Articles in PresS. J Appl Physiol (August 25, 2011). doi:10.1152/japplphysiol.00921.2011

Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes

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Running Title: Interval training in type 2 diabetes

Abstract word count: 255

Main text word count: 3593

Figures: 5

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Abstract

Low-volume high-intensity interval training (HIT) is emerging as a time-efficient exercise strategy for improving health and fitness. This form of exercise has not been tested in type 2 diabetes and thus we examined the effects of low-volume HIT on glucose regulation and skeletal muscle metabolic capacity in patients with type 2 diabetes. Eight patients with type 2 diabetes (63±8 yr, BMI 32±6 kg/m^2, HbA1C 6.9±0.7%) volunteered to participate in this study. Participants performed six sessions of HIT (10 x 60 s cycling bouts eliciting ~90% maximal heart rate, interspersed with 60 s rest) over 2 wk. Before training and from ~48-72 h after the last training bout, glucose regulation was assessed using 24-h continuous glucose monitoring under standardized dietary conditions. Markers of skeletal muscle metabolic capacity were measured in biopsy samples (*vastus lateralis*) before and after (72 h) training. Average 24-h blood glucose concentration was reduced after training (7.6±1.0 vs 6.6±0.7 mmol/L) as were the sum of the 3-h postprandial areas under the glucose curve for breakfast, lunch and dinner (both $p<0.05$). Training increased muscle mitochondrial capacity as evidenced by higher citrate synthase maximal activity (~20%) and protein content of Complex II 70 kDa subunit (~37%), Complex III Core 2 protein (~51%), and Complex IV subunit IV (~68%, all $p<0.05$). Mitofusin 2 (~71%) and GLUT4 (~369%) protein content were also higher after training (both $p<0.05$). Our findings indicate that low-volume HIT can rapidly improve glucose control and induce adaptations in skeletal muscle that are linked to improved metabolic health in patients with type 2 diabetes.

Keywords
Exercise; Continuous Glucose Monitoring; Mitochondria; GLUT4; Glycemic Control

63 **Introduction**

64 Regular exercise is an effective strategy for the prevention and treatment of type 2
65 diabetes (T2D) (6). Most studies examining the therapeutic effects of exercise in T2D
66 involve continuous, low- to moderate-intensity exercise such as walking, jogging or
67 cycling for ≥30 minutes per session [reviewed in (6)]. Although the optimal strategy has
68 not been established, higher intensity exercise may be more effective for improving
69 glycemic control in patients with T2D (2, 25). Recently revised guidelines from the ADA
70 advocate at least 150 minutes of moderate to vigorous exercise per week (6). High-
71 intensity interval training (HIT), which involves repeated bursts of vigorous exercise
72 interspersed with periods of rest, may be an attractive option to implement higher
73 intensity exercise training in T2D. The utility of HIT for improving disease outcomes has
74 been demonstrated in patients with metabolic syndrome, heart failure, and chronic
75 obstructive pulmonary disease [reviewed in (7)]. However, the potential benefits of HIT
76 on disease parameters in T2D have yet to be established.

77 We (8, 14, 16) and others (1, 21) have shown that HIT elicits physiological
78 remodeling comparable to moderate-intensity continuous training in healthy adults,
79 despite a substantially lower time commitment and reduced total exercise volume. As
80 little as 6 sessions of low-volume HIT over 2 wk increases skeletal muscle mitochondrial
81 capacity (8), which may be of clinical relevance for T2D given that reduced content (22)
82 or biogenesis (18) of mitochondria have been implicated in insulin resistance and T2D.
83 Two weeks of low-volume HIT has also been shown to improve glucose tolerance (1)
84 and enhance insulin sensitivity (21) in healthy adults. These findings are intriguing
85 because they suggest that low-volume HIT may result in many of the same health

86 benefits as traditional exercise training with substantially reduced exercise volume and
87 time commitment. Low-volume HIT may therefore represent a potent, time-efficient
88 exercise strategy to improve skeletal muscle metabolic control and glycemic regulation
89 in patients with T2D.

90 The primary purpose of this pilot investigation was to examine the effects of low-
91 volume HIT on glucose regulation and skeletal muscle metabolic capacity in individuals
92 with T2D. Based on accumulating evidence indicating that postprandial hyperglycemia
93 plays a predominant contributing role in diabetic complications (5), we utilized
94 continuous glucose monitoring (CGM) to examine the effects of HIT on overall glycemic
95 exposure and postprandial glucose fluctuations. We hypothesized that 2 wk of low-
96 volume HIT would reduce hyperglycemia and increase mitochondrial capacity and
97 GLUT4 content measured in skeletal muscle biopsy samples.

98

99 **Methods**

100 Participants

101 Participants were recruited through local diabetes clinics, community diabetes
102 information sessions and poster advertisement. All participants were diagnosed with
103 T2D at least 3 months prior by a clinician according to standard criteria, including a
104 fasting glucose ≥7.0 mmol/l and/or 2-hr OGTT blood glucose concentration ≥11.1
105 mmol/l, were not taking insulin and had no history of end-stage liver or kidney disease,
106 neuropathy, retinopathy, hypertension that could not be controlled by standard
107 medication, cardiovascular disease or other contraindication to exercise. Eight
108 individuals (mean age 62.5±7.6 yr, BMI 31.7±5.8 kg/m^2, hemoglobin A1C [Hb_{A1C}]

109 6.9±0.7% [range 6.4-8.5%]) volunteered to participate in this study. Six participants
110 were sedentary, which was defined as ≤2 exercise sessions of 30 minutes per week.
111 Two participants reported engaging in ~30 minutes of low-intensity walking exercise on
112 3-5 days per week, in accordance with guidelines provided from their diabetes care
113 team. Six subjects were taking blood glucose lowering medications but had Hb_{A1C}
114 values ≤8.5% and were not on exogenous insulin therapy. Four patients were treated
115 with metformin only, one patient with gliclazide only, and one patient with a combination
116 of metformin, pioglitazone, sitagliptin and repaglinide. Due to the short duration of the
117 intervention, participants did not adjust their medications and were instructed to
118 maintain their typical dietary and activity patterns throughout. All participants provided
119 written informed consent. The study protocol was approved by the Hamilton Health
120 Sciences/McMaster University Faculty of Health Sciences Research Ethics Board.

121 Experimental Design

122 The experimental design consisted of i) medical clearance and familiarization, ii)
123 baseline testing, iii) a 2 wk training intervention, and iv) post-testing.

124 *Medical clearance and familiarization*. Height and weight were recorded and a
125 maximal exercise test on a recumbent cycle ergometer (Corival, Lode BV, Groningen,
126 The Netherlands) was performed with pre- and post-exercise 12-lead electrocardiogram
127 (ECG) collection to confirm the absence of any underlying contraindications to vigorous
128 exercise participation. The test started at 30 W and increased by 15 W/min until
129 volitional exhaustion. Peak power output (W_{max}) and maximal heart rate (HR_{max}) were
130 recorded. Following ECG clearance by a study physician, participants completed 1-2

131 familiarization sessions to become acquainted with low-volume HIT. These sessions
132 were also used to determine the interval power output that elicited ~90% HR_{max}.
133 *Baseline testing*. Prior to training, participants performed a 15-min walking test to
134 examine the cardiovascular response and ratings of perceived exertion (RPE) during
135 exercise. Speed was self-selected by each participant during an initial 5-min warm-up.
136 Heart rate was measured by telemetry (Polar) and RPE was measured using the 0-10
137 continuous/interval scale.
138 At least 2 d following the walk test, participants reported to the laboratory for
139 CGM device insertion (CGMS® iPro™, Medtronic, Northridge, CA, USA). Participants
140 were given a glucose meter (OneTouch® UltraMini®, Lifescan, Inc., Milpitas, CA, USA)
141 with instructions for both calibration capillary blood sampling and individualized control
142 diets. The following day served as a dietary control day for 24-h CGM data collection.
143 Subjects returned to the laboratory 2 d later for removal of the CGM device and
144 collection of a resting skeletal muscle biopsy sample as we have previously described
145 (8). Briefly, muscle samples were obtained under local anesthesia (1% Lidocaine) from
146 the *vastus lateralis* using a Bergstrom needle adapted with suction. Muscle samples
147 were quickly blotted to remove excess blood, sectioned into several pieces, and placed
148 in separate vials before snap freezing in liquid nitrogen for subsequent analyses.
149 *Training.* Approximately 5 d following the muscle biopsy procedure, subjects
150 commenced training. The HIT protocol involved a total of 6 supervised sessions over 2
151 wk (Monday, Wednesday, Friday each week). Each session consisted of 10 x 60 s
152 cycling intervals interspersed with 60 s of recovery based on our recent work (14).
153 Training was performed on a cycle ergometer (LifeCycle C1 or R1, Life Fitness, Schiller

154 Park, IL, USA) set in constant watt mode at a pedal cadence of 80-100 revolutions per
155 minute. Individual workloads were selected to elicit a heart rate of ~90% HR_{max} during
156 the intervals. During recovery, participants were allowed to rest or pedal slowly against
157 a resistance of 50 W. Each training session included a 3-min warm-up and 2-min cool-
158 down at 50 W, for a total of 25 min. Therefore, the training protocol involved a total of 30
159 min of high-intensity exercise within a total time commitment of 75 min per week,
160 including warm-up, cool-down and the recovery interval between high-intensity efforts.
161 *Post-testing.* CGM data were collected for a 24-h period starting ~48 h following
162 the final training session. Diet was controlled to be the same as pre-training. Resting
163 muscle biopsy samples were obtained ~72 h following the final training session.
164 Approximately 2-4 d following the biopsy, the walk test (performed at the same speed
165 as pre-training) and maximal exercise test were performed using the same procedures
166 as baseline testing. Perceived enjoyment of low-volume HIT was assessed by asking
167 participants how enjoyable they would find engaging in a) a single bout of HIT (10 x 1-
168 min) and b) HIT at least 3 times per week for the next 4 weeks using a 9-point likert
169 scale ranging from 1 (not enjoyable at all) to 9 (very enjoyable).
170 Continuous Glucose Monitoring
171 Average blood glucose concentration and area under the glucose curve were
172 calculated from CGM data for a 24-h period before and after training. CGM data were
173 also used to analyze the 3 h postprandial areas under the glucose curve for breakfast,
174 lunch and dinner. On CGM data collection days, participants consumed their habitual
175 breakfast but were provided with standardized snacks (e.g., almonds, fruit, vegetables)
176 based on their personal preferences and habits. Lunch and dinner were standardized

177 for all participants by providing vouchers to a local sandwich restaurant. Subjects
178 completed detailed dietary logs to record the timing and quantity of all food consumed
179 during the pre-training day. For post-testing, these dietary logs, along with all snacks
180 and vouchers for lunch and dinner were provided with instructions for diet replication
181 under free-living conditions over the 24-h CGM data collection period. As per
182 manufacturer's recommendations, capillary blood glucose samples were obtained at 4
183 points during the day at a time when blood glucose would be expected to be stable (i.e.,
184 upon awakening, before lunch, before dinner, and before bed) and were automatically
185 stored in the glucose meters provided to participants. These 4 values were used during
186 CGM downloading to construct 24-h blood glucose curves based on interstitial glucose
187 recordings averaged every 5 minutes by the CGM device using the associated software
188 algorithm (Solutions Software, Medtronic, Northridge, CA). CGM data were exported
189 and analyzed using SigmaPlot (Statsoft, Chicago, IL, USA). Reproducibility of the CGM
190 device in our lab was verified in 5 volunteers who wore the monitor on 2 occasions
191 separated by 1 week under identical dietary conditions. The CV for the 24 h blood
192 glucose measurements was 2.8% (data not shown).

193 Muscle Analyses

194 *Citrate synthase enzyme activity.* One piece of muscle (~20 mg) was
195 homogenized using a glass tissue grinder (Kimble/Kontes 885300-0002) in 10 volumes
196 of buffer containing 70 mM sucrose, 220 mM mannitol, 10mM HEPES (pH 7.4)
197 supplemented with protease inhibitors (Complete Mini®, Roche Applied Science, Laval,
198 PQ, Canada) and used to determine the maximal activity of citrate synthase (CS) as we
199 have previously described (8, 16). Protein concentration of homogenates was

200 determined using a commercial assay (BCA Protein Assay, Pierce, Rockford, IL, USA)
201 and enzyme activity is expressed as mmol·kg of protein^{-1}·hr^{-1} wet weight (w.w.).
202 *Western blotting.* A second piece of muscle (~30 mg) was homogenized in RIPA
203 buffer for Western blot analyses using techniques described previously (8, 16). Briefly,
204 protein concentration of homogenates were determined as above and equal amounts of
205 protein (5-20 µg) were prepared in 4X Laemmli's buffer and heated to 95°C before
206 being separated by 10-12.5% SDS-PAGE and electrotransferred to nitrocellulose
207 membranes. Ponceau S staining was performed following transfer to visualize equal
208 loading and transfer. Following 1 h blocking in 5% fat-free milk Tris-buffered saline 0.1%
209 Tween® 20 (TBS-T), membranes were incubated in primary antibodies overnight at 4°C
210 or at room temperature (RT) for 2 h in 3% fat-free milk TBS-T or 3% BSA TBS-T
211 depending on previously determined optimization conditions. After 3 X 5-min washes in
212 TBS-T, membranes were incubated in the appropriate species-specific secondary
213 antibody diluted (1:10000) in 3% fat-free milk TBS-T for 1 hr at RT, washed in TBS-T for
214 3 X 15-min, and visualized by chemiluminescence (SuperSignal West Dura, Pierce)
215 using a FluorChem® SP Imaging System (Alpha Innotech Corporation, San Leandro,
216 CA, USA). ImageJ software (NIH) was used to quantify the optical density of protein
217 bands. Alpha-tubulin (Cell Signaling Technology, #2125), which did not change
218 following training (p=0.91), was used as a loading control. Primary antibodies for the
219 following proteins of interest were used: NDUFA9 (Mitosciences, MS111), Complex II
220 70 kDa subunit (Mitosciences, MS204), Complex III Core 2 protein (Mitosciences,
221 MS304), cytochrome *c* oxidase (COX) subunit II (MitoSciences, MS405), COX subunit
222 IV (Mitosciences, MS408), ATP synthase α subunit (Mitosciences, MS507), CS (kind gift

223 from Dr. Brian Robinson, The Hospital for Sick Children, Toronto, CANADA), mitofusin
224 (Mfn) 2 (Sigma, M6319), and GLUT4 (Millipore, AB1345).

225 Statistical analyses

226 All data were analyzed using paired Student's *t* tests with significance set at
227 $p \leq 0.05$ (Sigma Stat v3.10). Values are means±SD in the text and on figures.

228

229 **Results**

230 Descriptive characteristics of training

231 All participants completed all prescribed intervals during training with no
232 complications. Interval intensity averaged across all intervals for all subjects
233 corresponded to 95±14% of W_{max}, elicited 88±3% HR_{max}, and RPE was 6.4±1.3 (0-10
234 scale). The response to each interval averaged across all 6 training sessions for all
235 subjects is depicted in Figure 1. Training had no effect on body mass (pre: 93±19 kg vs.
236 post: 92±18 kg, p=0.28). On average, perceived enjoyment of HIT was rated high by
237 this group of participants (single session, 8.1±1.0; three times per wk, 7.9±1.0)

238 Continuous Glucose Monitoring

239 Average blood glucose concentration over 24-h was reduced from 7.6±1.0 to
240 6.6±0.7 mmol/L following training (Figure 2A, p=0.01). Area under the 24-h blood
241 glucose curve was also lower following HIT (pre: 11066±1703 vs. post: 9572±995
242 mmol/L·day, p=0.02). The sum of the 3-h postprandial area under the glucose curves
243 for breakfast, lunch and dinner was significantly lower post-training (pre: 965±483 vs.
244 post: 679±437 mmol/L·9 h, p=0.01). Pre- and post-training 24-h blood glucose curves
245 for a representative subject are shown in Figure 2B.

246 Adaptations in skeletal muscle

247 The maximal activity of CS was elevated following training (Figure 3A, p=0.04).
248 Training also increased skeletal muscle mitochondrial protein content as evidenced by
249 changes in Complex II 70 kDa subunit (p=0.03), Complex III Core 2 protein (p=0.04),
250 and COX subunit IV (p=0.02) measured by Western blotting (Figure 3B). The protein
251 content of CS (~57%, data not shown), NDUFA9, COX subunit II (~53%, data not
252 shown), and ATP synthase α subunit also increased, but did not reach statistical
253 significance (p=0.06-0.12, Figure 3B). The protein content of Mfn2 was elevated
254 following training (~71%, p=0.02, Figure 4), as was total GLUT4 protein (~369%,
255 p=0.003, Figure 5).

256 Functional Exercise Performance

257 Maximal workload achieved on the ramp cycling test was increased by ~10%
258 following training (pre: 111±36 vs. post: 124±37 W, p=0.03). Training reduced heart rate
259 (pre: 73±7 vs. post: 66±6 $\%HR_{max}$, p<0.001) and RPE (pre: 2.4±0.7 vs. post: 1.3±1.2,
260 p=0.01) during the walk test.

261

262 **Discussion**

263 The present study demonstrates that low-volume HIT can rapidly reduce
264 hyperglycemia and increase skeletal muscle oxidative capacity in patients with T2D.
265 These improvements were realized despite a small total volume of exercise that
266 consisted of 6 training sessions over 2 wk. The training protocol involved a total of only
267 30 min of high-intensity exercise and a total time commitment of only 75 min per week.
268 This is much lower than current physical activity guidelines for T2D that recommend a

269 total of 150 min of moderate to vigorous intensity exercise each week (6). Given that the
270 majority of individuals with and without T2D do not accumulate sufficient exercise to
271 achieve health benefits (6) and the most common cited barrier to regular exercise is
272 lack of time (26), our results suggest that low-volume HIT may be a viable, time-efficient
273 strategy to improve health in patients with T2D.

274 Low-volume HIT and glycemic control

275 Glycemic control is an important aspect of T2D treatment and is an independent
276 risk factor for the development of diabetic complications (24, 27). We utilized CGM to
277 assess the effects of short-term low-volume HIT on overall glycemic exposure and
278 postprandial glucose responses. CGM provides information about direction, magnitude,
279 and frequency of blood glucose excursions and may provide a sensitive means to
280 detect acute changes in blood glucose throughout the day (15). Although exercise is
281 regarded as an effective strategy to improve glycemic control (2, 6, 25), there are limited
282 data regarding the effect of exercise training on glucose control using CGM. Studies
283 using CGM technology in patients with T2D have reported that acute resistance
284 exercise reduces the prevalence of hyperglycemia (19) and acute endurance exercise
285 reduces 24-h average blood glucose concentration (17). In the only training study
286 conducted to date, Cauza et al. (4) reported a greater reduction in 24-h average blood
287 glucose concentration following 4 months of resistance training compared to endurance-
288 type training in individuals with type 2 diabetes, but interpretations on the effects of
289 exercise are potentially limited by significant changes in body composition and an
290 apparent lack of dietary control.

291 To our knowledge, this is the first study to examine the effects of HIT on glycemic
292 regulation using CGM. Average blood glucose concentration and area under the
293 glucose curve measured under standardized dietary conditions from ~48-72 h following
294 the final training session were significantly lower than pre-training, indicating that short-
295 term, low-volume HIT improved glycemic control, particularly glycemic excursions after
296 meals. Although reducing fasting hyperglycemia is a significant aspect of T2D
297 treatment, increasing evidence suggests lowering postprandial hyperglycemia is as
298 important, if not more important, for achieving targeted Hb_{A1C} levels (27). Additionally,
299 elevated post-meal blood glucose excursions have been implicated in the development
300 and progression of T2D related co-morbidities such as cardiovascular disease (5, 27).
301 Following HIT, the sum of the postprandial areas under the glucose curve for breakfast,
302 lunch and dinner was significantly lower than pre-training, highlighting the potency of
303 HIT to lower post-meal glucose excursions. These findings demonstrate that low-
304 volume HIT may be an effective strategy for improving glycemic regulation in individuals
305 with T2D and suggest that CGM may be a sensitive technique to measure the effects of
306 exercise on glucose control.
307 The mechanisms mediating the improvement in glycemic control following HIT
308 remain to be determined. Training had no effect on body mass and while not assessed
309 directly, it is unlikely that such a short exercise intervention would lead to any
310 substantial changes in body composition. Therefore, it is tempting to speculate that
311 adaptations in skeletal muscle were involved. Since reduced mitochondrial capacity in
312 skeletal muscle has been reported in insulin resistance and T2D (22) and muscle
313 oxidative capacity has been shown to be a significant predictor of insulin sensitivity (3),

314 it is possible that the rapid increase in skeletal muscle mitochondrial content following
315 low-volume HIT may be a contributing factor related to reduced insulin resistance and
316 improved glycemic control. However, the notion that mitochondrial deficiency mediates
317 insulin resistance has been questioned recently (12), indicating that other adaptations in
318 skeletal muscle may be more important. The training-induced increase in GLUT4
319 protein content likely plays a role in improving glucose regulation. Studies in rodents
320 indicate that the exercise-induced increase in GLUT4 protein is directly related to the
321 increase in muscle glucose uptake at any given insulin concentration (20). Thus, even in
322 the face of insulin resistance, an increase in skeletal muscle GLUT4 could facilitate
323 greater muscle glucose uptake and contribute to improved glycemic regulation. In
324 addition to skeletal muscle adaptations, training-induced alterations in hepatic glucose
325 output cannot be ruled out. The effect of exercise training on hepatic insulin resistance
326 in T2D has not been directly assessed in humans although there is evidence to suggest
327 that endurance exercise training improves hepatic insulin signaling and glycemic control
328 in rodents (10).

329 We did not directly assess the effects of training on insulin sensitivity using
330 hyperinsulinemic-euglycemic clamps and therefore cannot conclude whether low-
331 volume HIT improves muscle insulin sensitivity. CGM assesses exposure to
332 hyperglycemia as well as glycemic excursions throughout the day. Exposure to
333 hyperglycemia over time may be a better indicator of diabetic complications than insulin
334 sensitivity *per se* (5) and therefore CGM may provide greater insight into the clinical
335 benefits of exercise training. Changes in Hb_{A1C} are commonly used to assess the

336 effectiveness of glucose-lowering interventions in T2D but due to the short duration of
337 the current study was not measured.

338 Low-volume HIT and skeletal muscle mitochondrial adaptations

339 Individuals with insulin resistance and T2D have been shown to have reduced
340 mitochondrial content (22), impaired *in vivo* mitochondrial function (23), and/or reduced
341 markers of mitochondrial biogenesis (18) in skeletal muscle. These findings have led to
342 the hypothesis that reduced mitochondrial capacity or impaired regulation of
343 mitochondrial biogenesis in skeletal muscle may play a role in the pathogenesis of T2D
344 (18, 22, 23). Although it is currently unclear whether skeletal muscle mitochondrial
345 impairment causes insulin resistance (12), interventions that increase muscle
346 mitochondrial content may be effective for the treatment and prevention of T2D (9, 13).
347 Given the potency of low-volume HIT to induced mitochondrial biogenesis in young,
348 healthy subjects (8, 16), we hypothesized that HIT might also increase mitochondrial
349 capacity in skeletal muscle of individuals with T2D. Low-volume HIT was a potent
350 stimulus to increase mitochondrial capacity in the current study, as evidenced by
351 increased enzyme activity of CS as well as elevated protein content of several subunits
352 from complexes in the electron transport chain.

353 Another novel observation in the present study was that low-volume HIT
354 increased the protein content of Mfn2. The primary role of Mfn2 is in mitochondrial
355 fusion, although it also appears to regulate the expression of electron transport chain
356 subunits and influence mitochondrial bioenergetic capacity (28). Our findings provide
357 evidence that elevated Mfn2 may be involved in regulating the increase in mitochondrial
358 capacity following low-volume HIT. A role for Mfn2 in the pathogenesis of T2D is

359 supported by studies reporting reduced Mfn2 expression in skeletal muscle of patients
360 with T2D (11), suggesting that alterations in mitochondrial fusion/fission may contribute
361 to mitochondrial impairment. Whether a training-induced increase in muscle Mfn2 is
362 linked to improved metabolic health is unknown. Further studies are needed to clarify
363 the role of mitochondrial dynamics and examine the effects of exercise and other
364 interventions on Mfn2 skeletal muscle.

365 Conclusions

366 Two weeks of low-volume HIT — involving only 30 min of vigorous exercise
367 within a total time commitment of 75 min per week — lowered 24-h average blood
368 glucose concentration, reduced post-meal blood glucose excursions, and increased
369 markers of skeletal muscle mitochondrial capacity in individuals with T2D. The total
370 weekly training time commitment in the present study was 50% lower than recently
371 revised guidelines that call for 150 min of moderate to vigorous exercise per week.
372 While longer-term comparative studies are clearly warranted, our findings indicate that
373 low-volume HIT may represent a time-efficient exercise strategy for the treatment of
374 T2D. Future research is needed to examine the long-term influence of HIT and to
375 comprehensively examine how this type of training compares to traditional therapeutic
376 exercise strategies.

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378

379

Author Contributions

JPL, MJG, JBG, MAT, and ZP designed the study. MAT and ZP oversaw medical clearance and procedures. JPL, JBG, and MP supervised exercise testing and training sessions. JPL and JBG conducted continuous glucose monitoring analyses. JPL, AS, JBG, and MP performed muscle analyses. JPL wrote the manuscript with critical review, discussion, and interpretation from all authors.

Acknowledgments

The Canadian Diabetes Association and Natural Sciences and Engineering Council (NSERC) of Canada provided the funding for this project (Principal Investigator: MJG). JPL was supported by an NSERC doctoral scholarship. AS was supported by a Canadian Institutes of Health Research doctoral scholarship. We thank Medtronic of Canada for in-kind contribution of continuous glucose monitors and sensors.

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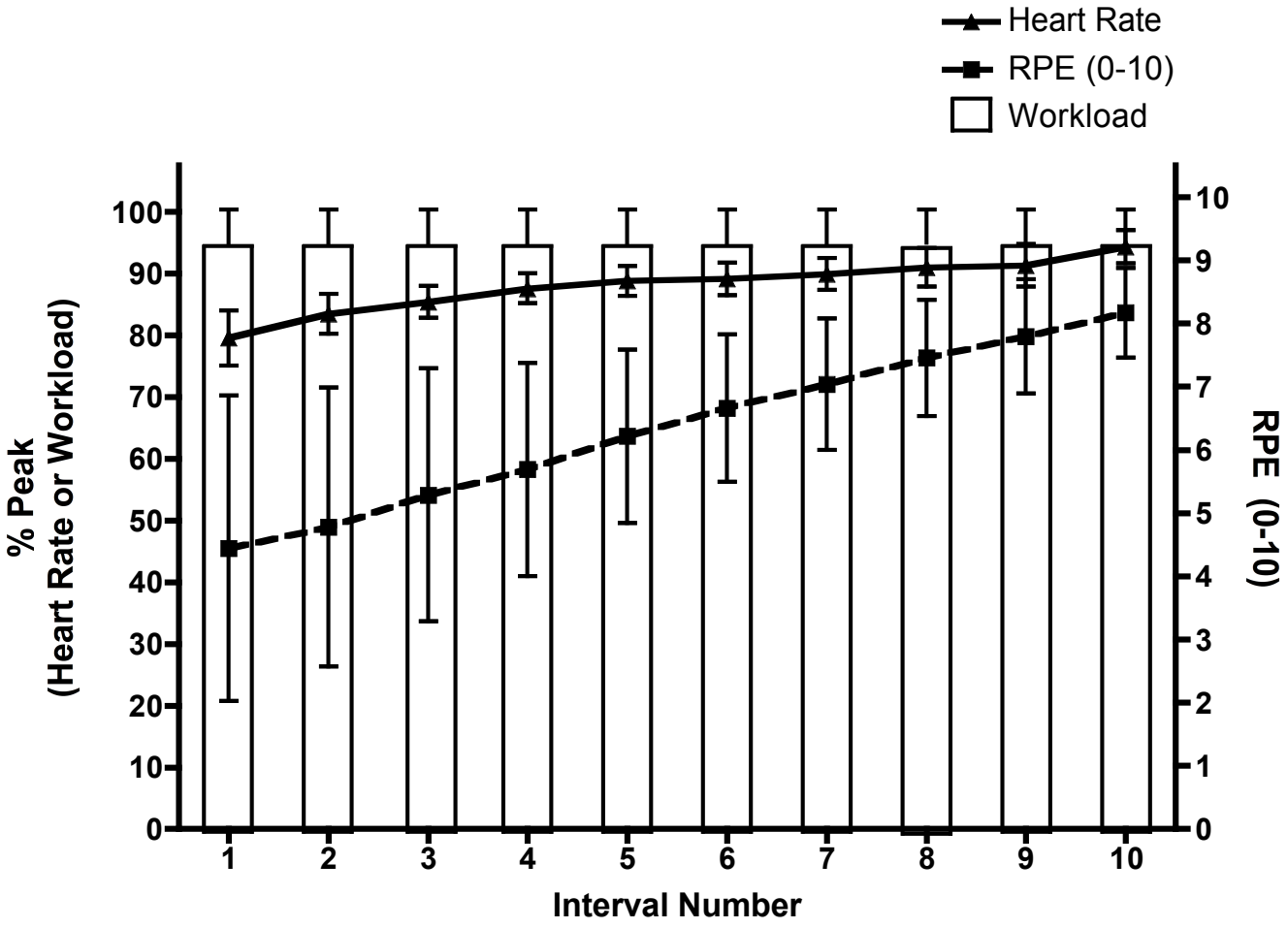
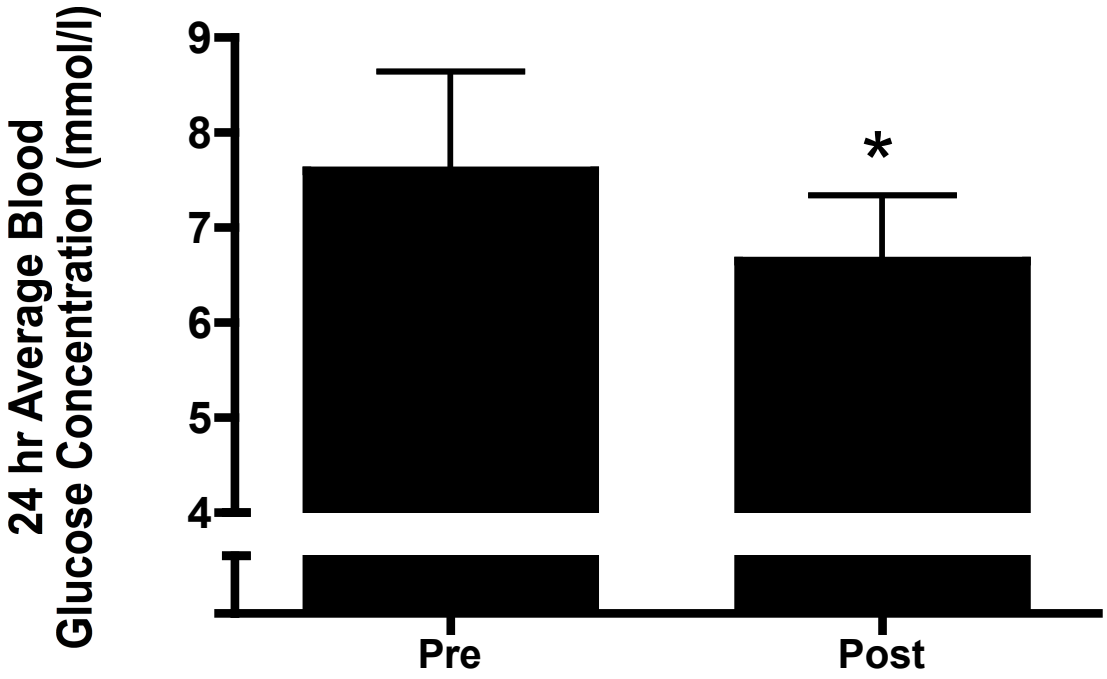
486
487

488

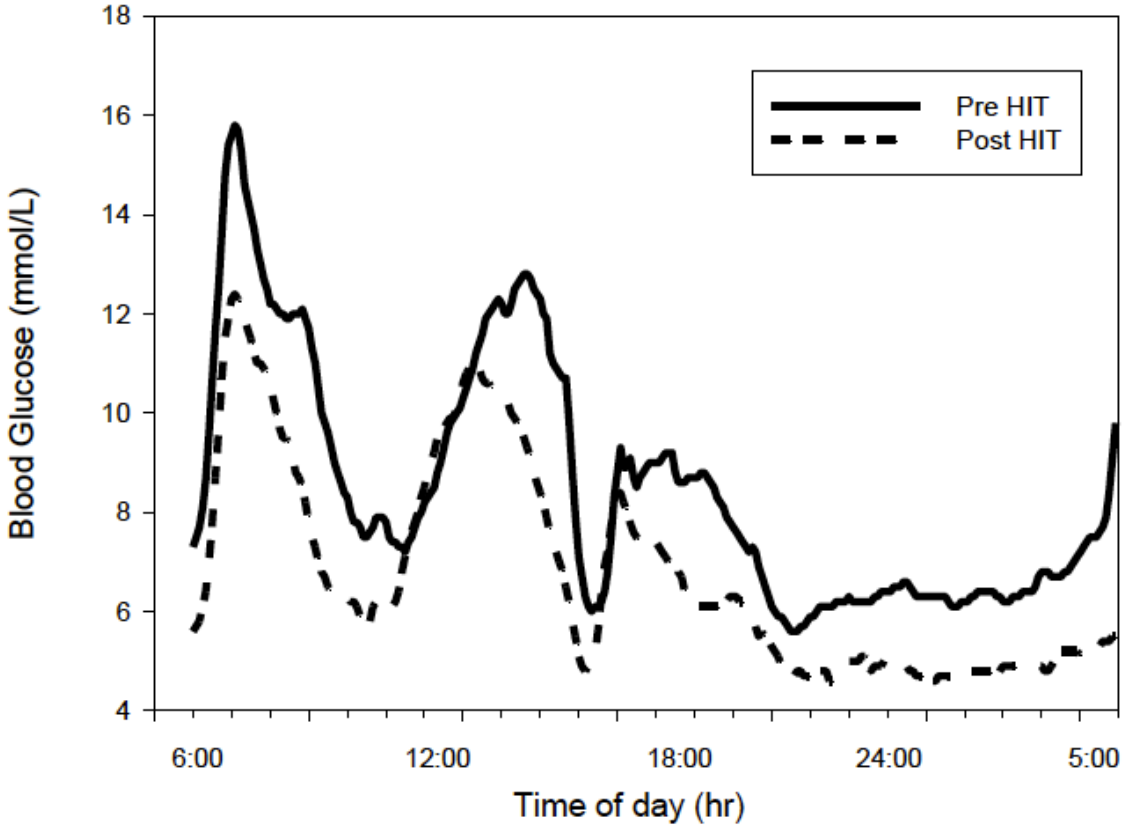
489 **Figure Legends**
490
491 **Figure 1. Characterization of high-intensity interval training protocol.** Training
492 intensity expressed as a percentage of peak workload (bars), peak heart rate (solid
493 line), and rating of perceived exertion (dashed line) averaged across all 6 sessions for
494 all subjects. Values are means±SD (N=8).
495
496 **Figure 2. Two weeks of high-intensity interval training improves glycemic control.**
497 **A,** Average blood glucose concentration measured by continuous glucose monitoring
498 (CGM) over a 24-hr period before (Pre) and after (Post) 2 weeks of training. **B**, Blood
499 glucose concentration assessed by CGM over 24 hours before (Pre; solid line) and after
500 (Post; dashed line) training in a representative subject. Post-training CGM data was
501 collected from ~48-72 hr following the final training session. Values are means±SD
502 (N=7). *p<0.05
503
504 **Figure 3. Summary of metabolic adaptations in skeletal muscle following two**
505 **weeks of high-intensity interval training**. **A,** Maximal activity of citrate synthase (CS)
506 measured in skeletal muscle biopsy samples before (Pre) and after (Post) 2 weeks of
507 training. **B,** Protein content of one subunit from each complex of the electron transport
508 chain measured in skeletal muscle biopsy samples obtained before (Pre) and after
509 (Post) training. Representative Western blots for each protein for two individual subjects
510 are also shown. Values are means±SD (N=7). *p<0.05
511
512 **Figure 4. Two weeks of high-intensity interval training increases mitofusin 2**
513 **(Mfn2) protein content**. Mitofusin 2 (Mfn2) protein content measured in skeletal muscle
514 biopsy samples obtained before (pre) and after (post) training. Representative Western
515 blots from two subjects are shown. Values are means±SD (N=7). *p=0.02
516
517 **Figure 5. Two weeks of high-intensity interval training increases GLUT4 protein**
518 **content**. **A,** Glucose transporter 4 (GLUT4) protein content measured in skeletal
519 muscle biopsy samples obtained before (Pre) and after (Post) training. Representative
520 Western blots from two subjects. Values are means±SD (N=7). *p=0.003
521


Heart Rate
RPE (0-10)
Workload
% Peak
(Heart Rate or Workload)
RPE (0-10)
Interval Number
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
6
7
8
9
10

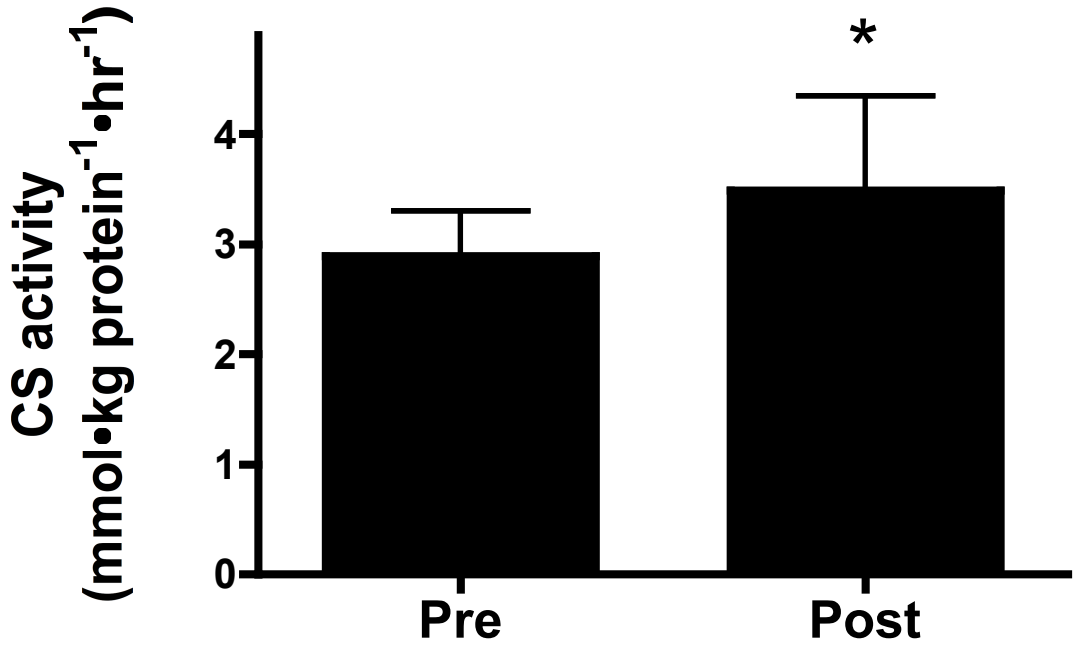
A


24 hr Average Blood Glucose Concentration (mmol/l)
9
8
7
6
5
4
*
Pre
Post

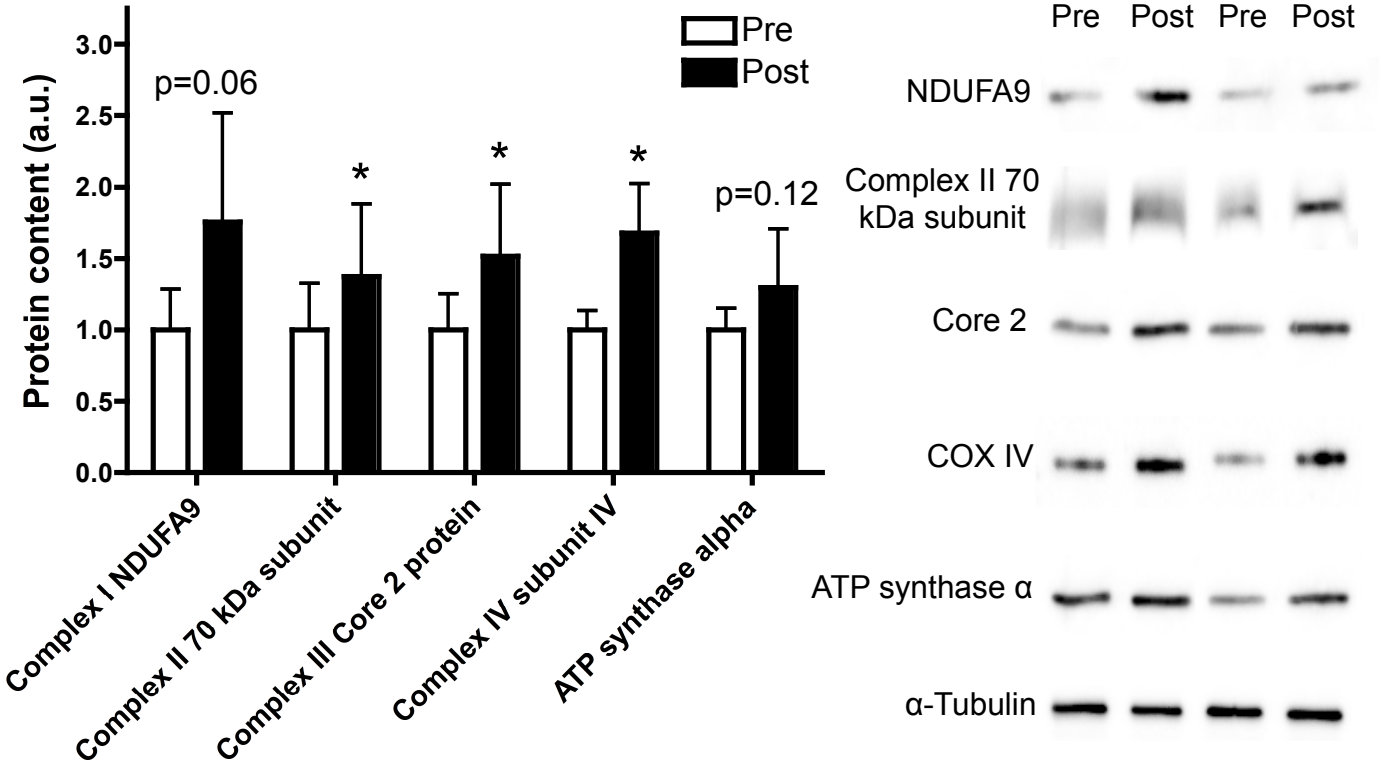
B

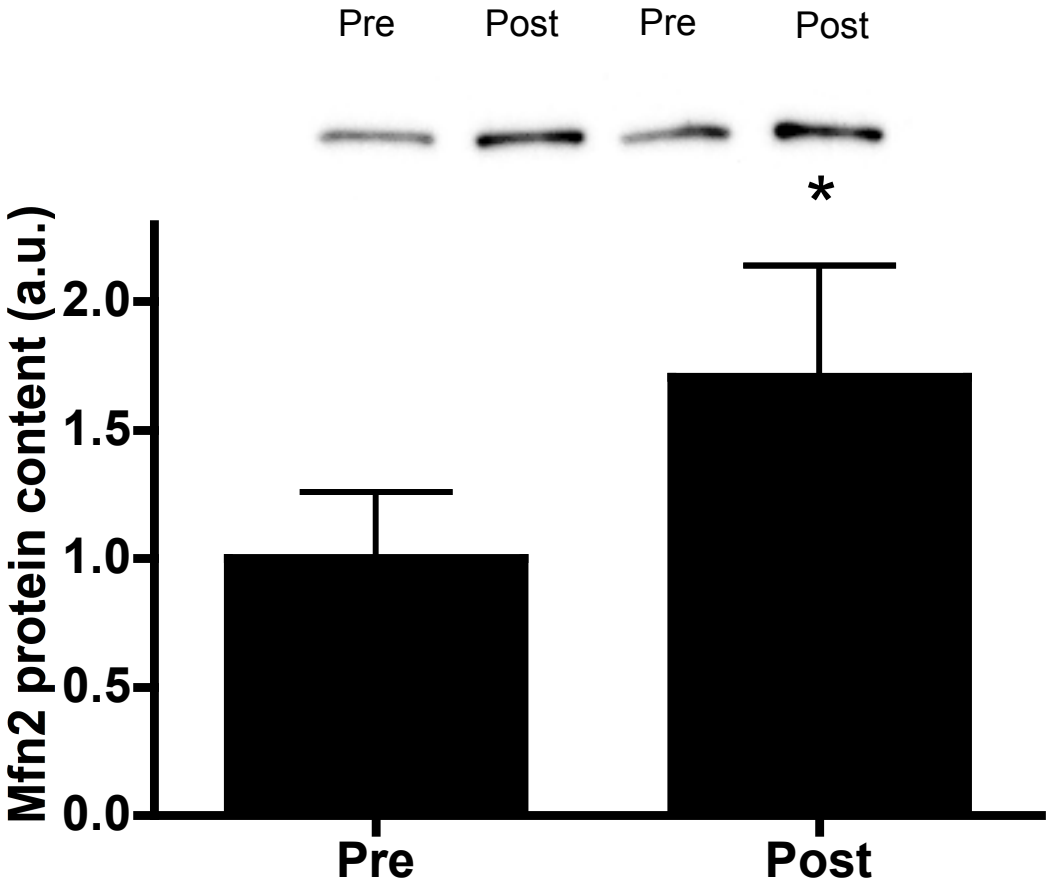

Blood Glucose (mmol/L)
18
16
14
12
10
8
6
4
Pre HIT
Post HIT
6:00
12:00
18:00
24:00
5:00
Time of day (hr)

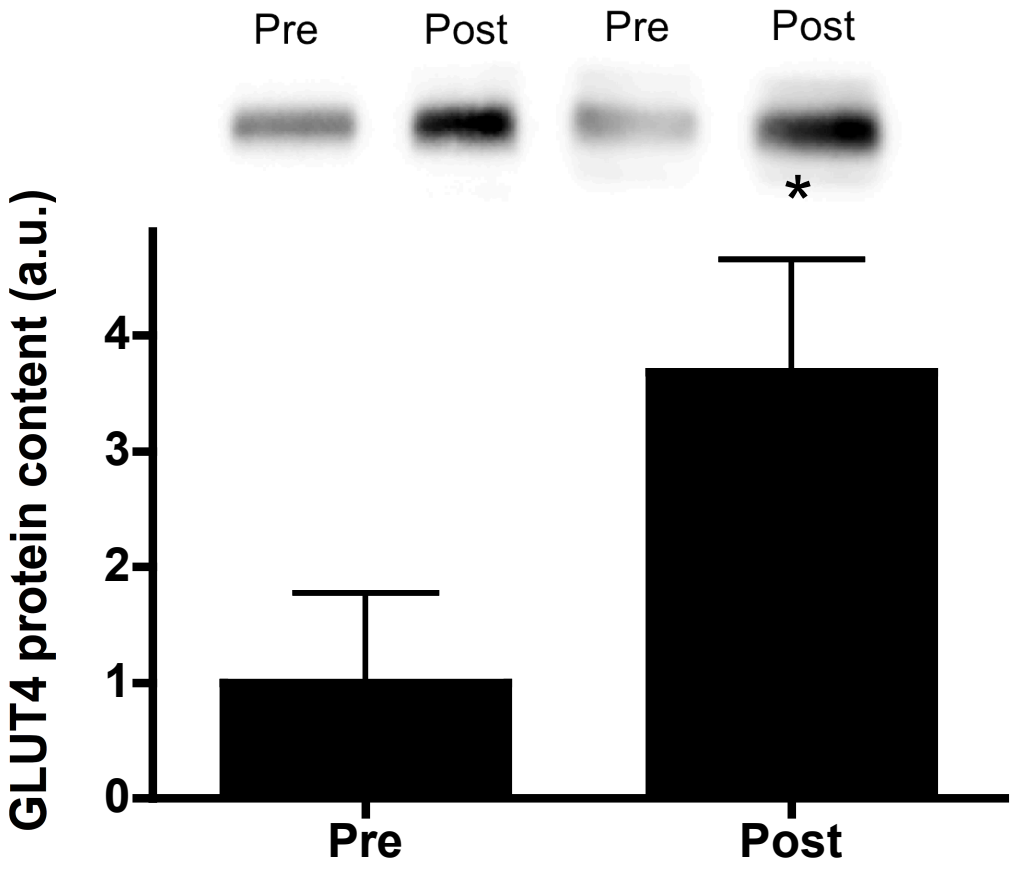
A


CS activity
(mmol•kg protein-1•hr-1)
0
1
2
3
4
*
Pre
Post

B


Protein content (a.u.)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Pre
Post
p=0.06
*
*
*
p=0.12
Complex I NDUFA9
Complex II 70 kDa subunit
Complex III Core 2 protein
Complex IV subunit IV
ATP synthase alpha
Pre
Post
Pre
Post
NDUFA9
Complex II 70 kDa subunit
Core 2
COX IV
ATP synthase α
α-Tubulin


Pre
Post
Pre
Post
*
Mfn2 protein content (a.u.)
2.0
1.5
1.0
0.5
0.0
Pre
Post


Pre
Post
Pre
Post
*
GLUT4 protein content (a.u.)
0
1
2
3
4
Pre
Post