Research Report

# Effects of Aerobic Exercise on Energy Metabolism in the Hypertensive Rat Heart

Background and Purpose. In order to explore the possible effects of physical therapy interventions on patients with hypertension, we evaluated the effects of aerobic exercise training on myocardial energy metabolism in an animal model of hypertension. Subjects. We used 36 female spontaneously hypertensive rats (rats with genetically induced hypertension) and 12 normotensive Wistar-Kyoto rats. Methods. The normotensive rats were sedentary and formed the CONsed group. The spontaneously hypertensive rats were randomly divided into 3 experimental groups (12 rats per group). Hypertensive rats that were sedentary formed the HTNsed group, those that received 8 weeks of exercise training formed the HTN×8 group, and those that received 16 weeks of exercise training formed the HTN×16 group. We measured systolic blood pressure, heart wet weight, maximal activities of cardiac energy metabolism enzymes, glucose transporter content, and total concentrations of protein, glycogen, and triglyceride. Results. Systolic blood pressure was greater than 200 mm Hg in the CONsed group at the time of testing. Exercise training modestly (~11–18 mm Hg) lowered blood pressure in the HTN×8 and HTN×16 groups. Fatty acid enzyme activity was greater in the CONsed group than in the HTNsed and HTN×8 groups, but activity was roughly equivalent between the CONsed group and the HTN×16 group. Glucose enzyme activity was greater in the HTN×16 group than in the CONsed group and the HTNsed group. Intracellular glycogen concentration was greater in the HTN×8 group than in the HTNsed group. Discussion and Conclusion. Results of this study suggest that aerobic exercise training may help to normalize cardiac energy metabolism in mammals with hypertension. [Kinney LaPier TL, Rodnick KJ. Effects of aerobic exercise on energy metabolism in the hypertensive rat heart. *Phys Ther.* 2001;81:1006–1017.]

**Key Words:** *Exercise, Heart, Hypertension, Spontaneously hypertensive rats.*

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Hypertension is a major health problem in the United States, affecting approximately 44% to 65% of the population over the age of 50 years.[1] Hypertension is a risk factor for the development of atherosclerosis and the subsequent sequelae of peripheral vascular disease, coronary artery disease, cerebrovascular disease, nephropathy, and retinopathy.[2,3] Hypertension can also induce left ventricular hypertrophy, which is a risk factor for cardiac ischemia, myocardial infarction, arrhythmia, sudden death, ventricular dysfunction, and congestive heart failure.[4–6] Physical therapists commonly examine, evaluate, and treat patients with these hypertension-related conditions. Another important role for the physical therapist is the primary prevention of impairments and functional limitations in patients with hypertension.

Hypertension, by means of pressure overload, stimulates adaptations in cardiac morphology, energy metabolism, and function.[4,7] With hypertension, pressure overload can produce concentric cardiac hypertrophy in which increases in ventricular mass are out of proportion to increases in chamber volume.[4] Cardiac hypertrophy can induce a shift in energy substrate preference that may contribute to reduced myocyte adenosene triphosphate (ATP) levels and to impaired myocardial function, with subsequent progression to heart failure.[7] Normally, myocytes utilize primarily fatty acids in the production of high-energy phosphates.[7]

Hypertension with concomitant cardiac hypertrophy alters myocyte energy substrate preference from predominantly fatty acids to glucose.[7–10] The uptake and use of glucose is elevated and the uptake and use of fatty acids is diminished in hypertrophied hearts of animals and humans.[7–11] With hypertension, left ventricular glycolytic enzyme activities increase and oxidative enzyme activities decrease.[11–15] These changes in cardiac energy metabolism with concentric cardiac hypertrophy may be related to reductions in coronary blood flow secondary to decreased capillary density.[15]

Aerobic exercise, by means of volume overload, also stimulates adaptations in cardiac morphology, energy metabolism, and function.[4,16–21] With aerobic exercise, volume overload can produce eccentric cardiac hypertrophy in which increases in ventricular mass are proportional to increases in chamber volume.[4] Cardiac glycogen stores and glucose uptake have been shown to increase with aerobic exercises.[16–18] Aerobic exercises

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generally do not alter cardiac glycolytic or oxidative enzyme systems.[16,17,19] Exercise-induced cardiac hypertrophy is associated with improved cardiac function especially during maximal workloads.[20,21] Normal capacity for myocardial blood flow is maintained with exercise-induced cardiac hypertrophy, but the mechanisms behind this adaptation are unclear.[19,20,22] Therefore, although both hypertension and exercise produce overload stimuli that induce cardiac hypertrophy, the adaptations in cardiac energy metabolism, function, and perfusion differ.

Despite reports on the modest (~10–20 mm Hg) blood pressure-lowering effects of aerobic exercise training,[23] little is known about the effects of exercise on energy metabolism in the hypertensive heart.[21] Although aerobic exercise is not usually associated with adaptations in cardiac energy metabolism in normotensive hearts,[16,17,19] the exercises may attenuate some of the metabolic changes that occur in hypertensive hearts. Therefore, the purpose of our study was to evaluate the effects of aerobic exercise on myocardial energy metabolism in an animal model of a hypertensive heart. We hypothesized that indexes of cardiac energy metabolism in exercise-trained rats with hypertension would be more like those of rats without hypertension. In this study, we used an animal model of hypertension because of the invasive nature of studying cardiac metabolism. The spontaneously hypertensive rat is a genetic strain of rats that develop high blood pressure without experimental manipulation.

## Methods

To evaluate the effects of hypertension on myocardial energy metabolism, we measured enzyme activities, glucose transporter (GLUT) content, and intracellular substrate stores in spontaneously hypertensive rats. To characterize myocardial energy metabolism, we used enzymatic markers of glycolysis (hexokinase [HK]), aerobic metabolism (citrate synthase [CS]), and fatty acid oxidation (carnitine O-palmitoyltransferase [CPT] and 3-hydroxyacyl-coenzyme A dehydrogenase [HOAD]). Hexokinase is a cytosolic enzyme that catalyzes the phosphorylation of glucose upon entry into the cell, and CS is a mitochondrial enzyme involved in the tricarboxylic acid cycle. CPT and HOAD are mitochondrial enzymes that participate in the transportation of fatty acids through the mitochondrial membrane and in the beta oxidation of fatty acids in the mitochondrial matrix, respectively.[24] In addition, GLUT content was measured as an indirect index of the capacity for glucose uptake and subsequent utilization. The GLUT 1 isoform is found in the cell membrane under basal conditions and is noninsulin regulatable. Conversely, the GLUT 4 isoform is found in submembranous vesicles under resting conditions and is inserted into the cell membrane in response to insulin or contractile stimulation.[25] Lastly, we measured intracellular glycogen and triglyceride stores as indirect indicators of the potential for uptake, storage, or utilization of glucose and free fatty acids, respectively. Figure 1 illustrates the major components of the metabolic pathways involved in myocyte ATP production.[26]

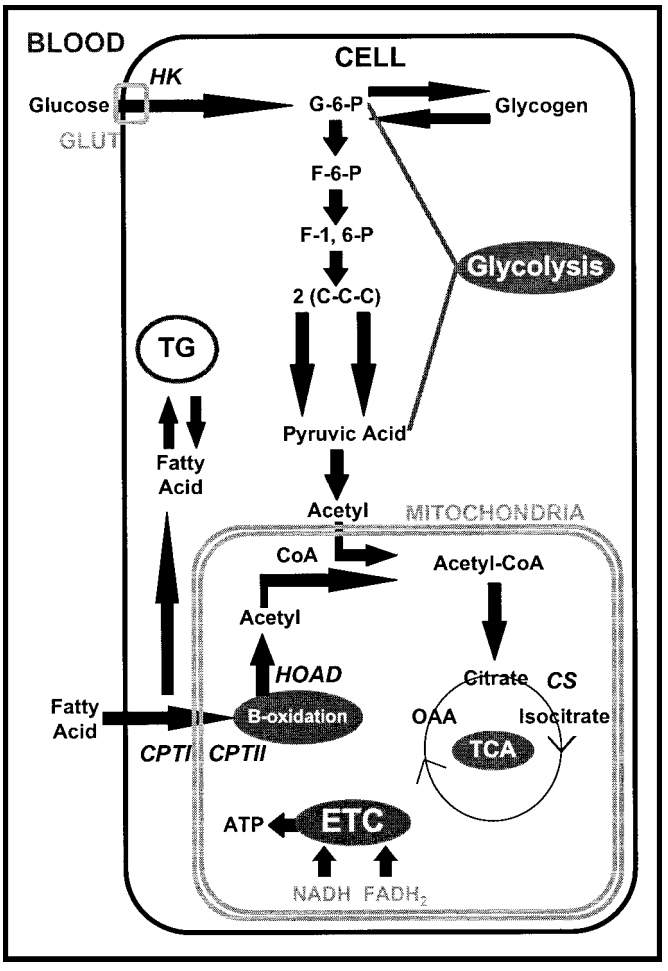


**Figure 1.**
Pathways involved in myocyte energy production. G=glucose; P=phosphate; C=carbon; CPT=carnitine O-palmitoyltransferase*; CS=citrate synthase*; NADH/$FADH_2$=electron carriers; ETC=electron transport chain; F=fructose; GLUT=glucose transporter*; HK=hexokinase*; HOAD=3-hydroxyacyl-coenzyme A dehydrogenase*; OAA=oxalacetic acid; TCA=tricarboxylic acid cycle; and TG=triglyceride.* *=measured in this study.

### *Experimental Animals and Treatments*

We purchased 12 female normotensive Wistar-Kyoto rats and 36 female spontaneously hypertensive rats at 4 weeks of age.* After 1 week to acclimate to the Animal Research Facility at Idaho State University (Pocatello, Idaho), we placed the Wistar-Kyoto rats in a sedentary control group (CONsed group) and randomly assigned

* Taconic Farms Inc, 273 Hover Ave, Germantown, NY 12526.

the spontaneously hypertensive rats to 1 of 3 groups (n=12 per group). Hypertensive rats that were sedentary formed the HTNsed group, those that received 8 weeks of exercise training formed the HTN×8 group, and those that received 16 weeks of exercise training formed the HTN×16 group. To ensure that all animals were the same age at the time the measurements were made, the HTN×8 group began exercising at 14 weeks of age, and the HTN×16 group began exercising at 6 weeks of age.

Animals were maintained at 22±2°C with a fixed 12-hour light-dark cycle (lights on from 7:00 AM to 7:00 PM). Animals had free access to food (Teklad 22/5 Rodent Diet #8640)[†] and tap water at all times. We housed the sedentary rats individually in metal hanging cages (28 × 21 × 19 cm) and the exercising rats in exercise wheel cages that were modified so that the rats remained in the wheel at all times and had continuous access to food and water.[27] All exercising rats ran voluntarily for the 8- or 16-week period. Total running distance was recorded from a revolution counter attached to the wheel axle and is expressed in meters per day. We weighed all rats and recorded values to the nearest gram at least once a week.

### *Blood Pressure Measurement*

We measured systolic blood pressure noninvasively in conscious resting animals using the tail-cuff method. The validity of measurements obtained with this method has been established previously.[28] The blood pressure measurement system consisted of a electrosphygmograph (Model 29 amplifier),[‡] a sensor (Model B60 [⅜-in and 7⁄16-in]),[‡] and a flatbed recorder (Model 45L),[‡] which housed channels for pressure and pulse. We took blood pressure measurements at the same time of day (between 10:00 AM and 2:00 PM) once a week to allow the animals to become accustomed to the tail-cuff procedure. Each day, we calibrated the blood pressure measurement system before use with an aneroid sphygmomanometer. Prior to blood pressure measurements, the rats were placed in acrylic holders (Model 82 and 83)[‡] and maintained at 28°C for 30 minutes. On each testing occasion, we took 2 to 4 blood pressure measurements on each animal. By the week before terminal experiments, the rats remained fairly motionless in the holders during the tail-cuff procedure, and we were able to obtain stable blood pressure readings. We measured blood pressure on 3 separate days during the week prior to terminal experiments, averaged these readings, and reported them as the final blood pressure value.

### *Tissue Collection*

When the rats were 22 weeks of age, we anesthetized them with an intraperitoneal injection of sodium pentobarbital (50 mg/kg of body weight), rapidly excised the heart, and rinsed it in ice-cold isotonic saline (0.9% weight per volume) to remove intraluminal blood. We then sectioned the heart into the right ventricular free wall, the interventricular septum, and the left ventricular free wall. Total ventricular weight was calculated as the sum of the weight of the interventricular septum and left ventricular free wall. Sections were blotted dry, weighed, clamped frozen with aluminum tongs at the temperature of liquid nitrogen, wrapped in aluminum foil, and stored at −70°C. Relative ventricular mass was calculated by dividing total ventricular, left ventricular free wall, interventricular septum, or right ventricular free wall mass by body weight. A portion of the left ventricle was not frozen and was immediately assayed for CPT activity. The soleus and plantaris muscles were also removed, weighed, clamped frozen, and stored at −70°C.

### *Determination of Maximal Enzyme Activities*

For each assay, frozen samples of the left ventricular free wall (~50–100 mg) were weighed and homogenized in 9 volumes of ice-cold extraction buffer (pH=7.4) using motor-driven ground-glass homogenizers (Duall Type 22)[§] except where noted. We measured enzyme activities spectrophotometrically (Lamda 6 UV/VIS)[‖] for 5 minutes under saturating substrate and cofactor concentrations. All samples were maintained at 25°C by a thermostatically controlled recirculating water bath.[#] For all assays, we zeroed measurements to a blank cuvette, and total assay volume was 1.0 mL. We conducted biochemical assays in duplicate or triplicate and then averaged the values. In each case, enzyme activities are expressed as micromoles of substrate converted to product per minute per gram of tissue wet weight. In all cases, we purchased analytical grade enzymes and biochemicals.**

The activity of HOAD (Enzyme Commission [EC] 1.1.1.35) was measured in left ventricular whole homogenates diluted 1:20 (weight per volume) in extraction buffer, consisting of 40 mmol 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).[29] The reaction mixture consisted of 40 mmol HEPES, 1 mmol of ethylenediaminetetraacetic acid (EDTA), 1 mmol potassium cyanide (KCN), 0.15 mmol NADH (the reduced form of nicotinamide adenine dinucleotide), and 0.1 mmol acetoacetyl-coenzyme A (pH=7.4). The reaction was initiated by adding 10 μL of ventricular homoge-

[†] Harlan Teklad, PO Box 44220, Madison, WI 53744-4220.
[‡] IITC Inc, 23924 Victory Blvd, Woodland Hills, CA 91367-1253.
[§] Kontes Glass Co, 537 Crystal Ave, Vineland, NJ 08360.
[‖] Perkin-Elmer Instruments, 761 Main Ave, Norwal, CT 06859-0001.
[#] NESLAB Instruments, 25 Nimble Hill Rd, Newington, NH 03801.
** Sigma Chemical Co, 6050 Spruce St, St Louis, MO 63103.

nate, and the change in absorbance was recorded at a wavelength of 340 nm.

Total CPT (EC 2.3.1.21) and CPT I activity (the portion of total CPT inhibited by malonyl-coenzyme A) was measured in mitochondrial fractions of left ventricular homogenates.[30] We homogenized tissue[§] in 9 volumes of ice-cold 20 mmol HEPES, 250 mmol sucrose, 1 mmol ethylene glycol-bis[2-aminoethyl ether]-N,N,N′,N′-tetraacetic acid (EGTA), and 10 mg/mL bovine serum albumin (BSA). Homogenates were kept ice-cold during processing. To isolate mitochondria, we centrifuged (Model MR 22I)[††] the whole homogenate at 3,000*g* for 1 minute, extracted the supernatant, centrifuged the supernatant at 20,000*g* for 1 minute, and discarded the supernatant. Next, we resuspended the mitochondrial pellet in 1 mL of 20 mmol HEPES, 300 mmol sucrose, 1 mmol EGTA, and 1% BSA (pH=7.4). We centrifuged the resuspended pellet at 20,000*g* for 1 minute, discarded the supernatant, and resuspended the pellet in 4.5 volumes of 20 mmol HEPES, 300 mmol sucrose, and 1 mmol EGTA (pH=7.4). The reaction mixture consisted of 220 mmol sucrose, 40 mmol potassium chloride (KCl), 20 mmol HEPES, 1 mmol EGTA, 0.13% BSA, and 0.1 mmol 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (pH=7.2). To 0.9 mL of the reaction mixture, we added 40 μL of the sample and 20 μL of water or 0.5 mmol malonyl-coenzyme A (final concentration 10 μmol) to measure total CPT and CPT II activity, respectively. We incubated this at 25°C for 5 minutes, added 20 μL of 2 mmol palmitoyl-coenzyme A (16:0) (final concentration 40 μmol), and measured baseline enzyme activity for 5 minutes. Next, we added 20 μL of 50 mmol carnitine (final concentration 1 mmol) and the change in absorbance was recorded at a wavelength of 412 nm. CPT I activity was calculated as the difference between total CPT and CPT II activities. We measured total mitochondrial protein content in these samples as described below.

The activity of CS (EC 4.1.3.7) was measured in left ventricular and plantaris muscle whole homogenates diluted 1:200 (weight per volume) in extraction buffer of 20 mmol HEPES and 1 mmol EGTA.[31] The reaction mixture consisted of 20 mmol HEPES, 1 mmol EGTA, 220 mmol sucrose, 40 mmol KCl, 0.1 mmol DTNB, 0.05 mmol acetyl-coenzyme A (pH=8.0). The homogenates were taken through a freeze-thaw cycle to disrupt mitochondrial membranes, and then 10 μL was added to each cuvette. The reaction was initiated by adding 50 μL of 2 mmol oxaloacetic acid (50 μmol final concentration) to the cuvette and the change in absorbance was recorded at a wavelength of 412 nm.

[††] Jouan Inc, 170 Marcel Dr, Winchester, VA 22602-4843.

The activity of HK (EC 2.7.1.1) was measured in left ventricular whole homogenates diluted 1:20 (weight per volume) in extraction buffer. The extraction buffer consisted of 40 mmol HEPES, 1 mmol EDTA, 2 mmol magnesium chloride ($MgCl_2$), 2 mmol dithiothreitol (DTT).[32] The reaction mixture consisted of 40 mmol HEPES, 0.8 mmol EDTA, 7.5 mmol $MgCl_2$, 1.5 mmol KCl, 2.5 mmol ATP (2 Na), 10 mmol creatine phosphate (2 Na), 0.9 international units (IU)/mL creatine phosphokinase (from rabbit muscle), 0.7 IU/mL glucose-6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*), and 0.4 mmol β-nicotinamide adenine dinucleotide phosphate (NADP) (pH=7.4). Next, 20 μL of ventricular homogenate was added to the cuvette. The reaction was initiated by adding 0.1 mL of 10 mmol D-glucose (1.0 mmol final concentration), and the change in absorbance was recorded at a wavelength of 340 nm.

### *Measurement of GLUT 1 and GLUT 4 Content*

We homogenized frozen samples (~25–50 mg) of the left ventricular free wall in 9 volumes of ice-cold filtered (0.22 μmol) hydroxyethyl starch (HES) buffer (pH=7.4), which contained 20 mmol HEPES, 1 mmol EDTA, and 250 mmol sucrose, using motor-driven ground-glass homogenizers. We diluted homogenates (~40 μg of protein) in filtered (0.22 μmol) Laemmli buffer containing 2% sodium dodecyl sulfate (SDS). Next, we electrophoresed (Mini-PROTEAN II Electrophoresis Cell)[‡‡] these samples on 4%-polyacrylamide stacker and 10%-polyacrylamide resolving gels at 200 V for 40 minutes. Each sample was run in duplicate on different gels for both GLUT 1 and GLUT 4 protein determination. The apparent molecular weights of GLUTs were confirmed from the mobility of a prestained molecular weight marker (Fumarase: 60,800 $M_r$) in an adjacent lane of each gel. We then electrophoretically transferred[‡‡] the proteins at 300 mA for 60 minutes onto to polyvinylidene fluoride (PVDF) microporous membrane (Immobilon-P Transfer Membrane).[§§]

The transfer buffer contained 20% (volume per volume) methanol, 192 mmol glycine, and 25 mmol Trizma base (pH=8.3). After transfer, PVDF membranes were blocked overnight at 4°C with 5% nonfat dry milk in filtered (0.22 μmol) phosphate-buffered saline (PBS) containing 0.02% sodium azide (pH=7.4).

We performed immunoblotting using polyclonal rabbit antibodies against either GLUT 1 (lot A970212)[‖‖] or

[‡‡] Bio-Rad Laboratories, 1000 Alfred Nobel Dr, Hercules, CA 94547.
[§§] Millipore Corp, 80 Ashby Rd, Bedford, MA 01730.
[‖‖] Biogenesis Ltd, 7 New Fields, Stinsford Rd, Poole, England, BH17 0NF, United Kingdom.

GLUT 4 protein (RALRGT, lot 819/4299).[##] The antibodies were generated by immunizing rabbits with synthetic peptides of the carboxyl-terminal end of the GLUT proteins. We washed the PVDF membranes 3 times for 15 minutes in PBS containing 1% Triton X-100 after removal from the blocking agent and after both incubation steps that follow. We performed Western blotting by incubating the PVDF membranes for 1 hour at room temperature (~22°–24°C) in anti–GLUT 1 serum (diluted 1:1,000) or anti–GLUT 4 serum (diluted 1:1,500) in filtered (0.22 μmol) PBS containing 1% powdered milk. After washing, we then incubated the PVDF membranes for 1 hour at room temperature in blotting grade goat anti-rabbit IgG (H + L) horseradish peroxidase conjugate (170-6515)[‡‡] diluted in PBS containing 0.1% BSA in ratios of 1:1,800 and 1:9,000 for GLUT 1 and GLUT 4 determination, respectively. After the final washing, we exposed the PVDF membranes to enhanced chemiluminescence detection reagents*** for 1 minute and then to Hyperfilm (Hyperfilm-MP)*** for 20 to 90 seconds.

We analyzed the autoradiographs containing the GLUT 1 and GLUT 4 blots by scanning densitometry (Gel Pro Analyzer 2.0).[†††] We expressed GLUT protein content relative to left ventricular muscle samples from a Sprague-Dawley rat (arbitrarily set at 1.0) run on each gel. The intragel and intergel variability for this technique were approximately 6% and 30%, respectively.

### *Determination of Total Protein, Glycogen, and Triglyceride Concentration*

We measured cardiac muscle concentrations of total protein, glycogen, and triglyceride spectrophotometrically using a regression curve developed from known concentrations of standards. According to the manufacturer, reliability and validity of the measurements is acceptable if the instructions in the kit are followed.** All assays were conducted at room temperature. For all assays, we zeroed measurements to a blank cuvette. We conducted biochemical assays in duplicate and then averaged the values. In each case, values are expressed as concentrations per tissue wet weight.

We measured total protein concentration on the same left ventricular homogenates that were used for determination of GLUT content diluted 1:200 (volume per volume) in water. To quantify total protein concentration, we added 25 μL of sample or protein standard (BSA) to 1 mL of bicinchoninic acid solution (Sigma Procedure No. TPRO-562). After incubation for 30 minutes at 50°C, we recorded absorbance at a wavelength of 562 nm.

We weighed and, using motor-driven ground-glass homogenizers,[§] homogenized frozen samples of the interventricular septum (~50–75 mg) in 5 volumes of ice-cold 0.03 N hydrochloric acid (HCl). Homogenates were then incubated[‡‡‡] for 5 minutes at 100°C, diluted 1:3 (volume per volume) in 1.0 N HCl, and incubated again for 4 hours at 100°C.[33] Following acid hydrolysis of glycogen, we measured the resulting glucose concentration enzymatically by adding 20 μL of homogenate or glucose standard to 2 mL of Trinder Reagent (Sigma Procedure No. 315). After incubation for 18 minutes at room temperature, we recorded absorbance at a wavelength of 505 nm.

First, we isolated triglyceride using a procedure based on the methods described by Folch et al[34] and Carr et al.[35] We weighed and, using motor-driven ground-glass homogenizers,[§] homogenized frozen samples of the left ventricular free wall and interventricular septum (~75–90 mg) in 30 volumes of ice-cold 2:1 (volume per volume) chloroform-methanol. Next, we centrifuged (Adams Analytical Centrifuge No. 0151)[§§§] samples at 1,300*g* for 1 minute, removed the supernatant, and mixed the supernatant with 1 ml of 0.6% (weight per volume) sodium chloride (NaCl). We then centrifuged this mixture at 1,300*g* for 1 minute, discarded the supernatant, and measured the total volume of the remaining lower phase. We added 1 mL of the lower phase or triglyceride standard (triolein) to 1 mL of 1% Triton X-100 solution (volume per volume diluted in chloroform) and dried this mixture under nitrogen gas[‡‡‡] at 45°C for 10 to 15 minutes. After drying, we added 0.5 mL of water to each tube, capped the tube, and placed it in a reciprocally shaking water bath[‖‖‖] at 50 cycles/min for 30 minutes at 37°C. At this point, samples were frozen for up to 3 days at −70°C prior to measurement of triglyceride concentration.

We measured triglyceride content enzymatically by adding 10 μL of sample or standard to 1 mL of Triglyceride INT Reagent (Sigma Procedure No. 336). After incubation for 30 minutes at room temperature, we recorded absorbance at a wavelength of 500 nm.

### *Statistical Analysis*

All values are expressed as means ± standard deviation. All statistical analyses were performed with n=12 for all groups except the analyses of CPT activity (n=7) and triglyceride concentration (n=11). We used a 1 × 4

[##] Charles River PharmServices, PO Box 727, Southbridge, MA 01550.
*** Amersham International, Amersham Pl, Little Chalfont, Buckinghamshire, England, HP7 9NA United Kingdom.
[†††] Media Cybernetics, 8484 Georgia Ave, Ste 200, Silver Spring, MD 20990.
[‡‡‡] Fisher Scientific, 2000 Park Ln, Pittsburgh, PA 15275.
[§§§] Clay Adams, Div of Becton, Dickinson and Co, Parsippany, NJ 07054.
[‖‖‖] Haake Fisons, 53 W Century Rd, Paramus, NJ 07652.

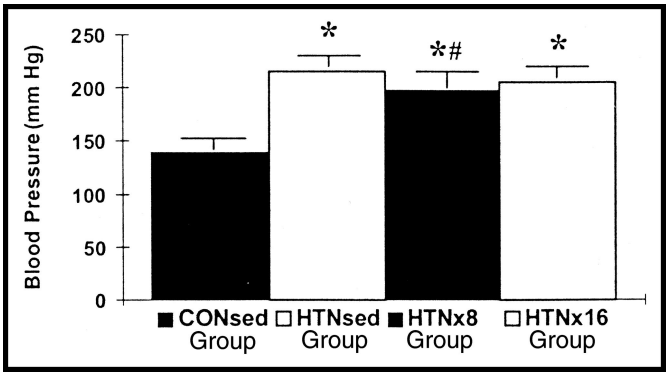


**Figure 2.**
Systolic blood pressure in the CONsed group (Wistar-Kyoto rats that were normotensive and sedentary), HTNsed group (spontaneously hypertensive rats that were sedentary), HTN×8 group (spontaneously hypertensive rats that received 8 weeks of exercise training), and HTN×16 group (spontaneously hypertensive rats that received 16 weeks of exercise training). Values are means ± SD. *Significant difference from the CONsed group. #Significant difference from the HTN×16 group.

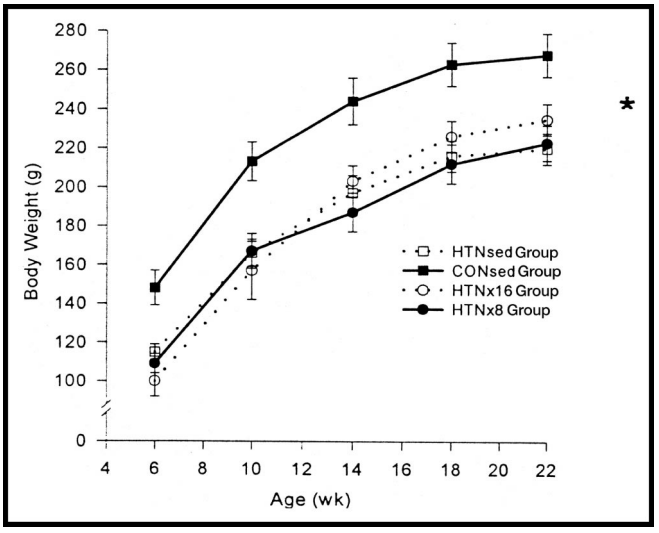


**Figure 3.**
Body weights of the CONsed group (Wistar-Kyoto rats that were normotensive and sedentary), HTNsed group (spontaneously hypertensive rats that were sedentary), HTN×8 group (spontaneously hypertensive rats that received 8 weeks of exercise training), and HTN×16 group (spontaneously hypertensive rats that received 16 weeks of exercise training). Values are means ± SD. *Statistical differences were found among all groups except between the HTNsed and HTN×8 groups.

single-factor analysis of variance (ANOVA) to examine differences among the CONsed, HTNsed, HTN×8, and HTN×16 groups for most of the variables measured. When differences were found, we performed Tukey *post hoc* tests to further analyze differences in group means. We used an unpaired *t* test to compare running distances between the HTN×8 and HTN×16 groups. We also used unpaired *t* tests to determine whether soleus muscle wet weight and plantaris muscle CS activity were greater in the spontaneously hypertensive rats that received exercise than in the spontaneously hypertensive rats that were sedentary. The alpha level was set at .05 for all analyses. We performed all statistical procedures using Excel for Windows 95 version 7.0.[###]

## Results

Blood pressure and body weight data are presented in Figures 2 and 3, respectively. Blood pressure was lower in the CONsed group (139±12 mm Hg) than in the HTNsed group (216±13 mm Hg), the HTN×8 group (198±22 mm Hg), or the HTN×16 group (205±10 mm Hg). Furthermore, blood pressure was lower in the HTN×8 group than in the HTNsed group. The body weights for the CONsed group were greater than those for all experimental groups, and the HTN×16 group had slightly higher body weights than the HTNsed and HTN×8 groups. The progression of running activity in the HTN×8 and HTN×16 groups is illustrated in Figure 4. Running distance averaged across all weeks (7,260±1,832 m/day for the HTN×8 group, 6,514±1,451 m/day for the HTN×16 group) was not different between groups.

[###] Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399.

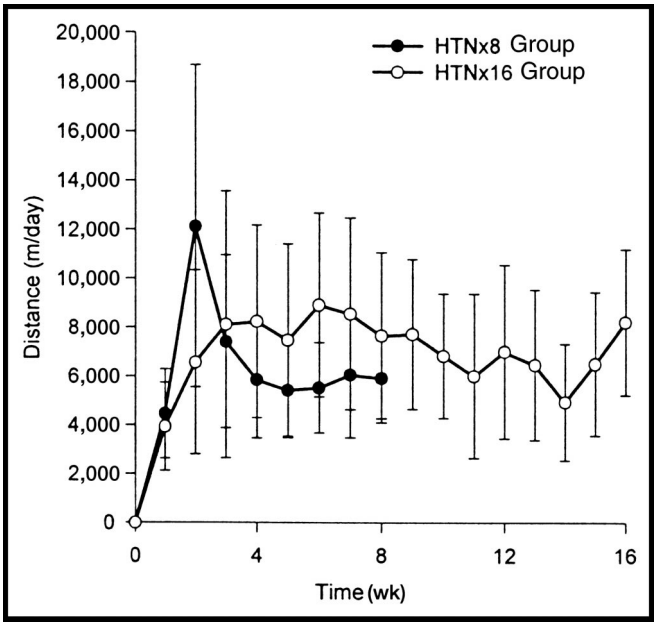


**Figure 4.**
Running distances of the HTN×8 group (spontaneously hypertensive rats that received 8 weeks of exercise training) and the HTN×16 group (spontaneously hypertensive rats that received 16 weeks of exercise training) throughout the experimental period. No significant differences were found between groups for values averaged across all weeks. Values are means ± SD.

Heart wet weights expressed relative to body weights are listed in Table 1. We found that relative total and left ventricular weights were different among all groups except the groups receiving exercise (HTN×8 and

**Table 1.**
Relative Heart Wet Weights (Tissue Wet Weight:Body Weight [in Grams]) in the CONsed Group (Wistar-Kyoto Rats That Were Normotensive and Sedentary), HTNsed Group (Spontaneously Hypertensive Rats That Were Sedentary), HTN×8 Group (Spontaneously Hypertensive Rats That Received 8 Weeks of Exercise Training), and HTN×16 Group (Spontaneously Hypertensive Rats That Received 16 Weeks of Exercise Training)[a]

| Heart Section | CONsed Group | | HTNsed Group | | HTN×8 Group | | HTN×16 Group | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| TV ($\times 10^{-3}$) | 2.85 | 0.48 | 3.99 | 0.17[b] | 4.38 | 0.44[b,c] | 4.48 | 0.18[b,c] |
| LV ($\times 10^{-3}$) | 1.38 | 0.29 | 2.01 | 0.23[b] | 2.19 | 0.33[b,c] | 2.30 | 0.20[b,c] |
| IVS ($\times 10^{-3}$) | 0.96 | 0.16 | 1.31 | 0.15[b] | 1.35 | 0.21[b] | 1.33 | 0.16[b] |
| RV ($\times 10^{-3}$) | 0.71 | 0.46 | 0.67 | 0.14 | 0.83 | 0.09 | 0.85 | 0.12 |

[a] TV=total ventricular weight, LV=left ventricle, IVS=interventricular septum, RV=right ventricle.
[b] Significant difference from CONsed group (F[3,11], $\alpha$=.05).
[c] Significant difference from HTNsed group (F[3,11], $\alpha$=.05).

**Table 2.**
Left Ventricular Enzyme Activities (in Arbitrary Units [U]) for the CONsed Group (Wistar-Kyoto Rats That Were Normotensive and Sedentary), HTNsed Group (Spontaneously Hypertensive Rats That Were Sedentary), HTN×8 Group (Spontaneously Hypertensive Rats That Received 8 Weeks of Exercise Training), and HTN×16 Group (Spontaneously Hypertensive Rats That Received 16 Weeks of Exercise Training)[a]

| | CONsed Group | | HTNsed Group | | HTN×8 Group | | HTN×16 Group | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| HOAD (U/g) | 16.2 | 1.6 | 15.5 | 2.4 | 15.3 | 2.0 | 16.3 | 1.3 |
| CPT total (mU/g) | 51.7 | 7.7 | 32.8 | 5.0[b] | 27.6 | 6.4[b] | 35.0 | 5.1[b] |
| CPT I (mU/g) | 35.3 | 5.6 | 24.7 | 5.6[b] | 22.2 | 4.8[b] | 31.0 | 3.5[d] |
| CS (U/g) | 105.1 | 7.5 | 103.2 | 10.0 | 104.3 | 8.7 | 97.8 | 9.7 |
| HK (U/g) | 3.3 | 0.4 | 3.4 | 0.5 | 3.7 | 0.5 | 4.0 | 0.4[b,c] |

[a] HOAD=3-hydroxyacyl-coenzyme A dehydrogenase, CPT=carnitine O-palmitoyltransferase, CS=citrate synthase, HK=hexokinase.
[b] Significant difference from CONsed group (F[3,11], $a$=.05).
[c] Significant difference from HTNsed group (F[3,11], $a$=.05).
[d] Significant difference from HTN×8 group (F[3,11], $a$=.05).

HTN×16 groups). Relative total and left ventricular weights were greater in the HTNsed group than in the CONsed group and greater in both groups receiving exercise (HTN×8 and HTN×16 groups) than in groups that were sedentary (CONsed group and HTNsed group). Relative interventricular septal weight was greater in all experimental groups than in the CONsed group. We found no difference in relative right ventricular weight among groups.

Skeletal muscle characteristics of the experimental groups, which were used as indexes of an exercise effect, included soleus muscle wet weight and CS activity in the plantaris muscle. Soleus muscle wet weight was greater in the HTN×8 group (0.181±0.013 g) and the HTN×16 group (0.221±0.028 g) than in the HTNsed group (0.167±0.013 g). Plantaris muscle CS activity was greater in the HTN×16 group (27.1±7.4 arbitary units [U]/g), but not in the HTN×8 group (21.5±3.6 U/g), as compared with the HTNsed group (20.5±3.8 U/g).

Maximal activities for cardiac muscle HOAD, CPT, CS, and HK for all groups are presented in Table 2. We found no differences in HOAD activity among groups. Total CPT activity was greater in the CONsed group than in all of the experimental groups, and no differences were found among the experimental groups. CPT I activity was higher in the CONsed group than in the HTNsed and HTN×8 groups. We did not find a difference in CPT I activity between the CONsed group and the HTN×16 group. Furthermore, we found greater CPT I activity in the HTN×16 group compared with the HTN×8 group. We found similar results when CPT activity was expressed per milligram of mitochondrial protein. We found no difference in CS activity among groups. The HTN×16 group demonstrated greater HK activity than both sedentary groups (the CONsed and HTNsed groups).

Cardiac muscle GLUT 1 and GLUT 4 protein content for all groups is shown in Figure 5. We found no differences in GLUT 1 or GLUT 4 protein content among all 4 groups.

Cardiac muscle total protein, glycogen, and triglyceride concentrations for all groups are presented in Table 3. We found no differences in total protein or intracellular triglyceride concentration among the groups. Intracellular glycogen concentration was greater in the HTN×8 group than in the HTNsed group. In Table 4,

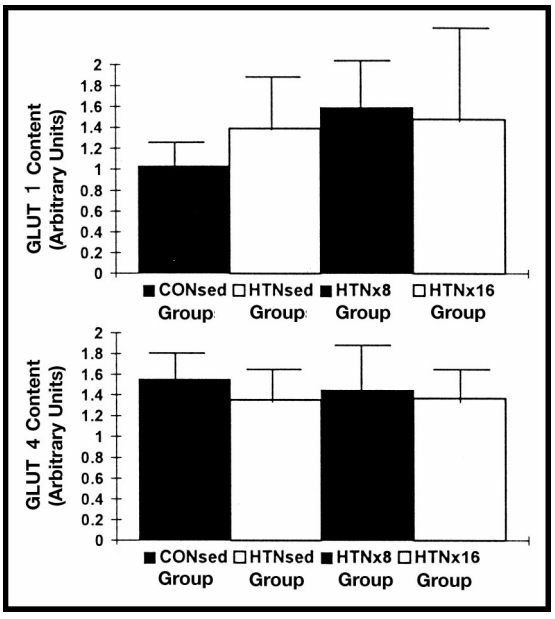


**Figure 5.**
Cardiac muscle GLUT 1 and GLUT 4 protein content for the CONsed group (Wistar-Kyoto rats that were normotensive and sedentary), HTNsed group (spontaneously hypertensive rats that were sedentary), HTN×8 group (spontaneously hypertensive rats that received 8 weeks of exercise training), and HTN×16 group (spontaneously hypertensive rats that received 16 weeks of exercise training). No significant differences were found. Values are means ± SD.

we summarize the statistical results of this study for all variables measured and analyzed.

## Discussion

We found that total CPT and CPT I activity in the left ventricle were reduced in the sedentary hypertensive group (HTNsed group) compared with the normotensive sedentary group (CONsed group), suggesting impaired fatty acid oxidation in the hypertensive heart. Because CPT I is considered to be the rate-limiting step in mitochondrial oxidation of long-chain fatty acids, it may be a particularly good indicator of cardiac fatty acid oxidative capacity.[36] We are not aware of other studies that have quantified CPT activity in hypertensive hearts, but reduced activity of other enzymes involved in fatty acid oxidation has been reported.[11–13]

The results of our study indicate that, with hypertension, exercise training may increase fatty acid oxidation and enhance glucose utilization in the left heart relative to sedentary normotensive controls. We found that CPT I activity in the spontaneously hypertensive rats that ran for 16 weeks, but not in the spontaneously hypertensive rats that ran for 8 weeks, was closer to that found in rats without hypertension. This suggested to us that exercise training may attenuate the impaired ability of hypertensive hearts to oxidize fatty acids. We also found that HK activity was greater in the spontaneously hypertensive rats that ran for 16 weeks (HTN×16 group), but not the spontaneously hypertensive rats that ran for 8 weeks (HTN×8 group), than in both sedentary groups. This may indicate an enhanced cardiac glucose uptake capacity with exercise training despite similar GLUT 1 and 4 content, because glucose phosphorylation may be a rate-limiting step in glucose metabolism.[37] Both cardiac adaptations in CPT I and HK activity were found only in the group that began running at an earlier age and ran for a longer period of time, indicating that a threshold for exercise training duration or initiation age may exist for altering cardiac metabolism in hypertension. Surprisingly, we found that intracellular glycogen content was elevated in the left ventricle after 8 weeks of exercise training but not after 16 weeks of exercise training in spontaneously hypertensive rats. Other researchers have found similar elevations in cardiac glycogen content after 2 to 8 weeks of exercise training.[16,18] We believe it is possible that increased cardiac glycogen storage did not occur in the rats that ran for 16 weeks, because the exercise training stimulus in this situation produced adaptations that increased capacity for fatty acid oxidation, such as greater CPT I activity or myocardial blood flow.[19,20] Because HOAD and CS activity were similar among groups but CPT I activity increased, there appears to be selective regulation of mitochondrial enzyme expression with exercise training.

With voluntary running, we found greater relative total and left ventricular wet weights in female spontaneously hypertensive rats. Both hypertension and exercise training are known to produce cardiac enlargement.[21,22] We contend that our results suggest that the overload stimuli produced by hypertension and exercise are additive, because the relative cardiac weight of the spontaneously hypertensive rats that exercised was greater than that for the spontaneously hypertensive rats that were sedentary, which, in turn, was greater than that for the control rats that were normotensive.

We found that longer durations of exercise training resulted in greater increases in relative cardiac wet weight, suggesting that the degree of hypertrophy is related to the magnitude of the total overload stimulus. Although not quantified in this study, the hypertrophy produced by hypertension and exercise training are not thought to be morphologically equivalent. With hypertension, increases in ventricular mass are out of proportion to increases in chamber volume, whereas, with aerobic exercise training, increases in ventricular mass are proportional to increases in chamber volume.[4] Research that examines cardiac morphology, especially the wall thickness:ventricular diameter ratio, in response to concomitant hypertension and chronic exercise is needed to understand the changes in cardiac mass that we have documented.

**Table 3.**
Cardiac Muscle Total Protein, Glycogen, and Triglyceride Concentrations for the CONsed Group (Wistar-Kyoto Rats That Were Normotensive and Sedentary), HTNsed Group (Spontaneously Hypertensive Rats That Were Sedentary), HTN×8 Group (Spontaneously Hypertensive Rats That Received 8 Weeks of Exercise Training), and HTN×16 Group (Spontaneously Hypertensive Rats That Received 16 Weeks of Exercise Training)

| | CONsed Group | | HTNsed Group | | HTN×8 Group | | HTN×16 Group | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| Total protein (mg/g) | 173.4 | 10.5 | 177.9 | 11.8 | 178.1 | 11.4 | 175.1 | 11.0 |
| Glycogen ($\mu$mol/g) | 15.5 | 6.0 | 10.0 | 4.3 | 16.8 | 7.1[a] | 12.6 | 5.2 |
| Triglyceride (mg/g) | 2.8 | 0.7 | 3.1 | 0.7 | 2.7 | 0.8 | 2.4 | 0.7 |

[a] Significant difference from the HTNsed group ($F[3,11]$), $\alpha$=.05).

We believe that the exercise stimulus in our study was of an appropriate mode (walking/running), frequency (7 days/week), duration (8 and 16 weeks), and total load (~7,000 m/day) to produce adaptations in cardiac metabolic energy systems. Voluntary wheel running is frequently used as a model to evaluate chronic adaptation of exercise training in rats because noxious stimuli are unnecessary, the running pattern is relatively natural, and food and water are available during exercise.[27] The daily running distances that we recorded were similar to those previously reported for female spontaneously hypertensive rats, female Dahl salt-sensitive rats, and male Sprague-Dawley rats.[27,38,39] Overton et al[38] found that resting heart rate was reduced and maximum oxygen consumption was increased in wheel-running versus sedentary female spontaneously hypertensive rats, indicating physiologic adaptations consistent with aerobic exercise training. We found that soleus muscle wet weight was greater in both groups of spontaneously hypertensive rats that ran as compared with the age-matched spontaneously hypertensive rats that were sedentary. Furthermore, CS activity in the plantaris muscle was greater in the spontaneously hypertensive rats that ran for 16 weeks than the age-matched spontaneously hypertensive rats that were sedentary. The soleus and plantaris muscles are recruited during wheel running in rats. Both an increase in soleus muscle wet weight and plantaris muscle CS activity are indexes of aerobic training in wheel running rats.[38,39]

The changes that we observed following exercise training do not appear to be due to the independent effects of weight loss or pressure reduction. We found no changes in body weight with voluntary running in female spontaneously hypertensive rats. Other researchers have also documented the maintenance of body weight in female exercising rats but not in male exercising rats.[17,39] When evaluating the effects of exercise training on blood pressure, maintenance of body weight is important because weight loss has an antihypertensive effect that is independent of exercise.[40] In addition, pharmacological intervention does not appear to produce the same effects on cardiac enzyme activity as exercise training, despite greater reductions in blood pressure.[41]

Several clinical implications may be extrapolated from this study. Our results may indicate that patients with hypertension have an impaired ability to use fatty acids as an energy substrate for ATP production in the heart because of a reduced capacity for fatty acid entry into mitochondria. Furthermore, these findings indirectly suggest that aerobic exercise training normalizes cardiac energy metabolism in patients with hypertension, providing some support for aerobic exercise training as an intervention in the primary prevention of hypertension-related sequelae. Our findings in this rat model suggest that accentuation of hypertrophy in the hypertensive heart following aerobic exercise training is not necessarily detrimental. We believe that caution must be used when applying the results of this study to patients with hypertension. The animal model of hypertension and exercise training is not identical to the pathophysiology and exercise intervention that occur in patients with hypertension. Further research is needed to determine the relationship between cardiac energy metabolism impairment and direct indexes of cardiac function.

## Summary

We evaluated the effects of aerobic exercise training on cardiac energy metabolism in an animal model of hypertension to allow for greater experimental control and more invasive measurements than would be possible in patients with hypertension. Our findings in a rat model suggest to us that, in addition to modest reductions in systolic blood pressure, aerobic exercise training may also make cardiac energy metabolism in patients with hypertension more like that in people without hypertension. Our animal model, however, has limitations, and it is arguable whether our results can be applied to humans. Our study possibly provides additional evidence supporting aerobic exercise training as an intervention in the primary prevention of sequelae, such as angina and cardiovascular pump dysfunction or failure, in patients with hypertension. Our results suggest that a threshold for the duration of aerobic exercise training or for the point of initiation may exist for altering cardiac metabolism in the presence of hypertension.

**Table 4.**
Summary of Statistical Findings for the CONsed Group (Wistar-Kyoto Rats That Were Normotensive and Sedentary), HTNsed Group (Spontaneously Hypertensive Rats That Were Sedentary), HTN×8 Group (Spontaneously Hypertensive Rats That Received 8 Weeks of Exercise Training), and HTN×16 Group (Spontaneously Hypertensive Rats That Received 16 Weeks of Exercise Training)[a]

| | CONsed Group | HTNsed Group | HTN×8 Group | HTN×16 Group |
|---|---|---|---|---|
| Blood pressure (BP) | X | X | | |
| | X | | X | |
| | X | | | X |
| | | X | X | |
| Body weight (BW) | X | X | | |
| | X | | X | |
| | X | | | X |
| | | X | | X |
| | | | X | X |
| Total ventricular weight (TV) | X | X | | |
| | X | | X | |
| | X | | | X |
| | | X | X | |
| | | X | | X |
| Left ventricular weight (LV) | X | X | | |
| | X | | X | |
| | X | | | X |
| | | X | X | |
| | | X | | X |
| Intraventricular septum weight (IVS) | X | X | | |
| | X | | X | |
| | X | | | X |
| Right ventricular weight (RV) | No differences | | | |
| 3-hydroxyacyl-coenzyme A dehydrogenase (HOAD) | No differences | | | |
| Carnitine O-palmitoyltransferase (CPT) | X | X | | |
| | X | | X | |
| | X | | | X |
| Carnitine O-palmitoyltransferase I (CPT I) | X | X | | |
| | X | | X | |
| | | | X | X |
| Citrate synthase (CS) | No differences | | | |
| Hexokinase (HK) | X | | | X |
| | | X | | X |
| Glucose transporter 1 (GLUT 1) | No differences | | | |
| Glucose transporter 4 (GLUT 4) | No differences | | | |
| Total protein | No differences | | | |
| Glycogen | X | | X | |
| Triglyceride | No differences | X | X | |

[a] Significant differences between group pairs are indicated with X connected by dashed lines ($F[3,11]$, $\alpha=.05$).

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