Body-weight-support treadmill training improves blood glucose regulation in persons with incomplete spinal cord injury

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The life expectancy of persons who have suffered a traumatic spinal cord injury (SCI) is now approaching that of the able-bodied population. The primary causes of morbidity and mortality in persons with SCI are the same as those in the able-bodied population (2–4, 14, 15), with the exception that some diseases and associated risk factors (i.e., diabetes and dyslipidemias) arise earlier in life and with greater prevalence in persons with SCI (2–4). For these reasons, similar advice regarding lifestyle factors that can reduce risks for these diseases should be directed at persons with SCI (2–4, 14, 15).

With respect to the risk for development of non-insulin-dependent diabetes mellitus (Type 2 diabetes), one major determinant of insulin action is physical activity (for review, see Ref. 1). However, exercise involving large muscle masses, such as the legs, is often not possible in persons with SCI. Traumatic spinal cord injury results in loss of skeletal muscle mass, reduced or complete loss of capacity for voluntary neural activation of skeletal muscle, and a reduced oxidative potential of skeletal muscle (i.e., reduced mitochondrial content) below the level of the lesion. Muscle fiber size is reduced, which appears to occur relatively early and reach a nadir within a year postinjury (8, 31). Additionally, muscle oxidative capacity and the capacity for glucose transport and phosphorylation are also lower in persons with SCI (9, 10, 25, 28, 31, 37). Studies in which limbs have been immobilized or neural input to muscle has been ablated have also shown reductions in fiber size and metabolic capacity, as well as capacity for glucose transport (24, 33, 39). Because of a lack of neural input, possibly combined with reduced voluntary contractile activity, individuals with SCI also show reduced glucose tolerance and relative hyperinsulinemia during oral glucose loads (9, 10, 25, 28, 31, 37). Because hyperglycemia and hyperinsulinemia are hallmarks of Type 2 diabetes and contribute to the metabolic syndrome, it would be beneficial to develop therapies for these conditions aimed toward persons with SCI (19).

In persons with a complete SCI, programs of nonvoluntary electrical surface stimulation [functional electrical stimulation (FES)] have been shown to increase muscle glucose transporter (GLUT-4) content and improve whole body insulin action (9, 25, 27, 37). These results indicate that paralyzed skeletal muscle responds to involuntary activity with the induction of the skeletal muscle GLUT-4 transporter protein, which is due to a local contractile stimulus. Moreover, the chronic increase in muscle GLUT-4 transporter content induced by FES is insulin responsive (9, 25, 37). In persons with an incomplete SCI, some forms of voluntary exercise are still possible. Moreover, persons with SCI frequently identify functional ambulation as one of their rehabilitation goals. Body-weight-supported treadmill (BWST) training (Laufband therapy) has been shown to improve cardiovascular fitness (11) in SCI, with increased muscle glucose transporter protein content (12, 17). The primary purpose of this study was to determine if voluntary exercise in SCI improves blood glucose regulation.

In SCI, the hallmark of reduced voluntary neural activity is loss of voluntary contractile activity. The hallmark of reduced voluntary contractile activity is that muscle remains metabolically capable of oxidative metabolism (10). However, in SCI, there is also a decrease in the amount of muscle glycogen available for oxidation (10). To accomplish this goal, we examined the effects of a 6-mo body-weight-supported treadmill (BWST) training (Laufband therapy) has been shown to improve cardiovascular fitness (11) in SCI, with increased muscle glucose transporter content (12, 17).

The impact of a 6-mo body-weight-supported treadmill training program on glucose homeostasis and muscle metabolic characteristics was investigated. Nine individuals (31 ± 3 yr, 8.1 ± 2.5 yr postinjury; means ± SE) with incomplete spinal cord injury trained three times weekly for a total of 6 mo. Training session duration and intensity (velocity) increased by 54 ± 10% (P < 0.01) and 135 ± 20%, respectively. Muscle biopsies and a modified glucose tolerance test (100 g glucose with [U-13C]glucose) were performed before (Pre) and after training (Post). Training resulted in a reduction in area under the curve of glucose × time (~15 ± 4%) and insulin × time (~33 ± 8%; both P < 0.05). Oxidation of exogenous (ingested) glucose increased as a result of training (Pre = 4.4 ± 0.7 g/h, Post = 7.4 ± 0.6 g/h; P < 0.05), as did oxidation of endogenous (liver) glucose (Pre = 3.8 ± 0.3 g/h, Post = 5.2 ± 0.3 g/h; P < 0.05). Training resulted in increased muscle glycogen (80 ± 23%; P < 0.05) and GLUT-4 content and hexokinase II enzyme activity (126 ± 34 and 49 ± 4%, respectively, both P < 0.01). Resting muscle phosphorylase content also increased after training (Pre = 62.1 ± 4.3, Post = 78.7 ± 3.8, both mmol/kg dry wt and P < 0.05). Six months of thrice-weekly body-weight-supported treadmill training in persons with an incomplete spinal cord injury improved blood glucose regulation by increasing oxidation and storage of an oral glucose load. Increases in the capacity for transport and phosphorylation glucose in skeletal muscle likely play a role in these adaptations.

GLUT-4; non-insulin-dependent diabetes mellitus; insulin action; glucose control
been shown to restore a degree of ambulatory capacity in some individuals with incomplete SCI (48, 49). No information is currently available, however, on how BWST training affects oral glucose tolerance or insulin sensitivity.

The purpose of this investigation was threefold. First, because no information is currently available, we wished to test the hypothesis that BWST training would improve glucose homeostasis by reducing area under the curves (AUC) for glucose and insulin × time. Second, we wished to assess, using U-13C-labeled glucose, the oxidative fate of ingested glucose, with the hypothesis that BWST training would be of sufficient intensity to induce an increase in the peripheral (i.e., muscle) oxidative disposal of glucose, as it does in able-bodied persons (6). Finally, we examined muscle biopsies with the working hypothesis that BWST training would induce an increase in glucose transporter content and muscle glucose phosphorylation capacity, which would be of benefit in inducing an increase in insulin-stimulated glucose uptake (9, 27, 37).

METHODS

Subjects. All participants were recruited through local advertisements in newspapers and by contact with medical staff at The Central West Regional SCI Rehabilitation Program in Hamilton, Ontario (Chedoke Hospital), where some participants were receiving standard outpatient rehabilitative treatment. All subjects were advised of the purposes of the study and associated risks, and all gave written, informed consent before participating. The project was approved by the Research Ethics Board of Hamilton Health Sciences and conforms to guidelines involving use of human subjects as outlined in the Helsinki declaration. In total, nine subjects, eight men and one woman, were recruited to participate in the training study. All subjects were neurologically stable, and their average time since injury was 8.1 ± 2.5 yr (mean ± SE). All lesions (range C4–T12) were incomplete, and all subjects were classified as having an American Spinal Injury Association (ASIA) score of C. All subjects utilized wheelchairs on a regular basis, but some subjects were also able to ambulate with assistive devices (walkers) over short distances (<5–10 m). Subjects were not actively engaged in any form of exercise training and were not regularly participating in any form of structured physical activity at the time of the BWST training protocol. No subject performed any form of strenuous physical activity outside of the BWST training during the training program. For detailed subject characteristics, see Ref. 46.

Study design. A longitudinal training study design was utilized with subjects being evaluated both before (Pre) and after training (Post). The study spanned 68 exercise sessions (2.8 ± 0.2 training sessions/wk) and took 6.0 ± 0.3 mo for the subjects to complete. All subjects completed all 68 training sessions. All Post measures, of our modified glucose tolerance test (see Modified OGTT), were made 7 days after the last BWST training session, whereas biopsies were taken 3 days after the last BWST training session.

BWST training exercise protocol. Upright walking was completed on a motor-driven treadmill (Woodway, Foster, CT) while a harness, suspended from an overhead pulley system, supported the subject’s body weight. Initial body weight support and speed of the treadmill were chosen according to the individual abilities of each of the subjects. In brief, subjects began training with 65 ± 3% of their body weight supported and walked at a treadmill speed of <0.6 km/h. Depending on the amount of assistance required by each subject, it was usual to have one assistive therapist on either side of the subject to assist with leg movement. Assistance was particularly important in bending the knee and hip joint during the swing phase of the gait pattern to ensure full knee extension and stabilization during the stance phase of the gait cycle. Subjects were instructed to place their body weight over their fully extended leg during the stance phase of the walking cycle. Treadmill velocity and the amount of body weight supported by the subject’s own legs were individualized according to each subject’s own rate of improvement. In addition, time on the treadmill was adjusted according to each subject’s self-reported level of fatigue.

Muscle needle biopsy. At least 2 wk before initiating the training program, and within 3 days of completing their final training session, all subjects had a percutaneous needle biopsy taken from the vastus lateralis of a randomly selected leg. Needle (5 mm Bergström) biopsy punches (100–150 mg) were obtained from each subject under local anesthesia (2% lidocaine) by use of a needle custom modified for manual suction. The biopsies were taken from the middle portion of the vastus lateralis on the same leg before and after the BWST training program. Fat and connective tissue, if present, were immediately removed from each biopsy sample, under low-power (×10) magnification. The “clean” biopsy sample was then divided into three portions. Two of the portions were snap frozen in liquid nitrogen and reserved for enzyme (−30 mg) and Western blot (−50 mg) analysis, respectively (stored at −80°C before analysis). The third portion (−50 mg) was reserved for fiber size and type determination, as reported elsewhere (46).

Dual-energy X-ray absorptiometry. A separate portion of this study involved the subjects receiving a whole-body dual-energy X-ray absorptiometry (DXA) scan to assess changes in body composition and bone mineral content and density. These scans were only taken pretraining and after 12 mo of training. Briefly, subjects reported to the testing laboratory in an overnight fasted state, donned a thin cotton gown, and lay still, palms down, on the DXA scanner table (Hologic 4500A densitometer, Bedford, MA) for the time taken for the scan. We determined test and retest coefficients of variation for this scanner and found them to be <2.6% for lean mass and <3.4% for fat mass with repeated scans.

Western blot. Western blots were performed to determine GLUT-4 protein abundance. Frozen muscle was hand homogenized in 0.5% SDS-buffered saline (10 mM Tris, 0.1 mM EDTA, 2 mM PMSF). Crude muscle homogenates were stored in aliquots at −70°C until analysis. The protein contents of the homogenates were determined by using the Bradford protein assay (Bio-Rad, Hercules, CA), and equivalent amounts of protein were loaded in each lane in the gel. All samples were run in triplicate, and the coefficient of variation was <5% for each sample. Proteins were separated by using a 4% SDS-polyacrylamide stacking gel and a 12% SDS-polyacrylamide separating gel. Before electrophoresis, each homogenate was mixed with a Tris buffer containing 10% SDS and 2.5% β-mercaptoethanol. Pretraining and posttraining samples for each subject were loaded in adjacent lanes on the same gel, along with a broad range molecular weight standard (Bio-Rad). Gels were run with the power supply set at 100 V for 1 h at room temperature. After electrophoresis, the proteins were transferred to a synthetic PVDF membrane (Bio-Rad). The PVDF membrane was placed in a 3% gelatin solution dissolved in Tween-Tris-buffered saline (TTBS; 500 mM NaCl, 20 mM Tris, HCl, pH 7.5) with 0.1% Tween (Bio-Rad) to block for 1 h at 4°C. This was followed with a 3 × 7 min wash in TTBS and a second block in 5% skim milk powder dissolved in TTBS. The second block was followed again by a 3 × 7 min wash in TTBS before the primary antibody was applied. The rabbit anti-human GLUT-4 primary antibody (H-61, polyclonal IgG, Santa Cruz Biotechnologies, Santa Cruz, CA) was then incubated onto the membrane at a 1:500 dilution (in TTBS) for 2 h at room temperature. This was followed by a 4 × 10 min wash in TTBS, and the application of the secondary antibody [Bio-Rad, goat anti-rabbit IgG (H+L)-AP conjugate]. The goat anti-rabbit secondary antibody and a biotinylated-streptavidin alkaline phosphatase enzyme (Bio-Rad, horse radish peroxidase) were incubated onto the membrane at a 1:3,000 dilution (in TTBS) for 1 h at room temperature. The membrane was then washed before being exposed to immunostar substrate (Bio-Rad) and Kodak film, which was subsequently devel-
oped, digitized by using the Epi-Illumination UV Darkroom, and analyzed for band density.

**Hexokinase activity.** Approximately 10 mg (wt) of frozen muscle were hand homogenized in 425 µl of ice-cold phosphate homogenizing buffer (pH 7.4, containing 0.02% bovine serum albumin, 5 mM β-mercaptoethanol, 0.5 mM EDTA). The homogenate was diluted 1:125 in phosphate homogenizing buffer and added to 100 µl of the reaction buffer (100 mM Tris·HCl, 5 mM glucose, 5 mM ATP, 2 mM MgCl₂, 0.5 mM NAD⁺, 0.5% Triton X-100, 0.05% bovine serum albumin, and 2 U/ml glucose-6-phosphate dehydrogenase). The reaction tubes were incubated at 37°C for 1 h, after which, to stop the reaction, 10 µl of 1 N HCl were added and it was incubated at 95°C for 3 min. To the stopped reaction, 1 ml of fluorometric reaction buffer was added (50 mM imidazole, 100 µM NAD⁺, 30 mM ammonium acetate, 5 mM MgCl₂, 1 mM EDTA, and 0.1 U/ml phosphogluconate dehydrogenase), and the reaction was allowed to proceed for 15 min at room temperature (22°C). The hexokinase (HK) activity was calculated on the basis of comparison with known standards of glucose-6-phosphate and expressed as micromoles per minute per gram wet weight of muscle. All pre- and posttraining muscle samples were analyzed together. The intra-assay coefficient of variation was <4.0% for the assay.

**Model A: OGTT.** Subjects reported to the Exercise and Metabolism Research Laboratory at McMaster University having abstained from any strenuous activity for at least 48 h and in an overnight fasted state having abstained from eating and drinking since 2100 on the previous night. During each of the oral glucose tolerance tests (OGTT), subjects ingested 1,000 ml of room-temperature water containing 100 g of U-13C-enriched glucose (see below). The drink was given in four equal volumes of 250 ml ingested at 30-min intervals after the initial baseline blood sample (i.e., before the drink). Blood samples were taken at 10 min, at 20 min, and every 20 min thereafter until 180 min after ingestion of the initial drink. Breath samples were collected at baseline and at 60 min after the first drink and every 30 min thereafter to assess breath enrichments. All observations were made between 0800 and 1100 and were made at the same time of day Pre and Post.

For blood sampling, a 20-gauge catheter was inserted into an antecubital vein and was kept patent with a 0.9% NaCl drip. Samples were taken at time 0 (before the drink), 10 min, 20 min, 40 min, and every 20 min thereafter until 180 min. Blood samples (5 ml) were drawn into test tubes containing lithium heparin. Whole blood (20 µl) was immediately analyzed for glucose concentration by using a calibrated automated glucose analyzer (YSI 2300 Stat Plus, Yellow Springs, OH). The intra-assay coefficient of variation (CV; n = 10 samples) for glucose concentration determination with this analyzer was <3%. The heparinized blood was then spun in a refrigerated centrifuge (4°C for 2.800 g) for 15 min to separate the plasma. Plasma was then aliquoted into tubes and stored at −80°C until analysis of insulin concentration and blood glucose enrichment by mass spectrometry.

Breath samples were taken at baseline (0 min) and after 60, 90, 120, 150, and 180 min after ingestion of the glucose drink. Breath samples were extracted from Douglas bags that were directly online with the outflow from the metabolic gas analyzers. Breath samples were collected into 60-ml additive-free syringes that had been flushed three times with the expired, and well mixed, breath sample. Expired gases were then delivered via a 26-gauge needle from the 60-ml syringe into 10-ml additive-free Vacutainers and were immediately analyzed for breath 13CO₂-to-12CO₂ ratio (13CO₂/12CO₂) by automated isotope ratio mass spectrometry (see Mass spectrometry).

**Glucose drink.** Natural unlabeled glucose (Sigma, St. Louis, MO) was enriched with [U-13C]glucose (99%; Cambridge Isotopes, Andover, MA) to achieve a final isotopic composition greater than +135‰. This value of enrichment was measured experimentally by mass spectrometry to be +122.7% Pre and +110.3% Post. The high enrichment of this signal was deemed necessary to “obscure” any background changes in endogenous ¹³CO₂ breath enrichment during the OGTT and to detect small changes in glucose oxidation induced by the training protocol by achieving a high signal-to-noise ratio. The drink for the entire study was made at one time for all subjects and frozen as a 40% glucose solution in aliquots that were defrosted and made up to 1,000 ml the day before the study. This was done to ensure a consistent enrichment of the ingested drink between subjects and from Pre to Post. Combustion analysis of ¹³CO₂/¹²CO₂ of aliquots of all the drinks ingested by the subjects revealed a CV in the enrichment (n = 9) of ±0.3%.

**Glucose oxidation.** Carbohydrate and triglyceride oxidation were calculated from indirect calorimetry. Expired gases were analyzed with an AEI Moxus II gas-analysis system (Pittsburgh, PA). The system was calibrated with two points and gases with CO₂ and O₂ concentrations within the physiological range. Breath-sampling periods were 8 min long, or longer, to achieve at least 3 min of stable O₂ uptake (VO₂) and CO₂ production (VCO₂) readings. Protein oxidation was calculated as the urinary urea produced over the 3-h period (6). Hence, carbohydrate and triglyceride oxidation rates were computed from VO₂ and VCO₂, corrected for protein oxidation according to the stoichiometric equations and caloric equivalents as presented by Burelle et al. (6).

The oxidation of ingested glucose (Glu_{ing}) was computed as described (6, 42) and according to the following equation:

\[
\text{Glu}_{\text{ing}} = \frac{\text{V}_{\text{CO}_2} \times [(\text{R}_{\text{exp}} - \text{R}_{\text{bkg}}) / (\text{R}_{\text{exp}} - \text{R}_{\text{bkg}})] \times (k \cdot c)}
\]

where R_{exp} is the ¹³CO₂/¹²CO₂ in expired breath, R_{bkg} is the background ¹³CO₂/¹²CO₂ in the breath before ingestion of the labeled glucose, R_{ing} is the ¹³CO₂/¹²CO₂ of the ingested glucose solution, k is the volume of CO₂ produced as a result of the complete oxidation of glucose (0.7426 l/g) (6, 42), and c is the bicarbonate retention factor, which accounts for the retention of labeled CO₂ and was assumed to be 0.81 (32, 43). The delay in excretion of the retained ¹³C label (Fig. 1A) meant that glucose oxidation was only calculated during the last 60 min of the protocol, as previously described (6).

By using the isotopic composition of the plasma glucose (R_{plu}), the percentage of plasma glucose coming from the ingested glucose (F_{ing}) and the oxidation rate of blood-borne glucose (Glu_{blood}) can be calculated as described (6), presented below:

\[
\text{F}_{\text{ing}} = \frac{[(\text{R}_{\text{plu}} - \text{R}_{\text{plu-bkg}})] / (\text{R}_{\text{plu}} - \text{R}_{\text{plu-bkg}})] \times 100}
\]

and

\[
\text{Glu}_{\text{blood}} = \text{V}_{\text{CO}_2} \times [(\text{R}_{\text{bkg}} - \text{R}_{\text{exp}}) / (\text{R}_{\text{bkg}} - \text{R}_{\text{plu}})] / (k \cdot c)
\]

where R_{plu-bkg} is the isotopic composition of plasma glucose at baseline, before ingestion of the labeled glucose solution. Glucose that was either directly, or via conversion to lactate, oxidized from glycogen in muscle was computed as the difference between the total amount of carbohydrate oxidized (from indirect calorimetry) and G_{blood} (Eq. 3). Also, the amount of glucose released from the liver that was oxidized was calculated as the difference between G_{blood} (Eq. 3) and G_{plu} (Eq. 1).

**Mass spectrometry.** Breath samples were analyzed by using an automated ¹³CO₂ breath-analysis system, BreathMat Plus (Thermo Finnigan, San Jose, CA). Briefly, from the 10-ml Vacutainer, 2 ml of the expired breath sample were introduced into the gas chromatograph. Gases not of interest (N₂ and O₂) as well as water were removed online via a continuous-flow diffusion pump. Pure CO₂ was introduced to the isotope ratio mass spectrometer (IRMS) and was analyzed at mass-to-charge ratios of 44/45. Samples were compared with a known (PDB-1) standard and expressed as % difference vs. the PDB-1 Chicago standard. Comparison of the BreathMat Plus analysis of three subjects’ breath samples against samples analyzed by use of a nonautomated IRMS, described previously (43), and using cryodistillation to trap pure CO₂ showed excellent agreement across a range of concentrations.
of $-24\%$ to $+45\%$ ($r = 0.998$, $P < 0.001$). The CVs for repeated samples ($n = 6$) at rest and at peak breath enrichment were $<0.5\%$.

The enrichment of glucose in plasma was measured by first deproteinizing 1 ml of plasma with 1.5 ml each of 0.3 N Ba(OH)$_2$ and ZnSO$_4$. Precipitated proteins were separated from the supernatant by centrifugation for 20 min (3,300 g at 4°C). The supernatant was then passed over ion-exchange resins to isolate glucose. The sample (~3 ml) was washed over 0.5-cm-radius disposable chromatography columns (Fisher Scientific, Markham, ON, Canada) with ~3 cm each, in series, of AG50W-X8-H$^+$ (200–400 mesh; Bio-Rad) and AG 1-X8-Cl$^-$ (200–400 mesh; Bio-Rad) ion-exchange resins that had been prewashed (7 ml) with 4 N HCl and washed repeatedly (until neutral by pH paper) with distilled-deionized water. The samples were applied to these resins and were eluted with distilled-deionized water (~10 ml). The eluant was collected in test tubes and were eluted with distilled-deionized water.

Breath and blood glucose. Breath glucose enrichments increased throughout the final hour of the sampling period (Fig. 1A) and were significantly higher Post vs. Pre (Pre = 34.8 ± 4.0 vs. Post = 27.3 ± 4.3‰ δ$^{13}$C PDB-1; Fig. 1A). Blood glucose enrichments were relatively constant during the final hour of the modified OGTT; however, they were significantly lower Post vs. Pre (Pre = 141.0 ± 4.2 vs. Post = 127.3 ± 5.9‰ δ$^{13}$C PDB-1; Fig. 1B). The percentage of blood glucose that was derived from ingested glucose was also significantly lower Post (69.9 ± 2.2%) than Pre (61.8 ± 1.7%; Fig. 1B).

Muscle metabolites. Frozen wet muscle samples (~10 mg) were freeze-dried, powdered, dissected free of nonmuscle elements, and stored at ~80°C. Aliquots of freeze-dried muscle were extracted with 0.5 M perchloric acid, neutralized with 2.2 M KHCO$_3$, and assayed for glycogen, ATP, phosphocreatine (PCr), and creatine by standard enzymatic methods (22, 41). All pre- and posttraining samples from a given subject were analyzed at the same time, and all metabolite measurements were adjusted to the highest total creatine value to account for differences in blood or connective tissue content between samples.

**Blood and urine analysis.** Plasma was assayed for insulin concentration by using a commercially available radioimmunoassay kit from Diagnostic Products (Los Angeles, CA). Intra-assay CV for this procedure was <5%.

Urineary urea was analyzed by using a commercially available end-point assay kit that used a urease hydrolysis of urea and the sodium nitroprusside procedure (no. 640, Sigma Diagnostics, St. Louis, MO). The intra-assay CV for this procedure was <6%.

**Statistical analysis.** Data were analyzed by use of Statistica (v. 6.0, Statsoft, Tulsa, OK). Variables for which only Pre and Post measures were taken were analyzed by paired t-tests. Variables in which time and training were factors were analyzed by a repeated-measures analysis of variance. When a significant F ratio was observed, post hoc analysis using Tukey’s test was performed to locate the differences. Significance was set at $P < 0.05$. Data are presented as means ± SE.

**RESULTS**

**Treadmill performance.** Subjects showed a substantial decrease in the externally supported proportion of their body weight from 65 ± 3 to 23 ± 5% ($P < 0.001$) after the training protocol. The participant-selected velocity at which subjects ambulated on the BWST also increased from 0.54 ± 0.11 to 1.27 ± 0.13 km/h ($P < 0.01$). The subjects’ time per training session increased from pre- to posttraining (Pre = 22.8 ± 3.8 min, Post = 35.3 ± 5 min, $P < 0.001$). Although it is not possible to quantify the contribution from the assistants while the subjects were on the treadmill, the performance changes we observed were substantial and not due to increased assistant-initiated support while our subjects were ambulating. In fact, all participants progressed during their training to the point that less assistance was necessary, with some showing improvements to the point that completely minimal or no assistance was required at all.

**Blood glucose and insulin.** Figure 2 shows that blood glucose concentration increased on ingestion of the glucose drink but that the pattern of change over time was affected by training (training × time interaction $P < 0.01$). Blood glucose concentrations were significantly ($P < 0.05$) lower at 20, 40, 60, 80, and 140 min during the protocol Post compared with Pre. Calculation of the cumulative AUC, which was calculated by using the trapezoid rule, of the glucose × time curve (Fig.
showed that training induced a 15 ± 4% (range 6–26%) reduction in this variable (Fig. 2, inset).

The blood insulin response following ingestion of 100 g of glucose mirrored the changes in blood glucose, in that there were significant reductions in insulin concentration at 120 and 180 min of the protocol Post vs. Pre (Fig. 3). The cumulative AUC for the insulin × time curve showed that training resulted in a 33 ± 8% (range 17–47%) reduction in this variable ($P < 0.01$). By multiplying the AUC for glucose and insulin, one can obtain an insulin sensitivity index, which in this case would be specific to our modified OGTT. The insulin sensitivity index was 44 ± 5% (range 27–50%) lower (data not shown) after training.

**Substrate and glucose oxidation.** Calculations from indirect calorimetry showed that there were no significant differences in substrate oxidation (Fig. 4A) Pre vs. Post. The amount of carbohydrate oxidized tended ($P = 0.092$) to be higher after training.

The fate of the ingested glucose in terms of oxidation is presented in Fig. 4B. We observed a greater oxidation of the ingested glucose as well as liver-derived glucose Post as opposed to Pre ($P < 0.05$). There was no effect of training on peripheral (i.e., glycogen-derived glucose) unit oxidation.

**Muscle GLUT-4 content and HK activity.** Training induced an increase in muscle GLUT-4 content (Fig. 5, A and B). The increase was uniform across all subjects and increased on average by 126 ± 34% (range 22–320%). Muscle HK activity also showed a training-induced increase (49 ± 4%) that was also seen in all subjects (Fig. 5C).

**Muscle metabolites.** Muscle glycogen content increased as a result of training by 80 ± 23% (range 10–185%; Fig. 6A). Resting muscle ATP content was also elevated (17%, $P = 0.060$) as a result of training. In addition, resting muscle PCr content also increased after training (27%, range 6–54%; $P = 0.002$).

**Body composition.** A separate portion of this study involved the subjects receiving a whole-body DXA scan to assess changes in body composition and bone mineral content and density. These scans were taken pretraining and after 12 mo of training. Given that the present study took biopsies before and after 6 mo of training, we are not reporting the body composition data here. However, for the purposes of clarification and...
to elucidate some of the mechanistic factors responsible for the changes in glucose tolerance, we report here that the body mass of the nine subjects that participated in this study was 75.7 ± 6.2 kg pretraining and 76.7 ± 6.5 kg after 12 mo of training (P = 0.48). Fat bone-free mass increased from 46.8 ± 2.0 to 48.8 ± 2.1 kg (P = 0.05), consistent with the hypertrophy we reported previously (46). Fat mass remained unchanged from 18.6 ± 2.3 kg at pretraining to 19.4 ± 2.4 kg after 12 mo of training (P = 0.91). From Pre to Post, subjects’ body weight remained unchanged (Pre = 75.5 ± 6.3 kg, Post = 75.7 ± 6.2 kg, P = 0.67).

**Discussion**

We report here that a program of BWST training in persons with incomplete SCI, which we reported resulted in muscle fiber hypertrophy (46), also improves glycemic regulation. We also showed not only that a greater fraction of glucose that was ingested was oxidized but that over the course of the training period the capacity for nonoxidative disposal of glucose (i.e., storage) was enhanced, as evidenced by increased muscle-specific HK activity and muscle GLUT-4 and glycogen content. Additionally, the training program brought about an increase in muscle PCR content.

Physical activity is a prime determinant of insulin-stimulated glucose uptake (1, 34, 35). Individuals with a complete SCI have been shown to have an increased insulin-stimulated glucose uptake as a result of programs of FES (27, 37). Therefore, it is not only voluntary activity that can induce positive changes in glycemic regulation (9, 25, 27, 37). The improved insulin sensitivity in persons with SCI who have undergone FES is most likely due to increases in muscle GLUT-4 content (9, 25, 37, present results) and possibly also HK activity (10, 28, present results) and other adaptations. It is also possible that an overall increase in muscle mass (i.e., hypertrophy of the stimulated muscles) would also account for some the increased glucose uptake (37), because muscle accounts for almost 85% of insulin-stimulated glucose disposal (12). However, the increases in muscle mass we observed (+4.3 ± 1.1% after 1 yr of training) are small relative to the changes in insulin-mediated glucose uptake (37), which makes it unlikely that the training-induced muscle mass increases are playing a large role in the increase in insulin-stimulated glucose uptake. A more likely explanation of why our subjects showed improved glucose regulation after training is likely due in part to an increase in insulin-responsive muscle GLUT-4 content (13, 34, 35) that we and others (9, 25, 37) have observed. In addition, changes in fat mass and body fat percentage were unremarkable after 1

**Fig. 5.** A: representative Western blot results for subjects 1–4 (S1–S4) for muscle GLUT-4 content Pre (all odd-numbered lanes) and Post (all even-numbered lanes). Mean and individual subject (superimposed lines) muscle GLUT-4 content (B) and muscle-specific hexokinase activity (C) both Pre and Post are also shown. Values are means ± SE (n = 9). *Significantly different (P < 0.05).

**Fig. 6.** Muscle glycogen (A), ATP (B), and phosphocreatine (PCR; C) content both Pre and Post. All values are in millimoles per kilogram dry weight (DW) of muscle. Values are means ± SE (n = 9). *Significantly different (P < 0.05).
yr of BWST training and hence do not appear to be playing a large role. We cannot rule out changes in regional adiposity that might be contributing in part to the changes in insulin sensitivity; however, given that total adiposity did not change after 1 yr of BWST training, we hypothesize that this too is of minor importance in the changes we observed after a much shorter period of BWST training.

We made all of our Post measurements, of glucose tolerance, 7 days after the subjects’ last BWST training session, which we believe means it is unlikely that the changes we observed are due to an acute bout of exercise. It is well documented that an acute effect of exercise in improving insulin sensitivity can extend for up to 48–72 h after the last exercise bout (reviewed in Ref. 23); hence, the changes we observed in GLUT-4 and HK activity and other muscle-based variables may have been influenced (because the biopsy was taken 3 days postexercise) by the last exercise bout. At the same time, because we performed our modified glucose tolerance test 7 days after the last exercise bout, we attribute the changes there to a chronic training-induced adaptation. In fact, it may be that, in taking our muscle biopsies at 3 days posttraining and conducting the modified OGTT at 7 days posttraining, we underestimated the true beneficial effects of the training protocol (40, 47). Previous studies have shown in highly trained athletes that a period of inactivity of as short as 6 days can reduce muscle GLUT-4 content (−17%; Ref. 47) and result in reduced insulin sensitivity (40, 47). However, taking relatively inactive men and training them with a mild exercise training program, it was shown that even after 7 days of inactivity glucose effectiveness and insulin sensitivity were still improved (38). Furthermore, it is not known how the insulin signaling pathways would change with training cessation (23). It is possible that the different time points postexercise for the two groups of variables means that the improved glycemic regulation seen at 7 days postexercise was less a result of increases in GLUT-4 and HK, which have a short half-life (40, 47), seen at 3 days postexercise and more to changes in insulin signaling pathways.

Another possible mechanism underlying the training-induced improvement in glucose regulation that we observed could be the muscle fiber transition from a greater percentage of IIa to IIx fibers that our laboratory has reported previously (46). In vivo, insulin action during euglycemic clamping has been shown to have a negative correlation with the percentage of IIx fibers (29). The correlation with fiber type and insulin action may have more to do with the GLUT-4 content of more oxidative fibers, which has been clearly demonstrated in rat muscle (33). There are only minor differences in human skeletal muscle GLUT-4 content between fibers, however (11, 18). Therefore, the activity level of the muscle fiber, as opposed to its myosin heavy chain content (i.e., fiber type), appears to play a greater role in determining the GLUT-4 content of the fiber (11). Consequently, our results of improved glucose tolerance may have more to do with an exercise-induced increase in expression of GLUT-4, rather than a fiber-type shift.

The main difference between the increases in GLUT-4 content that we observed here, compared with other reports (9, 25, 27, 37), is that our data are from persons with an incomplete (ASIA C) vs. complete (ASIA A) SCI. Additionally, the changes we report here were induced by voluntary neural input and not a local electrically delivered stimulus (37). Despite the differences in subject populations (i.e., complete vs. incomplete injuries), our increases in GLUT-4 content are similar to those induced by FES in some (9, 37) but not all studies (25). The increases in GLUT-4 content that we observed were associated with a marked reduction in AUC in both the glucose and insulin × time curves, resulting in an improvement in the insulin sensitivity index (AUC glucose × AUC insulin curves) in all subjects. Our findings demonstrate that a prolonged lack of voluntary neural input is not a determinant of the ability of skeletal muscle to respond to an contractile activity; others have reached a similar conclusion (9, 25, 28, 37). In fact, it has been recently demonstrated (5) that in response to electrical stimulation both able-bodied persons and persons with SCI show remarkably similar changes in mRNAs encoding markers known to be responsive to increased skeletal muscle loading. A possible change in voluntary activation of muscles as a result of BWST training cannot be assessed from the present or from our laboratory’s previous data (46); however, evidence suggests that BWST training can bring about changes in muscle activation patterns (for reviews, see Refs. 16, 17). Moreover, we have showed that BWST training can induce hypertrophy (46), which would have necessitated an increased loading of the limb skeletal muscle likely due to an increased voluntary muscle activation.

Our modified OGTT was used to assess handling of a glucose load as well as to trace the oxidative fate of the ingested glucose (6). We observed that the present program of BWST training was of sufficient intensity and duration to induce an increase in the rate of exogenous oxidation of ingested as well as liver-derived glucose, similar in nature (but not magnitude) to that seen when sedentary and trained subjects were compared (6). We also observed that nonoxidative disposal of glucose was improved as a result of the training, as evidenced by the increase in muscle glycogen content. It should be noted that this increase in glycogen occurred in the absence of any significant change in either energy or carbohydrate intake, which was assessed from 3-day diet records (data not shown). A number of studies have shown that programs of endurance training result in able-bodied persons having elevated muscle glycogen content (20, 44, 45), which has been shown to be due entirely to training-induced increases in GLUT-4 content and HK activity, because the differences in neither percent glycogen synthase 1 (i.e., active), plasma insulin, nor glucose explain this phenomenon (see Ref. 26 for review).

Overall, the changes in GLUT-4 content and HK activity, along with an increased muscle oxidative capacity that our laboratory has reported previously (46), indicate that BWST training produces a muscle phenotype that is compatible with an increased capacity for glucose uptake, phosphorylation, and subsequently oxidative disposal of glucose. Given that skeletal muscle plays such a large role in insulin-mediated glucose disposal (12), our interpretation is that BWST training induces a beneficial adaptation in terms of the capacity for glucose uptake in persons with SCI. None of the subjects in this study had what would be considered impaired fasting glucose levels (Fig. 2); however, some subjects (n = 3) did have blood glucose levels of >8.5 mM at later time points (i.e., 100 and 120 min) during the modified OGTT protocol. Because our protocol was not a conventional OGTT, it is difficult to interpret these observations compared with other data (9, 37).
The general observation of lower blood glucose and insulin would indicate that BWST training, if regularly performed, would result in reduced risk for Type 2 diabetes and/or metabolic syndromes that are associated with progression of Type 2 diabetes. Because all causes of morbidity and mortality in persons with SCI are similar to those seen in the able-bodied population (2–4, 14, 15), our findings are relevant for those with a SCI.

Evidence has shown that casting immobilization reduces muscle PCr (24, 30) and ATP (30) content. In addition, even periods of relative inactivity have been shown to reduce muscle PCr and ATP content (21). We observed that the 6 mo of BWST training resulted in an increase in resting muscle PCr content (Fig. 6, B and C). Although the ATP content of the muscle was not significantly increased at $P = 0.06$ (Fig. 6C), we believe that the nonsignificant increase in ATP is more than likely a type II error. To our knowledge, our data are the first report of reduced high-energy metabolites in skeletal muscle from humans with SCI. However, given that immobilization and inactivity both result in reduced muscle ATP and PCr content (21, 24, 30), our results are perhaps not unexpected. The increase in muscle PCr content may be due to increased contractile activity, possibly coupled with enhanced blood flow; the true significance of these changes remains to be elucidated.

Previously, “control” groups of able-bodied persons have been used to demonstrate the variability in certain measures such as muscle fiber phenotype (7, 8). However, it is not known whether the variability in muscle fiber size and type, as well as GLUT-4 content or HK activity, are the same in able-bodied as in persons with SCI. Hence, use of an able-bodied group as a control may not be entirely appropriate. For several reasons we did not include a control group but still believe that our results are real and have clinical significance. Our measures of change in muscle phenotype and glucose regulation greatly exceeded what is seen due to biological variability for these analyses. We have conducted a repeated-measures study in which our primary variables of interest showed a remarkably uniform pattern of change between subjects, which is a strong indication that our results are not due to a systematic error or, as one reviewer states, the result of a “spontaneous” postinjury evolution (uncontrolled time effect) that is independent of the training intervention. All of our subjects were at least 2 yr postinjury (Ref. 46; mean 8.1 ± 2.5 yr), a time when variables such as fiber size and muscle oxidative capacity reach a stable nadir (10, 36, 37). Given the strong link between muscle oxidative capacity and GLUT-4 content, we would also expect that variables such as muscle GLUT-4 content, HK activity, and glycemic regulation would also be at a stable nadir in all of our subjects; hence, a spontaneous (and yet consistent) increase in the variables we measured (i.e., GLUT-4, HK activity, glycemic regulation) seems unlikely. Additionally, many of the muscle phenotypic changes that we observed with our voluntary form of exercise are consistent with what has been observed with involuntary electrically evoked contractions in persons with complete SCI (10, 36, 37). Although it may have been desirable to have a group of sex- and age-matched control subjects who also had spinal cord injuries of similar type (i.e., lesion level, duration, severity, and residual muscle spasticity), it would have been exceptionally difficult to match subjects on the basis of these variables, all of which could have had an impact on many of the outcome measures we documented.

Six months of thrice-weekly BWST training in persons with incomplete SCI resulted in improved glycemic regulation and changes in the handling of an oral glucose load, specifically increased glucose oxidation and storage. Muscle capacity for insulin-mediated glucose transport and phosphorylation were increased as a result of training. In addition, training resulted in an increased content of ATP and PCr. The improved glycemic control brought about as a result of this training regime could possibly confer a long-term benefit in terms of reducing the higher incidence of Type 2 diabetes and metabolic syndrome in people with a SCI.

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