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

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Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. J Appl Physiol 111: 1554–1560, 2011. First published August 25, 2011; doi:10.1152/japplphysiol.00921.2011.—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, body mass index 32 ± 6 kg/m2, HbA1C 6.9 ± 0.7%) volunteered to participate in this study. Participants performed six sessions of HIT (10 × 60-s cycling bouts eliciting ~90% maximal heart rate, interspersed with 60 s rest) over 2 wk. Before training and for ~48 to 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 was 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.

METHODS

Participants

Participants were recruited through local diabetes clinics, community diabetes information sessions, and poster advertisement. All participants were diagnosed with T2D at least 3 mo prior by a clinician according to standard criteria, including a fasting glucose ≥7.0 mmol/l and/or 2-h oral glucose tolerance test blood glucose concentration ≥11.1 mmol/l, were not taking insulin, and had no history of end-stage liver or kidney disease, neuropathy, retinopathy, hypertension that could not be controlled by standard medication, cardiovascular disease, or other contraindication to exercise. Eight individuals [mean age 62.5 ± 7.6 yr, body mass index 31.7 ± 5.8 kg/m2, hemoglobin A1C (HbA1C) 6.9 ± 0.7% (range 6.4–8.5%)]...
volunteered to participate in this study. Six participants were sedentary, which was defined as less than or equal to two exercise sessions of 30 min/wk. Two participants reported engaging in ~30 min of low-intensity walking exercise on 3–5 days/wk, in accordance with guidelines provided from their diabetes care team. Six subjects were taking blood glucose lowering medications but had HbA1c values ≤8.5% and were not on exogenous insulin therapy. Four patients were treated with metformin only, one patient with glitazide only, and one patient with a combination of metformin, pioglitazone, sitagliptin, and repaglinide. Due to the short duration of the intervention, participants did not adjust their medications and were instructed to maintain their typical dietary and activity patterns throughout. All participants provided written informed consent. The study protocol was approved by the Hamilton Health Sciences/McMaster University Faculty of Health Sciences Research Ethics Board.

Experimental Design

The experimental design consisted of 1) medical clearance and familiarization, 2) baseline testing, 3) a 2-wk training intervention, and 4) posttesting.

Medical clearance and familiarization. Height and weight were recorded, and a maximal exercise test on a recumbent cycle ergometer (Corival, Lode BV, Groningen, The Netherlands) was performed with pre- and postexercise 12-lead electrocardiogram (EKG) collection to confirm the absence of any underlying contraindications to vigorous exercise participation. The test started at 30 W and increased by 15 W/min until volitional exhaustion. Peak power output (Pmax) and maximal heart rate (HRmax) were recorded. Following ECG clearance by a study physician, participants completed one to two familiarization sessions to become acquainted with low-volume HIT. These sessions were also used to determine the interval power output that elicited ~90% HRmax.

Baseline testing. Prior to training, participants performed a 15-min walking test to examine the cardiovascular response and ratings of perceived exertion (RPE) during exercise. Speed was self-selected by each participant during an initial 5-min warm-up. Heart rate was measured by telemetry (Polar), and RPE was measured using the 0–10 continuous/interval scale.

At least 2 days after the walk test, participants reported to the laboratory for CGM device insertion (CGMS iPro, Medtronic, Northridge, CA). Participants were given a glucose meter (OneTouch UltraMini, Lifescan, Milpitas, CA) with instructions for both calibration and capillary blood sampling and individualized control diets. The following day served as a dietary control day for 24-h CGM data collection. Subjects returned to the laboratory 2 days later for removal of the CGM device and collection of a resting skeletal muscle biopsy sample as we previously described (8). Briefly, muscle samples were obtained under local anesthesia (1% Lidocaine) from the vastus lateralis using a Bergstrom needle adapted with suction. Muscle samples were quickly blotted to remove excess blood, sectioned into several pieces, and placed in separate vials before snap freezing in liquid nitrogen for subsequent analyses.

Training. Approximately 5 days after the muscle biopsy procedure, subjects commenced training. The HIT protocol involved a total of six supervised sessions over 2 wk (Monday, Wednesday, Friday each week). Each session consisted of 10 × 60-s cycling intervals interspersed with 60 s of recovery based on our recent work (14). Training was performed on a cycle ergometer (LifeCycle C1 or R1, Life Fitness, Schiller Park, IL) set in constant watt mode at a pedal cadence of 80–100 revolutions/min. Individual workloads were selected to elicit a heart rate of ~90% HRmax during the intervals. During recovery, participants were allowed to rest or pedal slowly against a resistance of 50 W. Each training session included a 3-min warm-up and 2-min cool-down at 50 W, for a total of 25 min. Therefore, the training protocol involved a total of 30 min of high-intensity exercise within a total time commitment of 75 min/wk, including warm-up, cool-down, and the recovery interval between high-intensity efforts.

Posttesting. CGM data were collected for a 24-h period starting ~48 h after the final training session. Diet was controlled to be the same as pretraining. Resting muscle biopsy samples were obtained ~72 h following the final training session. Approximately 2–4 days after the biopsy, the walk test (performed at the same speed as pretraining) and maximal exercise test were performed using the same procedures as baseline testing. Perceived enjoyment of low-volume HIT was assessed by asking participants how enjoyable they would find engaging in 1) a single bout of HIT (10 × 1 min) and 2) HIT at least 3 times/wk for the next 4 wk using a 9-point Likert scale ranging from 1 (not enjoyable at all) to 9 (very enjoyable).

Continuous Glucose Monitoring

Average blood glucose concentration and area under the glucose curve were calculated from CGM data for a 24-h period before and after training. CGM data were also used to analyze the 3 h postprandial areas under the glucose curve for breakfast, lunch, and dinner. On CGM data collection days, participants consumed their habitual breakfast but were provided with standardized snacks (e.g., almonds, fruit, vegetables) based on their personal preferences and habits. Lunch and dinner were standardized for all participants by providing vouchers to a local sandwich restaurant. Subjects completed detailed dietary logs to record the timing and quantity of all food consumed during the pretraining day. For posttesting, these dietary logs, along with all snacks and vouchers for lunch and dinner were provided with instructions for diet replication under free-living conditions over the 24-h CGM data collection period. As per manufacturer’s recommendations, capillary blood glucose samples were obtained at four points during the day at a time when blood glucose would be expected to be stable (i.e., upon awakening, before lunch, before dinner, and before bed) and were automatically stored in the glucose meters provided to participants. These four values were used during CGM downloading to construct 24-h blood glucose curves based on interstitial glucose recordings averaged every 5 min by the CGM device using the associated software algorithm (Solutions Software, Medtronic, Northridge, CA). CGM data were exported and analyzed using SigmaPlot (Statsoft, Chicago, IL). Reproducibility of the CGM device in our lab was verified in five volunteers who wore the monitor on two occasions separated by 1 wk under identical dietary conditions. The coefficient of variation for the 24 h blood glucose measurements was 2.8% (data not shown).

Muscle Analyses

Citrate synthase enzyme activity. One piece of muscle (~20 mg) was homogenized using a glass tissue grinder (Kimble/Kontes 885500–0002) in 10 volumes of buffer containing (in mM) 70 sucrose, 220 mannitol, 10 HEPES (pH 7.4) supplemented with protease inhibitors (Complete Mini, Roche Applied Science, Laval, PQ, Canada) and used to determine the maximal activity of citrate synthase (CS) as we previously described (8, 16). Protein concentration of homogenates was determined using a commercial assay (BCA Protein Assay, Pierce, Rockford, IL), and enzyme activity is expressed as millimoles per kilogram of protein per hour wet weight.

Western blotting. A second piece of muscle (~30 mg) was homogenized in RIPA buffer for Western blot analyses using techniques described previously (8, 16). Briefly, protein concentration of homogenates were determined as above and equal amounts of protein (5–20 μg) were prepared in 4× Laemmli’s buffer and heated to 95°C before being separated by 10–12.5% SDS-PAGE and electrotransferred to nitrocellulose membranes. Ponceau S staining was performed following transfer to visualize equal loading and transfer. Following 1 h blocking in 5% fat-free milk Tris-buffered saline 0.1% Tween 20 (TBS-T), membranes were incubated in primary antibodies overnight at 4°C or at room temperature for 2 h in 3% fat-free milk TBS-T or

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following training (\textit{P}-Tubulin (Cell Signaling Technology, \#2125), which did not change). NIH was used to quantify the optical density of protein bands. Imaging System (Alpha Innotech, San Leandro, CA). ImageJ software minescence (SuperSignal West Dura, Pierce) using a FluorChem SP from 7.6

Continuous Glucose Monitoring

session, 8.1 HIT was rated high by this group of participants (single 92

Training had no effect on body mass (pre: 93

six training sessions for all subjects is depicted in Fig. 1. Continuous Glucose Monitoring

Statistical Analyses

All data were analyzed using paired Student’s \( t \)-tests with significance set at \( P \leq 0.05 \) (Sigma Stat v3.10). Values are means \( \pm \) SD in the text and on figures.

RESULTS

Descriptive Characteristics of Training

All participants completed all prescribed intervals during training with no complications. Interval intensity averaged across all intervals for all subjects corresponded to 95 \( \pm \) 14% of \( W_{\text{max}} \), elicited 88 \( \pm \) 3% \( HR_{\text{max}} \), and RPE was 6.4 \( \pm \) 1.3 (0–10 scale). The response to each interval averaged across all six training sessions for all subjects is depicted in Fig. 1. Training had no effect on body mass (pre: 93 \( \pm \) 19 kg vs. post: 92 \( \pm \) 18 kg, \( P = 0.28 \)). On average, perceived enjoyment of HIT was rated high by this group of participants (single session, 8.1 \( \pm \) 1.0; 3 times/wk, 7.9 \( \pm \) 1.0).

Continuous Glucose Monitoring

Average blood glucose concentration over 24 h was reduced from 7.6 \( \pm \) 1.0 to 6.6 \( \pm \) 0.7 mmol/l after training (Fig. 2A, \( P = 0.01 \)). Area under the 24-h blood glucose curve was also lower following HIT (pre: 11,066 \( \pm \) 1,703 vs. post: 9,572 \( \pm \) 995 mmol·l\(^{-1}\)·day\(^{-1} \), \( P = 0.02 \)). The sum of the 3-h postprandial area under the glucose curves for breakfast, lunch, and dinner was significantly lower posttraining (pre: 965 \( \pm \) 483 vs. post: 679 \( \pm \) 437 mmol·l\(^{-1}\)·h\(^{-1} \), \( P = 0.01 \)). Pre- and posttraining 24-h blood glucose curves for a representative subject are shown in Fig. 2B.

Adaptations in Skeletal Muscle

The maximal activity of CS was elevated following training (Fig. 3A, \( P = 0.04 \)). Training also increased skeletal muscle mitochondrial protein content as evidenced by changes in Complex II 70 kDa subunit (\( P = 0.03 \)), Complex III Core 2 protein (\( P = 0.04 \)), and COX subunit IV (\( P = 0.02 \)) measured by Western blotting (Fig. 3B). The protein content of CS (\( \sim 57\% \); data not shown), NDUFA9, COX subunit II (\( \sim 53\% \); data not shown), and ATP synthase \( \alpha \)-subunit also increased, but did not reach statistical significance (\( P = 0.06–0.12 \); Fig. 3).

3\% BSA TBS-T depending on previously determined optimization conditions. After 3 \( \times \) 5-min washes in TBS-T, membranes were incubated in the appropriate species-specific secondary antibody diluted (1:10,000) in 3\% fat-free milk TBS-T for 1 h at room temperature, washed in TBS-T for 3 \( \times \) 15 min, and visualized by chemiluminescence (SuperSignal West Dura, Pierce) using a FluorChem SP Imaging System (Alpha Innotech, San Leandro, CA). ImageJ software (NIH) was used to quantify the optical density of protein bands. \( \alpha \)-Tubulin (Cell Signaling Technology, \#2125), which did not change following training (\( P = 0.91 \)), was used as a loading control. Primary antibodies for the following proteins of interest were used: NDUFA9 (Mitosciences, MS111), Complex II 70 kDa subunit (Mitosciences, MS204), Complex III Core 2 protein (Mitosciences, MS304), cytochrome \( \epsilon \) oxidase (COX) subunit II (Mitosciences, MS405); COX subunit IV (Mitosciences, MS408), ATP synthase \( \alpha \)-subunit (Mitosciences, MS507), CS (kind gift from Dr. Brian Robinson, The Hospital for Sick Children, Toronto, Canada), mitofusin (Mfn) 2 (Mitosciences, MS111), Complex II 70 kDa subunit (Mitosciences, MS204), cytochrome \( \epsilon \) oxidase (COX) subunit II (MitoSciences, MS405), COX subunit IV (Mitosciences, MS408), ATP synthase \( \alpha \)-subunit (Mitosciences, MS507), CS (kind gift from Dr. Brian Robinson, The Hospital for Sick Children, Toronto, Canada), mitofusin (Mfn) 2 (Sigma, M6319), and GLUT4 (Millipore, AB1345).
The protein content of Mfn2 was elevated following training (~71%, P = 0.02; Fig. 4), as was total GLUT4 protein (~369%, P = 0.003; Fig. 5).

**Functional Exercise Performance**

Maximal workload achieved on the ramp cycling test was increased by ~10% following training (pre: 111 ± 36 vs. post: 124 ± 37 W, P = 0.03). Training reduced heart rate (pre: 73 ± 7 vs. post: 66 ± 6%HRmax, P < 0.001) and RPE (pre: 2.4 ± 0.7 vs. post: 1.3 ± 1.2, P = 0.01) during the walk test.

![Fig. 3. Summary of metabolic adaptations in skeletal muscle following 2 wk of high-intensity interval training. A: maximal activity of citrate synthase (CS) measured in skeletal muscle biopsy samples before and after 2 wk of training. B: protein content of one subunit from each complex of the electron transport chain measured in skeletal muscle biopsy samples obtained before and after training. Representative Western blots for each protein for two individual subjects are also shown. Values are means ± SD (N = 7). *P < 0.05.](image1)

![Fig. 4. Two weeks of high-intensity interval training increases mitofusin 2 (Mfn2) protein content. Mfn2 protein content measured in skeletal muscle biopsy samples obtained before and after training. Representative Western blots from 2 subjects are shown. Values are means ± SD (N = 7). *P = 0.02.](image2)

![Fig. 5. Two weeks of high-intensity interval training increases GLUT4 protein content. Glucose transporter 4 (GLUT4) protein content measured in skeletal muscle biopsy samples obtained before and after training. Representative Western blots from 2 subjects. Values are means ± SD (N = 7). *P = 0.003.](image3)
DISCUSSION

The present study demonstrates that low-volume HIT can rapidly reduce hyperglycemia and increase skeletal muscle oxidative capacity in patients with T2D. These improvements were realized despite a small total volume of exercise that consisted of six training sessions over 2 wk. The training protocol involved a total of only 30 min of high-intensity exercise and a total time commitment of only 75 min/wk. This is much lower than current physical activity guidelines for T2D that recommend a total of 150 min of moderate to vigorous intensity exercise each week (6). Given that the majority of individuals with and without T2D does not accumulate sufficient exercise to achieve health benefits (6) and the most common cited barrier to regular exercise is lack of time (26), our results suggest that low-volume HIT may be a viable, time-efficient strategy to improve health in patients with T2D.

Low-Volume HIT and Glycemic Control

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

To our knowledge, this is the first study to examine the effects of HIT on glycemic regulation using CGM. Average blood glucose concentration and area under the glucose curve measured under standardized dietary conditions from ~48 to 72 h after the final training session were significantly lower than pretraining, indicating that short-term, low-volume HIT improved glycemic control, particularly glycemic excursions after meals. Although reducing fasting hyperglycemia is a significant aspect of T2D treatment, increasing evidence suggests lowering postprandial hyperglycemia is as important, if not more important, for achieving targeted HbA1C levels (27). Additionally, elevated postmeal blood glucose excursions have been implicated in the development and progression of T2D related comorbidities such as cardiovascular disease (5, 27). Following HIT, the sum of the postprandial areas under the glucose curve for breakfast, lunch, and dinner was significantly lower than pretraining, highlighting the potency of HIT to lower postmeal glucose excursions. These findings demonstrate that low-volume HIT may be an effective strategy for improving glycemic regulation in individuals with T2D and suggest that CGM may be a sensitive technique to measure the effects of exercise on glucose control.

The mechanisms mediating the improvement in glycemic control following HIT remain to be determined. Training had no effect on body mass, and, while not assessed directly, it is unlikely that such a short exercise intervention would lead to any substantial changes in body composition. Therefore, it is tempting to speculate that adaptations in skeletal muscle were involved. Since reduced mitochondrial capacity in skeletal muscle has been reported in insulin resistance and T2D (22) and muscle oxidative capacity has been shown to be a significant predictor of insulin sensitivity (3), it is possible that the rapid increase in skeletal muscle mitochondrial content following low-volume HIT may be a contributing factor related to reduced insulin resistance and improved glycemic control. However, the notion that mitochondrial deficiency mediates insulin resistance has been questioned recently (12), indicating that other adaptations in skeletal muscle may be more important. The training-induced increase in GLUT4 protein content likely plays a role in improving glucose regulation. Studies in rodents indicate that the exercise-induced increase in GLUT4 protein is directly related to the increase in muscle glucose uptake at any given insulin concentration (20). Thus, even in the face of insulin resistance, an increase in skeletal muscle GLUT4 could facilitate greater muscle glucose uptake and contribute to improved glycemic regulation. In addition to skeletal muscle adaptations, training-induced alterations in hepatic glucose output cannot be ruled out. The effect of exercise training on hepatic insulin resistance in T2D has not been directly assessed in humans, although there is evidence to suggest that endurance exercise training improves hepatic insulin signaling and glycemic control in rodents (10).

We did not directly assess the effects of training on insulin sensitivity using hyperinsulinemic-euglycemic clamps and therefore cannot conclude whether low-volume HIT improves muscle insulin sensitivity. CGM assesses exposure to hyperglycemia as well as glycemic excursions throughout the day. Exposure to hyperglycemia over time may be a better indicator of diabetic complications than insulin sensitivity per se (5) and therefore CGM may provide greater insight into the clinical benefits of exercise training. Changes in HbA1C are commonly used to assess the effectiveness of glucose-lowering interventions in T2D but due to the short duration of the current study was not measured.

Low-Volume HIT and Skeletal Muscle Mitochondrial Adaptations

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

Another novel observation in the present study was that low-volume HIT increased the protein content of Mfn2. The primary role of Mfn2 is in mitochondrial fusion, although it also appears to regulate the expression of electron transport chain subunits and influence mitochondrial bioenergetic capacity (28). Our findings provide evidence that elevated Mfn2 may be involved in regulating the increase in mitochondrial capacity following low-volume HIT. A role for Mfn2 in the pathogenesis of T2D is supported by studies reporting reduced Mfn2 expression in skeletal muscle of patients with T2D (11), suggesting that alterations in mitochondrial fusion/fission may contribute to mitochondrial impairment. Whether a training-induced increase in muscle Mfn2 is linked to improved metabolic health is unknown. Further studies are needed to clarify the role of mitochondrial dynamics and examine the effects of exercise and other interventions on Mfn2 skeletal muscle.

Conclusions

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

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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