Prevention of Glycogen Supercompensation Prolongs the Increase in Muscle GLUT4 After Exercise

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Running Title: Posttranscriptional regulation of GLUT4 expression

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ABSTRACT

Exercise induces an increase in GLUT4 in skeletal muscle with a proportional increase in glucose transport capacity. This adaptation results in enhanced glycogen accumulation, i.e. “supercompensation”, in response to carbohydrate feeding after glycogen depleting exercise. The increase in GLUT4 reverses within 40h after exercise in carbohydrate fed rats. The purpose of this study was to determine if prevention of skeletal muscle glycogen supercompensation after exercise results in maintenance of the increases in GLUT4 and the capacity for glycogen supercompensation. Rats were exercised by means of three daily bouts of swimming. GLUT4 mRNA was increased ~3-fold and GLUT4 protein was increased ~2-fold 18h in epitrochlearis muscle after exercise. These increases in GLUT4 mRNA and protein reversed completely within 42h after exercise in rats fed a high carbohydrate diet. In contrast, the increases in GLUT4 protein, insulin-stimulated glucose transport and increased capacity for glycogen supercompensation persisted unchanged for 66h, in rats fed a carbohydrate-free diet that prevented glycogen supercompensation after exercise. GLUT4 mRNA was still elevated at 42h, but had returned to baseline by 66h, after exercise in rats fed the carbohydrate-free diet. Glycogen-depleted rats fed carbohydrate 66h after exercise underwent muscle glycogen supercompensation with concomitant reversal of the increase in GLUT4. These findings provide evidence that prevention of glycogen supercompensation after exercise results in persistence of exercise-induced increases in GLUT4 protein and enhanced capacity for glycogen supercompensation.

Keywords: carbohydrate feeding; insulin responsiveness; muscle glucose transport
INTRODUCTION

Exercise training induces an increase in the GLUT4 isoform of the glucose transporter in skeletal muscle (11, 13, 14, 40). Insulin- and contraction-stimulated glucose transport increase in proportion to the increase in GLUT4 (29, 40), resulting in marked potentiation of the rate and magnitude of muscle glycogen accumulation, above the normal fed level, in response to carbohydrate feeding after exercise (15, 34). This post-exercise glycogen “supercompensation” phenomenon is self-limiting, because the rapid influx of glucose into muscle causes inhibition of insulin- and contraction-stimulated glucose transport, via the glucose toxicity type of insulin resistance (20, 25, 27-30, 42). Another factor involved in the rapid reversal of the exercise training-induced increase in muscle glucose transport capacity is the return of GLUT4 protein content to the pretraining level within 40h after exercise in carbohydrate fed animals (26).

Previous studies have provided evidence suggesting that prevention of glycogen supercompensation, by not feeding carbohydrate after exercise, results in persistence of an increase in insulin-stimulated glucose transport (5, 9, 46). This raises the possibility that, after glycogen-depleting exercise, muscle cells maintain the adaptations that make possible faster and greater glycogen accumulation until glycogen accumulation actually occurs. Exercise induces an increase in muscle insulin sensitivity (12, 41). It is not clear from available data whether the persistent increase in insulin action in the carbohydrate-depleted state after exercise is due to a persistent increase in insulin sensitivity (5) or also involves enhanced insulin responsiveness mediated by maintenance of the increase in GLUT4. In this context, the present study was undertaken to determine whether persistent increases in GLUT4 and insulin responsiveness are involved in a mechanism that enables trained, glycogen-depleted muscles to maintain their capacity for enhanced glycogen accumulation.
MATERIALS AND METHODS

**Materials.** 2-[1,2-$^3$H]-Deoxy-D-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO), and mannitol, D-[1-$^{14}$C] was obtained from NEN Life Science Products (Boston, MA). Purified porcine insulin (Iletin II) was purchased from Eli Lilly. A polyclonal antibody specific for the GLUT-4 glucose transporter was the generous gift of Dr. Mike Mueckler (Washington University, St. Louis). Horseradish peroxidase-conjugated donkey anti-rabbit IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). A mouse anti-human cytochrome oxidase subunit I (COXI) monoclonal antibody was purchased from Molecular Probes (Eugene, OR, USA). Reagents for enhanced chemiluminescence were obtained from Amersham (Arlington Heights, IL). All other reagents were obtained from Sigma Chemical (St. Louis, MO).

**Animal care.** This research was approved by the Animal Studies Committee of Washington University School of Medicine. Male Wistar rats (body wt 165-185 g) were obtained from Charles Rivers Laboratories, housed in individual cages and fed a diet of Purina rodent laboratory chow and water ad libitum. Animals were randomly assigned to either an exercise group or a sedentary control group. Rats in the exercise group were accustomed to swimming for 10 min/day for 2 days. They were then exercised on 3 successive days using a swimming protocol, described previously (34, 40) that involves two 3-h-long swimming sessions separated by a 45-min-long rest period during which the rats are kept warm and given food and water. After completion of the swimming on the 3rd day, food was withheld from one group of exercised animals, and the remaining exercised animals were fed either chow or lard ad libitum. The exercised, fasted rats and groups of exercised, high carbohydrate diet and sedentary animals were sacrificed 18h after the last exercise bout. Groups of chow-fed (high carbohydrate diet) and lard-fed (carbohydrate free diet) rats were sacrificed 42h post exercise. Additional groups of carbohydrate free diet rats were studied either 66h after exercise or 18h after being switched from a lard to a chow diet 66h after the last bout of exercise. The animals were anesthetized
with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt), and the epitrochlearis and triceps muscles were dissected out.

Muscle incubations. Epitrochlearis muscles were incubated with shaking for 60 min at 30°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) in Erlenmeyer flasks gassed continuously with 95% O₂-5% CO₂. The epitrochlearis is a small, thin muscle of the forelimb that, in rats of the size used in this study, is suitable for measurement of sugar transport in vitro (21, 45). The KHB was supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% radioimmunoassay-grade BSA, in the presence or absence of 2 mU/ml purified porcine insulin. This concentration of insulin maximally activates glucose transport in this muscle preparation (43). To remove glucose, muscles were then washed for 10 min at 30°C, in KHB containing 40 mM mannitol, 0.1% BSA, plus insulin if it was present in the previous incubation, and used for measurement of glucose transport activity.

Measurement of glucose transport activity. Glucose transport activity was measured by using the glucose analog 2-DG as described previously (16, 45). After the wash, epitrochlearis muscles were incubated at 30°C for 20 min in 1.0 ml KHB containing 4 mM [1,2-³H]-2-DG (1.5 µCi/ml), 36 mM [¹⁴C]mannitol (0.2 µCi/ml), 0.1% BSA, and insulin if present in previous incubations. Extracellular space and intracellular 2-DG concentration were determined as previously described (45).

Analytical methods. Glycogen was measured in perchloric acid extracts of the epitrochlearis muscle using the amyloglucosidase method (38). Plasma glucose concentrations were determined using the glucose oxidase method, with a Beckman Glucose Analyzer II (Beckman Instrument, Fullerton, CA). Plasma insulin was measured by radioimmunoassay. Serum free fatty acid concentration was measured using a kit (Wako, NEFA C –ACS-ACOD Method).

Western Blot Analysis. Epitrochlearis muscle GLUT-4 content was determined by Western blotting as described previously (18) using a rabbit polyclonal antibody directed against the
COOH terminus of GLUT-4, followed by horseradish peroxidase-conjugated anti-rabbit immunoglobulin G. Antibody-bound transporter protein was visualized using enhanced chemiluminescence. For evaluation of COX1 protein content, triceps muscle was homogenized in a buffer containing 20 mM HEPES, 1 mM EDTA, and 250 mM sucrose, pH 7.4. Protein content was measured using bicinchoninic acid (Pierce). Aliquots of homogenate were solubilized in Laemmli sample buffer and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked in PBS containing 5% nonfat dry milk. Blots were probed with a monoclonal antibody against COXI. Blots were then incubated with anti-mouse immunoglobulin G conjugated to horseradish peroxidase. Antibody bound protein was detected by ECL.

**Competitive RT-PCR.** The GLUT4 RNA competitors used in our assays were generated using the streamlined PCR-based approach described by Moller and Edvinsson (32, 33), which uses commercially available kits for most steps of the procedure. In the first round of PCR, a fragment representing the native GLUT4 mRNA was generated by conventional RT-PCR, purified on a gel and eluted using an extraction kit (Quianx II). The purified first round product was subjected to a second round of PCR, using a hybrid forward primer, that results in a product in which the forward and reverse primer sites are ~100 bp closer to each other, i.e. a ~100 bp deletion. The forward and reverse primers and the hybrid forward competitor primers used in this study are listed below. The competitor fragment was cloned into a PGEM-T Easy vector containing T7 promoters. After linearization of the plasmid and Klenow treatment, sense-competitor RNA was transcribed in vitro using a Riboprobe kit (Promega), and the resulting RNA copies were purified from the plasmid template by DNase 1 digestion. The identity of the RNA and extent of the deletion were verified by sequencing.

GLUT4   D84345  
Forward  5'GTGTGGTCAATACCGTCTTCACG3'  
Reverse  5'CCATTTTGCCCTCAGTCATTCC3'
Competitor Forward 5’GTGTGGTCAATACCGTCTTCACGATCTTGATGACGGTGGCTCTGC3’

Total RNA was isolated from ~20 mg of triceps muscle by the method of Chomczynski and Sacchi (6) and suspended in DEPC-treated H$_2$O containing 0.1 mM EDTA. For RNA quantization by competitive RT-PCR, RT reactions were performed with a constant amount of tissue mRNA and different amounts of the competitor mRNA. Once optimal initial concentrations of tissue mRNA and competitor mRNA were established final RT-PCR was performed. The RT involves the use of the Reverse Transcription System (Promega). Aliquots of each RT reaction were added to a PCR Master Mix (Promega) mixture containing Taq DNA Polymerase, dNTPs, MgCl$_2$, reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR and 10 pmol of both sense and antisense primers. The reaction medium was subjected to PCR amplification. After warming the lid at 105°C and 120 s at 94°C, the PCR mixtures were subjected to 35 cycles of PCR amplification with a cycle profile, including denaturation for 60 s at 94°C, hybridization for 60 s at 56.5°C, and elongation for 60 s at 72°C.

The PCR products were separated by electrophoresis on 2% agarose, stained with ethidium bromide, photographed and analyzed by densitometry. The ratio of sample to competitor band densities was then calculated.

Statistics. Values are expressed as means ± SE. Statistically significant differences were determined using ANOVA. When ANOVA showed significant differences, the Student-Newman-Keuls post hoc test was performed to discern statistical by significant differences.
RESULTS

*Muscle GLUT4 protein.* As in previous studies (26, 29, 30, 40) the exercise induced a ~2-fold increase in GLUT4 protein in epitrochlearis muscles (Fig. 1). In keeping with previous results (26), this adaptive increase in GLUT4 protein reversed completely within 42h after cessation of exercise in the high carbohydrate diet fed animals (Fig. 1). In contrast, the increase in GLUT4 protein was still present 66h after exercise in muscles of rats fed the carbohydrate-free diet after exercise. The increase in GLUT4 reversed completely within 18h after rats that were fed the carbohydrate free diet for 66h after exercise were switched to the high carbohydrate diet (Fig. 1).

*Glucose transport activity.* The increase in glucose transport activity in response to a maximal insulin stimulus was ~2-fold higher in epitrochlearis muscles of the exercised rats 18h after the last bout of exercise than in muscles of sedentary, fasted animals (Fig. 2). This enhancement of insulin-stimulated glucose transport activity had reversed completely 42h after exercise in muscles of rats fed the high carbohydrate diet. These findings confirm the results of previous studies (26, 29, 30). In contrast, the ~2-fold greater increase in glucose transport activity in response to insulin was still present 66h following exercise in epitrochlearis muscles of rats fed the carbohydrate-free diet (Fig. 2). The persistence of the increases in GLUT4 protein and insulin-stimulated 2DG transport were closely correlated (Fig. 2).

*Muscle glycogen concentration.* Epitrochlearis muscle glycogen concentration in rats fed the high carbohydrate diet for 18h after exercise was increased ~2-fold above that found in the high carbohydrate diet fed sedentary control animals (Fig. 3). This increase in muscle glycogen persisted unchanged 42h after exercise. The post-exercise increase in muscle glycogen was prevented by feeding the rats the carbohydrate-free diet for 42 or 66h after exercise. One of the hypotheses that we were testing in this study was that the exercise-induced increase in the ability of muscle to accumulate glycogen persists until the glycogen supercompensation actually occurs. This hypothesis appears to be correct, as the increase in
epitrochlearis muscle glycogen was as great in rats maintained in the glycogen-depleted state for 66h and then fed the high carbohydrate diet as in muscles of rats fed the high carbohydrate diet for 18h immediately after exercise (Fig. 3). This increase in glycogen was associated with a decrease in GLUT4 protein back to the control, sedentary level (Fig. 1).

**GLUT4 mRNA.** The increase in muscle GLUT4 protein induced by exercise is preceded, and probably mediated, by an increase in GLUT4 mRNA (40). In the present study, GLUT4 mRNA was increased ~3-fold 18h after exercise. This increase in GLUT4 mRNA reversed completely between 18h and 42h after exercise in the high carbohydrate diet rats (Fig. 4). GLUT4 mRNA concentration decreased less rapidly in the carbohydrate free diet group and was still elevated 42h post exercise in the animals fed the carbohydrate free diet. However, between 42h and 66h after exercise, GLUT4 mRNA returned to the baseline, control level despite prevention of glycogen supercompensation by the carbohydrate free diet.

**Cytochrome oxidase subunit I.** The exercise program used in this study also induces increases in the expression of mitochondrial proteins (2). COXI, which was used as a mitochondrial marker protein, was increased 18h after last exercise bout (Fig. 5). There was no reversal of the increase in COXI protein in muscles of rats fed carbohydrate for 42h beginning shortly after the exercise or for 18h beginning 66h post exercise. This is in contrast to the rapid reversal of the increase in GLUT4 in response to carbohydrate feeding and muscle glycogen supercompensation.
DISCUSSION

Exercise has three major effects on muscle glucose uptake (22). The first is an insulin independent stimulation of glucose transport (23, 44) mediated by translocation of GLUT4 to the cell surface (8). The second is an increase in insulin sensitivity that develops in the immediate post exercise period as the acute stimulation of glucose transport wears off (12, 41). The third is an adaptive increase in GLUT4 (11, 13, 40), with a proportional increase in insulin responsiveness (22, 40), that occurs after a prolonged bout of exercise. This adaptive increase in GLUT4 results in markedly enhanced muscle glycogen accumulation in response to carbohydrate feeding after glycogen depleting exercise (15, 34). The increase in GLUT4 expression reverses rapidly after the increase in muscle glycogen occurs (26). The results of previous studies have suggested that prevention of glycogen supercompensation following glycogen depleting exercise results in persistence of an exercise-induced increase in insulin action on muscle glucose transport (5, 9, 46).

In this context, we tested the hypothesis that muscles maintain their capacity for increased glycogen accumulation after exercise for as long as glycogen supercompensation is prevented after glycogen depleting exercise. Our findings show that this hypothesis is correct. They provide evidence that maintenance of the capacity for glycogen supercompensation is mediated by persistence of the adaptive increase in GLUT4. As in our previous study (26), the exercise-induced increase in muscle GLUT4 expression reversed completely within 42h after exercise in rats fed a high carbohydrate diet that resulted in glycogen supercompensation. In contrast, the increases in GLUT4, insulin responsiveness, and increased capacity for glycogen accumulation persisted unchanged for 66h, the longest period studied, in muscles of rats fed a carbohydrate-free diet that prevented glycogen supercompensation after exercise. Feeding the glycogen depleted rats carbohydrate 66h after exercise resulted in muscle glycogen supercompensation similar in magnitude to that observed in the animals fed chow immediately after exercise. Concomitantly, muscle GLUT4 protein concentration returned to the baseline,
control level, providing evidence that rapid glucose influx and/or glycogen accumulation are involved in mediating reversal of the exercise-induced increase in GLUT4.

Research interest in the exercise-induced increase in muscle GLUT4 is currently focused on the role that this adaptation can play in ameliorating insulin resistance associated with type 2 diabetes and obesity. However, it seems improbable that countering of insulin resistance is the biological function that led to the evolutionary selection of this adaptive response, as obesity and diabetes are extremely rare under natural conditions, i.e. in wild animals. On the other hand, muscle glycogen is necessary for strenuous exercise, and depletion of glycogen stores results in fatigue that makes vigorous exercise impossible (1, 3, 7). Therefore, rapid muscle glycogen repletion can be essential for survival in a fight or flight situation that requires prolonged, vigorous exercise. The major factor limiting glycogen accumulation in muscle appears to be the rate of glucose uptake (10, 17, 39, 40). Muscle glucose uptake is limited by both the availability of a high carbohydrate diet and muscle glucose transport activity. The rate of glucose transport into muscle is limited by glucose transport capacity, which is normally proportional to muscle GLUT4 content (19, 31, 40).

In this context, the rapid increase in GLUT4 expression induced by exercise could provide a survival advantage during prolonged flight or fight situations by making possible faster and greater glycogen repletion between exercise bouts or during intervals of less intense exercise. The rapid reversal of the exercise-induced increase in GLUT4 in response to carbohydrate feeding and glycogen supercompensation could serve to prevent glycogen accumulation to the point that it causes muscle stiffness and impaired function. The present results show that additional adaptations are present in glycogen depleted muscles that prevent this rapid decrease in GLUT4 protein and, thus, keep the muscles poised for rapid glycogen supercompensation until carbohydrate becomes available.
The increase in GLUT4 expression is a component of an adaptive response to endurance exercise that also involves an increase in mitochondrial biogenesis (2). The signals responsible for this adaptive response appear to be the increases in cytosolic Ca\(^{2+}\), leading to activation of Ca\(^{2+}\)-calmodulin dependent protein kinases, and the decrease in high energy phosphates, leading to activation of AMP kinase, during contractile activity (24, 35-37, 47). Following cessation of exercise, the time course of the reversal of the adaptive increase in mitochondrial proteins appears to be determined by the half-lives of the proteins which, for respiratory chain and citrate cycle enzymes, appears to be ~8 days (4). Thus, the adaptive increase in mitochondrial enzymes is gradually lost over a period of about one month after cessation of training in rats fed a chow diet (4). In the present study, switching rats from a carbohydrate-free to a high carbohydrate diet that resulted in glycogen supercompensation 66h after exercise had no effect on COXI protein level in muscle. In contrast, in rats fed carbohydrate, the increase in GLUT4 protein reverses completely within 40h (26). The decrease in muscle GLUT4 protein in glycogen depleted rats fed carbohydrate 66h after exercise was even more rapid, occurring within 18h. Two possible explanations for this rapid reversal may be that the GLUT4 protein normally has a short half-life, and that rapid glucose influx and glycogen synthesis may be associated with the specific activation of GLUT4 proteolysis.

Our finding in the present study that no decrease in GLUT4 protein occurred over a 66 hr period after exercise in glycogen depleted muscles could, theoretically, be due to either a persistent increase in GLUT4 protein synthesis or an inhibition of GLUT4 proteolysis. Our results suggest that both of these processes may have played a role. Muscle GLUT4 mRNA content had returned to the baseline control value 42h after exercise in the high carbohydrate diet fed rats. However, in the animals fed a carbohydrate free diet the persistent decrease in muscle glycogen was associated with maintenance of an increase in GLUT4 mRNA that was still evident 42h after exercise. Thus, the persistent increase in GLUT4 mRNA could have
played a role in the maintenance of an exercise-induced increase in GLUT4 protein synthesis. However, an increase in GLUT4 protein content of the glycogen-depleted muscle was still present 66h after exercise, by which time GLUT4 mRNA had returned to the sedentary control level. This is in contrast to the decline in both GLUT4 mRNA and GLUT4 protein to the sedentary control levels between 18h and 42h after exercise in the carbohydrate fed rats. It, therefore, seems possible that inhibition of GLUT4 proteolysis also played a role in the persistent increase in GLUT4 protein in the glycogen-depleted muscles.

In the high carbohydrate diet fed animals, GLUT4 protein was still elevated 18h after the onset of muscle glycogen repletion, while in the rats maintained in the glycogen depleted state, muscle GLUT4 protein content fell to the sedentary control level within 18h after the start of glycogen repletion. This difference may be explained by the finding that GLUT4 mRNA was elevated during glycogen repletion starting immediately after exercise, whereas GLUT4 mRNA had fallen to the sedentary control level at the time glycogen repletion was initiated in the rats maintained for 66h in the glycogen depleted state.

In conclusion, the results of this study provide evidence that prevention of glycogen supercompensation after exercise results in persistence of the increased capacity for muscle glycogen accumulation after exercise for at least three days. The mechanism responsible for this phenomenon appears to be a prolongation of the exercise-induced increase in muscle GLUT4 protein.
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REFERENCES


FIGURE LEGENDS

Figure 1. Effects of 3 daily bouts of exercise followed by a high carbohydrate diet or a carbohydrate-free diet on epitrochlearis muscle GLUT4 protein content. Rats were exercised by means of swimming on 3 consecutive days. Following the last bout of exercise, groups of rats were either fasted, fed a high carbohydrate or fed a carbohydrate-free (lard) diet for the times indicated. One group was switched to a high carbohydrate diet after eating the carbohydrate free diet for 66h. Epitrochlearis muscle GLUT4 content was determined by Western blot analysis. Values are means ± SE for 6 to 10 muscles per group. *P<0.05 versus Sedentary, Hi Carb 42h, and Carb Free 66h + Hi Carb 18h.

Figure 2. Insulin-induced increase in glucose transport activity parallels GLUT4 content in epitrochlearis muscles. Rats were exercised on 3 consecutive days followed by either fasting, a high carbohydrate diet or a carbohydrate-free diet for the times indicated. Epitrochlearis muscles were incubated with or without 2 mU/ml insulin for 60 min followed by measurement of 2DG transport as described in “Materials and Methods”. Epitrochlearis muscle GLUT4 protein content was evaluated by Western blot analysis. Values are means ± SE for 8 to 12 muscles per group. *P<0.05 versus Sedentary and Hi Carb 42h groups.

Figure 3. Muscle glycogen concentrations. Epitrochlearis muscle glycogen concentrations were measured in the same groups, described in the legend for Figure 1., in which muscle GLUT4 content was determined. Values are means ± SE for 8 to 10 muscles per group. *P<0.05 versus Sedentary, Fasted 18h, Carb Free 42h and Carb Free 66h †P<0.05 versus Fasted 18h, Carb Free 42h and Carb Free 66h.
**Figure 4.** Epitrochlearis muscle GLUT4 mRNA. GLUT4 mRNA was measured, in epitrochlearis muscles of the groups of rats described in Figure 1, using competitive RT-PCR as described under Materials and Methods. Values are means ± SE for 5 to 7 muscles. *P<0.05 versus Sedentary, Hi Carb 42h, Carb Free 66h, and Carb Free 66h + Hi Carb 18h.

**Figure 5.** Cytochrome oxidase subunit I protein (COXI). Rats were exercised on 3 consecutive days. COXI protein content of epitrochlearis muscles was determined by Western blot analysis in muscles of the Sedentary, Fasted 18h, Hi Carb 42h, Carb Free 42h and Carb Free 66h + Hi Carb 18h groups. Values are means ± SE for 4 to 6 muscles per group. *P<0.05 vs. all other groups.
FIGURE 1

GLUT4 protein (arbitrary units)

Exercise

Sedentary  
Fasted 18h  
Hi Carb 18h  
Hi Carb 42h  
CarbFree 42h  
Carb Free 66h  
Carb Free 66h + Hi Carb 18h

* *
FIGURE 2

Epitrochlearis Muscle 2-DG Transport (µmol * ml⁻¹ * 20min⁻¹)

- 2-Deoxyglucose transport
- GLUT4 protein

Exercise

Sedentary | Fasted 18h | Hi Carb 42h | Carb Free 66h

* Indicates significance
FIGURE 3

Muscle glycogen (µmol/g)

- Sedentary
- Fasted 18h
- Hi Carb 18h
- Hi Carb 42h
- Carb Free 42h
- Carb Free 66h
- Carb Free 66h + Hi Carb 18h

Exercise
FIGURE 4

GLUT4 mRNA (arbitrary units)

Sedentary  Fasted 18h  Hi Carb 18h  Hi Carb 42h  Carb Free 42h  Carb Free 66h  Carb Free 66h + Hi Carb 18h

Exercise
FIGURE 5

COX subunit 1 protein (arbitrary units)

- Sedentary
- Fasted 18h
- Hi Carb 42h
- Carb Free 42h
- Carb Free 66h + Hi Carb 18h

Exercise