

ScholarWorks@GSU

Effect of a Repeated Bout of Eccentrically-Biased Contractions on Insulin Resistance

Authors	Green, Michael Stephen
Citation	Green, Michael Stephen. "Effect of a Repeated Bout of Eccentrically-Biased Contractions on Insulin Resistance." Dissertation, Georgia State University, 2008. https://doi.org/10.57709/1059145
DOI	https://doi.org/10.57709/1059145
Rights	I hereby certify that, if appropriate, I have obtained and attached hereto a written permission statement from the owner(s) of each third party copyrighted matter to be included in my thesis, dissertation, or project report, allowing distribution as specified below. I certify that the version I submitted is the same as that approved by my advisory committee. I hereby grant to Georgia State University or its agents the non-exclusive license to archive and make accessible, under the conditions specified below, my thesis, dissertation, or project report in whole or in part in all forms of media, now or hereafter known. I retain all other ownership rights to the copyright of the thesis, dissertation or project report. I also retain the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or project report.
Download date	2026-03-08 21:33:33
Link to Item	https://hdl.handle.net/20.500.14694/9903

ACCEPTANCE

This dissertation, EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED CONTRACTIONS ON INSULIN RESISTANCE, by MICHAEL S. GREEN, was prepared under the direction of the candidate's Dissertation Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Education, Georgia State University.

The Dissertation Advisory Committee and the student's Department Chair, as representatives of the faculty, certify that this dissertation has met all standards of excellence and scholarship as determined by the faculty. The Dean of the College of Education concurs.

J. Andrew Doyle, Ph.D.
Committee Chair

Jeffrey C. Rupp, Ph.D.
Committee Member

Christopher P. Ingalls, Ph.D.
Committee Member

Dan Benardot, Ph.D.
Committee Member

Date

J. Andrew Doyle, Ph.D.
Chair, Department of Kinesiology and Health

R. W. Kamphaus, Ph.D.
Dean and Distinguished Researcher Professor
College of Education

AUTHOR'S STATEMENT

By presenting this dissertation as a partial fulfillment of the requirements for the advanced degree from Georgia State University, I agree that the library of Georgia State University shall make it available for inspection and circulation in accordance with its regulations governing materials of this type. I agree that permission to quote, to copy from, or to publish this dissertation may be granted by the professor under whose direction it was written, by the College of Education's director of graduate studies and research, or by me. Such quoting, copying, or publishing must be solely for scholarly purposes and will not involve potential financial gain. It is understood that any copying from or publication of this dissertation which involves potential financial gain will not be allowed without my written permission.

Michael S. Green

NOTICE TO BORROWERS

All dissertations deposited in the Georgia State University library must be used in accordance with the stipulations prescribed by the author in the preceding statement. The author of this dissertation is:

Michael S. Green
432 West College Street
Troy, AL 36081

The director of this dissertation is:

Dr. J. Andrew Doyle
Department of Kinesiology and Health
College of Education
Georgia State University
Atlanta, GA 30302-3975

VITA

Michael Stephen Green

ADDRESS: 432 West College Street
Troy, Alabama 36081

EDUCATION:

Ph.D. 2008 Georgia State University
Sport Science
M.S. 2002 Troy University
Sport and Fitness Management
B.S. 2000 Troy University
Sport and Fitness Management

PROFESSIONAL EXPERIENCE:

2008-Present Assistant Professor
Department of Kinesiology and Health Promotion
Troy University, Troy, AL
2004-2008 Graduate Research Assistant
Department of Kinesiology and Health
Georgia State University, Atlanta, GA
2002-2003 Director
Health and Performance Sciences Center
McCollough Institute, Gulf Shores, AL
2001-2002 Graduate Teaching Assistant
Department of Kinesiology and Health Promotion
Troy University, Troy, AL

PROFESSIONAL SOCIETIES AND ORGANIZATIONS:

2003-Present Member, American College of Sports Medicine
2001-Present Member, Southeast American College of Sports Medicine

PUBLISHED PAPERS:

Green, M.S., Corona, B.T., Doyle, J.A., Ingalls, C.P. Carbohydrate-protein drinks do not enhance recovery from exercise-induced muscle injury. *International Journal of Sport Nutrition and Exercise Metabolism*, 18(1), 1-18.

PUBLISHED BOOK CHAPTERS:

Doyle, J.A., Papadopoulos, C., Green, M.S. (2008). Utilization of carbohydrates in energy production. In I. Wolinsky & J. Driskell (Eds.), *Sports nutrition: Energy metabolism and exercise*. Boca Raton, FL: CRC Press.

PUBLISHED ABSTRACTS:

Green, M.S., Corona, B.T., Doyle, J.A., Ingalls, C.P. (2007). A carbohydrate-protein drink does not enhance recovery from exercise-induced muscle injury. *Medicine and Science in Sports and Exercise*, 39(5), S363.

Doyle, J.A., Green, M.S., Corona, B.T., Simone, J., Dennison, D. (2007). Validation of an electronic pedometer in a field-based setting. *Medicine and Science in Sports and Exercise*, 39(5), S186.

Rupp, J.C., Green, M.S., Corona, B.T., Heimbürger, K.D., Pullen, P.R., Everest, A.S. (2007). Predictors of 10k running performance in well trained athletes matched for ventilatory threshold. *Medicine and Science in Sports and Exercise*, 39(5), S207.

Corona, B.T., Green, M.S., Doyle, J.A., Rupp, J.C., Ingalls, C.P. (2006). Exercise-induced muscle injury results in elevations in aerobic and anaerobic metabolism during submaximal treadmill running. *Medicine and Science in Sports and Exercise*, 38(5), S1523.

Doyle, J.A., Dennison, D.A., Green, M.S., Corona, B.T., Kimball, A. (2006). Validity and reliability of an electronic pedometer in a laboratory setting. *Medicine and Science in Sports and Exercise*, 38(5), S2855.

PRESENTATIONS:

Green, M.S., Doyle, J.A., Ingalls, C.P., Benardot, D., and Corona, B.T. (2008). "Effect of a Repeated Bout of Eccentric Contractions on Insulin Resistance." Paper presented at the Southeast American College of Sports Medicine Regional Meeting, Birmingham, AL.

Green, M.S., Corona, B.T., Doyle, J.A., and Ingalls, C.P. (2007). "A Carbohydrate-Protein Drink Does Not Enhance Recovery from Exercise-Induced Muscle Injury." Paper presented at the Southeast American College of Sports Medicine Regional Meeting, Charlotte, NC.

Green, M.S., Corona, B.T., Kimball, A., Dennison, D.A., and Doyle, J.A. (2006). "Validity and Reliability of an Electronic Pedometer in a Laboratory Setting." Paper presented at the Southeast American College of Sports Medicine Regional Meeting, Charlotte, NC.

Green, M.S. (2005). "Cardiorespiratory Fitness: Considerations for Accurate Prediction of Maximal Oxygen Consumption." Paper presented at the Alabama State Association of the American Alliance for Health, Physical Education, Recreation and Dance Fall Conference, Birmingham, AL.

Green, M.S. and Tatum, L. (2001). "The Property Interest Rights of an HIV-Positive Individual Denied Participation in a Professional Contact Sport." Paper presented at the Sport Administration and Physical Education Conference, Florida State University, Tallahassee, FL.

ABSTRACT

EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED CONTRACTIONS ON INSULIN RESISTANCE

by
Michael S. Green

This study determined if insulin resistance (IR), induced by an acute bout of eccentrically-biased contractions that resulted in muscle injury, was attenuated following a repeated bout of contractions. Female subjects ($n = 10$, age 24.7 ± 3.0 yr, weight 64.9 ± 7.4 kg, height 1.67 ± 0.02 m, body fat 29.1 ± 1.9 %) performed two 30 minute bouts of downhill treadmill running (DTR 1 and DTR 2, -12 % grade, 8.0 mph) separated by 14 days. Oral glucose tolerance tests (OGTT) were administered at baseline and 48 hours following DTR 1 and DTR 2, with IR assessed by calculation of insulin and glucose areas under the curve (AUC). Maximum isometric quadriceps strength (MVC), muscle soreness (SOR), and serum creatine kinase (CK) were assessed pre-, immediately post-, and 48 hours post-DTR 1 and DTR 2 to determine the presence of muscle injury. Compared to baseline OGTT, insulin and glucose AUC (37.6 ± 8.4 and 21.4 ± 4.7 % increase, respectively), and peak insulin (44.1 ± 5.1 vs. 31.6 ± 4.0 $\text{uU}\cdot\text{mL}^{-1}$) and glucose (6.5 ± 0.4 vs. 5.5 ± 0.4 $\text{mmol}\cdot\text{L}^{-1}$) were elevated following DTR 1. These same insulin and glucose measures showed no increase above baseline 48 hours following DTR 2 ($p > 0.05$). MVC was reduced to a greater degree immediately following DTR 1 (16.7 ± 2.6 vs. 8.6 ± 1.2 % decline) and, although demonstrating a significant degree of recovery, remained reduced by 9.4 ± 2.7 % 48 hours following exercise. In contrast, MVC made a

full recovery back to baseline values 48 hours after DTR 2. SOR was elevated to a greater degree 48 hours following DTR 1 than after DTR 2 (48.08 ± 6.16 vs. 12.70 ± 3.24 mm). There was a tendency for an attenuated serum CK response 48 hours following DTR 2 (812.8 ± 365.1 vs. 162.5 ± 42.5 U·L⁻¹, $p = 0.08$). In conclusion, a novel bout of eccentrically-biased contractions resulting in a moderate degree of muscle injury confers a protective effect, whereby a subsequent bout of contractions 14 days later results in complete elimination of the IR observed following the initial bout.

EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED
CONTRACTIONS ON INSULIN RESISTANCE

by
Michael S. Green

A Dissertation

Presented in Partial Fulfillment of Requirements for the
Degree of
Doctor of Philosophy
in
Sport Science
in
the Department of Kinesiology and Health
in
the College of Education
Georgia State University

Atlanta, GA
2008

Copyright by
Michael S. Green
2008

ACKNOWLEDGEMENTS

I would like to give my whole-hearted love to my wife Susan who has supported me without complaint throughout my entire graduate education. Her numerous sacrifices have allowed me to remain devoted to my research. I also want to express my deepest appreciation to my parents who have also stood by my side and provided endless encouragement as I pursued my goals and dreams, both in academia and life in general.

I would also like to express my sincere appreciation to the members of my dissertation committee. I thank Dr. J. Andrew Doyle, my dissertation chair and advisor, who encouraged, fostered, and supported my research from the outset. I thank Dr. Christopher P. Ingalls, whose analytical mind and intelligence helped me refine my laboratory etiquette. I appreciate the timely input provided by Dr. Jeffrey C. Rupp during the pursuit of my degree. I also thank Dr. Dan Benardot for his trust, belief, and encouragement of my research abilities.

I want to express sincere thanks to my colleague and now close personal friend Benjamin T. Corona. His assistance and critical input were instrumental in the completion of my research.

Finally, I am indebted to the voluntary participation of the research subjects who were willing to devote a significant amount of time performing injury-inducing exercise and having numerous blood samples removed.

TABLE OF CONTENTS

List of Tables	v
List of Figures	vi
Abbreviations	vii
CHAPTER 1	1
EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED CONTRACTIONS ON INSULIN RESISTANCE.....	1
Introduction.....	1
Sarcolemmal Glucose Transport.....	2
Glucose Transporters	3
Insulin Signaling Pathway	4
Exercise and Glucose Transport	4
Exercise-Induced Muscle Injury	5
Causes	5
Mechanism.....	5
Symptoms	7
Delayed Onset Muscle Soreness.....	7
Force Deficits.....	8
Elevations in Creatine Kinase.....	9
Muscle Damage	10
Inflammatory Response	11
Eccentric Contractions and Glucose Metabolism	12
Insulin Resistance	12
GLUT-4.....	18
Glycogen Synthesis.....	19
Skeletal Muscle Adaptation to Exercise-Induced Muscle Injury	24
Repeated Bout Effect	24
Mechanism.....	29
Conclusion	30
References.....	32
CHAPTER 2	38
EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED CONTRACTIONS ON INSULIN RESISTANCE.....	38
Introduction.....	38
Methods.....	39
Experimental Design.....	41
Injury Protocol	41

Muscle Strength	42
Ratings of Muscle Soreness	42
Oral Glucose Tolerance Test	43
Blood Analysis.....	43
Area Under the Curve	44
Statistical Analysis.....	44
Results.....	44
Insulin Response	44
Glucose Response	45
Maximal Isometric Strength	49
CK Activity.....	50
Muscle Soreness.....	50
Injury Protocol	51
Discussion	52
References.....	60
Appendixes	63

LIST OF TABLES

Table		Page
1	Subject characteristics.....	40
2	Fasting and Peak Insulin and Glucose Response to an OGTT.....	45

LIST OF FIGURES

Figure		Page
1	Insulin (A) and glucose (B) response to an OGTT administered at baseline (BASE) and 48 h following DTR 1 and DTR 2.....	46
2	Insulin (A) and glucose (B) AUC in response to an OGTT administered at baseline (BASE) and 48 h following DTR 1 and DTR 2	48
3	Maximal isometric torque measured at baseline (BASE), immediately after (Post-I), and 48 h after (Post-2d) DTR 1 and DTR 2.....	50
4	Soreness measured at baseline (BASE), immediately after (Post-I), and 48 h after (Post-2d) DTR 1 and DTR 2.....	51

ABBREVIATIONS

AUC	Area under the curve
$\text{b}\cdot\text{min}^{-1}$	Beats per minute
CK	Creatine Kinase
DOMS	Delayed onset muscle soreness
DTR	Downhill treadmill run
$\text{g}\cdot\text{d}^{-1}$	Grams per day
$\text{g}\cdot\text{kg}^{-1}\text{ bw}\cdot\text{d}^{-1}$	Grams per kilogram body weight per day
$\text{g}\cdot\text{kg}^{-1}\text{ bw}\cdot\text{h}^{-1}$	Grams per kilogram body weight per hour
GLUT	Glucose transporter
G6P	Glucose 6-phosphate
IRS	Insulin receptor substrate
kg	Kilogram
$\text{mg}\cdot\text{dL}^{-1}$	Milligrams per deciliter
$\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	Milligrams per kilogram per minute
$\mu\text{U}\cdot\text{mL}^{-1}$	Microunits per milliliter
$\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	Milliliters per kilogram per minute
mm	Millimeter
$\text{mmol}\cdot\text{kg}^{-1}\text{ ww}$	Millimoles per kilogram wet weight
$\text{mmol}\cdot\text{kg}^{-1}\text{ ww}\cdot\text{h}$	Millimoles per kilogram wet weight per hour

Nm	Newton meter
MVC	Maximal voluntary contraction
OGTT	Oral glucose tolerance test
PI3-kinase	Phosphatidylinositol 3-kinase
RPE	Ratings of perceived exertion
steps·min ⁻¹	Steps per minute
TNF- α	Tumor necrosis factor-alpha
U·L ⁻¹	Units per liter

CHAPTER 1
EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED
CONTRACTIONS ON INSULIN RESISTANCE

Introduction

Eccentric muscle contractions occur as the muscle lengthens while it produces force, in contrast to concentric contractions where the muscle shortens as it produces force. Eccentric contractions can result in a large degree of muscle injury compared to concentric contractions, as evidenced by immediate and prolonged declines in force production (15, 35), elevated blood concentrations of muscle proteins such as creatine kinase (13), delayed onset muscle soreness (10), and reduced glucose uptake by skeletal muscle cells (44). Of particular note in relation to sarcolemmal glucose transport is that eccentric contractions also result in transient insulin resistance (3, 41, 42, 44, 79) and a decreased number of glucose transporters (4). Subsequently, it appears that skeletal muscle exposed to prior eccentric contractions possesses a reduced ability to synthesize glycogen (16, 21, 66, 89).

Although susceptible to injury by novel eccentric muscular actions, skeletal muscle displays a readily observable “repeated bout effect” whereby muscles exposed to prior eccentric contractions exhibit reduced soreness and serum CK responses in addition to more rapid returns to full force producing capacity following a second eccentric bout (11, 15, 64). Indeed, such a repeated bout effect can occur as a consequence of a

relatively minor initial eccentric insult (14, 65) and may afford protection from exercise-induced muscle injury for an extended period of time (11, 64).

This literature review will provide for an examination of skeletal muscle glucose metabolism in the normal and post “eccentric contraction” state and describe the mechanism, symptoms, and time course of the recovery from exercise-induced muscle injury. In particular, an emphasis will be placed on describing the observed effects of exercise-induced muscle injury on sarcolemmal glucose transport, namely the propensity of eccentric contractions to induce transient insulin resistance, cause a decrease in skeletal muscle glucose transporter content, and presumably impair the synthesis of muscle glycogen. Finally, the components of the repeated bout effect will be examined and a model of attenuated insulin resistance following repeated eccentric contractions will be presented that warrants further original investigation.

Sarcolemmal Glucose Transport

Glucose transport into the skeletal muscle cell is required to provide glucose as an energy substrate for glycolysis and for the replenishment of glycogen stores. Glucose can be obtained from both the diet and the process of gluconeogenesis, but in the post-absorptive state the majority of glucose is derived from ingested carbohydrate (38).

In order for glucose to enter into cellular metabolism it must proceed from the 1) blood to the interstitium, 2) interstitium to the intracellular space, and 3) enter into metabolic pathways via phosphorylation to glucose 6-phosphate (G6P). Several factors related to each of the three steps in this pathway can determine the rate of glucose uptake, including 1) muscle blood flow, capillary recruitment, and endothelial permeability, 2) number of glucose transporters in the sarcolemma and glucose gradient, and 3) amount

and activity of muscle hexokinase II, respectively (87). During moderate intensity exercise the primary fate of blood glucose is oxidation for energy production. However, in the post-exercise state the glucose is channeled to glycogen under the control of the enzyme glycogen synthase. Under most circumstances, sarcolemmal glucose transport is considered to be the rate-limiting step in glucose utilization (46).

Glucose Transporters

Glucose transport across cell membranes occurs via a process of facilitated diffusion utilizing a family of glucose transporter carrier proteins (GLUT) (31). These carrier proteins are expressed in a tissue specific manner (6). Of note, skeletal muscle contains two members of the GLUT family responsible for glucose transport, namely GLUT-1 and GLUT-4 (37), as well as a low abundance of GLUT-5 (40), which is a high-affinity fructose transporter with only a very low capacity to transport glucose (9). Present in small amounts, GLUT-1 is not believed to play a major role in glucose transport across the cell membrane, and may be responsible for basal glucose uptake (71). GLUT-4 would thus seem to be the major determinant of glucose transport into the muscle cell and subsequent glycogen synthesis.

Under basal conditions GLUT-4 cycles slowly between the plasma membrane and intracellular storage locations. The majority of the GLUT-4 is located intracellularly with only small amounts present in the plasma membrane (88). Recruitment of GLUT-4 and subsequent glucose transport across the sarcolemma is generally mediated by insulin and muscle contraction (32).

Insulin Signaling Pathway

The insulin signaling pathway is initiated by binding of insulin to the extracellular α -subunits of the insulin receptor. This results in tyrosine autophosphorylation of the intracellular β -subunits and subsequent activation of the subunits' intrinsic tyrosine kinase (39). Subsequent to this, signal transduction is mediated through several insulin receptor substrates (IRS), in particular IRS-1 (12). IRS-1 ultimately acts on phosphatidylinositol 3-kinase (PI3-kinase), a well characterized intermediate effector molecule that plays an important role in the effect of insulin on GLUT-4 translocation, glucose transport, and glucose metabolism (83). The importance of PI3-kinase in this pathway is exemplified by the fact that administration of wortmannin (an inhibitor of PI3-kinase) completely blocks the insulin stimulated translocation of GLUT-4 (50).

Exercise and Glucose Transport

It is apparent that muscle contraction is able to significantly increase glucose transport across the sarcolemma independent of insulin (70). Exercise results in an increase in glucose transporter number in the sarcolemma, and specifically the t-tubules (72). Interestingly, contraction and insulin mediated translocation of GLUT-4 appear to utilize divergent pathways and may recruit GLUT-4 from separate intracellular pools (20).

The stimulatory effect of exercise on glucose transport can also persist following cessation of an acute bout of exercise. This is manifested as an initial period of glucose transport and glycogen synthesis considered to be insulin independent (phase 1), followed by a more prolonged and delayed insulin dependent phase (phase 2). Phase 1 is characterized by a high degree of sarcolemmal glucose permeability, elevated glycogen

synthase activity (29), and a rapid rate of muscle glycogen synthesis (68). Phase 2 commences as muscle glycogen concentrations approach normal levels, with glucose uptake not elevated in the absence of insulin (68). Thus, GLUT-4 translocated to the membrane during muscle contraction contributes to the elevated rates of glucose transport in the immediate post-contraction period. Once contraction-induced GLUT-4 is recycled continued high rates of glucose transport become dependent on adequate levels of circulating insulin.

Exercise-Induced Muscle Injury

Causes

Exercise-induced muscle injury can result from various modes of muscular activity, including prolonged exercise (33, 85), exercise to exhaustion at a high relative intensity (58, 74), and bouts of novel exercise (15). In particular, those activities that contain a bias towards eccentric contractions, such as downhill treadmill running (11), are well-suited to induce this type of muscle injury. Other common models of inducing exercise-induced muscle injury include eccentric stepping (61), elbow extension (15), and knee extension (16).

Mechanism

The change in length of a muscle during activation and force production (i.e. contraction) is dependent on the load placed on the muscle and the force that it develops (24). During an eccentric muscle contraction, a muscle lengthens while at the same time producing tension. This is in contrast to a concentric contraction during which the muscle shortens while it produces force. Alternatively, an isometric contraction occurs when the muscle as a whole remains the same length during the time it produces force.

During an eccentric contraction the maximal tension generated can be in excess of that produced during concentric and isometric contractions (81). This is despite the fact that fewer motor units are recruited to perform the contraction (7). Thus, this indicates that significantly higher forces are encountered by fewer muscle fibers. Such high forces have a propensity to cause muscle damage, the symptoms of which will be described below.

Immediately following eccentric contractions two prominent signs of muscle injury that may lead to the observed force deficits are disruption of sarcomeres and damage to the excitation-contraction coupling machinery (69). Regardless of the specific mechanism, mechanical stress, and not metabolic stress, is generally accepted as being the initiating event in exercise-induced muscle injury (26). One theory advanced to explain the subsequent muscle injury sequence posits that 1) an initial mechanical event inaugurates the injury process, 2) a loss of calcium homeostasis results in a calcium overload phase, 3) calcium overload activates various degradative autogenetic mechanisms at the site of injury, 4) an inflammatory phagocytic phase ensues, and 5) ultimately the injured fibers undergo a regenerative phase (1). Some of the symptoms described below lend support to various aspects of theories such as this. For example, worsening ultrastructural damage 2-3 days following eccentric exercise (60), loss of desmin (a specific target of the calcium activated protease calpain) (5, 51), and delayed release of creatine kinase as a result of possible disruption and increased permeability of the sarcolemma (26, 51) lend support to the initiation and progression of various processes of degradation within the muscle fiber. Secondary injury of this nature is correlated to macrophage infiltration into the muscle cell (52). As will be addressed,

repeated bouts of eccentric contractions appear to offer a significant degree of protection against many of these processes.

Symptoms

Exercise containing a bias towards eccentric contractions has been shown to result in several readily observable alterations to the structure and function of skeletal muscle. The assessment of the presence and severity of exercise-induced muscle injury can be determined by direct and indirect measures. Direct methods involve the assessment of cellular and ultrastructural damage by way of biopsy and imaging techniques. Indirect methods include measurement of the efflux of intramuscular enzymes into the blood, muscular force production, soreness, swelling, and range of motion. In general, eccentric contractions have been shown to result in delayed onset muscle soreness (14, 61, 75), immediate and prolonged declines in force production (22, 59), elevated blood concentrations of muscle proteins (e.g. creatine kinase [CK]) (14, 75), muscle damage (28, 60), and an inflammatory response (25, 82).

Delayed Onset Muscle Soreness

Delayed onset muscle soreness (DOMS) is one of the most readily apparent symptoms of exercise-induced muscle injury. Studies have consistently shown that eccentric contractions possess the propensity to induce DOMS. Soreness is not typically experienced immediately following the exercise bout, but appears several hours after the exercise and reaches a delayed peak 1-2 days later. For example, utilizing repeated concentric and eccentric stepping exercise, Newham et al. (61) noted that pain and tenderness, although only occurring in the eccentrically exercised leg, took eight hours to develop and reached a peak 48 hours after exercise. Comparing downhill and level

treadmill running, significant increases in muscle soreness have been observed only following downhill treadmill running which peaked 24-48 hours following exercise (75). Similarly, a peak in muscle soreness and pain sensation when attempting to straighten the arm two days following eccentric elbow extension exercise has been noted (15).

Force Deficits

Measurement of force loss following exercise-induced muscle injury is generally considered the most relevant and consistent determinant of muscle injury (86). Utilizing eccentric elbow extension, it has been observed that following 70 maximal eccentric contractions the isometric force generation capacity was significantly reduced and had not fully recovered five days following exercise. Alternatively, 24 maximal eccentric contractions demonstrated a return to full force by two days post-exercise (15). A 50% reduction in voluntary force production (MVC) has been noted immediately following 20 minutes of maximal eccentric contractions of the biceps muscle (59). At 24 hours post-exercise there had been a negligible improvement in MVC (still a 49 % reduction), and although there was a gradual improvement MVC remained below baseline for over 2 weeks.

Downhill treadmill running results in smaller decrements in force loss. For example, downhill treadmill running for 40 minutes resulted in an immediate loss in MVC of ~22 % which recovered slightly to a 15 % decrement one day post-exercise (22). Complete recovery of strength took place somewhere between three and five days after exercise.

Of note is that animal studies have demonstrated much higher and prolonged decreases in force production. Mouse extensor digitorum longus muscle subjected to 150

electrically induced, maximal, in vivo eccentric contractions displayed a reduction of maximal isometric tetanic force by 50 % immediately following the eccentric contraction protocol, remaining unchanged for the next five days (35). An indication of the severity of injury that can be induced by such protocols in animals is evident by the fact that maximal tetanic force remained 13 % below baseline values even after 14 days of recovery.

The general understanding that can be taken from these studies is that force deficits are maximal immediately following exercise, and in most cases commence recovery after 24-48 hours. In human studies, full recovery can take between 3-14 days and is likely dependent upon the degree of injury that is induced by the particular protocol.

Elevations in Creatine Kinase

The presence of intramuscular enzymes in the blood is often taken as an indication of the presence of exercise-induced muscle injury. Creatine kinase (CK) is a commonly measured enzyme, and typically displays a somewhat delayed increase following exercise-induced muscle injury. Baseline serum CK levels are typically between 50 and 250 U·L⁻¹ (16, 59). Peak serum CK levels of ~2000 U·L⁻¹ were measured five days following 70 maximal eccentric contractions of the elbow flexors (15). Of note is that in this particular study serum CK levels did not show an increase above baseline until three days post-exercise. Similarly, Schwane et al. (75) observed no increase in serum CK levels immediately after downhill treadmill running, but found a 351 % increase 24 hours following exercise. Serum CK activity was still elevated by 141 % 48 hours following exercise, but had returned to baseline levels following 72 hours of

recovery. A similar observation following downhill treadmill running was made by Sherman et al. (79), who noted a 2.8 fold increase in serum CK 48 hours post-exercise.

It should be noted that there is a considerable degree of individual variability in the serum CK response to injurious exercise (14). For example, a study conducted by Costill et al. (16) involving 100 maximal eccentric contractions of the knee extensor muscles measured three days post-exercise peak serum CK values of $6988 \text{ U}\cdot\text{L}^{-1}$. Pre-exercise values averaged $57 \text{ U}\cdot\text{L}^{-1}$. However, three days post-exercise three subjects had serum CK values well in excess of $8000 \text{ U}\cdot\text{L}^{-1}$, with one having a serum CK value of $30,196 \text{ U}\cdot\text{L}^{-1}$. For this reason, although serum CK is a common and convenient measurement utilized in many studies, its use is not considered the most accurate marker to indicate the degree of exercise-induced muscle injury and care must be used when interpreting findings associated with serum CK responses (86).

Muscle Damage

Evidence has been provided that heavy eccentric work results in morphological evidence of damage two and seven days after exercise (28). However, in this study eccentric exercise was not compared to a similar amount of work conducted via concentric contractions. Seeking to distinguish between the alterations that may also result from concentric contractions, the study of Newham et al. (60) examined morphological changes in vastus lateralis muscles subjected to either concentric or eccentric stepping. Bilateral muscle biopsies revealed that muscles in the leg that performed concentric contractions possessed no morphological abnormalities either immediately or 24-48 hours post-exercise. However, abnormalities were noted in the muscle samples taken from the eccentrically exercised leg, being present both

immediately post-exercise and becoming more pronounced 24-48 hours after exercise. In particular, light microscopy showed that immediately post-exercise 16, 16, and 8 % of the counted fibers showed focal, extensive, and very extensive changes, respectively, while the remaining 58 % of the fibers appeared normal. Electron microscopy revealed sarcomere disruption including the presence of disorganized sarcomeres and streaming of Z-line material. Samples taken an average of 30 hours following eccentric contractions showed that the damage had become more marked and involved a greater percentage of fibers. The percentage of fibers appearing as normal had fallen to 45 %, with 6, 23, and 28 % of fibers showing focal, extensive, and very extensive changes, respectively. More recent observations in animals have indicated that there may be a rapid loss of cytoskeletal proteins such as desmin in response to eccentric contractions (5, 51).

Inflammatory Response

Muscle that has undergone eccentric contractions and subsequent exercise-induced muscle injury exhibits a distinct inflammatory response. As described by Tidball (82), such a response is dominated by an influx of neutrophils and macrophages from the peripheral circulation into the affected muscle. In general, it is thought that neutrophils invade injured muscle tissue within 1 h of muscle injury, remaining elevated for up to 5 d (25). It is likely that the presence of neutrophils results in a necessary proteolytic process that may actually augment the process of exercise-induced muscle injury (52). Indeed, neutrophils seem capable of cell membrane lysis, and a delayed appearance of macrophages may play a further role in the degradation and removal of the injured muscle tissue, although macrophages may also play a role in muscle repair and remodeling (82). Of note is that the initiation and amplification of the acute inflammatory

response is mediated at least in part by the de novo production of cytokines (19). In particular, at the onset of inflammation there is the production and release into the blood of several pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α) (19).

Eccentric Contractions and Glucose Metabolism

Eccentric contractions have been shown to result in transient insulin resistance (3, 41, 42, 44, 79), decreased glucose transporter number (4), and a reduced ability of the muscle to synthesize glycogen (8, 16, 21, 47, 66, 76, 89). The effect of eccentric contractions on these three factors will be examined below.

Insulin Resistance

An attenuated physiological response to normal levels of insulin characterizes insulin resistance (43). Insulin resistance has been documented following activities that result in exercise-induced muscle injury (3, 41, 42, 44, 79).

Seeking to clarify the effects of prior exercise on insulin secretion in untrained subjects, Kirwan et al. (42) compared the secretion of insulin following a bout of prolonged, intense exercise to the response that took place without prior exercise. Untrained males underwent a hyperglycemic clamp procedure to determine rates of insulin secretion 12 hours after performing intermittent treadmill running to exhaustion. During the hyperglycemic clamp procedure blood glucose was raised and maintained at 180 mg·dL⁻¹. Subsequently, the area under the curve (AUC) for both insulin and C-peptide was calculated for the early (0-10 minutes) and late (15-180 minutes) phases of the clamp. Peak insulin was significantly higher post-exercise in comparison to that measured following rest (81 ± 8 vs. 59 ± 9 $\mu\text{U}\cdot\text{mL}^{-1}$). During the early phase, the AUC

for insulin and C-peptide was significantly higher following exercise, whereas during the late phase only the AUC for C-peptide was significantly different (being higher after exercise). The utility of measuring C-peptide is that unlike insulin it is not degraded by the liver and can therefore be used as a surrogate measure of insulin release when insulin levels in the blood may not be indicative of its pancreatic secretion. This allows for a better understanding of insulin secretion (rate of appearance) during times when the clearance of insulin (rate of disappearance) may be increasing.

Interpretation of these findings indicates that prolonged, intense exercise caused increases in pancreatic insulin secretion during the early phase of the hyperglycemic clamp, which appeared to continue during the late phase concurrent with increases in insulin clearance. Measurement of the blood glucose response indicated no differences between the clamps, suggesting that the rate of glucose disposal was not altered but that it required more insulin to result in the same glucose disposal rates. On the surface these are unexpected findings, since it is generally accepted that post-exercise (both acute and chronic) the insulin response typically shows no changes or is actually reduced (i.e. an increase in insulin sensitivity is observed). However, exercise was performed by untrained subjects and likely resulted in exercise-induced muscle injury (evidenced by elevated serum CK levels and complaints of muscle soreness prior to the hyperglycemic clamp). The results thus suggest that insulin resistance developed as a result of inadvertently induced muscle injury and manifested itself as an increased insulin secretion in order to maintain a normal rate of glucose disposal.

Similar findings to the study of Kirwan et al. (42) were obtained by King et al. (41) who specifically attempted to cause exercise-induced muscle injury via a bout of

eccentric knee flexions and extensions. Indeed, this eccentric exercise regime resulted in a 50-fold increase in serum CK levels and significant increases in subjective ratings of muscle soreness 36 hours post-exercise. Results of the hyperglycemic clamp administered 36 hours following exercise showed that during the early phase (0–10 minutes) of the clamp there was an exaggerated peak insulin (28 % greater than control) and C-peptide response, accompanied by an 83 % increase in the insulin AUC. During the late phase, the insulin response was not different than control, whereas the C-peptide response was exaggerated, being 34 % greater following eccentric exercise. Again, the elevated C-peptide levels in the presence of non-elevated insulin levels following eccentric exercise suggest that there may be an increased fractional extraction of insulin by the liver. Thus, these two early studies (41, 42) introduced the relationship between increased insulin secretion and muscle injury.

As a direct result of the questions raised by their previous study (42), Kirwan et al. (44) investigated further the observations that exercise resulting in symptoms of exercise-induced muscle injury leads to an increased insulin response without a concomitant increase in glucose disposal. Insulin action under conditions of hyperinsulinemia and euglycemia (euglycemic-hyperinsulinemic clamp) 48 hours following exercise was assessed in untrained subjects. Insulin action following downhill treadmill running (eccentrically-biased exercise) was contrasted with the response following cycle ergometer exercise (i.e. concentrically biased exercise) and rest. Muscle soreness and serum CK measured 48 hours following downhill treadmill running were significantly elevated above the values for cycling and rest, thus indicating that it was likely that muscle injury had been successfully induced. Importantly, soreness and serum

CK following cycling were not different than resting control conditions, thus showing that the concentric exercise did not result in muscle injury. Fasting plasma glucose and insulin, as well as plasma insulin during the clamp, were not different between conditions. However, glucose disposal during the final 30 min of the clamp was significantly reduced following downhill treadmill running. Glucose disposal rates were 3.47 ± 0.51 , 5.55 ± 0.94 , and 5.48 ± 1.0 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ following eccentric, concentric, and resting conditions, respectively. The euglycemic-hyperinsulinemic clamp used in this study specifically controls the glycemic and insulinemic stimuli for glucose disposal and therefore permits more conclusive evidence to be collected than with the hyperglycemic clamp technique.

While the hyperglycemic clamp procedure utilized in the study of Kirwan et al. (42) suggested an increased insulin response to prolonged, intense exercise, the study of Kirwan et al. (44) was able to categorically determine that eccentric exercise resulted in insulin resistance. This was evidenced by the 37 % reduction in insulin-mediated whole body glucose disposal 48 hours after the exercise bout. Kirwan et al. (44) suggested the decreased glucose uptake could be explained by decreased insulin binding to skeletal muscle insulin receptors and interference with the translocation of glucose transporters, although it was also questioned whether the localized muscle trauma was sufficient to account for the large magnitude of the reduction in whole body glucose disposal.

Further evidence of insulin resistance following exercise was provided by Sherman et al. (79). Insulin and glucose responses to an oral glucose tolerance test (OGTT) were assessed in untrained subjects following no exercise (control) and 48 hours following downhill treadmill running, level treadmill running, or isokinetic leg exercise.

Downhill treadmill running induced muscle soreness 48 hours following exercise. Interestingly, and supporting the findings of previous studies (42, 44), downhill and level treadmill running both induced elevations in serum CK activity. Resting insulin and glucose levels were not different between treatments, nor were the blood glucose responses to the OGTT (indicating no alteration to glucose tolerance). Of particular note is that the peak insulin response to the OGTT was ~19% higher following downhill treadmill running. Additionally, the AUC for insulin was 37 % higher following the downhill run in comparison to the non-exercising control condition.

Combining the observations of a similar glucose response but elevated insulin response, it is apparent that downhill treadmill running induced an insulin resistant state 48 hours following exercise. Stated differently, a greater amount of insulin was required to maintain glucose homeostasis in response to the OGTT following downhill treadmill running.

An interesting finding in the study of Sherman et al. (79) was that level treadmill running resulted in elevated serum CK levels, but did not result in delayed onset muscle soreness as in the downhill treadmill running condition. The insulin response was elevated only following downhill treadmill running, suggesting that the increased bias towards eccentric contractions resulted in muscle soreness in addition to elevated serum CK levels. The development of delayed onset muscle soreness indicated a certain degree of exercise-induced muscle injury, and further strengthens the association between exercise-induced muscle injury and insulin resistance. Echoing the potential mechanism put forward by previous researchers (44), Sherman et al. (79) hypothesized that the decreased insulin effectiveness could be a result of disruptions to binding of insulin to its

receptor, a reduction in the availability of glucose transporters, or an alteration in the intracellular glucose disposal pathway.

Several authors have questioned whether the degree of skeletal muscle trauma resulting from eccentric contractions is sufficient enough to account for the large increases in insulin resistance and subsequent reduction in whole body glucose disposal (3, 44). Although skeletal muscle is highly regulated by insulin and accounts for approximately 85 % of glucose disposal following glucose ingestion (17, 84), it is difficult to conceive that such a focal amount of muscle injury can result in such a large degree of systemic insulin resistance.

It has been suggested that the release of pro-inflammatory cytokines such as TNF- α during the acute inflammatory response may be responsible for the systemic insulin resistance that typically ensues following eccentric exercise (18, 43). In support of this, in vivo administration of TNF- α in animals has been shown to impair systemic glucose uptake (48). TNF- α may somehow interfere with normal insulin signaling, and it has been proposed that elevations in the levels of TNF- α may result in phosphorylation of IRS-1 and impair its association with the insulin receptor (73). This could possibly help explain the systemic insulin resistance observed following eccentric contractions that may produce only relatively focal damage.

It should be noted that a finding of increased levels of TNF- α following eccentric contractions is not a universal finding (63, 80). However, if release of TNF- α during the acute phase inflammatory response is a plausible contributor to the observed insulin resistance, and it is also recognized that the magnitude of the acute response is dependent on the extent of muscle cell disruption (23), then it could be argued that a reduced

amount of muscle cell disruption following a repeated bout (see later) would result in less TNF- α release and subsequently less insulin resistance.

GLUT-4

As described previously, glucose transport across the sarcolemma occurs via the process of facilitated diffusion utilizing a family of glucose transporters. One member of this family, GLUT-4, plays a major role and is translocated from intracellular storage areas under the influence of insulin and muscle contraction.

In response to the research showing insulin resistance and reduced rates of post-exercise glycogen synthesis following eccentric exercise, possible changes in the concentration of GLUT-4 have been investigated in an attempt to explain these phenomena (4). Immediately after eccentric exercise of the quadriceps muscle there was no change in GLUT-4 protein concentration compared to the non-exercised control muscle. However, one and two days after exercise the eccentrically exercised muscle displayed 68 and 64 % reductions in GLUT-4 protein concentrations, respectively. GLUT-4 concentrations had returned to control values by four and seven days post-exercise. Interestingly, glycogen concentrations in the eccentrically exercised leg remained significantly lower than the control leg on days one and two, but returned to normal on days four and seven. Casting doubt on the theory put forward by Costill et al. (16), it was noted that there was no increase in inflammatory cells in the exercised leg at the time points when glycogen levels were reduced over control. Thus, the presence of inflammatory cells may not fully explain the reduced glycogen levels noted in the days following eccentric exercise.

The relationship between decreased GLUT-4 levels and insulin action following eccentric exercise has also been investigated (3). In this study the effects of insulin action both at the systemic and muscle level were monitored utilizing a euglycemic clamp technique in combination with arterial-venous catheterization. Clamp measurements were made two days post-exercise, since this is when GLUT-4 content has been shown to be lowest following eccentric exercise (4). In comparison to the non-exercising control leg, whole body and muscle insulin action were shown to be impaired. It was also noted that the reduced insulin action observed at the muscle level was indeed not sufficient to account for the reduced insulin action measured at the whole body, systemic level. It was suggested that this could be as a result of the release of a factor from the injured muscle that decreases systemic insulin response. The pro-inflammatory cytokine TNF- α , the potential effects of which were described previously, is a likely candidate for this factor.

Glycogen Synthesis

Following exercise-induced glycogen depletion there appears to be a rapid and slow phase of glycogen synthesis (29). In particular, there is an initial, rapid period (30-60 minutes) which is characterized by an insulin-independent translocation of GLUT-4 (54), and a more extended period (up to 48 hours) characterized by an insulin-dependent phase of glycogen synthesis at a slower rate (36).

A common finding in several studies has been a reduced ability of skeletal muscle to synthesize glycogen following exercise-induced muscle injury (8, 16, 21, 47, 66, 76, 89). Muscle biopsies taken following seven days of recovery from a marathon showed that although muscle glycogen had returned to a “normal” level for a trained muscle ($\sim 125 \text{ mmol}\cdot\text{kg}^{-1} \text{ ww}$), it had not returned to the supercompensated levels measured

immediately before the marathon ($\sim 196 \text{ mmol}\cdot\text{kg}^{-1} \text{ ww}$) (76). Additionally, and despite consuming 800 grams of carbohydrate in the 24 hour period following the marathon, muscle glycogen levels rose by only $55 \text{ mmol}\cdot\text{kg}^{-1} \text{ ww}$ during this period, which is a response more typical of untrained muscle (67).

O'Reilly et al. (66) found that eccentric cycle exercise in untrained males reduced pre-exercise glycogen levels by 33 % which then remained in this depressed state for ten days, suggesting that no glycogen synthesis took place whatsoever in the ten days following eccentric exercise. Subjects received 360 grams of carbohydrate per day, with no suggestion by the authors of attempts to provide immediate post-exercise carbohydrate. This amount of carbohydrate ($\sim 5.1 \text{ g}\cdot\text{kg}^{-1} \text{ bw}\cdot\text{d}^{-1}$) is not typically sufficient to promote adequate glycogen restoration following a significant degree of depletion, since it has been shown that carbohydrate provided in these amounts only modestly increases muscle glycogen even during a tapered training period (77), and must be as high as $10 \text{ g}\cdot\text{kg}^{-1} \text{ bw}\cdot\text{d}^{-1}$ to maintain glycogen stores during endurance training (78).

Kuipers et al. (47), investigating moderately trained male cyclists undergoing a single bout of concentric and two repeated bouts of eccentric cycling each separated by three weeks found that 24 hours following concentric cycling muscle glycogen had returned to normal levels, whereas muscle glycogen had actually fallen below baseline values 24 hours following eccentric cycling. This was true for both the first and second eccentric bout, and indicated that 24 hours following eccentric contractions muscle glycogen synthesis is impaired with glycogen demonstrating a decrease below baseline values.

Seemingly conflicting results to the study of Kuipers et al. (47) were found by Blom et al. (8) who determined that a bout of exhaustive running in trained and untrained runners allowed for a significant amount of glycogen to be synthesized in the 22 hour period following exercise. In support of Kuipers et al. (47), no increases in glycogen levels were found during the subsequent 48 hours. Provision of a significant amount of carbohydrate ($600 \text{ g}\cdot\text{d}^{-1}$; $\sim 8.4 \text{ g}\cdot\text{kg}^{-1} \text{ bw}$) following exercise in the study of Blom et al. (8) may explain these contrasting observations.

Costill et al. (16) implemented a single leg methodology in order to describe the pattern of muscle glycogen synthesis following eccentric exercise (100 eccentric contractions of the knee extensors of one leg) and subsequent endurance exercise (60 minutes of cycling with both legs) during the period in which subjects typically encounter the most muscle soreness (24-72 hours). Both legs increased their glycogen content during 24 hour of recovery, but between 24-72 hours of recovery it was observed that the eccentric leg showed only small and non-significant increases in glycogen content. Subjects experiencing the highest soreness and serum creatine kinase responses also experienced the least amount of glycogen resynthesis. One subject experienced a serum creatine kinase value of $30,196 \text{ U}\cdot\text{L}^{-1}$ and actually displayed a decrease in muscle glycogen of $\sim 38.3 \text{ mmol}\cdot\text{kg}^{-1} \text{ ww}$ in the first 24 hours of recovery with no subsequent storage in the following 48 hours. Muscle samples taken 24 hours after eccentric exercise contained frequent sites of leukocyte infiltration, and it was concluded that decreased glycogen storage was possibly related to the glucose consuming activity of the inflammatory response.

Widrick et al. (89) utilized the single leg methodology to characterize in more detail the time course of glycogen accumulation following eccentric exercise by measuring the rate of glycogen synthesis at 6, 24, and 72 hours post-exercise. Inclusion of the six hour time point was a major departure from previous studies. Eccentric exercise (eccentric knee flexion) was superimposed on previously glycogen depleted muscle, while the opposite leg served as a non-exercising control. A novel finding was that glycogen levels six hours post-exercise were the same in eccentric and control muscle. This was in stark contrast to the glycogen levels at 24 and 72 hours post-exercise, which were 15 and 24 % lower in the eccentrically exercised leg, respectively. It was suggested that altered glucose transport could be a causative factor in the lowered rates of glycogen synthesis seen in previously eccentrically exercised muscle.

Doyle et al. (21) conducted a comprehensive investigation of this topic with a study designed to allow for a timely analysis of glycogen synthesis (four hours post-exercise), control of initial glycogen levels, and use of an aggressive post-exercise carbohydrate consumption strategy ($1.6 \text{ g}\cdot\text{kg}^{-1} \text{ bw}\cdot\text{h}^{-1}$). Subjects underwent an initial injury induction protocol with one leg (with the other leg performing equal numbers of concentric contractions) that was superimposed on previously glycogen depleted muscle induced via stationary cycling and repeated sprints, much as in the study of Widrick et al. (89). A unique aspect was that following 48 hours of recovery subjects underwent a second bout of glycogen depleting exercise. Thus, two periods of glycogen depletion and subsequent synthesis were measured; one following depletion exercise and eccentric contractions and one 48 hours later following glycogen depleting exercise only. In support of earlier studies, no difference was observed in the rate of glycogen synthesis

over the initial four hour post-exercise period. However, four hours after the second bout of glycogen depleting exercise (itself taking place 48 hours after the eccentric and concentric contractions) revealed significantly decreased rates of glycogen synthesis. The rate of glycogen replenishment in the eccentric leg was 25 % lower than in the concentric leg (8.14 ± 0.68 vs. 10.97 ± 1.02 $\text{mmol}\cdot\text{kg}^{-1}$ $\text{ww}\cdot\text{h}^{-1}$, respectively), with this lower rate of glycogen synthesis in the eccentrically exercised limb taking place at a time corresponding to the development of muscle soreness in the eccentric limb and significantly elevated blood creatine kinase levels. Of note is that the insulin response to the identical carbohydrate load following the second bout of glycogen depleting exercise appeared to be somewhat elevated. This was particularly evident in the first two hours of recovery, although the authors provided no indication that this difference was statistically significant. Although speculative, it is possible that the trend towards a higher insulin response during the carbohydrate load administered 48 hours post-injury was indicative of a certain degree of insulin resistance. As indicated by the authors, insulin receptor binding and glucose transport are plasma membrane mediated events that could be disrupted as a result of membrane-damaging eccentric contractions.

The studies described above provide support for a temporal relationship between reduced glycogen synthesis rates and delayed onset muscle soreness, muscle damage, and inflammation. It is apparent that there is a coincidence of events that are initiated during the 24-48 hours post-exercise period, namely inflammation, infiltration of mononuclear cells, and insulin resistance. There exists the possibility that these events, alone or in combination, may be responsible for the reduction in glycogen synthesis following eccentric, injury-inducing skeletal muscle contractions.

The distinction between an initial insulin-independent and a delayed insulin-dependent phase of glucose transport would seem to support the findings of transient insulin resistance, decreased GLUT-4, and altered glycogen synthesis following eccentric exercise. Specifically, normal glycogen synthesis found in the hours immediately following eccentric exercise could be explained by elevated GLUT-4 remaining in the sarcolemma. Reduced glycogen synthesis rates measured 24-48 hours following such exercise can be interpreted as occurring at a time when glucose transport has become insulin dependent. Insulin resistance has been consistently observed during this time period. Thus, immediately following eccentric contractions glycogen synthesis is normal, even in the event insulin resistance is present, because this initial phase does not require insulin for translocation of GLUT-4 to the sarcolemma. It is only during the period that insulin is required for GLUT-4 translocation that reduced glycogen synthesis is observed.

Skeletal Muscle Adaptation to Exercise-Induced Muscle Injury

Repeated Bout Effect

Skeletal muscle possesses a considerable degree of plasticity. Befitting this is its ability to adapt to eccentric contractions such that an additional bout of eccentric exercise results in less markers of damage. This has been referred to as the “repeated bout effect” (62).

During eight weeks of consecutive eccentric training, Friden et al. (27) noted pronounced soreness and muscle fiber disruption only following the first 2-3 eccentric training sessions, with marked reductions in these variables for the remainder of the study. Similarly, several early investigators also noted diminished serum CK responses

following exercise training programs (34, 55). The specific time course and magnitude of such adaptations has also been investigated (11, 15, 59, 64).

Byrnes et al. (11) investigated delayed onset muscle soreness, serum CK activity, and serum myoglobin activity following repeated bouts of downhill treadmill running. Three groups of subjects performed two identical bouts of downhill treadmill running (30 min, -10° slope) at a speed that had previously elicited a heart rate of 170 beats per minute (bpm) during level treadmill running. The bouts were separated by three, six, or nine weeks. All groups demonstrated reduced heart rates (9 bpm) and oxygen uptakes ($2.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) during the second bout of exercise. Muscle soreness, which displayed a peak 42 hours following exercise, was significantly decreased compared to the first bout when the second bout was performed either three or six weeks later. Muscle soreness was not different between bouts when the second bout was conducted nine weeks later. Similarly, serum CK levels (expressed as both absolute and relative values) were significantly less compared to the first bout when the second bout was performed either three or six weeks, with no difference being observed when the second bout was delayed by nine weeks. The myoglobin response, when expressed relative to baseline values, showed a similar response to serum CK, being significantly depressed when bouts were separated by three or six weeks, but not being different when bouts were separated by nine weeks. MVC was not assessed during this study. Thus, a second bout of eccentric exercise was shown to result in an easily observable repeated bout effect when performed three or six weeks later, but not nine weeks later.

Eston et al. (22) performed a similar study to that of Byrnes et al. (11) but with the addition of strength measurements. Subjects performed two downhill treadmill

running protocols (7 mph, - 12 % grade) for 40 minutes separated by five weeks.

Elevations in serum CK and muscle tenderness, although still present, were significantly less following the second treadmill run. Interestingly, and in contrast to other studies of the repeated bout effect described below, maximal voluntary isometric force exhibited no repeated bout effect, displaying identical force decreases and patterns of recovery following both bouts of running.

Newham et al. (59), utilizing maximal eccentric contractions of the elbow flexors, provided supporting evidence for the study of Byrnes et al. (11). Subjects performed a single eccentric maximal voluntary contraction every 15 seconds for 20 minutes. This eccentric exercise protocol was performed on three separate occasions each separated by two weeks. MVC, plasma CK, and muscle tenderness were assessed before, immediately after, and at daily intervals after each exercise bout. Large (~50 %) decreases in MVC were noted immediately after the eccentric contractions, with no difference between bouts. Thus, the extent of force loss was identical immediately after each bout. However, whereas after the first bout of exercise there was no sign of recovery in the subsequent 24 hour period with strength deficits remaining close to 50 %, recovery following exercise bouts two and three displayed recoveries to 66 and 69 % of baseline, respectively. While recovery to full force producing capacity did not occur until one week after the third and final bout of exercise, there seemed to be a trend towards a more rapid partial recovery following the second and third bouts.

Possibly the most significant finding in the study of Newham et al. (59) was the elimination of an elevated serum CK response as a result of the first bout of exercise. As expected, following bout one there was a substantial and delayed increase in serum CK in

all subjects. However, following bouts two and three there were no significant increases in serum CK at any time point following eccentric exercise. Thus, the serum CK response was essentially completely eliminated as a result of a single bout of eccentric contractions. Muscle tenderness occurred following each bout, but displayed a progressive decline following subsequent bouts.

The above referenced studies indicated that a relatively severe bout of eccentric contractions induced a degree of adaptation that resulted in significantly less soreness and elevations in serum CK, as well as more rapid returns of force producing capacity. Clarkson et al. (15) investigated whether performance of a relatively low number of eccentric contractions resulting in only small changes in indicators of muscle damage would result in an appreciable adaptation. Utilizing the eccentric elbow extension model, subjects performed 70 maximal contractions with one arm and 24 maximal contractions with the other arm, followed two weeks later by 70 maximal contractions with the arm that had previously performed 24 contractions. Thus, it was possible to compare the response of 70 maximal contractions performed with no prior eccentric exercise with 70 maximal contractions performed with prior eccentric exercise.

Isometric force had not returned to baseline by five days post-exercise following the initial 70 contractions. In contrast, following 24 contractions force had returned to baseline values by two days post-exercise, with the subsequent set of 70 contractions demonstrating a return to baseline conditions by only one day post-exercise. Soreness ratings were significantly elevated following the initial 70 contractions, with much less soreness being experienced following either 24 or 70 subsequent contractions. Serum CK values were highest following the initial 70 contractions, and although displaying an

increase were significantly lower following 24 contractions. There was no elevation in serum CK following the second bout of 70 contractions.

The study of Clarkson et al. (15) demonstrated that a preliminary bout of eccentric contractions (i.e. 24 eccentric contractions) resulting in only a moderately small degree of muscle injury allows for an adaptation to take place. Such an adaptation allows the muscle to become more resistant to damage and allows for any damage that does occur to be repaired at a faster rate.

The study of Byrnes et al. (11) suggested that eccentric contractions conferred a protective effect on subsequent eccentric contractions lasting for up to six weeks, with the benefits being lost somewhere between six and nine weeks. Utilizing an eccentric elbow extension protocol it has been determined that the protective effect can last for up to six months, as evidenced by a faster recovery of maximal isometric force and attenuated increases in serum CK, soreness, and swelling (64). Amazingly, a faster recovery of maximal isometric force was also noted at nine months. There were no indications of a repeated bout effect for any of the measured variables at 12 months post-initial exercise. Thus, it was demonstrated that the repeated bout effect could last for up to six months for most measures of muscle injury, with the effects on strength recovery lasting up to nine months. The contrasting time frames of the repeated bout effect suggested by Byrnes et al. (11) and Nosaka et al. (64) could be related to the differing protocols used (downhill treadmill running vs. elbow extension), with it being likely that eccentric elbow flexion resulted in a significantly greater and more focal degree of muscle injury. Additionally, the fact that most individuals spend more time using their legs for purposes of walking

may result in an already present protective effect that is lacking in the less frequently used arms.

Mechanism

The studies outlined above provide evidence that an initial bout of eccentric exercise provides a protective effect on subsequent bouts of similar exercise. In most studies, the repeated bout effect is manifested by more rapid returns to full force producing capacity (15, 59, 64), decreased soreness ratings (11, 15, 22, 27, 59, 64), and significantly reduced serum CK responses (11, 15, 22, 34, 55, 59, 64) following a second bout of eccentric contractions. This effect may last for as long as 6-9 months (64). In addition, the initial bout does not have to be particularly severe to elicit this adaptation (15). The mechanism responsible for this adaptive process has not been fully elucidated, although several have been suggested. Concepts put forward to account for the adaptation following an initial bout of eccentric exercise can be grouped into neural, connective tissue, and cellular theories (56, 57).

The neural theory proposes that the initial damage stimulates an increased motor unit activity (45), slow-twitch fiber recruitment, and motor unit synchronization (30). Subsequently, forces encountered during a repeated bout of eccentric contractions are spread across more fibers (62). The connective tissue theory describes increases in the amount of intramuscular connective tissue (49) and remodeling of the intermediate filaments (27) in response to eccentric contractions. Cellular adaptation theories suggest a strengthening of cell membranes (15), removal of weak fibers (2), and longitudinal addition of sarcomeres (53) as likely candidates for the adaptive response.

Potential membrane related adaptations are particularly intriguing when reduced glycogen synthesis (8, 16, 21, 47, 66, 76, 89), transient insulin resistance (3, 41, 42, 44), and decreased glucose transporter number (3, 4) resulting from eccentric contractions are considered. These three events are intimately related to each other and the integrity of the membrane, and adaptations to the membrane following eccentric contractions may reduce their degree of severity. The significantly reduced level of serum CK measured after a repeated bout of eccentric exercise has been put forth by Clarkson et al. (15) as an indication that the cell membrane has been strengthened and is less likely to allow a loss of calcium homeostasis and subsequent necrosis of cellular components.

Conclusion

Exercise biased towards eccentric muscle contractions has a propensity to cause exercise-induced muscle injury, with the effects of a single bout of eccentric exercise evidenced by immediate and prolonged declines in the ability to produce maximal force (15, 35), elevated blood concentrations of skeletal muscle proteins (13), and muscle soreness (10). Eccentric contractions also disrupt glucose metabolism, which is typically manifested as an increased insulin response to obtain a given glucose uptake (79), or a reduction in the actual uptake of glucose by skeletal muscle cells (44). Likely candidates for this alteration in glucose uptake include reduced numbers of sarcolemmal glucose transporters (GLUT 4) (3, 4) and insulin resistance (3, 41, 42, 44, 79) following eccentric exercise.

Several studies have established the presence of a “repeated bout effect” with regard to the ability of a single eccentric insult to impart an accelerated strength recovery and an attenuated serum CK and soreness response (11, 15, 22, 59, 64). Thus, a bout of

novel eccentrically-biased exercise can confer a protective effective on skeletal muscle such that, within a certain period of time, a second bout of similar exercise seemingly results in less disruption to the muscle. This protective repeated bout effect has been illustrated to remain effective at reducing the magnitude of symptoms of exercise-induced muscle-injury for several weeks (11) or even months (64).

Although several studies have illustrated transient insulin resistance following an initial bout of eccentrically-biased exercise, there have been no studies investigating whether insulin resistance is attenuated following a second bout of eccentric contractions. It remains to be seen whether a second bout of eccentric contractions results in an attenuated degree of insulin resistance along the lines of an adaptive repeated bout effect as seen with many of the other symptoms of exercise-induced muscle injury.

References

1. **Armstrong RB**. Initial events in exercise-induced muscular injury. *Med Sci Sports Exerc* 22: 429-435, 1990.
2. **Armstrong RB, Ogilvie RW and Schwane JA**. Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol* 54: 80-93, 1983.
3. **Asp S, Daugaard JR, Kristiansen S, Kiens B and Richter EA**. Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects. *J Physiol* 494 (Pt 3): 891-898, 1996.
4. **Asp S, Daugaard JR and Richter EA**. Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. *J Physiol* 482 (Pt 3): 705-712, 1995.
5. **Barash IA, Peters D, Friden J, Lutz GJ and Lieber RL**. Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. *Am J Physiol Regul Integr Comp Physiol* 283: R958-R963, 2002.
6. **Bell GI, Burant CF, Takeda J and Gould GW**. Structure and function of mammalian facilitative sugar transporters. *J Biol Chem* 268: 19161-19164, 1993.
7. **Bigland-Ritchie B and Woods JJ**. Integrated electromyogram and oxygen uptake during positive and negative work. *J Physiol* 260: 267-277, 1976.
8. **Blom PC, Costill DL and Vollestad NK**. Exhaustive running: inappropriate as a stimulus of muscle glycogen super-compensation. *Med Sci Sports Exerc* 19: 398-403, 1987.
9. **Burant CF, Takeda J, Brot-Laroche E, Bell GI and Davidson NO**. Fructose transporter in human spermatozoa and small intestine is GLUT5. *J Biol Chem* 267: 14523-14526, 1992.
10. **Byrne C, Twist C and Eston R**. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. *Sports Med* 34: 49-69, 2004.
11. **Byrnes WC, Clarkson PM, White JS, Hsieh SS, Frykman PN and Maughan RJ**. Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol* 59: 710-715, 1985.
12. **Cheatham B and Kahn CR**. Insulin action and the insulin signaling network. *Endocr Rev* 16: 117-142, 1995.
13. **Clarkson PM, Byrnes WC, McCormick KM, Turcotte LP and White JS**. Muscle soreness and serum creatine kinase activity following isometric, eccentric, and concentric exercise. *Int J Sports Med* 7: 152-155, 1986.
14. **Clarkson PM and Ebbeling C**. Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clin Sci (Lond)* 75: 257-261, 1988.
15. **Clarkson PM and Tremblay I**. Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol* 65: 1-6, 1988.
16. **Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI and Pearson D**. Impaired muscle glycogen resynthesis after eccentric exercise. *J Appl Physiol* 69: 46-50, 1990.

17. **DeFronzo RA, Bonadonna RC and Ferrannini E.** Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 15: 318-368, 1992.
18. **Del Aguila LF, Krishnan RK, Ulbrecht JS, Farrell PA, Correll PH, Lang CH, Zierath JR and Kirwan JP.** Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E206-E212, 2000.
19. **Dinareello CA.** Role of pro- and anti-inflammatory cytokines during inflammation: experimental and clinical findings. *J Biol Regul Homeost Agents* 11: 91-103, 1997.
20. **Douen AG, Ramlal T, Rastogi S, Bilan PJ, Cartee GD, Vranic M, Holloszy JO and Klip A.** Exercise induces recruitment of the "insulin-responsive glucose transporter". Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *J Biol Chem* 265: 13427-13430, 1990.
21. **Doyle JA, Sherman WM and Strauss RL.** Effects of eccentric and concentric exercise on muscle glycogen replenishment. *J Appl Physiol* 74: 1848-1855, 1993.
22. **Eston RG, Lemmey AB, McHugh P, Byrne C and Walsh SE.** Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scand J Med Sci Sports* 10: 199-204, 2000.
23. **Evans WJ and Cannon JG.** The metabolic effects of exercise-induced muscle damage. *Exerc Sport Sci Rev* 19: 99-125, 1991.
24. **Faulkner JA.** Terminology for contractions of muscles during shortening, while isometric, and during lengthening. *J Appl Physiol* 95: 455-459, 2003.
25. **Fielding RA, Manfredi TJ, Ding W, Fiatarone MA, Evans WJ and Cannon JG.** Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am J Physiol* 265: R166-R172, 1993.
26. **Friden J and Lieber RL.** Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta Physiol Scand* 171: 321-326, 2001.
27. **Friden J, Seger J, Sjostrom M and Ekblom B.** Adaptive response in human skeletal muscle subjected to prolonged eccentric training. *Int J Sports Med* 4: 177-183, 1983.
28. **Friden J, Sjostrom M and Ekblom B.** A morphological study of delayed muscle soreness. *Experientia* 37: 506-507, 1981.
29. **Garetto LP, Richter EA, Goodman MN and Ruderman NB.** Enhanced muscle glucose metabolism after exercise in the rat: the two phases. *Am J Physiol* 246: E471-E475, 1984.
30. **Golden CL and Dudley GA.** Strength after bouts of eccentric or concentric actions. *Med Sci Sports Exerc* 24: 926-933, 1992.
31. **Goodyear LJ and Kahn BB.** Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 49: 235-261, 1998.
32. **Hayashi T, Wojtaszewski JF and Goodyear LJ.** Exercise regulation of glucose transport in skeletal muscle. *Am J Physiol* 273: E1039-E1051, 1997.
33. **Hikida RS, Staron RS, Hagerman FC, Sherman WM and Costill DL.** Muscle fiber necrosis associated with human marathon runners. *J Neurol Sci* 59: 185-203, 1983.
34. **Hunter JB and Critz JB.** Effect of training on plasma enzyme levels in man. *J Appl Physiol* 31: 20-23, 1971.

35. **Ingalls CP, Warren GL, Williams JH, Ward CW and Armstrong RB.** E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol* 85: 58-67, 1998.
36. **Ivy JL.** Muscle glycogen synthesis before and after exercise. *Sports Med* 11: 6-19, 1991.
37. **Ivy JL and Holloszy JO.** Persistent increase in glucose uptake by rat skeletal muscle following exercise. *Am J Physiol* 241: C200-C203, 1981.
38. **Jentjens R and Jeukendrup A.** Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Med* 33: 117-144, 2003.
39. **Kasuga M, Zick Y, Blith DL, Karlsson FA, Haring HU and Kahn CR.** Insulin stimulation of phosphorylation of the beta subunit of the insulin receptor. Formation of both phosphoserine and phosphotyrosine. *J Biol Chem* 257: 9891-9894, 1982.
40. **Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS, Eddy RL, Byers MG, Shows TB, Seino S and Bell GI.** Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). *J Biol Chem* 265: 13276-13282, 1990.
41. **King DS, Feltmeyer TL, Baldus PJ, Sharp RL and Nespor J.** Effects of eccentric exercise on insulin secretion and action in humans. *J Appl Physiol* 75: 2151-2156, 1993.
42. **Kirwan JP, Bourey RE, Kohrt WM, Staten MA and Holloszy JO.** Effects of treadmill exercise to exhaustion on the insulin response to hyperglycemia in untrained men. *J Appl Physiol* 70: 246-250, 1991.
43. **Kirwan JP and Del Aguila LF.** Insulin signalling, exercise and cellular integrity. *Biochem Soc Trans* 31: 1281-1285, 2003.
44. **Kirwan JP, Hickner RC, Yarasheski KE, Kohrt WM, Wiethop BV and Holloszy JO.** Eccentric exercise induces transient insulin resistance in healthy individuals. *J Appl Physiol* 72: 2197-2202, 1992.
45. **Komi PV and Buskirk ER.** Effect of eccentric and concentric muscle conditioning on tension and electrical activity of human muscle. *Ergonomics* 15: 417-434, 1972.
46. **Kubo K and Foley JE.** Rate-limiting steps for insulin-mediated glucose uptake into perfused rat hindlimb. *Am J Physiol* 250: E100-E102, 1986.
47. **Kuipers H, Keizer HA, Verstappen FT and Costill DL.** Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work. *Int J Sports Med* 6: 336-339, 1985.
48. **Lang CH, Dobrescu C and Bagby GJ.** Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology* 130: 43-52, 1992.
49. **Lapier TK, Burton HW, Almon R and Cerny F.** Alterations in intramuscular connective tissue after limb casting affect contraction-induced muscle injury. *J Appl Physiol* 78: 1065-1069, 1995.
50. **Lee AD, Hansen PA and Holloszy JO.** Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Lett* 361: 51-54, 1995.

51. **Lieber RL, Thornell LE and Friden J.** Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction. *J Appl Physiol* 80: 278-284, 1996.
52. **Lowe DA, Warren GL, Ingalls CP, Boorstein DB and Armstrong RB.** Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *J Appl Physiol* 79: 1260-1270, 1995.
53. **Lynn R and Morgan DL.** Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol* 77: 1439-1444, 1994.
54. **Maehlum S, Hostmark AT and Hermansen L.** Synthesis of muscle glycogen during recovery after prolonged severe exercise in diabetic and non-diabetic subjects. *Scand J Clin Lab Invest* 37: 309-316, 1977.
55. **Maxwell JH and Bloor CM.** Effects of conditioning on exertional rhabdomyolysis and serum creatine kinase after severe exercise. *Enzyme* 26: 177-181, 1981.
56. **McHugh MP.** Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports* 13: 88-97, 2003.
57. **McHugh MP, Connolly DA, Eston RG and Gleim GW.** Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med* 27: 157-170, 1999.
58. **Millard-Stafford M, Warren GL, Thomas LM, Doyle JA, Snow T and Hitchcock K.** Recovery from run training: efficacy of a carbohydrate-protein beverage? *Int J Sport Nutr Exerc Metab* 15: 610-624, 2005.
59. **Newham DJ, Jones DA and Clarkson PM.** Repeated high-force eccentric exercise: effects on muscle pain and damage. *J Appl Physiol* 63: 1381-1386, 1987.
60. **Newham DJ, McPhail G, Mills KR and Edwards RH.** Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* 61: 109-122, 1983.
61. **Newham DJ, Mills KR, Quigley BM and Edwards RH.** Pain and fatigue after concentric and eccentric muscle contractions. *Clin Sci (Lond)* 64: 55-62, 1983.
62. **Nosaka K and Clarkson PM.** Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc* 27: 1263-1269, 1995.
63. **Nosaka K and Clarkson PM.** Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med Sci Sports Exerc* 28: 953-961, 1996.
64. **Nosaka K, Sakamoto K, Newton M and Sacco P.** How long does the protective effect on eccentric exercise-induced muscle damage last? *Med Sci Sports Exerc* 33: 1490-1495, 2001.
65. **Nosaka K, Sakamoto K, Newton M and Sacco P.** The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *Eur J Appl Physiol* 85: 34-40, 2001.
66. **O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN and Evans WJ.** Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. *J Appl Physiol* 63: 252-256, 1987.
67. **Piehl K, Adolfsson S and Nazar K.** Glycogen storage and glycogen synthetase activity in trained and untrained muscle of man. *Acta Physiol Scand* 90: 779-788, 1974.

68. **Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI and Shulman RG.** Human muscle glycogen resynthesis after exercise: insulin-dependent and -independent phases. *J Appl Physiol* 76: 104-111, 1994.
69. **Proske U and Morgan DL.** Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 537: 333-345, 2001.
70. **Richter EA.** Glucose utilization. In: Handbook of physiology, Section 12: Exercise: Regulation and integration of multiple systems, edited by Rowell LB and Shepherd JT. New York: Oxford University Press, 1996, p. 912-951.
71. **Richter EA, Garetto LP, Goodman MN and Ruderman NB.** Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. *J Clin Invest* 69: 785-793, 1982.
72. **Roy D and Marette A.** Exercise induces the translocation of GLUT4 to transverse tubules from an intracellular pool in rat skeletal muscle. *Biochem Biophys Res Commun* 223: 147-152, 1996.
73. **Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A and White MF.** Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest* 107: 181-189, 2001.
74. **Saunders MJ, Kane MD and Todd MK.** Effects of a carbohydrate-protein beverage on cycling endurance and muscle damage. *Med Sci Sports Exerc* 36: 1233-1238, 2004.
75. **Schwane JA, Johnson SR, Vandenakker CB and Armstrong RB.** Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill running. *Med Sci Sports Exerc* 15: 51-56, 1983.
76. **Sherman WM, Costill DL, Fink WJ, Hagerman FC, Armstrong LE and Murray TF.** Effect of a 42.2-km footrace and subsequent rest or exercise on muscle glycogen and enzymes. *J Appl Physiol* 55: 1219-1224, 1983.
77. **Sherman WM, Costill DL, Fink WJ and Miller JM.** Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. *Int J Sports Med* 2: 114-118, 1981.
78. **Sherman WM, Doyle JA, Lamb DR and Strauss RH.** Dietary carbohydrate, muscle glycogen, and exercise performance during 7 d of training. *Am J Clin Nutr* 57: 27-31, 1993.
79. **Sherman WM, Lash JM, Simonsen JC and Bloomfield SA.** Effects of downhill running on the responses to an oral glucose challenge. *Int J Sport Nutr* 2: 251-259, 1992.
80. **Smith LL, Anwar A, Fragen M, Rananto C, Johnson R and Holbert D.** Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. *Eur J Appl Physiol* 82: 61-67, 2000.
81. **Stauber WT.** Eccentric action of muscles: physiology, injury, and adaptation. *Exerc Sport Sci Rev* 17: 157-185, 1989.
82. **Tidball JG.** Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 288: R345-R353, 2005.
83. **Vanhaesebroeck B, Leever SJ, Panayotou G and Waterfield MD.** Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci* 22: 267-272, 1997.

84. **Wallberg-Henriksson H.** Glucose transport into skeletal muscle. Influence of contractile activity, insulin, catecholamines and diabetes mellitus. *Acta Physiol Scand Suppl* 564: 1-80, 1987.
85. **Warhol MJ, Siegel AJ, Evans WJ and Silverman LM.** Skeletal muscle injury and repair in marathon runners after competition. *Am J Pathol* 118: 331-339, 1985.
86. **Warren GL, Lowe DA and Armstrong RB.** Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43-59, 1999.
87. **Wasserman DH and Halseth AE.** An overview of muscle glucose uptake during exercise: Sites of regulation. In: *Skeletal muscle metabolism in exercise and diabetes*, edited by Richter EA, Kiens B, Galbo H and Saltin B. New York: Plenum Press, 1998, p. 1-16.
88. **Watson RT and Pessin JE.** Intracellular organization of insulin signaling and GLUT4 translocation. *Recent Prog Horm Res* 56: 175-193, 2001.
89. **Widrick JJ, Costill DL, McConell GK, Anderson DE, Pearson DR and Zachwieja JJ.** Time course of glycogen accumulation after eccentric exercise. *J Appl Physiol* 72: 1999-2004, 1992.

CHAPTER 2
EFFECT OF A REPEATED BOUT OF ECCENTRICALLY-BIASED
CONTRACTIONS ON INSULIN RESISTANCE

Introduction

Skeletal muscle exposed to unaccustomed eccentric (lengthening) contractions exhibits decrements in maximal voluntary force production (MVC) (8, 10, 32), delayed swelling and soreness (7), cell membrane damage with release of muscle proteins (e.g. creatine kinase [CK]) into the bloodstream (9), and ultrastructural damage (29). These disruptions are evident immediately after the contractions and may persist for several days. Perturbations in glucose metabolism have also been observed following eccentric contractions, including decreased glucose transporter (GLUT) number (4) and a reduced ability of the muscle to synthesize glycogen (11, 13, 34, 40).

Transient insulin resistance has been demonstrated following eccentric contractions (3, 19, 20, 22, 37). Sherman et al. (37) administered oral glucose tolerance tests (OGTT) 48 hours following eccentric exercise and observed peak insulin and insulin area under the curve (AUC) responses that were significantly higher than control values. King et al. (19) administered hyperglycemic clamps 36 hours following eccentric exercise and demonstrated exaggerated peak insulin levels accompanied by increases in the insulin AUC (19). Utilizing euglycemic-hyperinsulinemic clamps, Kirwan et al. (22) reported reduced glucose disposal rates 48 h following eccentric exercise. Membrane

ultrastructural damage subsequent to high-force eccentric muscle contractions could be a plausible explanation for an impairment of insulin mediated glucose transport.

A “repeated bout effect” occurs in skeletal muscle exposed to prior eccentric contractions (31). A second bout of eccentric contractions performed at a later date results in less muscle soreness and CK release with a more rapid recovery of strength. Evidence of a protective repeated bout effect on measures of muscle injury has been demonstrated to extend for several weeks (8) or months (32), often as a result of relatively few novel eccentric contractions (10, 33).

While a single bout of eccentrically-biased exercise has been shown to induce insulin resistance, it is not known if a repeated bout of similar eccentrically-biased exercise results in the same degree of insulin resistance. Therefore, the purpose of this study was to determine whether the insulin resistance experienced following a novel bout of eccentrically-biased contractions would be diminished following a second bout of eccentric contractions in accordance with the concept of an adaptive repeated bout effect. Specifically, it was hypothesized that the AUC for insulin in response to an OGTT would be attenuated following a second bout of downhill treadmill running.

Methods

Subjects. Ten female recreational athletes participated in this research study (Table 1). A preliminary power analysis (with alpha set at 0.05 and power at 0.70) utilizing data from Sherman et al. (37) indicated that 10 subjects would be required to identify an attenuated insulin response of 20 % above baseline following a second downhill treadmill run. Female subjects were recruited because they may demonstrate

Table 1. Subject characteristics (n = 10)

	Mean \pm SD	Range
Age (yrs)	24.7 \pm 3.0	21 - 30
Weight (kg)	64.9 \pm 7.5	56.8 - 82.4
Height (m)	1.67 \pm 0.07	1.56 - 1.80
Body fat (%)	29.1 \pm 5.9	22.8 - 41.9

yrs = years; kg = kilogram; m = meter

larger initial force losses and faster recovery rates than males following exercise-induced muscle injury (35).

Criteria for participant selection included a low to moderate level of aerobic activity (1-5 hours per week), age between 18-35 years, and “low risk” classification as defined by the guidelines of the American College of Sports Medicine (1). Individuals previously trained (within the last 12 months) using eccentric contractions (e.g. plyometric training) were excluded from the study. Subjects were asked to abstain from the consumption of alcohol and caffeine for at least 12 hours prior to all testing sessions, to not engage in strenuous physical activity extraneous to the study, and to avoid treating any symptoms of exercise-induced muscle injury that may be experienced (e.g., cryotherapy, non-steroidal anti-inflammatory drugs, massage, etc.). Baseline VO₂ max tests were not conducted to avoid the potential initiation of muscle injury and subsequent repeated bout effect due to the physical stress of such a maximal effort test. Subjects were made aware of the purpose and potential risks of participation in the study prior to signing a written informed consent document approved by the Georgia State University Institutional Review Board.

Experimental Design. Insulin resistance was derived from the insulin AUC resulting from an OGTT administered after a bout of novel eccentrically-biased exercise (downhill treadmill running). The same protocol was repeated after 14 days to determine the degree of insulin resistance induced by a second bout of similar exercise.

Subjects reported to the laboratory on six separate occasions spanning a three week period. During the first visit, subject weight, height, and body composition (DEXA, Lunar Prodigy, General Electric, Madison, WI) were measured, and an isometric quadriceps strength familiarization trial was performed (500H Kincom isokinetic dynamometer, Chattanooga Group, Hixson, TN). A second visit 1-7 days later allowed for an additional strength familiarization trial, as well as assessment of baseline serum CK levels and insulin and glucose responses to an OGTT.

Subjects subsequently completed two downhill treadmill runs separated by 14 days. MVC torque and ratings of muscle soreness were assessed immediately pre and post-exercise, as well as 48 hours post-exercise. Serum CK and the insulin and glucose response to an OGTT were measured 48 hours following each treadmill run. With the exception of the OGTT visits, subjects attended each experimental visit 2-3 hours post-prandial at the same time of day. Macronutrient or caloric content of pre-visit meals were not controlled.

Injury Protocol. Subjects performed two intermittent downhill running protocols (DTR 1 and DTR 2) separated by 14 days on a motorized treadmill (Q65, Quinton Instrument Co., Bothell, WA) in order to induce muscle injury in the musculature of the lower extremities. Subjects ran down a -12 % grade at 8.0 mph for a total of 30 minutes (6 x 5 minutes with 2 minute standing rest intervals). Heart rate (Polar Electro, Kempele,

Finland) and oxygen consumption (TrueOne 2400 Metabolic Measurement System, Parvomedics, Inc., Sandy, Utah) were continuously recorded during the downhill treadmill runs. Ratings of Perceived Exertion (RPE) were assessed during the final minute of each interval (5). The number of steps taken during the fourth minute of each 5 minute interval were averaged and multiplied by a factor of 30 in order to estimate the total number of steps taken during the entire 30 minutes of running.

Muscle Strength. Functional changes in the quadriceps muscle group due to exercise-induced muscle injury were measured with a 500H Kincom isokinetic dynamometer (Chattanooga Group, Hixson, TN). Maximal isometric torque was determined immediately before, after, and 48 hours following each treadmill run as described previously (17). Briefly, subjects attempted three maximal voluntary isometric contractions (MVC) of five second duration at a knee angle of 80 and 90° with each leg (with 0° being full extension of the knee). Peak isometric torque measured at each knee angle was recorded for analysis. Strength tests taking place 48 hours following treadmill runs were performed immediately upon completion of the scheduled OGTT.

Ratings of Muscle Soreness. Subjective ratings of muscle soreness were assessed immediately pre-, post-, and 48 hours post-downhill run using a 100 mm visual analog scale (6). Standard instructions were given to rate muscle soreness only in the anterior thigh and posterior lower leg. Subjects reported their muscle soreness after stepping down from a bench that was 30.45 cm (1 ft.) high. Each subject stepped down a total of four times from the bench alternating the leading leg between attempts. A score of “0” corresponded to a perception of “no soreness”, while a score of “100” indicated a feeling

of “very, very sore”. The mean of the four soreness ratings was calculated and used for comparative analysis.

Oral Glucose Tolerance Test. Each subject underwent three OGTTs. The first OGTT was administered 48 hours prior to DTR 1, with the second and third OGTT administered 48 hours following DTR 1 and DTR 2, respectively. Subjects reported to the lab in the morning after an overnight (8-12 hours) fast for each OGTT. Following a baseline blood sample, subjects consumed 75 grams of glucose dissolved in water (Trutol 75, NERL Diagnostics, East Providence, RI) and blood samples were collected at regular intervals (30, 60, 90, and 120 minutes). Subjects remained in a seated position for the duration of the OGTT.

Blood Analysis. Blood samples (~3.5 mL) were collected via venipuncture of a superficial arm vein directly into tubes containing a polymer gel and clot activator (Vacutainer, Beckton Dickinson, Franklin Lakes, NJ) for collection of serum. A small aliquot of blood was immediately removed from each sample to allow for the determination of whole blood glucose in duplicate (YSI 2300 STAT Plus, YSI Inc., Yellow Springs, OH). Blood samples were allowed to clot at room temperature for 30 minutes before being centrifuged at 2,000-3,000 g for 15 minutes at $4 \pm 2^\circ \text{C}$. Separated serum was transferred to a freezable tube and stored at -20°C until analyzed for serum insulin and CK activity. Serum insulin levels were measured in duplicate with a microplate reader (Opsys MR, Dynex Technologies, Chantilly, VA) using a human insulin ELISA kit (Linco Research, St. Charles, MO). Serum CK activity was measured in duplicate with a spectrophotometer (Beckman DU 650, Beckman Coulter, Inc., Fullerton, CA) using a CK reagent kit (Pointe Scientific, Inc., Lincoln Park, MI). The

intra-assay coefficients of variation for duplicate measures of whole blood glucose, serum insulin, and serum CK were 1.6, 4.3, and 3.7 %, respectively.

Area Under the Curve (AUC). Area under the curve (AUC) for insulin and glucose were calculated utilizing the trapezoid method (SigmaPlot, Version 8.02, SPSS, Chicago, IL), and expressed as $\mu\text{U}\cdot\text{mL}^{-1}\cdot 120\text{ min}^{-1}$ and $\text{mmol}\cdot\text{L}^{-1}\cdot 120\text{ min}^{-1}$, respectively. The baseline for determination of the insulin and glucose AUC calculations was set at fasting levels. Use of the OGTT, along with calculation of the integrated area under the insulin curve, has been shown to be an accurate surrogate measure of insulin resistance (41).

Statistical Analysis. Maximal isometric quadriceps strength and lower extremity muscle soreness were analyzed with separate two-way (Bout x Time) ANOVA with repeated measures on time. Serum CK activity, basal and peak insulin and glucose, and insulin and glucose AUC data were analyzed with separate one-way ANOVA with repeated measures on time. Subjects' responses to the downhill treadmill runs (average oxygen consumption, heart rate, RPE, $\text{steps}\cdot\text{min}^{-1}$, and total steps) were analyzed with separate dependent samples t-tests. Statistical significance was determined at an alpha level of $p < 0.05$. When warranted, post hoc comparisons were made using Fisher's least significant difference test. All statistical analyses were performed using SPSS 12.0 (SPSS, Chicago, IL). Values are listed as mean \pm standard error of the mean (SEM) except where noted.

Results

Insulin Response. Fasting insulin was not different ($p = 0.69$) between baseline and 48 hours following DTR 1 or DTR 2 (Table 2 and Figure 1A). Compared to baseline,

Table 2: Fasting and Peak Insulin and Glucose Responses to an OGTT

	BASE	DTR 1	DTR 2
Insulin ($\mu\text{U}\cdot\text{mL}^{-1}$)			
Basal	5.52 ± 0.52	5.21 ± 0.72	5.06 ± 0.84
Peak	31.62 ± 3.99	$44.11 \pm 5.07^*$	38.35 ± 5.93
Glucose ($\text{mmol}\cdot\text{L}^{-1}$)			
Basal	4.04 ± 0.11	4.11 ± 0.12	4.03 ± 0.12
Peak	5.49 ± 0.35	$6.45 \pm 0.37^*$	5.66 ± 0.22

BASE = baseline OGTT; DTR 1 = OGTT 48 h following DTR 1; DTR 2 = OGTT 48 h following DTR 2. *Significantly different than BASE and DTR 2 ($p < 0.05$).

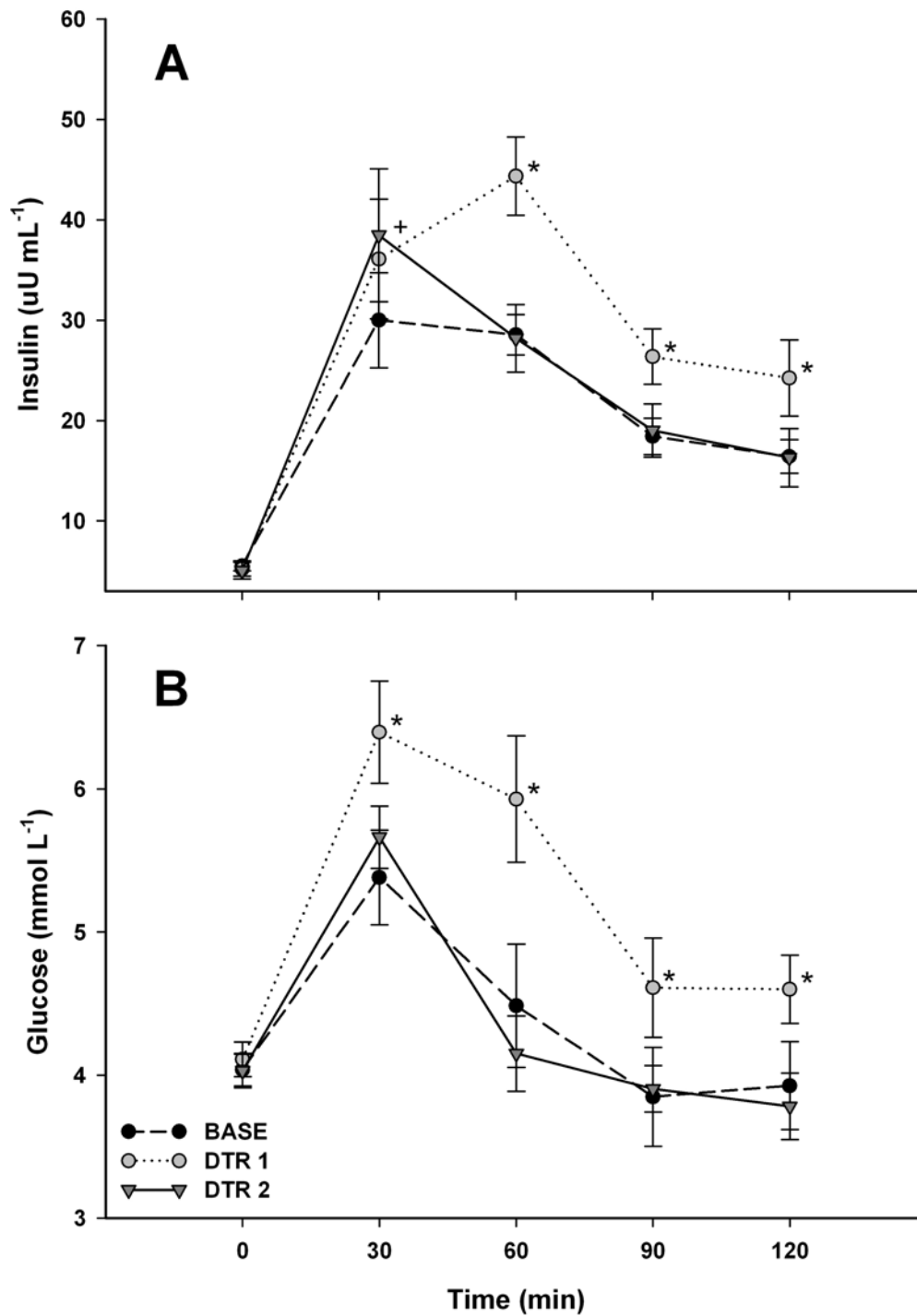


Figure 1. Insulin (A) and glucose (B) response to an OGTT administered at baseline (BASE) and 48 h following DTR 1 and DTR 2. *Significantly different than BASE and DTR 2 ($p < 0.05$). ⁺Significantly different than BASE ($p < 0.05$).

there was a significantly higher peak insulin response during the OGTT administered 48 hours following DTR 1 ($p < 0.01$), with no elevation above the baseline OGTT insulin response 48 hours following DTR 2 ($p = 0.08$) (Table 2 and Figure 1A). Insulin AUC was significantly higher than baseline AUC 48 hours following DTR 1 ($37.6 \pm 8.4\%$), but was not significantly higher than baseline AUC 48 hours following DTR 2 ($p = 0.28$) (Figure 2A).

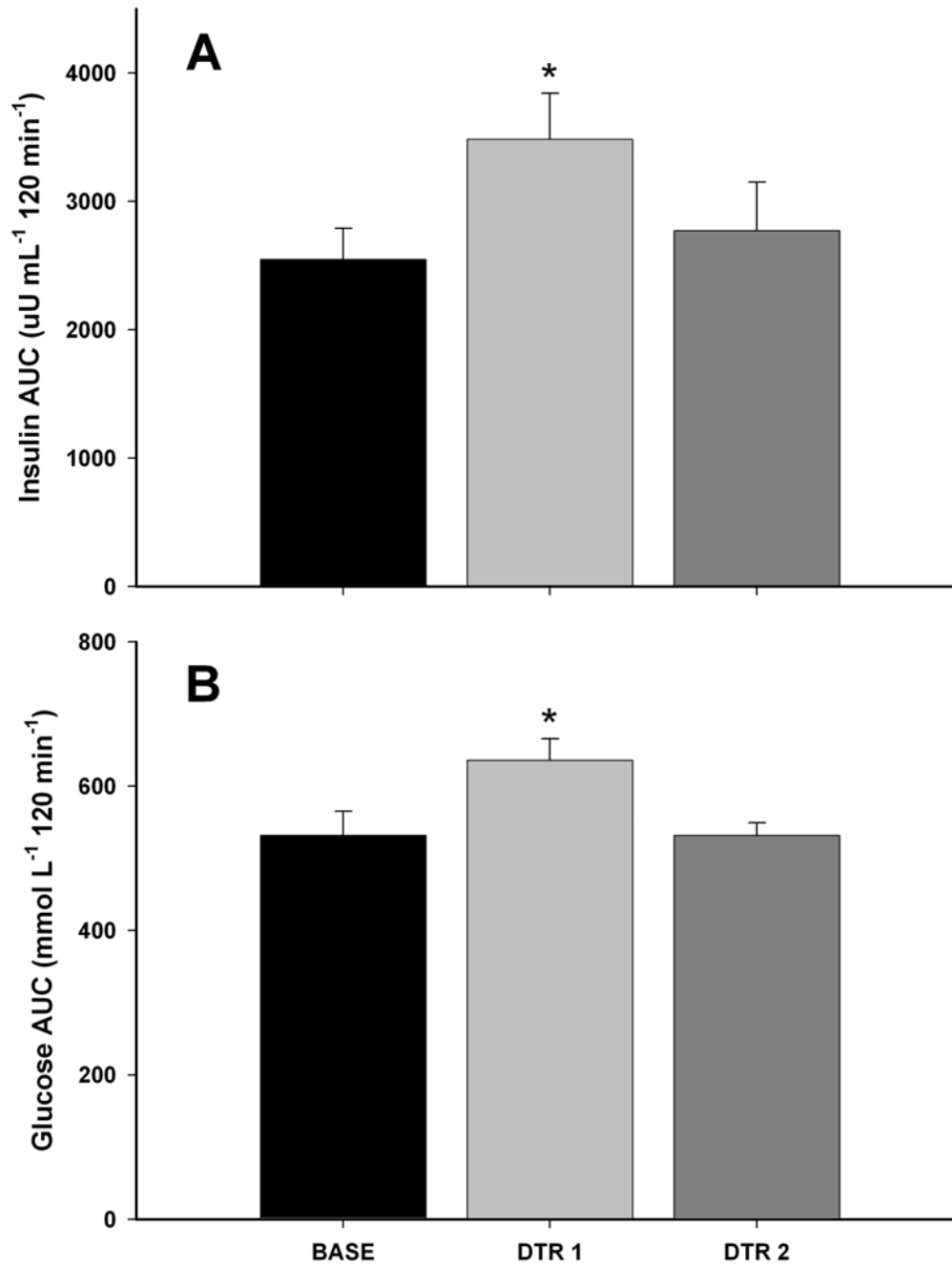


Figure 2. Insulin (A) and glucose (B) AUC in response to an OGTT administered at baseline (BASE) and 48 h following DTR 1 and DTR 2. *Significantly different than BASE and DTR 2 ($p < 0.05$).

Glucose Response. Fasting glucose was not different ($p = 0.59$) between baseline and 48 hours following DTR 1 or DTR 2 (Table 2 and Figure 1B). Compared to baseline, there was a significantly higher peak glucose response during the OGTT administered 48 hours following DTR 1 ($p < 0.05$), with no elevation above the baseline OGTT glucose response 48 hours following DTR 2 ($p = 0.60$) (Table 2 and Figure 1B). Glucose AUC was significantly higher than baseline AUC 48 hours following DTR 1 ($21.4 \pm 4.7\%$), but was not significantly higher than baseline AUC 48 hours following DTR 2 ($p = 0.99$) (Figure 2B).

Maximal Isometric Strength. Subjects produced similar torque values at the two angles measured ($p = 0.27$) and with both legs ($p = 0.10$) at each time point. Thus, comparisons at each time point are based on a composite torque value calculated as an average of the pooled results of both angles and legs. Subjects were familiarized with the strength testing procedure after the initial familiarization trial, as evidenced by no significant differences between strength measurements during the second familiarization trial and that measured immediately before the first downhill treadmill run (214.4 ± 11.6 vs. 222.7 ± 9.7 Nm, respectively; $p = 0.59$) and a correlation coefficient of 0.99 ($p < 0.01$). Baseline MVC torque immediately prior to DTR 1 and DTR 2 were similar (222.70 ± 9.69 vs. 221.03 ± 9.66 Nm, respectively; $p = 0.49$). The decline and subsequent recovery of MVC torque were different between treadmill runs (Figure 3). Immediately post-exercise, MVC torque declined to a greater degree following DTR 1 (DTR 1 vs. DTR 2, 16.7 ± 2.6 vs. $8.62 \pm 1.17\%$, respectively). Forty eight hours after DTR 1 MVC torque showed a significant degree of recovery but was still reduced by $9.40 \pm 2.73\%$

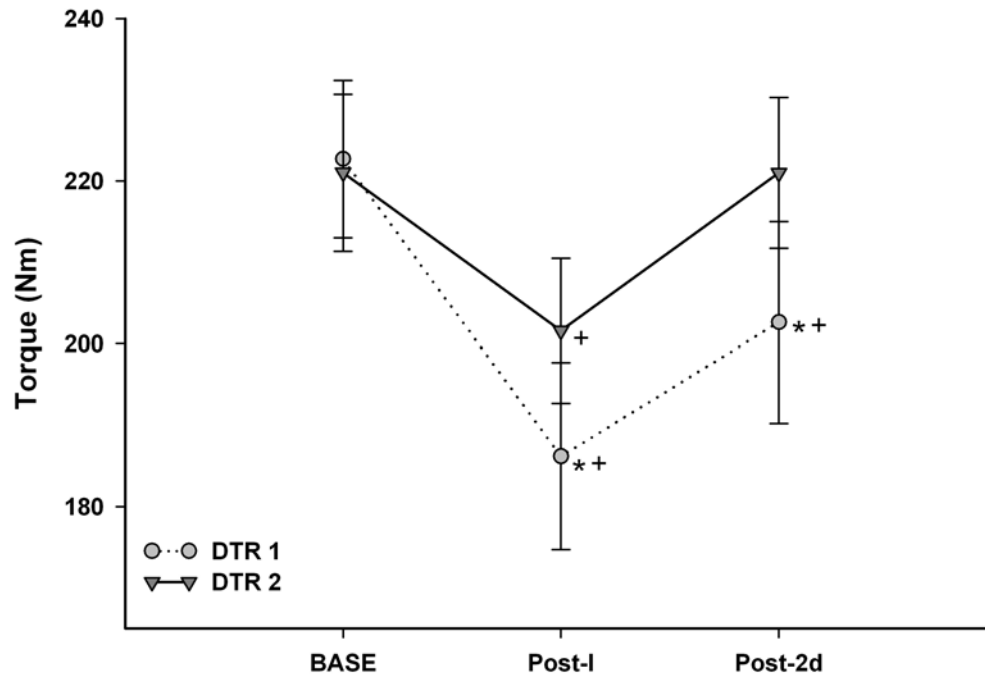


Figure 3. Maximal isometric torque measured at baseline (BASE), immediately after (Post-1), and 48 h after (Post-2d) DTR 1 and DTR 2 (values are means \pm SEM of both legs and angles). *Significantly different than DTR 2 ($p < 0.05$). +Significantly different than respective BASE ($p < 0.05$).

compared to baseline values. Forty-eight hours after DTR 2 MVC torque had fully recovered back to baseline values.

CK Activity. Serum CK values tended to be elevated 48 hours following DTR 1 (93.6 ± 21.6 vs. 812.8 ± 365.1 U \cdot L $^{-1}$; $p = 0.07$) and were elevated 48 hours following DTR 2 ($p < 0.05$). The CK response 48 hours following DTR 2 tended to be less than the response 48 hours following DTR 1 (DTR 1 vs. DTR 2, 812.8 ± 365.1 vs. 162.5 ± 42.5 U \cdot L $^{-1}$, respectively; $p = 0.08$).

Muscle Soreness. Subjects reported a similarly mild degree of muscle soreness in their lower extremities immediately following both treadmill runs ($p = 0.10$) (Figure 4). Forty eight hours following DTR 1 subjects reported a moderate degree of muscle

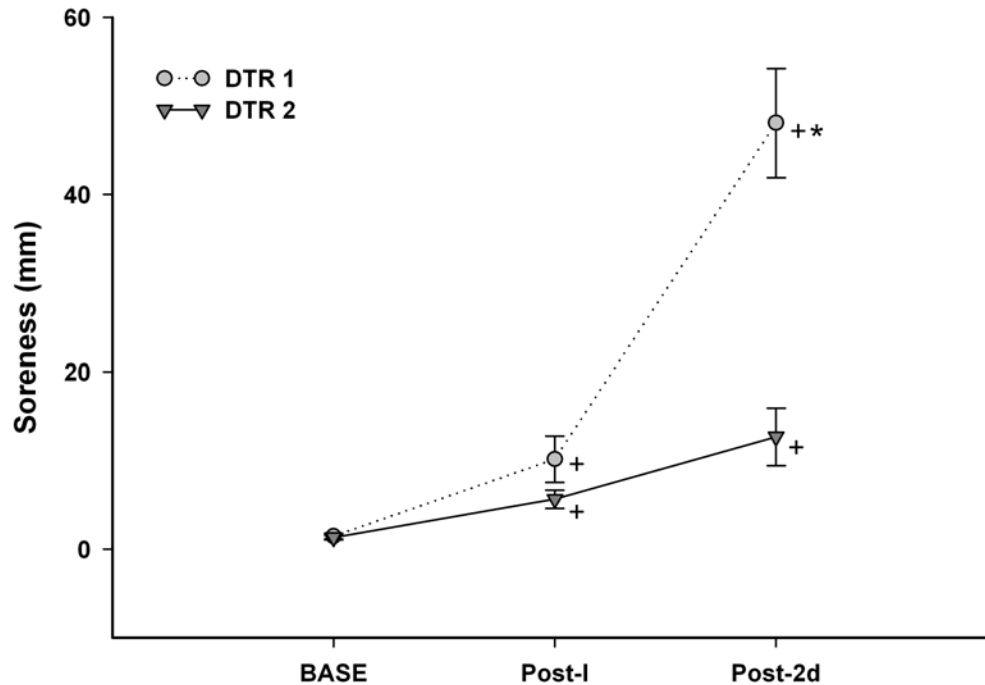


Figure 4. Soreness measured at baseline (BASE), immediately after (Post-1), and 48 h after (Post-2d) DTR 1 and DTR 2 (values are means \pm SEM of both legs). *Significantly different than DTR 2 ($p < 0.05$). ⁺Significantly different than respective BASE ($p < 0.05$).

soreness (48.08 ± 6.16 mm), while soreness remained at a mild level (12.70 ± 3.24 mm) 48 hours following DTR 2.

Injury Protocol. A similar number of total steps were taken during the downhill treadmill runs (DTR 1 vs. DTR 2, 5139.5 ± 77.6 vs. 5131.5 ± 81.8 steps, respectively; $p = 0.78$). There was a trend towards a lower average oxygen consumption during DTR 2 (27.1 ± 1.0 vs. 25.5 ± 0.7 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p = 0.07$). Subject body weight measured immediately before each treadmill run was not different (65.96 ± 2.60 vs. 66.39 ± 2.62 kg, $p = 0.16$). Both average heart rate (165.1 ± 4.8 vs. 158.2 ± 5.0 $\text{b}\cdot\text{min}^{-1}$) and RPE (12.2 ± 0.5 vs. 11.0 ± 0.5) were lower during DTR 2 ($p < 0.05$).

Discussion

The major findings of this study were that the elevated insulin and glucose responses indicating increased insulin resistance that occurred following a novel bout of eccentrically-biased exercise were completely eliminated following a second bout of eccentric exercise performed 14 days later. As has previously been demonstrated (7, 8, 10, 29, 32), the initial bout of eccentric exercise in this study resulted in the induction of muscle injury as evidenced by elevations in muscle soreness and serum creatine kinase, as well as immediate and prolonged decrements in MVC torque, all of which were diminished following the second bout of identical contractions.

A number of studies investigating exercise-induced muscle injury have illustrated the response of skeletal muscle to an acute bout of novel eccentric exercise. Delayed onset muscle soreness (DOMS) is one of the most readily apparent symptoms of exercise-induced muscle injury. It is not typically experienced immediately following exercise but appears several hours after the bout, reaches a peak 24-48 hours later, and then gradually resolves over the next few days (10, 30, 36). Force deficits have been found to be maximal immediately following exercise-induced muscle injury, and in most cases commence recovery after 24-48 hours, with full recovery being dependent upon the magnitude of injury (10, 28) but typically occurring within 3-14 days (10, 14, 28). Serum CK displays a delayed increase, typically peaking 2-5 days following exercise before returning to baseline within approximately seven days (9, 36, 37).

The response of subjects to a novel bout of eccentrically-biased exercise in the present study is in general agreement with those of previous investigations. Subjects reported only a very mild degree of lower extremity muscle soreness immediately

following DTR 1, which reached moderate levels 48 hours following exercise. Immediately following DTR 1, MVC torque was reduced by 17 % and exhibited only a partial recovery (10 % below baseline) in the subsequent 48 hours. Serum CK measured 48 hours following DTR 1 appeared to be elevated ~8-fold, although this increase failed to achieve statistical significance. It is possible that a true treatment effect was masked by the inherent variability of the CK response (9, 39). It has been documented that within a normal population there exists a small percentage of individuals who exhibit an inordinate CK response to unaccustomed exercise (11, 39). Two such subjects appear to be present in the current study, exhibiting ~15-fold increases in CK compared to the ~4-fold increases of all other subjects. Removal of these two subjects from the analysis reveals a statistically significant 4-fold increase in CK levels 48 hours following DTR 1. Examined together, the perturbations in soreness, MVC torque, and CK can be taken as indicators that a moderate degree of exercise-induced muscle injury was achieved as a result of the initial 30 minute bout of downhill treadmill running.

Although susceptible to injury by novel eccentric muscular actions, skeletal muscle displays a readily observable “repeated bout effect” whereby muscles exposed to prior eccentric contractions exhibit reduced soreness and CK responses in addition to more rapid returns to full force producing capacity when subsequently submitted to a second bout (8, 10, 28, 32, 33). Byrnes et al. (8) observed that muscle soreness and serum CK responses to a repeated bout of downhill treadmill running were reduced by approximately 80 and 40 %, respectively, while oxygen consumption and heart rate measured during exercise were also lower. The eccentric elbow flexion model has demonstrated a complete suppression of the serum CK response (28) and a significantly

accelerated rate of recovery for MVC torque (10, 28), with complete recovery taking place one day following a second bout compared to only partial recovery five days following an initial novel bout (10).

As has been previously demonstrated (8, 10, 28, 32), the present study revealed a repeated bout effect with reference to MVC torque and soreness. Although there was a trend for an attenuated CK response, it was perhaps clouded by the inherent individual variability of this measure.

The initial decline in MVC torque immediately following DTR 2 was significantly less than that observed following the first treadmill run, and MVC torque made a complete recovery back to baseline levels 48 hours following exercise. In contrast, 48 hours following DTR 1 MVC torque was still reduced by 9 %. These results are in slight contrast to observations in other studies where repeated eccentric contractions resulted in an identical decline in MVC torque immediately post-exercise, with the repeated bout effect only being evident as a more rapid recovery in the days that follow (10, 18, 28). However, such studies utilized eccentric contractions of the elbow flexors, which appear to result in a greater degree of muscle injury. Additionally, Eston et al. (14) failed to observe a repeated bout effect for MVC torque following repeated downhill treadmill running, although bouts were separated by five weeks compared to only two weeks in the present study. It is possible that the protective effect of downhill treadmill running on enhanced MVC torque recovery is diminished somewhere beyond two weeks, although this cannot be determined from the current study.

Lower extremity muscle soreness was rated as mild immediately following both downhill treadmill runs, but displayed a sharp increase 48 hours following DTR 1 only.

Heart rate and RPE were lower by $6.9 \pm 1.7 \text{ b}\cdot\text{min}^{-1}$ and 1.2 ± 0.2 points, respectively, and oxygen consumption tended to be lower by $1.1 \pm 0.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($p = 0.07$) during DTR 2 compared to DTR 1. These results are in agreement with the findings of Byrnes et al. (8) who observed reductions in heart rate and oxygen consumption of $9 \text{ b}\cdot\text{min}^{-1}$ and $2.1 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively, during a repeated bout of downhill treadmill running.

Defined as an attenuated physiological response to normal levels of insulin (21), insulin resistance following exercise-induced muscle injury has been documented by a number of studies (19, 20, 22, 37). Utilizing hyperglycemic clamp procedures 36 hours following eccentric knee flexions and extensions, King et al. (19) observed that eccentric exercise resulted in exaggerated peak insulin levels that were 28 % above control values during the early phase (0-10 minutes) of the clamp, accompanied by an 83 % increase in the insulin AUC. Utilizing euglycemic-hyperinsulinemic clamps 48 hours following downhill treadmill running, Kirwan et al. (22) were able to specifically measure glucose disposal rates and determined that they were reduced by 37 % compared to control values.

Further evidence of insulin resistance following eccentric exercise was provided by Sherman et al. (37). Insulin and glucose responses to an OGTT were assessed 48 hours following downhill treadmill running, level treadmill running, or isokinetic leg exercise. Downhill treadmill running resulted in peak insulin and insulin AUC responses that were ~19 and 37 % higher than control values, respectively. Although peak insulin and insulin AUC were elevated, the blood glucose response to the OGTT was not altered, suggesting no alteration in glucose tolerance. Stated differently, a greater amount of insulin was required to maintain glucose homeostasis following eccentric exercise. Level

treadmill running did not alter the insulin or glucose response to the OGTT, although it did result in an increase in serum CK. Echoing the potential mechanism put forward by others (22), it was hypothesized that decreased insulin effectiveness resulted from disruptions to binding of insulin to its receptor, reductions in the availability of glucose transporters, or alterations in the intracellular glucose disposal pathway.

In the current study, 48 hours following DTR 1 peak insulin during the OGTT was elevated by 43 % compared to baseline, with the insulin AUC exhibiting a 38 % increase. In contrast to the findings of Sherman et al. (37), peak glucose and glucose AUC were also significantly elevated (20 and 21 %, respectively) in response to the initial downhill treadmill running bout. Thus, the presence of elevated insulin levels was unable to maintain glucose homeostasis 48 hours following DTR 1. It is important to note that the study of Sherman et al. (37) utilized male subjects that were considerably heavier and leaner than the females used in the current study. Indeed, body fat differences aside subjects in the present study received glucose in amounts approximating $1.6 \text{ g}\cdot\text{kg}^{-1}$ lean bw, whereas those in the Sherman et al. (37) study received $1.0 \text{ g}\cdot\text{kg}^{-1}$ lean bw. Increased provision of glucose per unit body mass during the OGTT, combined with a greater percentage of body fat, could explain the contrasting findings of glucose intolerance following the initial downhill treadmill run in the present study.

Skeletal muscle can be highly regulated by insulin, and under resting conditions accounts for approximately 85 % of glucose disposal following glucose ingestion (12, 38). Failure to adequately dispose of blood glucose following eccentric exercise could help explain the reduced rates of skeletal muscle glycogen synthesis shown to occur following exercise-induced muscle injury (11, 13, 34, 40).

To our knowledge, there appear to have been no studies investigating the repeated bout effect as it may pertain to insulin resistance occurring after eccentric exercise. The results of the current study clearly demonstrate that the insulin resistance experienced following a repeated bout of eccentric contractions was not merely attenuated but was completely eliminated. This was despite the fact that the initial injury bout appeared to induce only a moderate degree of muscle injury. This should not be surprising, since it has been shown that performance of only a small number of novel eccentric contractions confers a significant protective effect, at least as it pertains to strength, soreness, and CK responses (10). Although elevated following DTR 1, peak insulin, glucose, and their respective AUC showed no such elevations above baseline levels following DTR 2. Therefore, the presence of a repeated bout effect for the altered insulin and glucose response was noted following the second bout of eccentric contractions.

Several mechanisms have been suggested to account for the adaptive processes that take place following eccentric contractions. Concepts put forward to account for the adaptation can be grouped into neural, connective tissue, and cellular theories (26, 27). Accordingly, the neural theory proposes that the initial damage stimulates an increased motor unit activity (23), slow-twitch fiber recruitment, and motor unit synchronization in subsequent eccentrically-biased exercise (16). Accordingly, forces encountered during a repeated bout of eccentric contractions are spread across more muscle fibers (31). The connective tissue theory describes an increased amount of intramuscular connective tissue (24) and remodeling of intermediate filaments (15). Cellular adaptation theories suggest a strengthening of cell membranes (10), removal of weak fibers (2), and longitudinal addition of sarcomeres (25) as likely candidates for the adaptive response.

The design of the current study does not allow for a direct determination of the specific mechanism or mechanisms that may be responsible for the attenuated insulin and glucose response to a repeated bout of eccentric contractions. However, potential membrane related adaptations are particularly intriguing when reduced glycogen synthesis (11, 13, 34, 40), transient insulin resistance (19, 20, 22, 37), and decreased glucose transporter number (3) resulting from eccentric contractions are considered. These events are intimately related to each other and to the integrity of the membrane, and alterations to the membrane following repeated eccentric contractions may reduce their degree of severity.

The significantly reduced level of serum CK measured after a repeated bout of eccentric exercise has been put forth by Clarkson et al. (10) as an indication that the cell membrane has been strengthened and is less likely to allow a loss of calcium homeostasis and subsequent necrosis of cellular components. A trend towards a lower CK response following DTR 2 in the present study suggests that there may have been less cell membrane damage.

Better maintenance of membrane integrity following eccentric contractions may be accompanied by reduced disruptions in binding of insulin to its receptor, increased availability of glucose transporters, and positive alterations in the intracellular glucose disposal pathway, which could explain the reduced insulin and glucose response following repeated eccentric exercise observed in the present study. Future studies are warranted to address the degree to which these mechanisms may have been operating.

In conclusion, a novel bout of downhill treadmill running resulted in muscle injury and an elevated insulin and glucose response. A second bout of downhill treadmill

running performed two weeks later was associated with a lesser degree of muscle injury. The unique finding of this study was that the insulin resistant state observed following a novel bout of downhill treadmill running was completely eliminated following a second bout. These results suggest that novel eccentric contractions induce adaptations that lead to the elimination of insulin resistance and a normalization of the glycemic response to subsequent eccentrically-biased exercise.

REFERENCES

1. **American College of Sports Medicine.** *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia: Lippincott Williams & Wilkins, 2006.
2. **Armstrong RB, Ogilvie RW and Schwane JA.** Eccentric exercise-induced injury to rat skeletal muscle. *J Appl Physiol* 54: 80-93, 1983.
3. **Asp S, Dugaard JR, Kristiansen S, Kiens B and Richter EA.** Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects. *J Physiol* 494 (Pt 3): 891-898, 1996.
4. **Asp S, Dugaard JR and Richter EA.** Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. *J Physiol* 482 (Pt 3): 705-712, 1995.
5. **Borg G.** Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 2: 92-98, 1970.
6. **Braun WA and Dutto DJ.** The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. *Eur J Appl Physiol* 90: 29-34, 2003.
7. **Byrne C, Twist C and Eston R.** Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. *Sports Med* 34: 49-69, 2004.
8. **Byrnes WC, Clarkson PM, White JS, Hsieh SS, Frykman PN and Maughan RJ.** Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol* 59: 710-715, 1985.
9. **Clarkson PM and Ebbeling C.** Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clin Sci (Lond)* 75: 257-261, 1988.
10. **Clarkson PM and Tremblay I.** Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol* 65: 1-6, 1988.
11. **Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI and Pearson D.** Impaired muscle glycogen resynthesis after eccentric exercise. *J Appl Physiol* 69: 46-50, 1990.
12. **DeFronzo RA, Bonadonna RC and Ferrannini E.** Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 15: 318-368, 1992.
13. **Doyle JA, Sherman WM and Strauss RL.** Effects of eccentric and concentric exercise on muscle glycogen replenishment. *J Appl Physiol* 74: 1848-1855, 1993.
14. **Eston RG, Lemmey AB, McHugh P, Byrne C and Walsh SE.** Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scand J Med Sci Sports* 10: 199-204, 2000.
15. **Friden J, Seger J, Sjostrom M and Ekblom B.** Adaptive response in human skeletal muscle subjected to prolonged eccentric training. *Int J Sports Med* 4: 177-183, 1983.
16. **Golden CL and Dudley GA.** Strength after bouts of eccentric or concentric actions. *Med Sci Sports Exerc* 24: 926-933, 1992.

17. **Green MS, Corona BT, Doyle JA and Ingalls CP.** Carbohydrate-protein drinks do not enhance recovery from exercise-induced muscle injury. *Int J Sport Nutr Exerc Metab* 18: 1-18, 2008.
18. **Ingalls CP, Wenke JC, Nofal T and Armstrong RB.** Adaptation to lengthening contraction-induced injury in mouse muscle. *J Appl Physiol* 97: 1067-1076, 2004.
19. **King DS, Feltmeyer TL, Baldus PJ, Sharp RL and Nespore J.** Effects of eccentric exercise on insulin secretion and action in humans. *J Appl Physiol* 75: 2151-2156, 1993.
20. **Kirwan JP, Bourey RE, Kohrt WM, Staten MA and Holloszy JO.** Effects of treadmill exercise to exhaustion on the insulin response to hyperglycemia in untrained men. *J Appl Physiol* 70: 246-250, 1991.
21. **Kirwan JP and Del Aguila LF.** Insulin signalling, exercise and cellular integrity. *Biochem Soc Trans* 31: 1281-1285, 2003.
22. **Kirwan JP, Hickner RC, Yarasheski KE, Kohrt WM, Wiethop BV and Holloszy JO.** Eccentric exercise induces transient insulin resistance in healthy individuals. *J Appl Physiol* 72: 2197-2202, 1992.
23. **Komi PV and Buskirk ER.** Effect of eccentric and concentric muscle conditioning on tension and electrical activity of human muscle. *Ergonomics* 15: 417-434, 1972.
24. **Lapier TK, Burton HW, Almon R and Cerny F.** Alterations in intramuscular connective tissue after limb casting affect contraction-induced muscle injury. *J Appl Physiol* 78: 1065-1069, 1995.
25. **Lynn R and Morgan DL.** Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol* 77: 1439-1444, 1994.
26. **McHugh MP.** Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports* 13: 88-97, 2003.
27. **McHugh MP, Connolly DA, Eston RG and Gleim GW.** Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med* 27: 157-170, 1999.
28. **Newham DJ, Jones DA and Clarkson PM.** Repeated high-force eccentric exercise: effects on muscle pain and damage. *J Appl Physiol* 63: 1381-1386, 1987.
29. **Newham DJ, McPhail G, Mills KR and Edwards RH.** Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* 61: 109-122, 1983.
30. **Newham DJ, Mills KR, Quigley BM and Edwards RH.** Pain and fatigue after concentric and eccentric muscle contractions. *Clin Sci (Lond)* 64: 55-62, 1983.
31. **Nosaka K and Clarkson PM.** Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc* 27: 1263-1269, 1995.
32. **Nosaka K, Sakamoto K, Newton M and Sacco P.** How long does the protective effect on eccentric exercise-induced muscle damage last? *Med Sci Sports Exerc* 33: 1490-1495, 2001.

33. **Nosaka K, Sakamoto K, Newton M and Sacco P.** The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *Eur J Appl Physiol* 85: 34-40, 2001.
34. **O'Reilly KP, Warhol MJ, Fielding RA, Frontera WR, Meredith CN and Evans WJ.** Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. *J Appl Physiol* 63: 252-256, 1987.
35. **Sayers SP and Clarkson PM.** Force recovery after eccentric exercise in males and females. *Eur J Appl Physiol* 84: 122-126, 2001.
36. **Schwane JA, Johnson SR, Vandenaeker CB and Armstrong RB.** Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill running. *Med Sci Sports Exerc* 15: 51-56, 1983.
37. **Sherman WM, Lash JM, Simonsen JC and Bloomfield SA.** Effects of downhill running on the responses to an oral glucose challenge. *Int J Sport Nutr* 2: 251-259, 1992.
38. **Wallberg-Henriksson H.** Glucose transport into skeletal muscle. Influence of contractile activity, insulin, catecholamines and diabetes mellitus. *Acta Physiol Scand Suppl* 564: 1-80, 1987.
39. **Warren GL, Lowe DA and Armstrong RB.** Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43-59, 1999.
40. **Widrick JJ, Costill DL, McConell GK, Anderson DE, Pearson DR and Zachwieja JJ.** Time course of glycogen accumulation after eccentric exercise. *J Appl Physiol* 72: 1999-2004, 1992.
41. **Yeni-Komshian H, Carantoni M, Abbasi F and Reaven GM.** Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. *Diabetes Care* 23: 171-175, 2000.

APPENDIX A

INFORMED CONSENT FORM

Georgia State University

Department of Kinesiology and Health

Informed Consent Form

Title: Effect of exercise-induced muscle injury on insulin resistance

Principal Investigators: Dr. J. Andrew Doyle, Michael Green, and Benjamin Corona

I. Introduction/Background/Purpose

You are invited to participate in a research study investigating the effects of exercise-induced muscle injury on insulin resistance. Common symptoms of exercise-induced muscle injury include muscle soreness and weakness, which may cause you some discomfort for about 5 days. For example, you may experience tenderness in your thigh muscles while walking down steps for a few days, but you will not be debilitated from the injury. In fact, exercise-induced muscle injury is often experienced following the performance of activities you have not done for a while, such as yard work, rock climbing, or playing soccer. In this research study muscle injury will be caused by either running downhill on a treadmill or a knee flexion protocol. You will be randomly assigned to only one of these methods, and you will be informed as to which method you have been assigned prior to reading and possibly signing this Informed Consent Form. Prior research looking at the effects of exercise-induced muscle injury has suggested a temporary tendency for people to become slightly resistant to the effects of insulin. We are therefore investigating the effectiveness of two different techniques to cause exercise-induced muscle injury as well as their ability to cause temporary insulin resistance.

II. Procedures

If you choose to participate in this research study, you will be required to report to the Applied Physiology Laboratory (Sports Arena; room #G18) on *seven* separate occasions ranging from 35-135 minutes per visit. Details and definitions of the activities you will perform during each of these visits are detailed below.

In order to participate in the research study you must be aged 18 to 35 years, low to moderately active (participating in any form of endurance exercise for 1 to 5 hours per week), and not currently taking physician prescribed or over the counter medicines. Also, you must be classified as "low risk" under the guidelines set by the American College of Sports Medicine risk stratification method. By completing the health history form, it will be determined if you meet the required subject characteristics of the research study.

During the *first visit* you will (1) review and possibly sign an informed consent form (this form), (2) complete contact information and health history forms, (3) have your body composition determined by a Dual Energy X-Ray Absorptiometry (DEXA) scan, (4) perform a familiarization trial of the quadriceps muscles' (muscles in the front of the thigh) strength protocol, and (5) schedule the upcoming six visits to complete the research study. The first visit will require approximately 35 minutes of your time. The DEXA scan uses a low-dose x-ray to determine your body composition and will require you to lie still on a flat surface for approximately 10 minutes. A medical doctor will not review the DEXA results. Therefore, this data will have no medical usefulness.

The strength protocol familiarization trial will require you to perform a total of 3 contractions with each leg as hard as you can with a short rest in between. When you perform each contraction you will produce as much force as possible against a pad, but the pad will not move. *For the remaining visits all of the strength protocols will be identical to the one performed during this visit. All strength assessments will occur in the Musculoskeletal Injury Research Laboratory (approximately 100m away from the Applied Physiology Laboratory). You will be escorted to and from the Musculoskeletal Injury Research Laboratory for each strength test.*

During the *second visit* you will (1) undergo an oral glucose tolerance test (OGTT # 1) and (2) perform a second familiarization trial with the strength testing protocol. The second visit is expected to require 135 minutes of your time. You will attend the OGTT in the morning after an overnight fast. At the beginning of the test you will consume 2.6 ounces of glucose dissolved in water. Additionally, at the beginning of the test and at regular intervals (every 30 minutes), small (one half of a tablespoon) blood samples will be collected from a vein in your arm. A sterile needle will be inserted into a vein in your arm, but once inserted will be removed allowing an

1



Consent Form Approved by Georgia State University IRB May 09, 2007 - February 14, 2008

intravenous catheter to remain in place in your vein for the entire 2 hour period. The entire procedure will require less than 4 teaspoons of your blood. For the full 2 hour duration of the procedure you will remain seated and allowed to read, study, or perform work on a laptop computer. You will be continuously monitored for any discomfort by a researcher. Following the final blood sample, the intravenous catheter will be removed and the area covered with a sterile dressing.

During the *third visit* you will (1) perform a 30-minute intermittent downhill treadmill run or eccentric knee flexion protocol in order to cause muscle injury, (2) complete a pre- and post-injury strength protocol, and (3) complete a pre- and post-injury soreness assessment protocol. The third visit is expected to require 60 minutes of your time. Details of the procedures occurring during the third visit are described below.

If you are randomly assigned to perform the **30-minute downhill treadmill run** you will run 6 intervals lasting 5 minutes each. Between each interval you will be given a 2-minute standing rest. You will run on the treadmill at a speed of 7.5 mph (8 minutes per mile pace) at a downhill grade of -12%. Heart rate will be recorded using a heart rate monitor. This requires you to place an elastic strap around your chest level with your breastbone.

If you are randomly assigned to perform the **eccentric knee flexion protocol** you will perform 10 sets of 10 repetitions per leg (for a total of 100 contractions per leg) on the same piece of equipment that you used for the strength protocol. You will perform the contractions as hard as you can with a short rest (10 seconds) in between each one, and a slightly longer rest (60 seconds) in between each set of 10. You will push as hard as possible against the pad for 2 seconds. The pad will not only resist your effort but will slowly move in the opposite direction to how you are pushing, causing your knee to bend even though you are attempting to cause it to straighten.

The pre- and post-injury strength protocol will be identical to that done on your first and second visits to the lab when you performed the two familiarization trials. You will be required to perform these 3 maximal contractions before and after the downhill running or eccentric knee flexion protocol.

The soreness assessment protocol involves you stepping down from a box that is one foot high and then marking your level of soreness on a line the length of 100 mm (about 4 inches). You will step down from the one-foot tall box a total of four times assessing your soreness in each leg twice.

The *fourth visit* will take place two days after the third (“injury-causing”) visit. During this visit you will (1) undergo a second OGTT (OGTT # 2), (2) complete a soreness assessment protocol, and (3) complete a strength protocol. The fourth visit is expected to require approximately 135 minutes of your time. All procedures performed during this visit will be identical to those described for earlier visits.

The *fifth, sixth, and seventh visits* will take place approximately two weeks after the fourth visit. You will not be required to perform any special activities during this two week interval. The *fifth, sixth, and seventh visits* are identical in nature to the second, third, and fourth visits described above. Briefly, the *fifth visit* (135 min) involves a third OGTT (OGTT # 3), soreness assessment, and strength measurement, the *sixth visit* (60 min) involves a repeat of the injury protocol you performed earlier (downhill treadmill run or eccentric knee flexion), and the *seventh visit* (135 min) involves a fourth OGTT (OGTT # 4), soreness assessment, and strength measurement.

Blood collection and visit summary table

Visit	Duration	Name	Volume (mL)	Volume (tspn)
1	35 min	Preliminary	--	--
2	135 min	OGTT # 1	15	< 4
3	60 min	Injury # 1	--	--
4	135 min	OGTT # 2	15	< 4
TWO WEEK INTERVAL				
5	135 min	OGTT # 3	15	< 4
6	60 min	Injury # 2	--	--
7	135 min	OGTT # 4	15	< 4

2



Consent Form Approved by Georgia State University IRB May 09, 2007 - February 14, 2008

*During the course of the study you will be asked not to consume caffeine and alcohol 12 hours prior to testing, not to perform any strenuous exercise outside of the study, and not to consume any over the counter medicines (e.g. anti-inflammatory pills). Prior to each OGTT you will be asked to **abstain from consuming breakfast** thus allowing you to attend the morning OGTT in a fasted state. Additionally, you will be asked not to treat the symptoms of muscle injury with medications, icing, heating, or any other treatment methods.*

You will report to the Applied Physiology Laboratory at the beginning of each visit and meet with Michael Green or Benjamin Corona.

III. Risks

The risks associated with the submaximal downhill running treadmill protocol used in this study are comparable to those encountered during moderate to high intensity exercise. There is a small risk for abnormal heartbeats and/or a very small risk of heart attack during the downhill running protocol. Even in maximal treadmill tests, of which this test is not, this risk is extremely low in the general population (risk of death is 1 in 10,000 to 20,000). During and following the treadmill running protocol, you may experience muscle fatigue, weakness, cramping, joint discomfort, light-headedness, and shortness of breath.

The risks associated with the eccentric knee flexion protocol used in this study are minimal in nature, but are comparable to those encountered during moderate to high intensity static exercise. During and following the eccentric knee flexion protocol you may experience muscle fatigue, weakness, cramping, joint discomfort, light-headedness, and shortness of breath.

Standard emergency procedures are in place in the Applied Physiology Laboratory and Musculoskeletal Injury Research Laboratory, and all tests will be monitored by at least two laboratory personnel who are familiar with the emergency procedures and are CPR certified.

The downhill running and eccentric knee flexion protocols are designed to induce a moderate amount of muscle injury. Following completion of either of these exercises you may experience muscle soreness, swelling, tenderness, decreased leg range of motion, muscle weakness, joint discomfort, and general discomfort. Generally, the soreness resulting from downhill running or eccentric knee flexion is short-lived and is far from debilitating – it is comparable to muscle soreness that may occur when you perform an intense exercise that you have not done for a while. You are asked not to treat these symptoms of muscle injury with medications, icing, heating, or other treatment methods, as these treatments may interfere with the measurements being made throughout the course of the study.

There is a small chance that you may experience minor gastrointestinal distress (such as bloating or flatulence) as a result of consumption of the OGTT glucose solution used in this study.

The risks associated with venipuncture and intravenous catheters are minor and include: excessive bleeding, fainting or feeling light-headed, hematoma (blood accumulating under the skin), and infection (a slight risk any time the skin is broken). In the event that complications arise due to the blood taking process, standard procedures will be followed as required by the specific situation. If you have questions as to what the exact procedures are, please direct any inquiries to Michael Green or Benjamin Corona (contact information listed below).

DEXA emits a low dose of x-ray to determine body composition. The x-ray exposure experienced by this method is approximately 0.02 to 0.05 mREM of radiation. For comparison, a standard hospital chest x-ray exposes the body to approximately 30.0 to 40.0 mREM of radiation; and an individual is exposed to approximately 0.5 to 0.75 mREM of non-medical background radiation daily from sources such as the sun, fluorescent lights, television and computer screens, and other radiation emitting devices. It would require approximately 600 to 1,500 DEXA scans to obtain the same amount of radiation exposure experienced by single chest x-ray, or approximately 25 DEXA scans to gain a single additional day of non-medical background radiation.

IV. Benefits

By participating in the research study, you will obtain valuable information about your physical fitness. In particular, you will be given information about your body composition derived from the DEXA scan (e.g. % fat mass and % lean mass). Also, by participating in this study you will assist in increasing the understanding of the body's response to exercise-induced muscle injury and the potential for studying its effects on insulin resistance.



V. Voluntary Participation and Withdrawal

Participation in research is voluntary. You have the right to refuse to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. You may skip questions or discontinue participation at any time. However, any information already used to the point when you withdraw consent may not be removed. Whatever you decide, you will not lose any benefits to which you are otherwise entitled.

VI. Confidentiality

We will keep your records private to the extent allowed by law. We will use a study number rather than your name on study records where we can. With the exception of the Informed Consent and Contact Information forms, all data collection sheets will be distinguishable solely by a randomly assigned number. Research data files will be located in a lockable filing cabinet in the Graduate Research Assistant's office (room # G20), located in the Applied Physiology lab (room # G18). The Informed Consent form will be retained in a lockable filing cabinet in Dr. Doyle's office (room # G04). These files will only be accessible to Dr. Andrew Doyle, Benjamin Corona, and Michael Green. Your name and other facts that might point to you will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

VII. Georgia State University Disclaimer

If you have any questions about this study, or believe you have suffered any injury (other than exercise-induced muscle injury) because of your participation in the study, you may contact Michael Green or Benjamin Corona at (404) 651-2831. You will have to contact your personal physician to arrange for appropriate management for any unnecessary physical or psychological injury resulting from this study. You will be responsible for any costs of treatment.

VIII. Contact Persons

Call Michael Green or Benjamin Corona at (404) 651-2831 if you have questions about this study. If you have questions or concerns about your rights as a participant in this research study, you may contact the Institutional Review Board (IRB), which oversees the protection of human research participants. Susan Vogtner in the office of research compliance can be reached at 404-463-0674.

IX. Copy of Consent Form to Subject

We will give you a copy of this consent form to keep.
If you are willing to volunteer for this research, please sign below.

Subject Signature

Date (mm:dd:yy)

Principal Investigator Signature

Date (mm:dd:yy)



APPENDIX B

HEALTH HISTORY FORM

Applied Physiology Laboratory
Department of Kinesiology and Health
Georgia State University

Health History

All information given is personal and confidential. The information will enable us to better understand your health status and fitness habits. Please direct any questions pertaining to this form to laboratory personnel. Please do not put your name in this form.

Subject Number _____ Date _____
(provided by researcher)
Address _____ Home Phone _____
City/State _____ Zip Code _____
E-mail _____ Bus. Phone _____
D.O.B. _____ Gender _____ Height _____ Weight _____ Race _____

I. Signs and Symptoms

Have you ever experienced any of the following:

(please circle yes or no)

- Yes No 1. Pain, discomfort, tightness or numbness in the chest, neck, jaw or arms.
- Yes No 2. Shortness of breath at rest or with mild exertion.
- Yes No 3. Dizziness or fainting.
- Yes No 4. Difficult, labored or painful breathing during the day or at night.
- Yes No 5. Ankle swelling.
- Yes No 6. Rapid pulse or heart rate.
- Yes No 7. Intermittent cramping.
- Yes No 8. Known heart murmur.
- Yes No 9. Unusual shortness of breath or fatigue with unusual activities.

If you answered yes to any of the above:

How often do you experience the symptom? _____

Have you ever discussed the symptom with a doctor? _____

Explain the symptom in more detail: _____

II. Major Risk Factors

- Yes No 1. Do you have a body mass index ≥ 30 or a waist girth > 100 cm (> 39 in.)? (*Body mass index is equal to your body weight in kilograms divided by your height squared in meters – kg/m²*).
- Yes No 2. Have you had a fasting glucose of ≥ 100 mg/dL confirmed by measurements on at least 2 separate occasions. (*This is a measurement of blood glucose taken after you have not eaten for 12 to 14 hours*).
- Yes No 3. Has your father or brother experienced a heart attack before the age of 55? Or has your mother or sister experienced a heart attack before the age of 65?
- Yes No 4. Do you currently smoke or have you quit smoking within the past 6 months?
- Yes No 5. Has your doctor ever told you that you have high blood pressure?
- Yes No 6. Do you have high cholesterol?
Total cholesterol: _____ HDL: _____ Date Tested: _____
- Yes No 7. Do you have a sedentary lifestyle? (sitting most of the day in your job with no regular physical activity)

III. Medical Diagnoses

Have you ever had any of the following? Circle all that apply:

asthma (<i>lung disease causing difficult breathing</i>)	coronary artery disease	heart surgery
angioplasty (<i>surgical opening of heart artery</i>)	angina (<i>chest pain</i>)	hypertension
heart clicks (<i>abnormal heart sounds</i>)	heart murmur	heart attack
emphysema (<i>lung disease</i>)	bronchitis (<i>lung inflammation</i>)	stroke
emboli (<i>abnormal blood particle</i>)	phlebitis (<i>inflammation of vein</i>)	anemia
cancer	emotional disorders	eating disorders
osteoporosis (<i>decreased bone mass/density</i>)		

Any special problems not listed above: _____

If any of the above diagnoses are circled, please give details and explain: _____

IV. General

Yes No 1. Do you have arthritis or any bone or joint problem?
If yes, please explain: _____

Yes No 2. Are you taking any medications, vitamins, or supplements?
Name them and their dosage (list both prescribed and over-the-counter
medications). _____

3. Menstruation history (if applicable):
a. Date of previous menstruation (mm:dd) _____

b. Predicted date of next menstruation (mm:dd) _____

V. Training Status

Yes No Do you currently exercise?
If yes, how long have you been exercising? _____

What do you do? _____

How often do you do those exercises? _____

How intense are the exercises? _____

How many hours per week do you exercise?

- 0-1
- 1-2
- 2-3
- 3-4
- 4-5
- 5+

Do you consider yourself to be of recreational athletic status? (Specify what you believe your training status to be and specify why you believe you are of this status)

Give a brief example of what your typical training week is like. _____

By checking the box below I certify that all of the above is true, to the best of my knowledge.

Check here Date: _____

RESEARCHER USE ONLY

Comments: _____

Stratification (circle one): Low Risk Moderate Risk High Risk

Resting blood pressure: _____ Resting Heart Rate: _____

Yes No Do medications affect BP or HR?

Date: _____ Initials: _____

APPENDIX C

RECRUITMENT FLYER

GEORGIA STATE UNIVERSITY RESEARCH STUDY FOR RECREATIONAL ATHLETES

*Effect of exercise-induced muscle injury
on insulin resistance*



- We are looking for males and females aged 18 to 35 years that are recreationally active (e.g. activity level equivalent to 1– 5 hours of aerobic exercise per week).
- Complete 2 downhill treadmill runs or knee flexion protocols, consume a glucose drink and give several small blood samples, and several leg strength measurements.
- Receive valuable body composition information: (% body fat, % muscle mass, total fat mass, total muscle mass, and body mass index).

Contact: Benjamin Corona/Michael Green (404) 651-2831 mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

Applied Physiology Laboratory
(404) 651-2831
mgreen12@gsu.edu

APPENDIX D
IRB PERMISSION LETTER



INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999

In Person: Alumni Hall
30 Courtland St, Suite 217

Phone: 404/463-0674
Fax: 404/654-5838

March 6, 2007

Principal Investigator: Doyle, James Andrew

Student PI: Michael S Green

Protocol Department: Kinesiology & Health

Protocol Title: Effect of Exercise-Induced Muscle Injury on Insulin Resistance

Submission Type: Protocol H07312

Review Type: Full Board Review

Approval Date: February 15, 2007

Expiration Date: February 14, 2008

The Georgia State University Institutional Review Board (IRB) reviewed and approved the above referenced study and enclosed Informed Consent Document(s) in accordance with the Department of Health and Human Services. The approval period is listed above.

Federal regulations require researchers to follow specific procedures in a timely manner. For the protection of all concerned, the IRB calls your attention to the following obligations that you have as Principal Investigator of this study.

1. When the study is completed, a Study Closure Report must be submitted to the IRB.
2. For any research that is conducted beyond the one-year approval period, you must submit a Renewal Application 30 days prior to the approval period expiration. As a courtesy, an email reminder is sent to the Principal Investigator approximately two months prior to the expiration of the study. However, failure to receive an email reminder does not negate your responsibility to submit a Renewal Application. In addition, failure to return the Renewal Application by its due date must result in an

automatic termination of this study. Reinstatement can only be granted following resubmission of the study to the IRB.

3. Any adverse event or problem occurring as a result of participation in this study must be reported immediately to the IRB using the Adverse Event Form.
4. Principal investigators are responsible for ensuring that informed consent is obtained and that no human subject will be involved in the research prior to obtaining informed consent. Ensure that each person giving consent is provided with a copy of the Informed Consent Form (ICF). The ICF used must be the one reviewed and approved by the IRB; the approval dates of the IRB review are stamped on each page of the ICF. Copy and use the stamped ICF for the coming year. Maintain a single copy of the approved ICF in your files for this study. However, a waiver to obtain informed consent may be granted by the IRB as outlined in 45CFR46.116(d).

All of the above referenced forms are available online at <https://irbwise.gsu.edu>. Please do not hesitate to contact Susan Vogtner in the Office of Research Integrity (404-463-0674) if you have any questions or concerns.

Sincerely,



Ann C. Kruger, IRB Chair

Federal Wide Assurance Number: 00000129

APPENDIX E

DATA COLLECTION FORMS

Downhill Run (-12%, 8mph)

Subject #: _____

Date: _____

Stage	Time	RPE	Steps 1 min.	Comments:
1	B:			
	E:			
2	B:			
	E:			
3	B:			
	E:			
4	B:			
	E:			
5	B:			
	E:			
6	B:			
	E:			

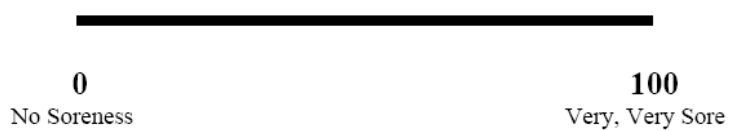
Rating of Exercise Induced – Muscle Soreness
Visual Analogue Scale: 100 mm

Subject #	
Date	
Time	
Trial Name	

R1



L1



Rating of Exercise Induced – Muscle Soreness
Visual Analogue Scale: 100 mm

R2



L2



Maximal Voluntary Isometric Contraction

5 - second contraction / 30 - second rest

Subject:

Leg Tested :

Lever (m):

Dominant Leg:

Seat:

		Trial: Practice I		Degree		Comments
Date:		Contraction #	90	80		
		1				
		2				
Time:		3				
		Peak				
		Trial: Practice II		Degree		Comments
Date:		Contraction #	90	80		
		1				
		2				
Time:		3				
		Peak				
		Trial: Pre Inj (imm)		Degree		Comments
Date:		Contraction #	90	80		
		1				
		2				
Time:		3				
		Peak				
		Trial: Post Inj (imm)		Degree		Comments
Date:		Contraction #	90	80		
		1				
		2				
Time:		3				
		Peak				
		Trial: Post Inj + 2d		Degree		Comments
Date:		Contraction #	90	80		
		1				
		2				
Time:		3				
		Peak				

APPENDIX F

PHYSICAL CHARACTERISTICS OF SUBJECTS

Subject	Age (yrs)	Weight (kg)	Height (m)	Body Fat (%)
1	24	62.4	1.63	32.7
2	23	70.9	1.68	32.8
3	23	64.7	1.80	25.8
4	27	59.6	1.68	28.3
5	24	58.4	1.67	22.8
6	24	82.4	1.64	41.9
7	22	66.0	1.78	25.8
8	30	66.5	1.65	23.1
9	29	61.6	1.56	32.5
10	21	56.8	1.64	25.5
Mean	24.70	64.93	1.67	29.12
SEM	0.94	2.35	0.02	1.87

APPENDIX G
AREA UNDER THE CURVE

INSULIN AREA UNDER THE CURVE
($\mu\text{U}\cdot\text{mL}^{-1}120\text{ min}^{-1}$)

Subject	BASE	DTR 1	DTR 2
1	2485.20	3238.80	2844.60
2	1378.80	1584.00	980.85
3	2620.20	3040.65	2121.60
4	2559.41	2973.30	2539.95
5	1871.10	3194.85	2724.30
6	4231.05	6046.80	5168.25
7	2328.75	3585.00	2918.40
8	2285.10	4340.85	1522.05
9	2516.10	3291.15	2864.55
10	3208.80	3540.30	4055.25
Mean	2548.45	3483.57	2773.98
SEM	240.88	358.45	375.26

GLUCOSE AREA UNDER THE CURVE
($\text{mmol}\cdot\text{L}^{-1}\cdot 120 \text{ min}^{-1}$)

Subject	BASE	DTR 1	DTR 2
1	429.45	604.50	509.10
2	649.20	678.45	567.30
3	473.70	576.45	480.00
4	410.57	553.50	508.28
5	448.35	611.55	533.70
6	677.85	705.38	641.48
7	689.70	768.90	551.93
8	567.00	780.90	538.65
9	481.20	504.68	432.36
10	487.58	572.85	552.38
Mean	531.46	635.72	531.52
SEM	33.55	29.52	17.58

INSULIN AND GLUCOSE AREA UNDER THE CURVE
(PERCENT INCREASE FROM BASELINE)

Subject	Insulin		Glucose	
	DTR 1	DTR 2	DTR 1	DTR 2
1	30.32	14.46	40.76	18.55
2	14.88	-28.86	4.51	-12.62
3	16.05	-19.03	21.69	1.33
4	16.17	-0.76	34.81	23.80
5	70.75	45.60	36.40	19.04
6	42.91	22.15	4.06	-5.37
7	53.95	25.32	11.48	-19.98
8	89.96	-33.39	37.72	-5.00
9	30.80	13.85	4.88	-10.15
10	10.33	26.38	17.49	13.29
Mean	37.61	6.57	21.38	2.29
SEM	8.44	8.29	4.74	4.85

APPENDIX H

FASTING AND PEAK INSULIN AND GLUCOSE

FASTING AND PEAK INSULIN
($\mu\text{U}\cdot\text{mL}^{-1}$)

Subject	Fasting Insulin			Peak Insulin		
	BASE	DTR 1	DTR 2	BASE	DTR 1	DTR 2
1	7.12	8.19	11.25	24.78	39.21	33.05
2	5.64	3.08	1.87	12.94	16.32	13.12
3	5.67	3.09	4.24	33.31	41.31	28.37
4	4.41	4.20	4.89	36.05	41.29	35.90
5	5.45	6.51	3.97	22.46	37.60	36.47
6	8.78	9.35	7.50	57.57	77.67	75.96
7	2.73	3.05	3.71	22.39	36.72	42.24
8	4.29	3.99	2.98	30.43	60.60	18.71
9	5.81	6.49	5.67	31.74	46.74	37.29
10	5.32	4.15	4.50	44.55	43.61	62.36
Mean	5.52	5.21	5.06	31.62	44.11	38.35
SEM	0.52	0.72	0.84	3.99	5.07	5.93

FASTING AND PEAK GLUCOSE
(mmol·L⁻¹)

Subject	Fasting Glucose			Peak Glucose		
	BASE	DTR 1	DTR 2	BASE	DTR 1	DTR 2
1	3.69	4.04	3.77	3.80	6.29	5.43
2	4.34	4.10	3.96	6.30	5.99	5.43
3	3.72	3.47	4.04	5.13	5.63	5.03
4	3.50	3.84	3.68	4.85	6.36	5.39
5	4.04	4.32	4.23	4.13	6.03	5.46
6	4.68	4.85	4.77	7.14	8.01	6.77
7	3.95	4.13	3.78	6.84	7.22	5.27
8	4.43	4.46	4.50	6.23	8.63	6.77
9	4.02	4.14	3.62	5.04	4.79	4.85
10	4.01	3.75	3.96	5.48	5.58	6.24
Mean	4.04	4.11	4.03	5.49	6.45	5.66
SEM	0.11	0.12	0.12	0.35	0.37	0.22

PEAK INSULIN AND GLUCOSE
(PERCENT INCREASE FROM BASELINE)

Subject	Insulin		Glucose	
	DTR 1	DTR 2	DTR 1	DTR 2
1	58.20	33.40	65.50	42.90
2	26.10	1.40	-4.90	-13.80
3	24.00	-14.80	9.70	-1.90
4	14.50	-0.40	31.10	11.10
5	67.40	62.40	46.00	32.20
6	34.90	31.90	12.20	-5.20
7	64.00	88.70	5.60	-23.00
8	99.10	-38.50	38.50	8.70
9	47.30	17.50	-5.00	-3.80
10	-2.10	40.00	1.80	13.90
Mean	43.34	22.16	20.05	6.11
SEM	9.42	11.83	7.57	6.38

APPENDIX I
CREATINE KINASE
(U·L⁻¹)

Subject	BASE	DTR 1	DTR 2
1	45.16	114.70	79.43
2	77.46	364.70	157.88
3	72.18	351.85	129.53
4	35.76	67.24	60.48
5	47.46	522.58	118.49
6	253.96	3272.93	527.85
7	63.61	184.74	78.44
8	101.02	183.26	129.37
9	68.56	397.66	172.88
10	170.73	2668.11	170.73
Mean	93.59	812.78	162.51
SEM	21.55	365.12	42.45

APPENDIX J

TORQUE

TORQUE
(PERCENT OF BASELINE)

Subject	DTR 1		DTR 2	
	POST	POST 2d	POST	POST 2d
1	77.30	86.10	87.92	101.71
2	80.87	90.32	84.37	101.09
3	88.59	91.58	89.78	99.23
4	96.26	97.35	96.51	99.55
5	71.67	76.46	93.51	102.10
6	76.56	98.72	89.10	101.80
7	90.33	104.40	94.39	103.76
8	92.21	90.23	91.92	91.70
9	77.35	78.65	91.21	101.00
10	82.04	92.17	95.09	100.00
Mean	83.32	90.60	91.38	100.19
SEM	2.55	2.73	1.17	1.03

TORQUE
(PERCENT DECLINE FROM BASELINE)

Subject	DTR 1		DTR 2	
	POST	POST 2d	POST	POST 2d
1	-22.70	-13.90	-12.08	1.71
2	-19.13	-9.68	-15.63	1.09
3	-11.41	-8.42	-10.22	-0.77
4	-3.74	-2.65	-3.49	-0.45
5	-28.33	-23.54	-6.49	2.10
6	-23.44	-1.28	-10.90	1.80
7	-9.67	4.40	-5.61	3.76
8	-7.79	-9.77	-8.08	-8.30
9	-22.65	-21.35	-8.79	1.00
10	-17.96	-7.83	-4.91	0.00
Mean	-16.68	-9.40	-8.62	0.19
SEM	2.55	2.73	1.17	1.03

APPENDIX K

SORENESS
(mm)

Subject	DTR 1			DTR 2		
	PRE	POST	POST 2d	PRE	POST	POST 2d
1	4.25	19.75	63.50	1.00	7.25	19.00
2	1.50	14.75	50.00	1.50	7.50	7.00
3	1.00	1.25	19.75	1.00	1.50	13.50
4	1.00	2.50	67.50	3.75	10.00	3.00
5	2.00	13.00	66.75	1.00	9.75	36.50
6	1.00	25.25	75.25	1.00	7.00	15.25
7	1.00	7.50	34.50	1.00	2.50	16.50
8	1.00	1.00	25.25	1.00	6.00	4.25
9	1.00	5.00	43.50	1.00	1.00	10.00
10	1.00	11.75	34.75	1.00	4.00	2.00
Mean	1.48	10.18	48.08	1.33	5.65	12.70
SEM	0.33	2.60	6.16	0.27	1.03	3.24

APPENDIX L
TREADMILL DATA

TREADMILL RUN AVERAGES

DTR 1							
Subject	VO ₂ (L·min ⁻¹)	VO ₂ (mL·kg ⁻¹ ·min ⁻¹)	RER	HR (b·min ⁻¹)	RPE	Steps (min ⁻¹)	Steps (total)
1	1.65	26.4	0.90	149.7	12.0	174.8	5245
2	2.00	28.3	0.93	175.2	14.0	162.0	4860
3	1.87	29.4	0.84	178.2	10.3	165.8	4975
4	1.40	23.7	0.95	133.2	9.8	165.5	4965
5	1.74	30.8	0.88	184.2	14.7	159.5	4785
6	1.78	21.4	0.95	178.8	14.2	174.2	5225
7	1.89	28.2	0.84	163.2	11.2	178.7	5360
8	1.69	25.1	0.93	161.8	12.3	185.0	5550
9	1.71	27.1	0.95	162.3	11.3	178.2	5345
10	1.78	30.7	0.91	164.8	12.3	169.5	5085
Mean	1.75	27.1	0.91	165.1	12.2	171.3	5139.5
SEM	0.05	1.0	0.01	4.8	0.5	2.6	77.6

DTR 2							
Subject	VO ₂ (L·min ⁻¹)	VO ₂ (mL·kg ⁻¹ ·min ⁻¹)	RER	HR (b·min ⁻¹)	RPE	Steps (min ⁻¹)	Steps (total)
1	1.66	25.9	0.90	148.0	11.5	173.8	5215
2	1.91	26.7	0.95	167.5	11.8	157.0	4710
3	1.75	26.9	0.89	163.3	9.5	171.5	5145
4	1.39	23.2	0.92	122.8	9.2	166.5	4995
5	1.64	29.1	0.91	176.8	12.3	159.5	4785
6	1.93	23.0	0.89	175.7	13.7	175.0	5250
7	1.59	24.0	0.89	162.2	10.3	175.7	5270
8	1.64	24.4	0.84	146.2	11.7	186.5	5595
9	1.70	26.7	0.93	157.7	9.0	176.3	5290
10	-	-	-	162.0	10.8	168.7	5060
Mean	1.69	25.5	0.90	158.2	11.0	171.1	5131.5
SEM	0.06	0.7	0.01	5.0	0.5	2.7	81.8

OXYGEN CONSUMPTION
PER STAGE
(L·min⁻¹)

DTR 1							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	1.61	1.62	1.64	1.64	1.68	1.69	1.65
2	1.85	1.92	2.03	2.07	2.06	2.04	2.00
3	1.88	1.84	1.85	1.87	1.90	1.90	1.87
4	1.36	1.37	1.38	1.40	1.41	1.45	1.40
5	1.60	1.55	1.73	1.83	1.87	1.86	1.74
6	1.74	1.72	1.80	1.80	1.81	1.82	1.78
7	1.69	1.81	1.91	1.98	1.95	2.00	1.89
8	1.61	1.68	1.73	1.72	1.72	1.70	1.69
9	1.62	1.65	1.72	1.78	-	1.80	1.71
10	1.70	1.77	1.79	1.85	1.76	1.82	1.78
Mean	1.67	1.69	1.76	1.79	1.80	1.81	1.75
SEM	0.05	0.05	0.05	0.06	0.06	0.05	0.05

DTR 2							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	1.59	1.62	1.65	1.70	1.69	1.68	1.66
2	1.86	1.82	1.91	1.95	1.93	2.00	1.91
3	1.61	1.73	1.78	1.81	1.76	1.81	1.75
4	1.35	1.37	1.37	1.39	1.41	1.42	1.39
5	1.55	1.62	1.67	1.67	1.66	1.69	1.64
6	1.79	1.85	1.92	1.96	1.97	2.07	1.93
7	1.42	1.49	1.57	1.66	1.65	1.73	1.59
8	1.54	1.61	1.63	1.69	1.68	1.69	1.64
9	1.57	1.65	1.75	1.75	-	1.80	1.70
10	-	-	-	-	-	-	-
Mean	1.59	1.64	1.69	1.73	1.72	1.77	1.69
SEM	0.05	0.05	0.06	0.06	0.06	0.06	0.06

OXYGEN CONSUMPTION
PER STAGE
(mL·kg⁻¹·min⁻¹)

DTR 1							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	25.8	26.0	26.3	26.2	26.9	27.1	26.4
2	26.2	27.2	28.8	29.4	29.3	29.0	28.3
3	29.5	28.9	29.0	29.4	29.8	29.8	29.4
4	23.1	23.2	23.4	23.8	23.9	24.5	23.7
5	28.3	27.4	30.6	32.4	33.1	32.8	30.8
6	20.9	20.6	21.6	21.6	21.8	21.8	21.4
7	25.2	27.1	28.5	29.5	29.1	29.8	28.2
8	23.7	24.9	25.6	25.4	25.5	25.2	25.1
9	25.6	26.0	27.2	28.1	-	28.4	27.1
10	29.3	30.5	30.9	31.9	30.3	31.3	30.7
Mean	25.8	26.2	27.2	27.8	27.7	28.0	27.1
SEM	0.9	0.9	1.0	1.1	1.2	1.1	1.0

DTR 2							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	24.9	25.4	25.9	26.6	26.4	26.3	25.9
2	25.9	25.4	26.6	27.3	27.0	27.9	26.7
3	24.7	26.5	27.3	27.8	27.1	27.7	26.9
4	22.6	23.0	22.9	23.3	23.6	23.9	23.2
5	27.4	28.8	29.6	29.5	29.4	29.9	29.1
6	21.3	22.1	23.0	23.4	23.5	24.7	23.0
7	21.5	22.6	23.8	25.1	25.0	26.2	24.0
8	23.0	24.0	24.3	25.1	25.0	25.2	24.4
9	24.6	25.8	27.4	27.4	-	28.1	26.7
10	-	-	-	-	-	-	-
Mean	24.0	24.8	25.6	26.2	25.9	26.7	25.5
SEM	0.7	0.7	0.8	0.7	0.7	0.6	0.7

HEART RATE
PER STAGE
(b·min⁻¹)

DTR 1							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	138	138	146	153	159	164	149.7
2	157	166	176	181	185	186	175.2
3	170	174	179	181	183	182	178.2
4	129	131	133	134	137	135	133.2
5	-	168	181	189	190	193	184.2
6	160	176	181	184	188	184	178.8
7	152	158	162	162	167	178	163.2
8	151	157	161	164	168	170	161.8
9	141	148	160	170	176	179	162.3
10	152	159	163	169	170	176	164.8
Mean	150.0	157.5	164.2	168.7	172.3	174.7	165.1
SEM	4.1	4.7	5.0	5.2	5.1	5.1	4.8
DTR 2							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	134	140	141	153	159	161	148.0
2	154	160	166	172	174	179	167.5
3	153	158	162	168	168	171	163.3
4	126	122	119	121	124	125	122.8
5	-	168	175	178	180	183	176.8
6	169	171	178	178	177	181	175.7
7	140	151	167	172	170	173	162.2
8	134	138	148	148	152	157	146.2
9	135	143	163	168	167	170	157.7
10	155	159	160	164	166	168	162.0
Mean	144.4	151.0	157.9	162.2	163.7	166.8	158.2
SEM	4.6	4.8	5.6	5.5	5.1	5.3	5.0

RATINGS OF PERCEIVED EXERTION
PER STAGE

DTR 1							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	11	11	12	12	13	13	12.0
2	13	13	14	14	15	15	14.0
3	7	9	10	11	12	13	10.3
4	7	8	9	11	12	12	9.8
5	10	12	15	16	17	18	14.7
6	12	13	13	15	15	17	14.2
7	7	10	11	12	13	14	11.2
8	11	12	12	13	13	13	12.3
9	11	11	12	12	11	11	11.3
10	9	11	13	13	13	15	12.3
Mean	9.8	11.0	12.1	12.9	13.4	14.1	12.2
SEM	0.7	0.5	0.6	0.5	0.6	0.7	0.5

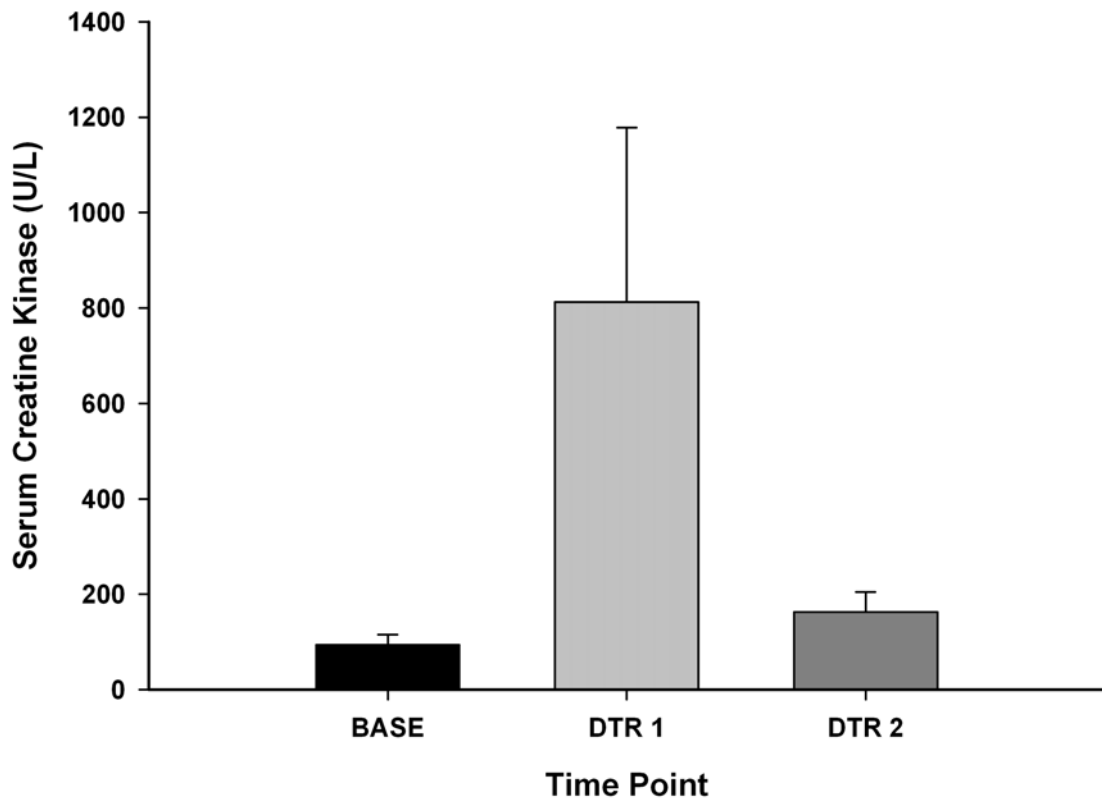
DTR 2							
Subject	Stage						Avg
	1	2	3	4	5	6	
1	11	11	11	12	12	12	11.5
2	10	11	12	12	13	13	11.8
3	7	7	9	10	12	12	9.5
4	7	8	9	10	10	11	9.2
5	7	9	12	14	15	17	12.3
6	12	12	13	13	15	17	13.7
7	7	9	10	11	12	13	10.3
8	11	11	12	12	12	12	11.7
9	9	9	9	9	9	9	9.0
10	9	9	10	12	12	13	10.8
Mean	9.0	9.6	10.7	11.5	12.2	12.9	11.0
SEM	0.6	0.5	0.5	0.5	0.6	0.8	0.5

TOTAL STEPS AND AVERAGE STEPS (steps·min⁻¹)
PER STAGE

DTR 1								
Subject	Stage						Avg	Total
	1	2	3	4	5	6		
1	173	175	174	175	175	177	174.8	5245
2	158	162	161	164	164	163	162.0	4860
3	161	166	168	166	166	168	165.8	4975
4	169	166	165	164	164	165	165.5	4965
5	161	159	161	161	158	157	159.5	4785
6	183	176	171	173	172	170	174.2	5225
7	176	178	180	177	181	180	178.7	5360
8	184	184	186	184	187	185	185.0	5550
9	178	181	179	179	174	178	178.2	5345
10	168	169	172	168	171	169	169.5	5085
Mean	171.1	171.6	171.7	171.1	171.2	171.2	171.3	5139.5
SEM	2.9	2.7	2.6	2.4	2.7	2.7	2.6	77.6

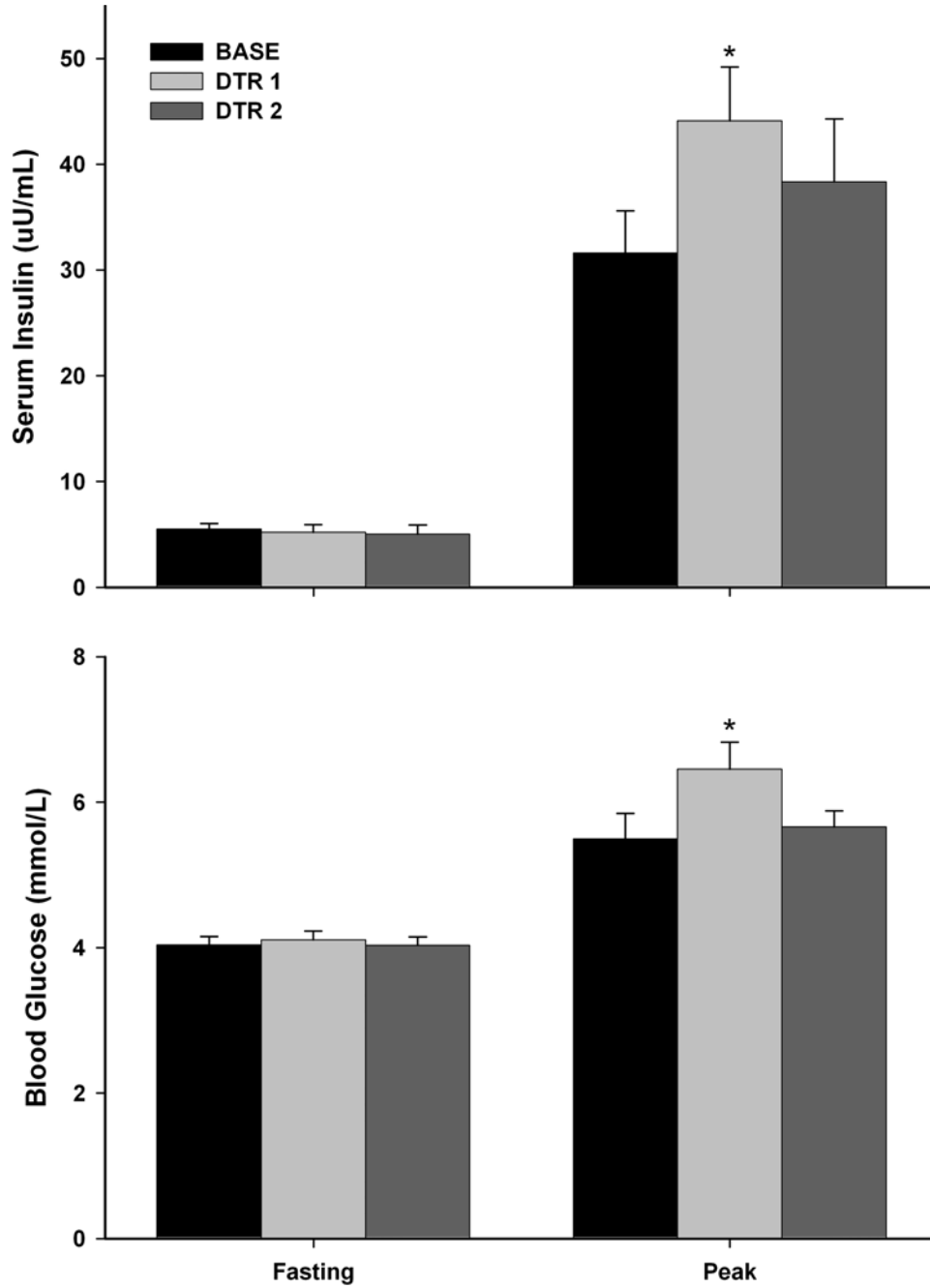
DTR 2								
Subject	Stage						Avg	Total
	1	2	3	4	5	6		
1	171	172	174	175	175	176	173.8	5215
2	154	156	154	158	161	159	157.0	4710
3	168	172	173	173	172	171	171.5	5145
4	168	165	166	166	167	167	166.5	4995
5	160	159	159	161	162	156	159.5	4785
6	179	179	176	171	170	175	175.0	5250
7	179	176	175	178	173	173	175.7	5270
8	184	186	187	188	190	184	186.5	5595
9	176	175	177	176	175	179	176.3	5290
10	166	169	167	169	172	169	168.7	5060
Mean	170.5	170.9	170.8	171.5	171.7	170.9	171.1	5131.5
SEM	2.9	2.9	3.0	2.7	2.6	2.7	2.7	81.8

APPENDIX M
CREATINE KINASE



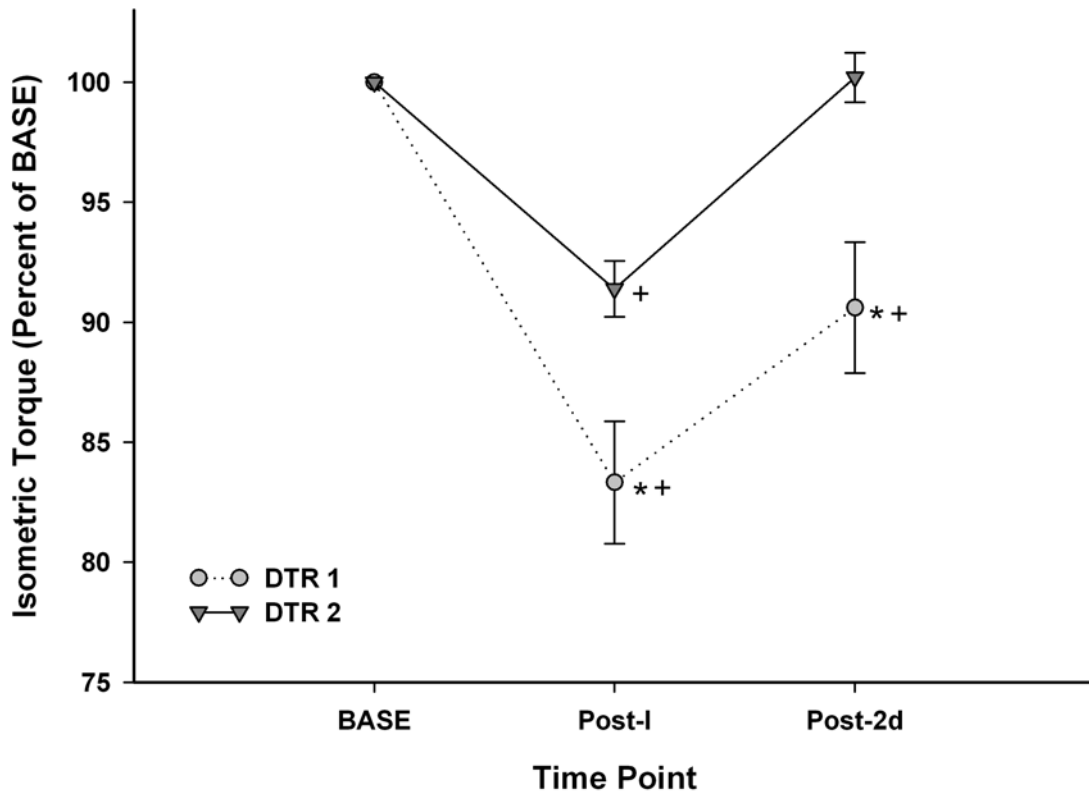
APPENDIX N

FASTING AND PEAK INSULIN AND GLUCOSE



APPENDIX O

Torque



APPENDIX P
INDIVIDUAL INSULIN AND GLUCOSE RESPONSES

