig. 5).
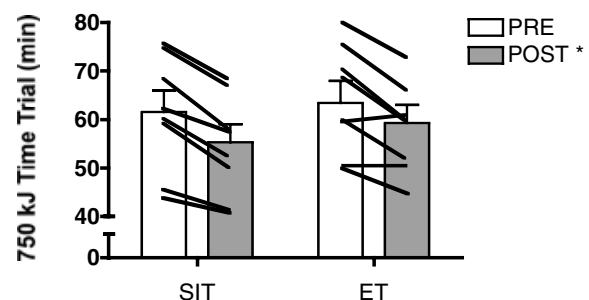

Figure 1. 750 kJ cycling time trial performance before (PRE) and after (POST) 6 sessions of sprint interval training (SIT) or endurance training (ET) over 2 weeks
*$P \leq 0.05$ *versus* pre-training (main effect for time). Lines denote individual data for 8 subjects in each group.

Discussion

The major novel finding from the present study was that six sessions of either low volume SIT or traditional high volume ET induced similar improvements in muscle oxidative capacity, muscle buffering capacity and exercise performance. To our knowledge this is the first study to directly compare interval *versus* continuous training using a research design that matched groups with respect to exercise mode (cycling), training frequency ($3 \times$ per week) and training duration (2 weeks), but differed in terms of total training volume and time commitment. Several previous studies have examined muscle metabolic and/or performance adaptations to interval *versus* continuous training (Henriksson & Reitman, 1976; Saltin *et al.* 1976; Eddy *et al.* 1977; Fournier *et al.* 1982; Gorostiaga *et al.* 1991; Edge *et al.* 2006), but the data are equivocal and in all cases the total volume of work was similar between groups. The present study was unique because, by design, the total training volume for the SIT group was only ~10% that of the ET group (i.e. 630 *versus* 6500 kJ). In addition, the total training time commitment over 2 weeks was ~2.5 h for the SIT group (including the work intervals and the recovery periods between intervals), whereas the ET group performed continuous exercise each training day for a total of ~10.5 h. Thus, while previously speculated by others (Coyle, 2005), to our knowledge this is the first study to demonstrate that SIT is indeed a very 'time efficient' training strategy.

Effect of short-term sprint or endurance training on exercise performance

We are aware of only one previous study that examined changes in volitional exercise performance after continuous or interval training. Eddy *et al.* (1977) had subjects perform cycle exercise training, 4 days per week for 7 weeks, using either a continuous (70% of $\dot{V}_{O_2peak}$) or interval method (repeated 1 min bouts at 100% $\dot{V}_{O_2peak}$

followed by 1 min of rest). The daily workload was matched between groups and increased progressively from ~100 kJ per session during week 1 to ~275 kJ per session during week 7. After training, subjects in both groups showed similar improvements during a matched-work exercise test, such that cycling time to exhaustion at 90% of $\dot{V}_{O_2peak}$ increased by an almost identical amount (~26 min) in both the continuous and interval groups. In the present study, subjects performed 50 and 750 kJ cycling time trials, which demanded work intensities equivalent to ~120 and ~65% of peak power output elicited during the $\dot{V}_{O_2peak}$ tests. Consistent with the work of Eddy *et al.* (1977), subjects in the interval and continuous training groups showed remarkably similar improvements in exercise performance. However, whereas Eddy *et al.* (1977) employed matched-work training protocols, the

Figure 3. Protein content of COX subunit II (middle panel) and IV (bottom panel) measured in resting muscle biopsy samples obtained before (PRE) and after (POST) 6 sessions of sprint interval training (SIT) or endurance training (ET) over 2 weeks
*$P \leq 0.05$ *versus* pre-training (main effect for time). A representative Western blot (top panel) based on one subject from each group is also presented for each subunit. Lines denote individual data for 8 subjects in each group.
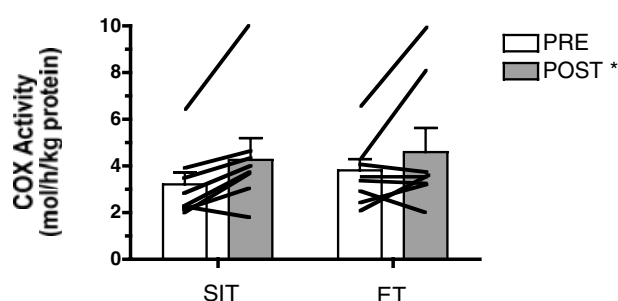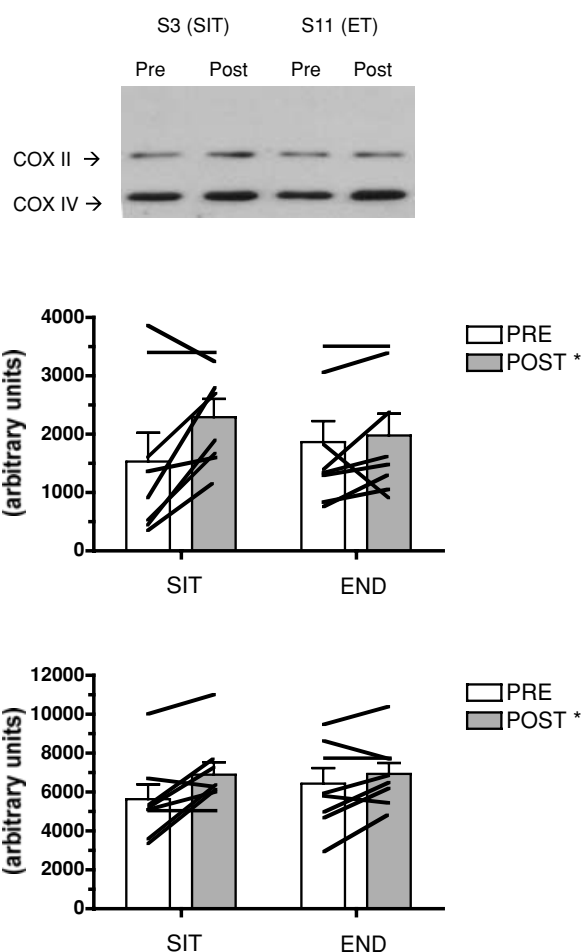

Figure 2. Maximal activity of COX measured in resting muscle biopsy samples obtained before (PRE) and after (POST) 6 sessions of sprint interval training (SIT) or endurance training (ET) over 2 weeks
*$P \leq 0.05$ *versus* pre-training (main effect for time). Lines denote individual data for 8 subjects in each group.

Table 4. mRNA data

mRNA	SIT group		ET group	
	Pre-TR	Post-TR	Pre-TR	Post-TR
COX II	0.84 ± 0.06	0.89 ± 0.04	0.79 ± 0.05	0.79 ± 0.04
COX IV	0.0053 ± 0.0004	0.0072 ± 0.0010	0.0061 ± 0.0009	0.0048 ± 0.0008

SIT, sprint interval training; ET, endurance training; COX, cytochrome oxidase.

SIT group in the present study performed only 2–3 min of intense exercise per training session (which lasted 18–27 min in total, including recovery periods between intervals), whereas the ET group performed 90–120 min of continuous exercise per session. While there was no control group in the present study, recent work from our laboratory has shown that control subjects drawn from the same population show no change in cycle endurance capacity (Burgomaster *et al.* 2005) or time trial performance (Burgomaster *et al.* 2006) when tested ~2 weeks apart with no training intervention.

Rapid muscle adaptations induced by sprint or endurance training

Obviously, the factors responsible for training-induced improvements in exercise capacity are extremely complex and determined by numerous physiological (e.g. cardiovascular, muscle metabolic, neural, respiratory, thermoregulatory) and psychological attributes (e.g. mood, motivation, perception of effort). We assessed changes in two parameters – muscle oxidative capacity and muscle buffering capacity – that are related to exercise tolerance (Hawley, 2002) and thus may have contributed to the observed improvement in time trial performance. Surprisingly, only a few previous studies have directly compared changes in mitochondrial capacity after interval or continuous training, and all employed matched-work training protocols that lasted several weeks (Henriksson & Reitman, 1976; Saltin *et al.* 1976; Fournier *et al.* 1982;

Gorostiaga *et al.* 1991). The results from these studies are equivocal, with two studies reporting similar increases in the maximal activities of mitochondrial enzymes after interval and continuous training (Henriksson & Reitman, 1976; Saltin *et al.* 1976), while two others reported increases after continuous training only (Fournier *et al.* 1982; Gorostiaga *et al.* 1991). The present study is the first to directly compare changes in muscle oxidative capacity after low-volume SIT and high-volume ET.

In accordance with one of our hypotheses, we observed a training-induced increase in the maximal activity of COX and the protein contents of COX subunits II and IV, but there were no differences between groups despite the marked differences in training volume. The present findings are consistent with recent work from our laboratory that showed muscle oxidative capacity was increased after a SIT protocol similar to that used in the present study, or ~15 min of intense exercise over six training sessions in 2 weeks (Burgomaster *et al.* 2005, 2006). While the time course for mitochondrial adaptations after short-term aerobic exercise training is equivocal (Green *et al.* 1992; Putman *et al.* 1998), our results are consistent with data from many laboratories showing increases in oxidative enzymes after six to seven sessions of prolonged moderate intensity exercise (Spina *et al.* 1996; Chesley *et al.* 1996; Green *et al.* 1999; Starrit *et al.* 1999; Youngren *et al.* 2001). Finally, while the design of the present human study was unique, our data are supported by previous work on rats that examined muscle adaptations to various forms of exercise training (Dudley
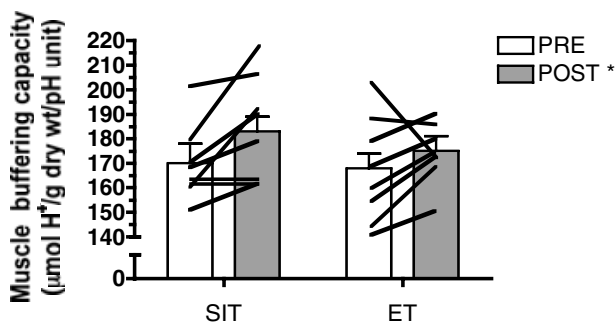

Figure 4. Skeletal muscle buffering capacity measured in resting muscle biopsy samples before (PRE) and after (POST) 6 sessions of sprint interval training (SIT) or endurance training (ET) over 2 weeks
*$P \leq 0.05$ *versus* pre-training (main effect for time). Lines denote individual data for 8 subjects in each group.
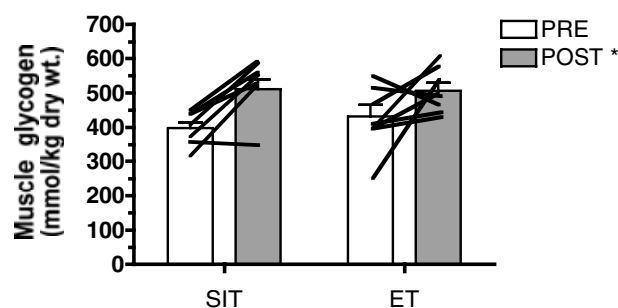

Figure 5. Resting muscle glycogen content before (PRE) and after (POST) 6 sessions of sprint interval training (SIT) or endurance training (ET) over 2 weeks
*$P \leq 0.05$ *versus* pre-training (main effect for time). Lines denote individual data for 7 subjects in SIT group and 8 subjects in ET group.

et al. 1982; Terada *et al.* 2001). Dudley *et al.* (1982) reported similar increases in COX maximal activity after 6 weeks of training with either short bouts of intense running or prolonged periods of continuous running at lower work intensities. Given the large difference in training volume between groups, the authors concluded: 'the typical endurance-training response of a biochemical change in mitochondrial content can be achieved at relatively intense exercise (i.e. exceeding $\dot{V}_{O_2 max}$) maintained for relatively short durations ... for the same adaptive response, the length of daily exercise necessary to bring about the change becomes less as the intensity of training increases' (Dudley *et al.* 1982). More recently, Terada *et al.* (2001) showed that 8 days of high-intensity, intermittent swim training (lasting < 5 min per day) increased citrate synthase maximal activity in rat skeletal muscle to a level similar to that induced by 6 h of daily low-intensity training.

In spite of a significant increase in both COX enzyme activity and protein content for COX subunits encoded by nuclear (COX IV) and mitochondrial (COX II) DNA, we did not find a corresponding increase in mRNA content. Previously, some groups have reported increased mRNA abundance for components of the electron transport chain and/or fatty acid metabolism following endurance training in humans (Tunstall *et al.* 2002; Schmitt *et al.* 2003; Timmons *et al.* 2005). There is also evidence that both the intensity and duration of exercise affect the transcriptional regulation of metabolic genes in a fibre type-specific manner, possibly reflecting the relative stress imposed by the exercise bout (Hildebrandt *et al.* 2003). Various factors including the specific mRNA species being studied, the duration of the training program, as well as the time of the biopsy following the last bout of exercise can influence skeletal muscle mRNA content. For example, after 9 days of endurance training, baseline mRNA content for fatty acid translocase (FAT/CD36) and carnitine palmitoyltransferase 1 (CPT1) was elevated, yet plasma membrane associated FA-binding protein (FABPpm) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) mRNA content was unchanged (Tunstall *et al.* 2002). Schmitt *et al.* (2003) observed higher CPTI mRNA content in endurance trained athletes compared with sedentary controls and Short *et al.* (2003) reported a 66% increase in COX IV mRNA abundance after 16 weeks of endurance exercise training, which suggests longer term training can influence mRNA content. Finally, our laboratory has previously found that the mRNA for several proteins involved in mitochondrial biogenesis and metabolism were elevated 3 h after an acute bout of endurance exercise, but not at 48 h (Mahoney *et al.* 2005). These data are consistent with the concept that initial signalling events associated with acute exercise induce transient pulses of increased mRNA abundance that eventually lead to an increase in protein content and enzyme activity, and that with longer term training there are increases in mRNA abundance for some but not all mitochondrial proteins (Hood, 2001; Mahoney & Tarnopolsky, 2005; Hawley *et al.* 2006).

The potency of SIT to elicit rapid changes in oxidative capacity comparable to ET is no doubt related to its high level of muscle fibre recruitment, and the potential to stress type II fibres in particular (Gollnick *et al.* 1973; Dudley *et al.* 1982). Contraction-induced metabolic disturbances in muscle activate several kinases and phosphatases involved in signal transduction; in particular, the adenosine monophosphate-activated protein kinase (AMPK), calcium–calmodulin-dependent protein kinase (CAPK), and mitogen-activated protein kinase (MAPK) cascades have been shown to play a role in promoting specific co-activators involved in mitochondrial biogenesis and metabolism (Hood, 2001; Hawley *et al.* 2006; Koulmann & Bigard, 2006). Studies in animals have shown that different exercise or stimulation protocols result in selective activation of specific intracellular signalling pathways, which may determine the specific adaptations induced by different forms of exercise training (Nader & Esser, 2001; Lee *et al.* 2002; Atherton *et al.* 2005; Terada *et al.* 2005). Recently, Terada *et al.* (2005) examined the effect of exercise intensity on exercise-induced expression of peroxisome proliferator-activated receptor γ co-activator-1 (PGC-1α) protein in rat skeletal muscle. PGC-1α has emerged as a critical factor coordinating the activation of metabolic genes required for substrate utilization and mitochondrial biogenesis, possibly via its interaction with several DNA binding transcription factors (Knutti & Kralli, 2001). Terada *et al.* (2005) measured PGC-1α protein content after a single session of exercise that consisted of either high intensity swimming (14×20 s intervals while carrying a load equivalent to 14% of body mass, with 10 s of rest between intervals), or low intensity swimming (2×3 h with no load, separated by 45 min of rest). Epitrochlearis muscle samples harvested after 2, 6 and 18 h following exercise revealed that PGC-1α increased to a similar extent in both groups (by 126–140% in the high intensity group and 67–95% in the low intensity group) compared with resting control muscle. Given their previous observation that 8 days of swim training using either the high-intensity or low-intensity protocol produced similar increased in muscle oxidative capacity (Terada *et al.* 2001), the authors concluded 'high-intensity exercise is a potent tool to increase mitochondrial biogenesis, probably through enhancing PGC-1α expression in rat skeletal muscle.' With regards to human muscle, several groups have described changes in the expression of PGC-1α and other metabolic transcriptional co-activators and transcription factors after acute exercise (Pilegaard *et al.* 2003; Russell *et al.* 2005; Coffey *et al.* 2006); however, no studies have directly compared adaptations induced by different types of exercise training.

With respect to our other marker of exercise tolerance, both the SIT and ET protocols induced similar increases in skeletal muscle buffering capacity, which was in contrast to one of our hypotheses. Previous studies have yielded equivocal data with respect to exercise training and buffering capacity (Nevill *et al.* 1989; Bell & Wenger, 1988; Weston *et al.* 1997) and to our knowledge only one group has specifically examined the issue of training intensity (Edge *et al.* 2006). Recently, Edge *et al.* (2006) studied the effect of 5 weeks of interval (4–10 repeats × 2 min at ~90–100% $\dot{V}_{O_2peak}$) or continuous training (~20 min at ~60–75% $\dot{V}_{O_2peak}$) on muscle buffering capacity in recreationally active women. Following training, buffering capacity in the interval training group increased by ~25% but there was no change in the group that performed continuous exercise. While these data (Edge *et al.* 2006) are in contrast to the present results, there are marked differences between studies in terms of the training stimulus. Edge *et al.* (2006) matched the interval and continuous training groups in terms of the total work performed during each session, which meant that the continuous training group performed only 20 min of moderate intensity exercise per session. The training stimulus in that study (Edge *et al.* 2006) was therefore modest compared with the exercise bouts performed by the ET group in the present study (90–120 min of cycling at ~65% $\dot{V}_{O_2peak}$ per session). The present study is the first to report improvements in muscle buffering capacity after short-term exercise training (<4 week) and we speculate that changes in this parameter might represent a relatively rapid muscle adaptation that contributes to the observed improvement in exercise capacity. It should be noted that the *in vitro* method employed in this study isolates for the physico-chemical buffering capacity of skeletal muscle, which is dominated by cytosolic phosphates and proteins/peptides. Histidine-related compounds appear to be the most important determinant of muscle buffering capacity, particularly across the physiological pH range (Abe, 2000), and thus in theory any protein or peptide with histidine residues that are exposed to the cytosol could contribute to buffering capacity.

Perspective: limitations and implications of the present work

The adaptive response to physical training is obviously influenced by a multitude of complex molecular, cellular and physiological changes, and the present data should not be interpreted to suggest that SIT is necessarily adequate preparation for prolonged endurance-type activities. The duration of the training program in the present study was relatively short (6 sessions over 2 weeks) and it remains to be determined whether similar adaptations are manifest after many weeks or months of interval and continuous training. It is possible that the time course for physio-logical adjustments differs between training protocols; the very intense nature of the SIT protocol may stimulate rapid changes, whereas the adaptations induced by lower intensity ET may occur more slowly. The present study examined only a few specific muscle parameters, and future studies should examine whether low volume SIT induces other physiological adjustments typically associated with high volume ET (e.g. increased maximal capacity for lipid oxidation, improvements in cardiorespiratory function, changes in blood health status markers, potential for weight loss, etc.). Although pulmonary oxygen uptake ($\dot{V}_{O_2}$) remains high during recovery between intervals, energy expenditure during a 20–30 min SIT session is nonetheless lower than a 90–120 min bout of endurance exercise. We estimate, based on calculations of heart rate reserve, that $\dot{V}_{O_2}$ during Wingate-based interval training averages ~65–70% of $\dot{V}_{O_2peak}$ over the course of each 30 s sprint and subsequent 4 min recovery period. Thus, based on the average duration of each training session in the present study, absolute energy expenditure in the SIT group was ≤25% of the ET group. A detailed discussion of the potential health benefits of various training strategies is beyond the scope of this paper; however, obesity experts have recognized a role for high intensity exercise in body weight management (Hunter *et al.* 1998). There is also a growing appreciation of the potential for intense, interval-based training to stimulate cardiovascular and muscular adaptations in various populations, including disease states (e.g. Rognmo *et al.* 2004; Vogiatzis *et al.* 2005).

In conclusion, the most striking finding from the present study was that two very diverse forms of training induced remarkably similar changes in exercise capacity and selected muscle adaptations that are related to exercise tolerance. Given the markedly lower training volume in the SIT group, our results suggest that intense interval training is indeed a time-efficient strategy to induce rapid muscle and performance adaptations comparable to traditional endurance training. Additional research is warranted to clarify the effect of different acute exercise 'impulses' on molecular signalling events in human skeletal muscle, and the precise time course and mechanisms responsible for the contraction-induced changes that facilitate the training adaptation.

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