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Effects of type 1 diabetes, sprint training and sex on skeletal muscle sarcoplasmic reticulum Ca^{2+} uptake and Ca^{2+}-ATPase activity

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Key points

- Skeletal muscle calcium resequestration and performance is increased in male rats with induced diabetes; and whilst muscle calcium resequestration is important during exercise, it has not been investigated in human diabetes or compared between sexes.
- We show that Ca^{2+}-ATPase activity and Ca^{2+} uptake are higher among people with type 1 diabetes (T1D) compared with matched non-diabetic controls (CON), but that performance during intense exercise was similar; Ca^{2+}-ATPase activity and Ca^{2+} uptake are also higher among men than women.
- We show that Ca^{2+}-ATPase activity is reduced during intense exercise in men but not women, and is reduced by high-intensity exercise training (HIET) in T1D and CON.
- Fatigue is commonly reported by people with diabetes, but our data show that muscle calcium resequestration and performance during intense exercise and after HIET is not impaired in T1D, and hence other physiological or psychological mechanisms for fatigue in diabetes must be sought.
- Sex influences muscle calcium regulation.

Abstract Calcium cycling is integral to muscle performance during the rapid muscle contraction and relaxation of high-intensity exercise. Ca^{2+} handling is altered by diabetes mellitus, but has not previously been investigated in human skeletal muscle. We investigated effects of high-intensity exercise and sprint training on skeletal muscle Ca^{2+} regulation among men and women with type 1 diabetes (T1D, $n = 8$, 3F, 5M) and matched non-diabetic controls (CON, $n = 8$, 3F, 5M). Secondarily, we examined sex differences in Ca^{2+} regulation. Subjects undertook 7 weeks of three times-weekly cycle sprint training. Before and after training, performance was measured, and blood and muscle were sampled at rest and after high-intensity exercise. In T1D, higher Ca^{2+}-ATPase activity (+28%) and Ca^{2+} uptake (+21%) than in CON were evident across both times and days ($P < 0.05$), but performance was similar. In T1D, resting Ca^{2+}-ATPase activity correlated with work performed until exhaustion ($r = 0.7$, $P < 0.01$). Ca^{2+}-ATPase activity, but not Ca^{2+} uptake, was lower (−24%, $P < 0.05$) among the women across both times and days. Intense exercise did not alter Ca^{2+}-ATPase activity in T1D or CON. However, sex differences were evident: Ca^{2+}-ATPase was reduced with exercise among men but increased among women across both days (time × sex interaction, $P < 0.05$). Sprint training reduced Ca^{2+}-ATPase (−8%, $P < 0.05$), but not Ca^{2+} uptake, in T1D and CON. In summary, skeletal muscle Ca^{2+} resequestration capacity was increased in T1D, but performance was not greater than CON. Sprint training reduced

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Ca^{2+}-ATPase in T1D and CON. Sex differences in Ca^{2+}-ATPase activity were evident and may be linked with fibre type proportion differences.

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Abbreviations BMI, body mass index; CI, confidence interval; CON, control group; HbA_{1c}, glycated haemoglobin; iCa, ionised (free) calcium; NCX, sodium–calcium exchanger; PostExh, post-training performance test to fatigue; PostM, post-training matched work blood and biopsy test; Pre, pre-training blood and biopsy test to fatigue; SERCA, sarcoplasmic reticulum calcium ATPase; SR, sarcoplasmic reticulum; STZ, streptozotocin; T_3, triiodothyronine; T1D, type 1 diabetes.

Introduction

High-intensity exercise challenges metabolic regulation in skeletal muscle. During intense exercise most cellular ATP is used by the ATPases (sodium–potassium pumps, actomyosin crossbridge interactions and calcium pumps) (Green, 1997). Muscle contraction and relaxation depends on the rates of sarcoplasmic reticulum (SR) Ca^{2+} release and resequestration and on other Ca^{2+} buffers that influence cytosolic and intra-SR $[Ca^{2+}]$; hence, SR Ca^{2+} cycling is integral to muscle performance. It has been suggested that there may be a causal link between impairment of muscle Ca^{2+} regulation and fatigue during exercise in human muscle (Li *et al.* 2002). During muscular activity propagation of an action potential along the sarcolemma and into the t-tubules causes conformational change of the dihydropyridine receptors, which then activate the ryanodine receptors resulting in release of Ca^{2+} from the SR and hence a rise in cytosolic $[Ca^{2+}]$, thereby allowing muscle contraction. Calcium removal is accomplished by the sarcoplasmic reticulum calcium (SERCA) pumps (or Ca^{2+}-ATPase) that transport Ca^{2+} ions against the concentration gradient into the SR (Allen *et al.* 2008). Rapid removal of intracellular Ca^{2+} is essential for muscle relaxation and to prevent proteolytic muscle cell damage (Lamb, 2009). During high-frequency electrical stimulation (a model for intense exercise) the sodium–calcium exchangers (NCXs), which can extrude or allow influx of Ca^{2+} depending on the cellular sodium gradient generated by the sodium–potassium ATPase (Na^+–K^+-ATPase), also contribute to intracellular Ca^{2+} handling (Germinario *et al.* 2008).

Altered cellular Ca^{2+} handling is evident in diabetes, and indeed is a fundamental disorder of the diabetic state (Levy *et al.* 1994), and hence affects cellular function. In skeletal muscle of male rats with streptozotocin (STZ)-induced diabetes, Ca^{2+}-ATPase activity, Ca^{2+} uptake and NCX activity were increased and muscle function was enhanced (Ganguly *et al.* 1986; Taira *et al.* 1991). In human diabetes, Ca^{2+} handling is also altered in various tissues including red cells, platelets, arteries and adipocytes (Levy *et al.* 1994). In platelets drawn from patients with diabetes, intracellular Ca^{2+} and Ca^{2+}-ATPase activity were higher than in non-diabetic controls and these changes have been linked to enhanced platelet aggregability (Mazzanti *et al.* 1990). However, despite the integral role of SR Ca^{2+} handling in regulating skeletal muscle function, no previous study has examined Ca^{2+} handling in skeletal muscle of patients with diabetes, and this is the first aim of the study.

Exercise training is a key component of diabetes management, especially given that people with diabetes report greater daily fatigue than people without diabetes (Fritschi & Quinn, 2010). High-intensity (sprint) exercise training improves objectively measured fatigue resistance during intense exercise (McKenna *et al.* 1997; Harmer *et al.* 2000). It is not known whether altered Ca^{2+} handling contributes to fatigue resistance during intense exercise after high-intensity exercise training or whether any effects of exercise training differ between people with or without diabetes. This is the second aim of the study.

Based on rodent muscle studies (Ganguly *et al.* 1986; Taira *et al.* 1991) and the limited data on human tissue other than muscle, we hypothesised that skeletal muscle SR Ca^{2+} uptake and ATPase activity would be higher in young patients with type 1 diabetes (T1D) compared with age-, body mass index (BMI)- and fitness-matched young non-diabetic controls. We also hypothesised that objectively measured fatigue resistance would be greater before and after high-intensity sprint training in the patients with diabetes compared with the controls. Although not part of our original hypotheses, after examination of our results from this study, we also compared responses to acute high-intensity exercise and sprint training between men and women to determine if sex differences in Ca^{2+} handling were significant.

Methods

Ethical approval

This study was approved by the Human Research Ethics Committees of The University of Sydney and Sydney South

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West Area Health Service, was conducted in accordance with the Declaration of Helsinki, and is registered with the Australia–New Zealand Clinical Trial Registry (ACTRN012606000368538-ANZCTR.org). Eight subjects with type 1 diabetes mellitus (T1D group; 3F, 5M) and eight healthy controls (CON group; 3F, 5M) were recruited. Each subject provided written informed consent after being provided with verbal and written information regarding the nature and risks of the experimental procedures. We have previously reported data for all but one of these subjects on muscle and blood potassium regulation (Harmer *et al.* 2006) and respiratory gas exchange and muscle metabolism (Harmer *et al.* 2008) during a single bout of high-intensity matched-work exercise, and on blood ion regulation during repeated 30 s sprint bouts (Harmer *et al.* 2007) before and after sprint training. Here we also include data from an extra male control subject from whom we obtained muscle for Ca^{2+} assays.

In subjects with T1D, duration of diabetes mellitus was 7.1 ± 4.0 years (mean ± SD), average daily insulin dose was 52 ± 11 U day^{-1} and glycated haemoglobin (HbA_{1c}) was 8.6 ± 0.8%. Control subjects (HbA_{1c}, 5.3 ± 0.3%) were closely matched with those with diabetes for age (CON, 23 ± 5; T1D, 25 ± 4 years), BMI (CON, 23.9 ± 4.6; T1D, 25.4 ± 3.2 kg m^{-2}) and peak oxygen uptake ($\dot{V}_{O_2peak}$: CON, 3.27 ± 0.71; T1D, 3.30 ± 0.97 l min^{-1}). Potential subjects with T1D were included if diabetes duration was ≥1.5 years and HbA_{1c} was <10%, and were excluded from the study if overt complications of diabetes were present. Subjects without diabetes had no family history of metabolic disorders. No subject smoked, took regular medication (other than insulin in the T1D group) or had previously engaged in high-intensity cycle training.

Experimental procedures

Testing was conducted in the fasted state. Subjects in the T1D group reduced their night-time dose of insulin by 1–2 U to prevent a hypoglycaemic episode on the morning of the tests, and delayed their morning insulin dose until after testing had been completed. Subjects abstained from alcohol consumption and vigorous exercise for 48 h prior to each test. Female subjects were tested in the follicular phase of the menstrual cycle (determined by subject feedback and subsequent plasma progesterone concentration) when possible. However, two women, one in each of the T1D and CON groups, had irregular menstrual cycles, and testing could not be accurately scheduled.

Exercise testing

Constant load sprint tests. (i) Prior to training, following an identical test (used for familiarisation) on a separate day, a sprint test (Pre) was conducted to exhaustion on an electronically braked cycle ergometer. After a 3-min warm up at 20 W, subjects pedalled at a power output equivalent to 130% $\dot{V}_{O_2peak}$ at 110 rev min^{-1} until exhaustion – defined as the inability to maintain a cadence ≥ 80 rev min^{-1}, despite strong verbal encouragement. Muscle and blood were sampled in this sprint test. This test has been described in more detail in our previous reports of data sampled from the subjects in the present study (Harmer *et al.* 2006, 2008). (ii) After completion of training a test was conducted in which the pre-training power output and exercise duration were intentionally replicated for each individual, i.e. the post-training work (PostM) was matched with Pre. Blood and muscle were sampled in this test. (iii) A second post-training test (conducted on a separate day) was performed at the same power output, but continued until exhaustion (PostExh). This test was conducted to assess performance (time to fatigue and work) after training and there was no blood or muscle sampled.

Exercise training

Subjects undertook seven weeks of three times-weekly supervised cycle sprint training. The training load was progressed from four to ten 30-s 'all out' sprints, separated by a 3- to 4-min rest period, as previously described (Harmer *et al.* 2006).

Muscle sampling and analyses

The skin overlying the vastus lateralis muscle was anaesthetised using 2% xylocaine (without adrenaline). Two percutaneous muscle biopsies ($n = 7$, CON; $n = 8$, T1D), obtained using an aseptic technique (Bergström 1962) with 6 mm biopsy needles (AB Stille-Werner, Stockholm, Sweden) with suction applied via a 50 ml syringe, were performed at rest and immediately after exercise (whilst still on the cycle ergometer) before (Pre test) and after training (PostM test). One female subject in the CON group declined muscle biopsy. The first muscle biopsy sample at each time was immediately immersed in liquid nitrogen for determination of muscle metabolites, high-energy phosphates and enzyme activities (Harmer *et al.* 2008). The second sample was used to determine Na^+–K^+-ATPase content (Harmer *et al.* 2006), Ca^{2+} uptake, Ca^{2+}-ATPase activity and protein content. The average muscle tissue wet weight used for the calcium assays was 51.8 ± 2.7 mg (range 20–108 mg). Muscle for Ca^{2+} assays was rapidly weighed and then homogenised in 10 volumes of a pre-cooled homogenizing buffer (40 mM Tris, 0.3 M sucrose, 5 mM dithiothreitol, pH 7.9). Homogenisation was performed in a 10 mm Kimble tube on ice using an Omni 2000 hand-held electric homogeniser for

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three bursts of 15 s (with 15-s rests between each burst) at 60% maximum power.

Muscle homogenate protein content

Homogenate protein content was determined in triplicate, using serum albumin as the standard (Precimat, Boehringer Mannheim, Mannheim, Germany), according to a published method (Markwell *et al.* 1978). SR Ca^{2+}-ATPase activity and Ca^{2+} uptake were corrected for muscle homogenate protein content and expressed as nmol min^{-1} (mg muscle protein)$^{-1}$.

SR Ca^{2+}-ATPase activity

The maximal *in vitro* rate of SR Ca^{2+}-ATPase activity was measured in triplicate as previously described by our laboratory (Ruell *et al.* 1995; Hunter *et al.* 1999; Thom *et al.* 2001). Assays were performed at 37°C using a spectrophotometer (UV-1601PC; Shimadzu, Tokyo, Japan) at 340 nm, which allowed the quantification of the progressive decrease in absorbance of NADH as it was converted to NAD. The reaction medium consisted of 18 mM HEPES buffer, pH 7.5, 180 mM KCl, 13 mM $MgCl_2$, 9 mM NaN_3, 1 mM EGTA, 0.3 mM NADH, 9 mM phospho*enol*pyruvate, 22 U ml^{-1} lactate dehydrogenase, 16 U ml^{-1} pyruvate kinase and 4 mM ATP. The calcium ionophore A-23187 (2.5 μM) was added to the reaction medium to permeabilise the SR membrane and prevent feedback inhibition of SR Ca^{2+}-ATPase activity. One millilitre of assay buffer was mixed with 1.4 mg tissue wet weight of muscle homogenate and the reaction was initiated with the addition of 1.1 mM $CaCl_2$, corresponding to a free Ca^{2+} concentration ($[Ca^{2+}]_f$) of ~12.1 μM (Green *et al.* 1996). After 90 s, 36 mM $CaCl_2$ was added to completely inhibit the Ca^{2+}-ATPase activity, thus allowing the measurement of basal ATPase activity, and the reaction was followed for a further 90 s. The maximal SR Ca^{2+}-ATPase activity was calculated by subtracting the basal ATPase (Ca^{2+}-independent) from the total (Ca^{2+}-dependent + Ca^{2+}-independent) ATPase activities.

Ca^{2+} uptake

The peak rate of oxalate-supported muscle homogenate SR Ca^{2+} uptake was measured at 37°C using ratiometric dual-emission spectrofluorometry, with continuous stirring, using the fluorescent Ca^{2+}-binding dye indo-1 (Ruell *et al.* 1995). The luminescence spectrophotometer (Aminco Bowman Series 2; SLM Instruments, Urbana, IL, USA) was set with an excitation wavelength of 349 nm, while the emission wavelength cycled between 410 and 485 nm (emission maxima for Ca^{2+}-bound and Ca^{2+}-free indo-1, respectively). Excitation bandpass width was set to 1 nm and emission bandpass width at 8 nm, with ratiometric data being collected every second. The reaction medium consisted of 20 mM HEPES, 150 mM KCl, 10 mM NaN_3, 6.8 mM oxalate, 5 μM *N*,*N*,*N*8,*N*8-tetrakis(2-pyridylmethyl)-ethane diamine, 4.5 mM MgATP and 1 μM indo 1, pH 7.0. Addition of extra $CaCl_2$ was not necessary because the Ca^{2+} in the assay buffers gave a starting $[Ca^{2+}]_f$ of ~1 μM. Calcium uptake was measured in triplicate after the addition of 50 μl of muscle homogenate to 2.2 ml of reaction medium. The reaction was allowed to proceed for ~100 s before the addition of 40 μl of 200 mM EGTA and 120 μl of 100 mM $CaCl_2$ for calibration purposes to give final concentrations of 3.5 and 5.0 mM, respectively. The dissociation constant for the Ca^{2+}–indo 1 complex in 150 mM KCl buffer and 20 mM HEPES, pH 7.0, was determined to be 170 nM by using precise mixtures of Ca^{2+}/EGTA. Alteration in the ratio of the emission signal at 410–485 nm reflects a change in the $[Ca^{2+}]$, which was calculated using a standard equation (Grynkiewicz *et al.* 1985). The maximal rate of Ca^{2+} uptake was determined by dividing the smoothed first derivative of $[Ca^{2+}]$ by the $[Ca^{2+}]$ *versus* time graph, such that Ca^{2+} uptake values were corrected to the same $[Ca^{2+}]$ of 1 μM (Ruell *et al.* 1995).

Blood sampling and analyses

A 22G flexible catheter (Optiva 225; Johnson & Johnson, Sydney, Australia) was inserted into a dorsal hand vein and the venous blood sampled at rest and during and after exercise (Pre and PostM) was arterialised and analysed as we previously described for these subjects (Harmer *et al.* 2006). Previously we reported the change in plasma glucose from the rest value (Harmer *et al.* 2006), whereas here we report absolute values. Plasma glucose was analysed using a commercial kit (Thermo Electron, Melbourne, Australia). Free (ionised) plasma calcium concentration (iCa) was determined using an automated blood gas analyser (Corning 865; Chiron Diagnostics, Medfield, MA, USA) and was analysed at the same time as our previously reported blood gas and plasma ion values (Harmer *et al.* 2006, 2008). A 5 ml blood sample, collected at rest before and after training from the six female subjects, was allowed to clot, then serum was drawn off and analysed for progesterone concentration with an automated immunoassay analyser (Abbott IMX, Abbott Diagnostics, Abbot Park, IL, USA) at Lidcombe-Bankstown Hospital.

Statistics

Blood and muscle data from Pre and PostM tests were analysed using repeated measures analysis of

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variance (ANOVA) with within-subjects factors: training status ('day'; before training, after training), time (rest, exercise); and between-subjects factor: group (diabetes, no diabetes). Performance data from the Pre and PostExh tests were analysed using repeated-measures ANOVA with within-subjects factor: training status, and between-subjects factor: group (diabetes, no diabetes). Based on *post hoc* observation of possible differences between men and women, secondary analyses that used the same ANOVA procedures, but with a between-subjects factor of sex, were conducted. Main effects and interaction effects are reported. Significant F ratios were further examined using an ANOVA contrast technique. Data are expressed as mean ± SEM. Correlations were determined using linear regression; and Pearson's r is reported. All statistical procedures were performed using IBM SPSS Statistics v 21 (IBM Corp).

Results

Performance – Pre and PostExh tests

Exercise to fatigue. While there was a tendency for those in the T1D group to have a longer time to fatigue before (Pre) and after (PostExh) training compared with the control group (CON, 74 ± 8; T1D 95 ± 8 s; $P = 0.08$), work performed until fatigue did not differ between groups (CON, 26 ± 3; T1D, 31 ± 3 kJ; $P = 0.33$). High-intensity training increased the work performed until fatigue by 43 ± 6% (PostExh; $P < 0.0001$) compared with Pre, with no difference between CON (+41 ± 8%) and T1D (+45 ± 9%; training status × group interaction, $P = 0.78$; Table 1).

Work performed to fatigue was less before and after training ($P = 0.001$) among the women (20 ± 3 kJ) than the men (34 ± 2 kJ) with a mean difference of 14 ± 3 kJ (95% confidence interval (CI) 7-21). Improvement in work to fatigue with training was also less among the women than the men (Women, +29 ± 5%; Men, +51 ± 8%; training status × sex interaction, $P < 0.01$; Table 1).

Table 1. Work (kJ) performed to fatigue before and after training

	n	Pre	PostExh*†
CON	8	21 ± 2	30 ± 4
T1D	8	25 ± 3	37 ± 4
Men	10	27 ± 2	41 ± 3
Women	6	17 ± 2	22 ± 3

Data are mean ± SEM. CON, non-diabetic control group; T1D, group with type 1 diabetes. Pre, pre-training test to fatigue; PostExh, post-training test to fatigue. *$P < 0.001$, main effect of training status, PostExh>Pre in CON and T1D; †$P = 0.001$, main effect of sex, Men>Women.

Muscle – Pre and PostM tests

Total protein content. Vastus lateralis muscle homogenate total protein content (wet weight) did not differ between T1D and CON groups (across both times and days, $P = 0.83$; Table 2), with exercise (main effect of time, $P = 0.86$), with exercise training (main effect of training status, $P = 0.36$), or between the sexes across both times and days ($P = 0.82$; Table 2).

Basal, total and Ca^{2+}-ATPase activity. Basal ATPase activity did not differ with time ($P = 0.55$), training status ($P = 0.79$), or between T1D and CON groups ($P = 0.40$). There was a tendency for basal ATPase to be higher in the women than the men across both times and days (Women, 14.4 ± 0.9; Men, 12.2 ± 0.7 nmol min^{-1} (mg $Pr)^{-1}$; $P = 0.08$).

Total ATPase activity was higher in the T1D group across both times and days (CON, 91.0 ± 6.3; T1D, 111.7 ± 5.9 nmol min^{-1} (mg $Pr)^{-1}$; $P < 0.05$) with a mean difference of 20.7 ± 8.7 nmol min^{-1} (mg $Pr)^{-1}$ (95% CI 2.0–39.4). Total ATPase activity was not altered by acute high-intensity dynamic exercise (Rest, 102.5 ± 5.5; Exercise, 100.2 ± 3.9 nmol min^{-1} (mg $Pr)^{-1}$; main effect of time, $P = 0.57$) but tended to be lower after sprint training (Before training, 104.9 ± 4.8; After training, 97.8 ± 4.5 nmol min^{-1} (mg $Pr)^{-1}$; main effect of training status, $P = 0.05$). Total ATPase activity was lower ($P < 0.05$) in the women (88.0 ± 7.6 nmol min^{-1} (mg $Pr)^{-1}$) than the men (109.0 ± 5.4 nmol min^{-1} (mg $Pr)^{-1}$) across both times and days. In response to acute high-intensity exercise, total ATPase activity was increased in women but reduced in men (Women, Rest 82.9 ± 8.0; Exercise 93.2 ± 8.1; Men, Rest 113.3 ± 5.6, Exercise 104.8 ± 5.7 nmol min^{-1} (mg $Pr)^{-1}$; time × sex interaction, $P < 0.05$).

Maximal *in vitro* Ca^{2+}-ATPase activity was 28% higher across both times and days in the T1D group (CON, 77.5 ± 6.7; T1D, 99.2 ± 6.3 nmol min^{-1} (mg $Pr)^{-1}$; $P < 0.05$; Fig. 1*A*) with a mean difference of 21.7 ± 9.2 nmol min^{-1} (mg $Pr)^{-1}$ (95% CI 1.9–41.6). Ca^{2+}-ATPase activity was not altered by acute high-intensity dynamic exercise (Rest, 89.6 ± 5.6; Exercise, 87.1 ± 4.1 nmol min^{-1} (mg $Pr)^{-1}$; main effect of time, $P = 0.47$). Although Ca^{2+}-ATPase activity was 8% lower across both times after sprint training (Before training, 92.0 ± 5.1; After training, 84.7 ± 4.7 nmol min^{-1} (mg $Pr)^{-1}$; $P < 0.05$; Fig. 1*A*), Ca^{2+}-ATPase activity at rest was lower in CON, but preserved in T1D after training (training status × time × group interaction, $P < 0.05$). Ca^{2+}-ATPase

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Table 2. Muscle total protein content (mg g^{-1} ww)

	Before training (Pre)		After training (PostM)	
Group	Rest	Exercise	Rest	Exercise
CON	144 ± 6	143 ± 4	143 ± 7	142 ± 4
T1D	142 ± 5	140 ± 4	147 ± 7	140 ± 4
Men	142 ± 5	144 ± 3	142 ± 6	141 ± 3
Women	145 ± 7	137 ± 4	151 ± 8	141 ± 5

Data are mean ± SEM. CON, $n = 7$, except Pre Exercise where $n = 6$; T1D, $n = 8$; Men, $n = 10$, except Pre Exercise where $n = 9$; Women, $n = 5$; ww, wet weight.

activity was 24% lower ($P < 0.05$) among the women ($n = 5$; 73.6 ± 7.9 nmol min^{-1} (mg Pr^{-1})) than the men ($n = 10$; 96.8 ± 5.6 nmol min^{-1} (mg Pr)$^{-1}$; Fig. 1*B*) across both times and days. Ca^{2+}-ATPase activity increased in women after acute high-intensity exercise but decreased in men (time × sex interaction, $P < 0.05$; Fig. 1*B*).

Ca^{2+} uptake. *In vitro* Ca^{2+} uptake was 21% higher in the T1D group across both times and days (CON, 10.0 ± 0.6; T1D, 12.1 ± 0.5 nmol min^{-1} (mg Pr)$^{-1}$; $P < 0.05$; Fig. 2*A*) with a mean difference of 2.1 ± 0.8 nmol min^{-1} (mg Pr)$^{-1}$ (95% CI 0.5–3.8). Ca^{2+} uptake was not significantly altered by acute high-intensity dynamic exercise (main effect of time, $P = 0.10$) or by sprint training (main effect of training status, $P = 0.47$; Fig. 2*A*). There was no significant effect of sex on Ca^{2+} uptake ($P = 0.35$; Fig. 2*B*); however, when the men were analysed separately there was a tendency for lower Ca^{2+} uptake after intense exercise (main effect of time, $P = 0.06$).

Ratio of Ca^{2+} uptake to Ca^{2+}-ATPase activity. The ratio of *in vitro* Ca^{2+} uptake to Ca^{2+}-ATPase activity did not differ between T1D and CON groups across both times and days (CON, 0.13 ± 0.01; T1D, 0.13 ± 0.01; $P = 0.75$) and was not significantly changed by acute high-intensity exercise across both days (Rest, 0.13 ± 0.01, Exercise, 0.12 ± 0.01; $P = 0.16$) or by sprint training (Before training, 0.13 ± 0.01, After training, 0.13 ± 0.01; $P = 0.47$). There was a higher ratio of Ca^{2+} uptake to Ca^{2+}-ATPase activity among the women than the men across both times and days (Women, 0.14 ± 0.01; Men, 0.12 ± 0.01; $P < 0.05$).

Correlations. Ca^{2+}-ATPase activity before exercise (at rest) was highly correlated with Ca^{2+}-ATPase activity at the end of exercise ($r = 0.73$; $P < 0.001$), and with Ca^{2+}uptake at rest ($r = 0.53$; $P = 0.01$) and at the end of exercise ($r = 0.44$; $P < 0.05$) across both days for all subjects. Ca^{2+}-ATPase activity at rest was correlated with work performed until exhaustion across both days for all subjects ($r = 0.53$, $r^2 = 0.28$, $P < 0.01$; Fig. 3, continuous line).

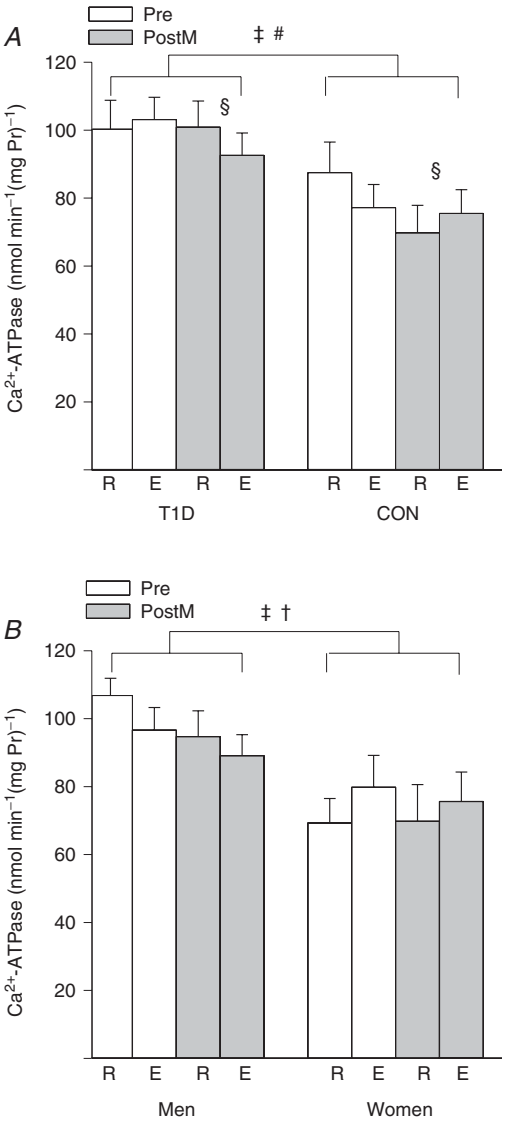

Figure 1. Maximal *in vitro* calcium ATPase activity
Maximal *in vitro* calcium ATPase activity (mean ± SEM) in vastus lateralis at rest (R) and immediately after high-intensity exercise (E) before (Pre; open bars) and after sprint training (PostM; grey bars). *A*, among the group with type 1 diabetes (T1D; $n = 8$) and the non-diabetic control group (CON; $n = 7$, except in Pre rest where $n = 6$ and Pre exercise where $n = 5$). ‡Main effect for group, T1D > CON, $P < 0.05$; §main effect of sprint training, Pre > PostM, $P < 0.05$; and #training status × time × group interaction, T1D rest unchanged and CON rest lower after training, $P < 0.05$. *B*, among the men ($n = 10$, except Pre rest where $n = 9$ and Pre exercise where $n = 8$) and the women ($n = 5$). ‡Main effect for sex, Men>Women, $P < 0.05$; and †time × sex interaction, E < R in men, but not women, $P < 0.05$.

Interestingly, when considered as separate groups, there was no correlation between Ca^{2+}-ATPase activity at rest and work performed to exhaustion for CON, women or men. However, for T1D only, Ca^{2+}-ATPase activity at rest

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was highly correlated with work performed to exhaustion ($r = 0.70$, $r^2 = 0.49$, $P = 0.002$; Fig. 3, dashed line).

Blood – Pre and PostM tests

Ionised (free) plasma calcium (iCa). Plasma iCa was lower across both days and all times in T1D compared with CON (CON 1.21 ± 0.01, T1D 1.16 ± 0.01 mmol l^{-1}; $P < 0.05$; Fig. 4). In both T1D and CON, iCa rose with high-intensity exercise (effect of time, $P < 0.001$) and remained above the resting value until 20 min of recovery; it was not affected by sprint training ($P = 0.40$). Sex did not affect iCa (Women, 1.19 ± 0.02; Men, 1.19 ± 0.01 mmol l^{-1}; $P = 0.90$).

Plasma glucose. Plasma glucose concentration was higher across both days and all times in T1D than CON (CON, 5.8 ± 0.8; T1D, 13.89 ± 0.8 mmol l^{-1}; $P < 0.001$; Fig. 5). In CON, across both days, plasma glucose rose with exercise, peaked at 6.4 ± 0.8 mmol l^{-1} at 10 min of recovery and returned to resting values by 45 min recovery. In T1D, across both days, plasma glucose also rose with exercise, but continued to rise unabated throughout recovery (time × group interaction, $P < 0.001$). There was no effect of sprint training ($P = 0.70$) or sex on plasma glucose concentration (Women, 10.7 ± 2.0; Men, 9.3 ± 1.5 mmol l^{-1}; $P = 0.59$).

Serum progesterone. Indicative of successfully testing four of the six females in the follicular phase of the menstrual cycle, progesterone concentrations were low before (1.8 ± 0.2 nmol l^{-1}) and after (2.6 ± 0.6 nmol l^{-1}) training. In the remaining two females (one each from CON and T1D groups), due to irregular menstrual cycles, one test was successfully conducted in the follicular phase

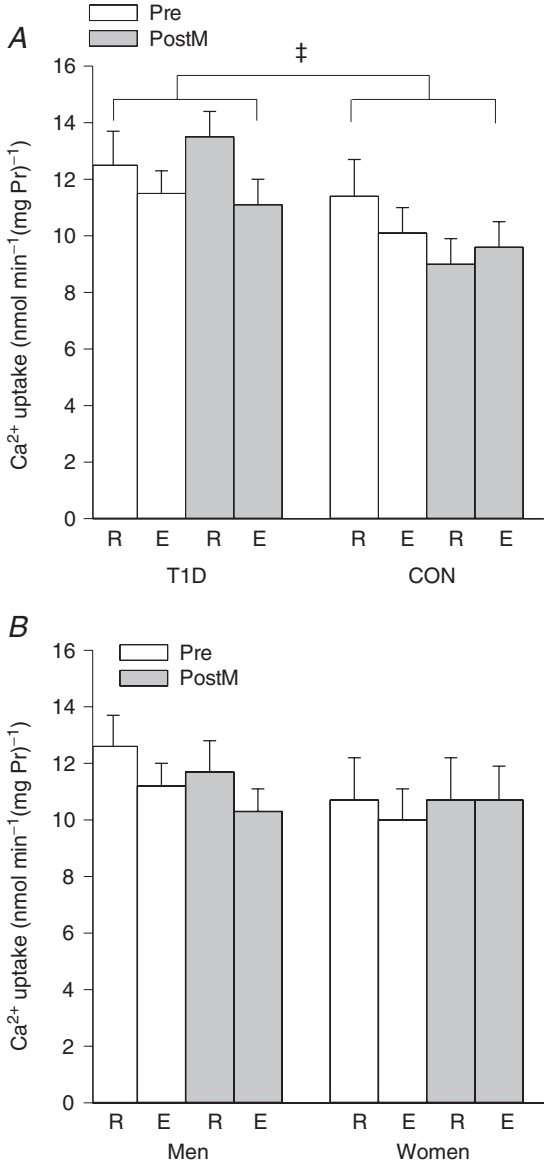

Figure 2. Maximal *in vitro* calcium uptake
Maximal *in vitro* calcium uptake (mean ± SEM) in vastus lateralis at rest (R) and immediately after high-intensity exercise (E) before (Pre; open bars) and after sprint training (PostM; grey bars). *A*, among the group with type 1 diabetes (T1D; $n = 8$) and the non-diabetic control group (CON; $n = 7$, except in Pre rest where $n = 6$ and Pre exercise where $n = 5$). ‡Main effect for group, T1D>CON, $P < 0.05$. *B*, among the men ($n = 10$, except Pre rest where $n = 9$ and Pre exercise where $n = 8$) and the women ($n = 5$).

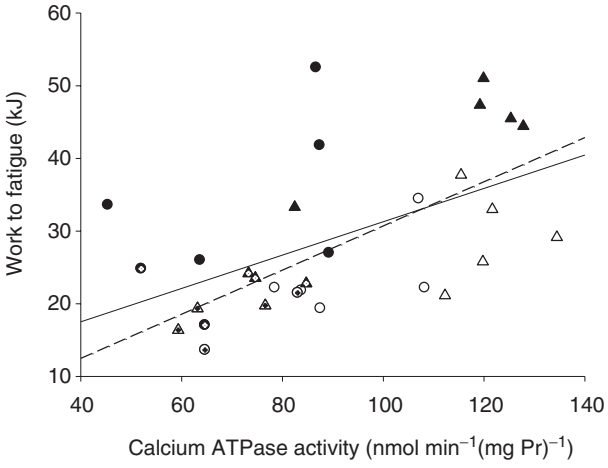

Figure 3. Correlation between maximal *in vitro* calcium ATPase activity at rest and the amount of work performed before fatigue
Maximal *in vitro* calcium ATPase activity at rest and the amount of work performed to fatigue among the group with type 1 diabetes (T1D) before (open triangles) and after sprint training (filled triangles) and the non-diabetic control group (CON) before (open circles) and after sprint training (filled circles). The women in each group are indicated by cross-hairs within the symbols. Solid line, regression for all subjects, pooled data: $r = 0.53$, $r^2 = 0.28$, $P < 0.01$. Dashed line, regression for the T1D group only: $r = 0.70$, $r^2 = 0.49$, $P = 0.002$.

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(2.0 and 4.8 nmol l^{-1}) whilst the other was during the luteal phase (18.7 and 33.4 nmol l^{-1}).

Discussion

The effects of acute high-intensity exercise and high-intensity (sprint) cycle exercise training on SR Ca^{2+}-ATPase activity, Ca^{2+} uptake and performance were investigated for the first time among young patients with T1D and age-, BMI- and fitness-matched control subjects without diabetes. The major findings were that (1) Ca^{2+}-ATPase activity and Ca^{2+} uptake were higher across both times and days in T1D than CON, (2) Ca^{2+}-ATPase activity was lower across both times and days in women than men, (3) Ca^{2+}-ATPase activity and Ca^{2+} uptake were preserved after acute high-intensity exercise in T1D and CON, although sex differences were evident: Ca^{2+}-ATPase activity was reduced in men but increased in women after high-intensity exercise across both days, and (4) sprint training reduced Ca^{2+}-ATPase activity but not Ca^{2+} uptake.

Higher Ca^{2+}-ATPase and Ca^{2+} uptake in patients with T1D

The present study demonstrates for the first time that Ca^{2+}-ATPase activity and Ca^{2+} uptake are higher in skeletal muscle of young patients with T1D than in non-diabetic controls. Consistent with our results, Ca^{2+} uptake and Ca^{2+}-ATPase activity (Ganguly *et al.* 1986; Taira *et al.* 1991) were higher in skeletal muscle of male rats with STZ-induced diabetes than in male non-diabetic control rats. Moreover, 2–4 weeks of insulin treatment returned Ca^{2+} uptake and ATPase activity to normal levels. Briefer periods of insulin treatment were not examined, and thus the time course of the response is unknown. Insulin regulates skeletal muscle Ca^{2+} pumps (SERCA1 and 2) via the insulin receptor substrates (IRS)-1 and -2 and the subsequent tyrosine phosphorylation cascade;

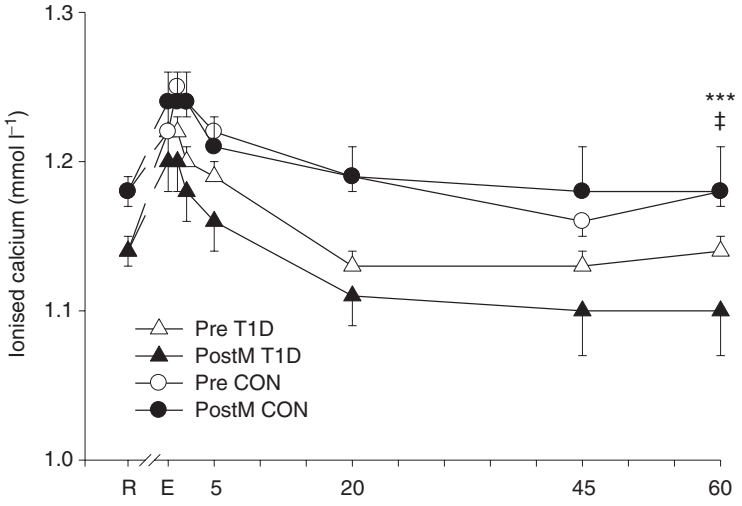

Figure 4. Plasma ionised calcium concentration
Plasma ionised calcium concentration (mean ± SEM) among the T1D (triangles; $n = 8$) and CON (circles; $n = 8$, except where $n = 7$ for CON post training at 5–60 min recovery) groups before (open symbols) and after (filled symbols) sprint training at rest (R), immediately after intense exercise (E), and at 1, 2, 5, 20, 45 and 60 min of recovery. ‡Main effect of group, T1D < CON, $P < 0.05$; and ***main effect of time, E, 1, 2 and 5>R, $P < 0.001$.

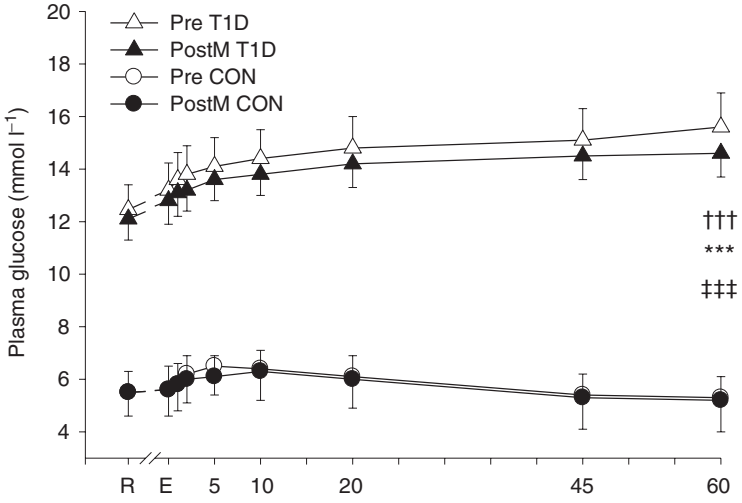

Figure 5. Plasma glucose concentration
Plasma glucose concentration (mean ± SEM) among the T1D (triangles; $n = 8$) and CON (circles; $n = 8$) groups before (open symbols) and after (filled symbols) sprint training at rest (R), immediately after intense exercise (E), and at 1, 2, 5, 10, 20, 45 and 60 min of recovery. ‡‡‡Main effect of group, T1D < CON, $P < 0.0001$; †††time × group interaction, T1D>CON at every time, $P < 0.001$; and ***main effect of time, $P < 0.001$.

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and in STZ diabetes, the association of IRS-1 and IRS-2 with SERCA1 and SERCA2 was reduced (Algenstaedt *et al.* 1997).

Patients with diabetes in the present study reduced their usual evening insulin dose by 1–2 U to prevent hypoglycaemia on the morning of testing and delayed their morning insulin dosage until laboratory testing was completed. We previously reported (Harmer *et al.* 2006) immunoreactive insulin concentrations in the T1D (free insulin) and CON groups and there was no difference between groups prior to exercise. However, as evidenced by the elevated mean blood glucose level before exercise (Fig. 5), the subjects in the T1D group were mildly relatively hypoinsulinaemic. In contrast, in the non-fasted state and when insulin has been administered, patients with T1D will probably have moderate basal hyperinsulinaemia because of the need to supply an insulin dose sufficient to normalize portal vein insulin levels and control hepatic glucose output. Whether Ca^{2+}-ATPase activity and Ca^{2+} uptake were chronically elevated in our patients or transiently elevated due to direct or indirect effects of mild relative hypoinsulinaemia cannot be determined from the present study. Interestingly, and probably relevant to the present study, red blood cell sodium–potassium ATPase activity was lower and magnesium ATPase activity higher in five patients with uncontrolled T1D than in non-diabetic controls, and activities were restored to control levels after a 24 h insulin infusion titrated to achieve euglycaemia (Rahmani-Jourdheuil *et al.* 1987). These data imply that cellular ATPases are rapidly responsive to changing concentrations of insulin (and/or glycaemia) in humans.

Other Ca^{2+}-regulating systems are also affected in diabetes and probably contribute to Ca^{2+} cycling to a lesser extent than Ca^{2+}-ATPase activity. NCXs in the plasma membrane can extrude or allow influx of Ca^{2+} to the muscle cell according to the sodium gradient established by the Na^+–K^+-ATPase. There is a paucity of data regarding the effects of insulin, hyperglycaemia or diabetes on skeletal muscle sodium–calcium exchange. However, NCX activity and Ca^{2+} influx were elevated in sarcolemmal preparations from diabetic rat muscle (Taira *et al.* 1991). Mild relative hypoinsulinaemia and consequent hyperglycaemia, as our patients with diabetes experienced during testing, may have affected Ca^{2+} exchange via the NCX and contributed to differences in Ca^{2+} regulation via alteration in muscle $[Ca^{2+}]$, but this remains to be tested.

STZ diabetes can also upregulate muscle calmodulin concentration and increase phospholipid membrane permeability to Ca^{2+}, thus potentially increasing Ca^{2+}-ATPase activity (Taira *et al.* 1988); however, this has not been examined in humans. Skeletal muscle SR Ca^{2+}-ATPase activity may also be altered via elevated or depressed levels of thyroid hormone (triiodothyronine, T_3; Simonides *et al.* 2001). In animals with STZ diabetes, insulin administration restored or elevated T_3 levels above normal (Sundaresan *et al.* 1984). However, among human patients with T1D, thyroid hormone levels are normal unless there is coincident thyroid disease (which, being autoimmune, is more common in T1D than in the general population – but is still uncommon). To our knowledge there is no evidence in humans that intermittent hyperinsulinaemia (due to insulin therapy) influences thyroid hormone levels. Conversely, the low levels of T_3 (and thyroxine (T_4) and thyrotropin (TSH)) evident in human diabetic ketoacidosis (Schmitz *et al.* 1981), one among many acute or chronic disease states that induce 'sick euthyroid syndrome' (or non-thyroidal illness syndrome) (Economidou *et al.* 2011), would be anticipated to depress SR Ca^{2+}-ATPase activity (Simonides *et al.* 2001); however, this has not been investigated. Although our diabetic patients reduced their evening insulin dose by one or two units and delayed their morning insulin administration until after testing was completed, none was in a state of ketoacidosis.

An interesting finding in the present study was lower iCa across all times and both days in T1D compared with CON. Lower iCa has previously been reported in T1D (Fogh-Andersen *et al.* 1982) and is positively related to lower bone mineral density (Hamilton *et al.* 2009); however, the relationship between iCa and muscle Ca^{2+} regulation is unknown.

Ca^{2+}-ATPase activity is lower in women than men

Ca^{2+}-ATPase activity was lower among the women than the men in the present study when data from the CON and T1D groups were combined. Only one previous study (Fano *et al.* 2001) has examined sex differences in skeletal muscle Ca^{2+} regulation. Consistent with results of the present study, albeit not statistically significant, Ca^{2+}-ATPase activity tended to be lower (~20% lower) in young women ($n = 5$) than young men ($n = 5$) (Fano *et al.* 2001). Two previous studies exclusively recruited women, and investigated effects of strength training in young compared with older women (Hunter *et al.* 1999) and effects of leg cast immobilisation (Thom *et al.* 2001). Maximal *in vitro* Ca^{2+}-ATPase activity among the women in the present study was 73.6 ± 7.9 nmol min^{-1} (mg Pr)$^{-1}$, which compares very closely to ~80 nmol min^{-1} (mg Pr)$^{-1}$ for the young women in Hunter *et al.* (1999) and 75.7 ± 4.5 nmol min^{-1} (mg Pr)$^{-1}$ in Thom *et al.* (2001). In contrast, in a number of studies that have exclusively recruited men, Ca^{2+}-ATPase activity varied between ~89 and 96 nmol min^{-1} (mg Pr)$^{-1}$ (Booth *et al.* 1997; Ørtenblad *et al.* 2000; Li *et al.* 2002) and these data compare very closely with our finding of 96.8 ± 5.6 nmol min^{-1} (mg Pr)$^{-1}$ among the men in the present study.

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A probable contributor to the 24% lower Ca^{2+}-ATPase activity in the women in the present study is the sex difference in muscle fibre type. Women have a higher type I proportional area (+6 to 19%) of the muscle than men (Simoneau & Bouchard, 1989; Roepstorff *et al.* 2006) and lower type II subgroup proportional areas (11% IIA Roepstorff *et al.* 2006; 3% IIB Simoneau & Bouchard, 1989). Human type II fibres have been estimated to have approximately 3- to 4-fold higher Ca^{2+}-ATPase activity (Li *et al.* 2002; Green *et al.* 2009) and 2-fold higher Ca^{2+} uptake (Li *et al.* 2002) than type I fibres; and a strong linear relationship ($r = 0.72$) exists between the proportion of type II fibres and Ca^{2+}-ATPase content (Madsen *et al.* 1994). Thus, it may be anticipated that women will have lower Ca^{2+}-ATPase activity – which we confirmed in the present study. Similarly, endurance-trained athletes had a higher percentage of type I fibres than either untrained or resistance-trained subjects and had correspondingly lower Ca^{2+}-ATPase activity (Li *et al.* 2002). Ca^{2+} uptake was not different amongst women and men in the present study, although this may be a type II error given our small number of women; or perhaps sensitivity of Ca^{2+}-ATPase to activation is higher in women than men given the higher ratio of Ca^{2+} uptake to Ca^{2+}-ATPase activity in women in the present study. The tendency for higher basal ATPase activity in the women in the present study is consistent with the higher basal ATPase activity in soleus (predominantly slow twitch) compared with white gastrocnemius (predominantly fast twitch) in rodent muscle (Dossett-Mercer *et al.* 1994); however, the physiological significance of this finding is not known.

To our knowledge, only one study (Liu *et al.* 2008) has examined the effects of sex hormones on skeletal muscle Ca^{2+} handling. In aged male rats, oestrogen injections had little effect on genioglossal muscle SR Ca^{2+}-ATPase activity (Liu *et al.* 2008). However, in whole heart preparations from ovariectomised female rats, Ca^{2+} uptake and SR Ca^{2+}-ATPase activity were suppressed; and with oestrogen and/or progesterone supplementation, Ca^{2+} sequestration capacity was restored (Bupha-Intr & Wattanapermpool, 2006). We attempted to test our female subjects, both before and after training, in the follicular phase of the menstrual cycle, and were successful in four of the five women from whom we obtained a muscle biopsy. Whether low progesterone levels contributed to lower skeletal muscle Ca^{2+}-ATPase activity in our female subjects is unknown; however, it may be plausible given the effects of progesterone in rodent heart muscle.

Ca^{2+}-ATPase activity and uptake were preserved with intense exercise, but the response differs by sex

Ca^{2+}-ATPase activity and Ca^{2+} uptake were preserved, in both T1D and CON across both days, immediately after acute high-intensity exercise that continued for a period of 70 ± 5 s. Hill *et al.* (2001), who induced fatigue during 7 min of maximal one-legged kicking, also found no reduction in Ca^{2+}-ATPase activity at fatigue; however, Ca^{2+} uptake was reduced. Li *et al.* (2002) reported marked reductions in both Ca^{2+}-ATPase activity and Ca^{2+} uptake after 50 maximal one-legged knee extensions. The differences between these studies and the present study may be attributable to different modes of exercise; however, they cannot be attributed to a lesser degree of muscle metabolic perturbation in the present study. Intracellular perturbation, as evidenced by glycogen depletion, lactate accumulation and ATP and phosphocreatine (PCr) depletion, was at least as great prior to training in subjects in the present study (Harmer *et al.* 2008) as in Hill *et al.* (2001) and Li *et al.* (2002).

However, it appears that the main difference between studies, with regard to maximal *in vitro* Ca^{2+}-ATPase activity, is explained by the greater inclusion of women in the present study, as demonstrated by the differing responses to acute exercise: preservation of activity in women and reduction of activity in men (Fig. 2). All eight untrained subjects and all but one of the 16 athletes in Li *et al.* (2002) and all but two subjects in Hill *et al.* (2001) ($n = 9$) were healthy young men. Prolonged moderate-intensity exercise consistently resulted in reduction of Ca^{2+} uptake (Booth *et al.* 1997; Leppik *et al.* 2004) and Ca^{2+}-ATPase activity (Booth *et al.* 1997; Green *et al.* 1998); and all but one of the subjects in these studies were men. Reduction in maximal Ca^{2+}-ATPase activity and Ca^{2+} uptake with acute intense exercise was related to proportion of type II fibres (Li *et al.* 2002), and given that women generally have a higher proportion of type I fibres than men (Simoneau & Bouchard, 1989; Roepstorff *et al.* 2006), this may partially explain the preservation of Ca^{2+}-ATPase activity with intense exercise in women in the present study. With regard to Ca^{2+} uptake, when data only from the men were analysed, there was a tendency for lower uptake after intense exercise ($P = 0.06$), suggesting a type II error.

Reduced Ca^{2+}-ATPase activity but preserved Ca^{2+} uptake with sprint training

The present study demonstrated lower Ca^{2+}-ATPase activity across both times, but no significant change in Ca^{2+} uptake, after sprint training. Interestingly, Ca^{2+}-ATPase activity at rest did not change after training in the T1D group, but was lower in CON. No previous study has examined Ca^{2+} handling in skeletal muscle of patients with diabetes, so these data are novel. The only previous study to investigate sprint training effects on Ca^{2+} handling in human skeletal muscle did not include post-exercise data, but demonstrated no change in Ca^{2+}

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uptake or Ca^{2+}-ATPase activity at rest, but increased density of SERCA1 and SERCA2 isoforms after 5 weeks of training (Ørtenblad *et al.* 2000).

With regard to other forms of exercise training and muscle Ca^{2+} regulation, 3 months of strength training in young men (Green *et al.* 1998) or women (Hunter *et al.* 1999) had no effect on resting Ca^{2+} uptake and/or ATPase activity; however, it did attenuate the reduction in Ca^{2+}-ATPase activity with moderate-intensity exercise in men (Green *et al.* 1998) and increased both resting Ca^{2+} uptake and Ca^{2+}-ATPase activity in elderly women (Hunter *et al.* 1999). Intensified training in moderately endurance-trained subjects did not alter Ca^{2+}-ATPase activity (Madsen *et al.* 1994), but values may have already been depressed by exercise training. Cross-sectional data demonstrate lower Ca^{2+} uptake, Ca^{2+}-ATPase and Ca^{2+} release in endurance-trained men compared with resistance-trained or untrained subjects, and lower Ca^{2+} uptake in resistance-trained subjects compared with untrained subjects (Li *et al.* 2002). Exercise training thus appears capable of inducing alterations in muscle Ca^{2+} handling at rest and during exercise, as confirmed by the present study; however, effects may be dose-related and affected by sex and/or age.

Performance during high-intensity exercise was similarly enhanced in both groups after sprint training in the present study. Work performed to fatigue was correlated with Ca^{2+}-ATPase activity at rest in the whole cohort, but this was due to the strong correlation among the T1D group ($r = 0.70$). This finding demonstrates that calcium handling is important in diabetes, but that other mechanisms are also of importance in fatigue given that performance did not differ among the groups with and without diabetes. Li *et al.* (2002) demonstrated an association between the fatigue index and the change in Ca^{2+} uptake and ATPase activity with intense fatiguing exercise among a cohort that included exercise-trained and untrained subjects.

In conclusion, this study demonstrated that young patients with T1D had similar performance, but higher Ca^{2+} uptake and ATPase activity than age-, BMI-, and fitness-matched non-diabetic controls. The mechanisms of these differences remain to be investigated, but may be related to (1) prior relative hyperinsulinaemia (from therapy) or (2) relative hypoinsulinaemia and/or hyperglycaemia during testing. The present study also demonstrated that young patients with T1D adapted equally well, compared with non-diabetic controls, to the marked stresses imposed by sprint training. Sex differences in Ca^{2+} resequestration capacity at rest and during exercise were notable, with the women exhibiting lower Ca^{2+}-ATPase activity and performing less work before fatigue, but showing preservation of Ca^{2+}-ATPase activity with high-intensity exercise. The mechanisms for the sex differences may include differences in muscle fibre type proportions and/or sex hormones. Sprint training improved performance during intense exercise, and although we cannot speculate regarding the mechanisms involved, training also reduced Ca^{2+}-ATPase activity.

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Additional information

Competing interests

None.

Author contributions

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