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EFFECT OF LOWER LIMB COMPRESSION (NORMATEC) ON GLYCOGEN
RESYNTHESIS

By:

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B.A. Colorado Mesa University
Grand Junction, CO 2008

Thesis presented in partial fulfillment of the
requirements for the degree of:

Master of Science
In Health and Human Performance, Exercise Science

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Spring 2012

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Exercise Science

Effect of Lower Limb Compression (NormaTec) on Glycogen Resynthesis

Chairperson: Brent C. Ruby, Ph.D.

Purpose: The purpose of this study was to investigate the effects of pneumatic compression pants on post-exercise glycogen resynthesis. **Methods:** Active male subjects (n=10) completed two trials consisting of a 90-minute glycogen depleting ride, followed by 4 hours of recovery with either a pneumatic compression device (PCD) or passive recovery (PR) in a random counterbalanced order. A carbohydrate beverage ($1.8 \text{ g}\cdot\text{kg}^{-1}$ bodyweight) was provided at 0 and 2 hours post exercise. Muscle biopsies (vastus lateralis) were obtained immediately and 4 hours post exercise for glycogen analyses. Blood samples were collected throughout recovery to measure glucose and insulin. Eight finger stick blood samples for lactate were collected in the last 20 minutes of the exercise period and during the initial portion of the recovery period. Heart rate was monitored throughout the entire trial. During the PCD trial subjects recovered using a commercially available recovery device (NormaTec PCD, Newton Center, MA) operational at 0-60 and 120-180 min into recovery period. The same PCD was worn during the passive recovery trial but was not turned on to create pulsatile pressures. **Results:** Muscle glycogen increased similarly over the recovery period for both trials (6.9 ± 0.8 and $6.9 \pm 0.5 \text{ mmol}\cdot\text{kg}^{-1} \text{ wet wt}\cdot\text{hr}^{-1}$ for the PR and PCD trials, respectively) additionally, blood glucose, insulin, and lactate concentrations changed in respect to time but were not different between trials ($p>0.05$). **Conclusion:** The use of PCD did not alter the rate of muscle glycogen resynthesis, blood lactate and the blood glucose and insulin concentrations associated with a post exercise oral glucose load.

Key Words

massage, insulin, glucose, carbohydrate, recovery

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INTRODUCTION

Recovery post exercise is a key component to repeated performances during subsequent exercise. After exercise the internal milieu of the body is disrupted (60). This disruption may result from creatine phosphate and adenosine triphosphate depletion as well as a decrease in muscle and blood pH, blood glucose concentration and muscle glycogen level (30, 37, 63) as well as increased inflammation (54) and fluid shifts from vasculature to the muscle (30).

Consequently, any recovery modality that returns the disrupted internal environment back to homeostatic levels should lead to the ability to perform with maximal effort in subsequent exercise sessions (60). Therefore many recovery modalities have become common such as, massage, compression garments, and active recovery (24). However, evidence as to whether they enhance between-training recovery is equivocal (4).

Proper recovery post exercise has the potential to lessen or even totally diminish delayed onset muscle soreness (DOMS), fatigue, decreased performance on subsequent work bouts, and overtraining. Lack of appropriate recovery may result in an athlete being unable to train at the required intensity or complete the required load at the next training session (4). Furthermore, full recovery is necessary for optimal performance in competition (4). If recovery is compromised, it may reduce the athlete's ability to train at the desired intensity due to reduced muscle glycogen (39, 89). Several factors have been shown to influence the rate of glycogen resynthesis following depletion. These include the amount (48), timing (47), and CHO composition ingested post exercise (46, 52, 53, 77). Additionally, it has been shown that muscle temperature also affects the rate of glycogen synthesis (66, 81, 82). Although these

variables are known to have an effect of glycogen resynthesis the effect of massage/compression is still equivocal.

STATEMENT OF PROBLEM

To date many of the current studies investigating these recovery modalities after exercise have been inconclusive. Most studies contain methodological limitations including insufficient duration of treatment, inadequate number of subjects, or the over/under working of muscles that limit practical conclusions (65). In addition, very limited research to date has reported the effects of either compression or massage on the rate of post-exercise glycogen synthesis (23).

PURPOSE

The purpose of this study was to evaluate the effect of a mechanical pneumatic compression/peristaltic pulsing applied by a commercially available portable compression device (PCD) on muscle glycogen recovery, blood glucose, insulin, lactate and heart rate after exercise and oral glucose feedings.

HYPOTHESES

1. Treatment with the NormaTec PCD will not alter rates of muscle glycogen resynthesis post exercise.
2. Treatment with the NormaTec PCD will have no effect on lactate concentration post exercise.

3. Treatment with the NormaTec PCD will have no effect on the blood glucose response after ingestion of a carbohydrate (CHO) drink post exercise.
4. Treatment with the NormaTec PCD will have no effect on the insulin response after ingestion of CHO drink post exercise.

SIGNIFICANCE OF THE STUDY

Other studies have evaluated the effects of both massage and compression on post exercise lactate concentration, but no known studies have evaluated their effect on glycogen resynthesis, blood glucose, and insulin post exercise. Therefore, this study was the first study to identify if compression has any effect on glycogen resynthesis, blood glucose, and insulin after exercise and in response to multiple oral glucose bolus feedings. Additionally, this was the first study to evaluate changes in post exercise blood lactate using pneumatic compression combined with peristaltic pulsing.

LIMITATIONS

1. The subjects had varying physical abilities but were be males between 18-40 years of age with a VO_2 peak of $> 40\text{ml/kg/min}$ and capable of completing 90 minutes of vigorous cycling without nutritional assistance.
2. Participants' lifestyle between the trials could not be controlled. In order to better control physical activity levels, a dietary and physical activity recall for the day prior to the exercise trial was recorded and repeated prior to the next trial.

3. The use of any instrumentation could have caused error. To limit this occurrence of instrumentation error, all researchers were trained and the equipment was carefully calibrated.
4. Participants were not randomly sampled and were recruited by convenience. However, random ordering of treatments will be utilized.

DELIMITATIONS

1. All participants in this study were active male cyclists with a VO_2 peak of $>40\text{ml/kg/min}$. Due to the effects of the menstrual cycle on fuel utilization, females were excluded from this study.
2. Participants were apparently healthy and were excluded from the study if they had any contraindications on the PAR-Q health questionnaire.

DEFINITION OF TERMS

VO_2 peak: The maximal amount of oxygen consumed during a specific mode of exercise.

Graded exercise test: An incremental maximal exercise stress test done to determine an individual's VO_2 peak as well as maximal wattage on a cycle ergometer.

Pneumatic compression device (PCD): An air driven unit which applies an external pressure gradient (30-90mmHg) to a person's skin.

Compression garment: A piece of clothing usually made from lycra and or nylon that is designed to apply an external pressure gradient ($\sim 18\text{-}23\text{mmHg}$) to a person's skin.

Active recovery: low intensity exercise used to aid in the recovery process.

Passive recovery: Sedentary or a state of non-activity following exercise.

REVIEW OF LITERATURE

Anecdotal information from coaches, athletic trainers, and athletes suggest that various types of recovery modalities including the use of active recovery (60) massage (14, 60, 92, 95), contrast bathing (29), and compression garments (95) may have a positive effect on returning the body's internal environment to homeostatic levels at an accelerated rate. While a review of some of these recovery modalities will be made to identify potential research directions some recovery modalities are beyond the scope of this article.

Active Recovery

It has been well documented that performing low intensity aerobic exercise immediately post exercise is more effective in accelerating lactate clearance than passive recovery (5, 31, 34, 40, 76, 85, 93). This increased lactate clearance is promoted by an increased metabolic rate and systemic blood flow, thereby accelerating lactate metabolism via oxidation and gluconeogenesis (12, 13, 34, 76). Therefore, increasing the rate that one may achieve homeostasis after exercise.

It is also important to note that several studies have shown that active recovery in a fasted state impairs glycogen resynthesis (9, 19, 27). Thus, active recovery may be detrimental to rapid glycogen resynthesis (4) and as a result passive recovery may be more beneficial in this sense.

Massage

Massage has been used for centuries in an attempt to prevent and cure injuries (11, 18, 87).

Massage is considered to enhance muscle relaxation (67, 96), reduce muscle tension, and soreness (83, 88), promote the healing process (86), and consequently, improve athletic performance (72, 91, 99). Massage is used extensively in the training of elite athletes and is commonly thought to decrease edema and pain, enhance blood lactate removal and alleviate DOMS largely by increasing muscle blood flow (92).

The most recent studies using Doppler ultrasound to measure blood flow and vessel diameter found no increase in muscle blood flow during massage with (41, 88) or without (79) preceding exercise. Additionally, Cafarelli et al. noted that previous studies concerning the effects of massage on skeletal muscle blood flow have been contradictory and difficult to compare due to differences in experimental designs, statistical analyses, and the massage techniques used (16). Based upon the lack of scientific evidence the efficacy of massage in promoting muscle blood flow is inconclusive.

Several studies have attempted to evaluate the effect of massage on blood lactate concentration. Despite efforts to determine the effects of massage on blood lactate, little empirical evidence has been found (92) even though participants reported less fatigue after massage application (35, 38, 64). Therefore, Martin et al., has concluded that currently there is a lack of controlled research to support the efficacy of sports massage on accelerating the rate of post-exercise blood lactate clearance (62). However Crane et al. found that massage appears

to reduce inflammation and promote mitochondrial biogenesis after exercise induced muscle damage (22).

Contrast Bathing

Contrast water bathing is increasingly being used by athletes to accelerate post exercise recovery (90). It is usually done by an athlete as soon as practical post exercise. The process typically involves alternating between hot and cold water immersion pools for a period of time lasting from 4-30 minutes total, with 30 second to 5 minute immersions per pool done repeatedly (97).

It is speculated that contrast bathing by promoting increased blood flow by alternating vasodilation and vasoconstriction (42). Anecdotally athletes have reported beneficial effect on subsequent exercise. However the use of contrast bathing is still inconclusive as there is insufficient evidence to support its use post exercise. This is evidenced by several studies that have found inconclusive evidence on subsequent performance enhancements when compared with a control (20, 25, 28, 36, 44, 56, 57, 74).

Compression Garments

There appears to be a lay acceptance that compression garments aid in post exercise recovery (4). Compression garments are considered to provide an external pressure gradient that theoretically reduces the space available for swelling, hemorrhage, or hematoma formation (68). To date, the evidence as to whether compression is effective at enhancing recovery

between exercise bouts remains to be fully elucidated (4). Additionally, limited research that has been published has produced conflicting results regarding the potential benefits of wearing compression garments during recovery (8, 59). Although evidence may be conflicting, what remains apparent through anecdotal reports is that recovery or regeneration interventions are commonly used among athletes.

Many studies have underlined a close association between lactate and exercise performance (71). Investigators have observed that work capacity and performance were adversely affected by elevated blood lactate levels (3, 55, 58, 84). However, a strong argument for lactate production not being a cause of metabolic acidosis has been made (73), additionally recent evidence suggests that acidosis has little effect of muscle contraction at physiological temperatures (17, 94). Therefore, lactate removal does not appear to be a valid indicator of recovery (4). But an increase of skeletal muscle blood flow may accelerate the rate at which lactate is shuttled to various sites of elimination, thereby promoting lactate clearance (16, 18), thus in this sense blood lactate is more of a “marker” of blood flow than it is a “marker” of recovery (4, 73).

It has been thought that using compression garments increases blood flow by providing a higher pressure gradient at the ankles than the more proximal regions of the leg (49). Similarly, compression garments have been noted by several studies to increase venous return (1, 43, 68). However, the results of these studies may not be applicable to a healthy population as some of these studies have been done on post-operative and bedridden patients (1) or in the

treatment of patients with chronic venous insufficiency (43). Additionally, this increase in blood flow is hypothesized to aid in the removal of lactate (24) to sites where it could be eliminated or converted and used as energy. However, as stated earlier in this manuscript the effects of compression on lactate removal remains inconclusive.

Glycogen Synthesis

Muscle glycogen is the primary fuel source during prolonged moderate to high intensity exercise (75). Fatigue during prolonged exercise is often associated with muscle glycogen depletion (6) and therefore high pre-exercise muscle glycogen levels are believed to be essential for optimal performance (15, 21, 45).

Glycogen synthesis following a glycogen depleting exercise occurs in two phases. Initially, there is a period of rapid synthesis that does not require the presence of insulin and lasts for 30-60 minutes (51). It has been suggested that the rapid phase only occurs when post exercise muscle glycogen concentration are lower than 128-150 mmol/kg dw (61, 69) and carbohydrate (CHO) is provided immediately after exercise (47). Following this rapid phase, muscle glycogen synthesis occurs at a much slower rate and can last for several hours. During this phase muscular contraction and insulin have been shown to increase the activity of glycogen synthase, the rate-limiting enzyme in glycogen synthesis (51).

As previously mentioned, several factors have been shown to influence the rate of glycogen synthesis following depletion. These include the amount (48), timing (47), and CHO

composition ingested post exercise (46, 52, 53, 77). Additionally, it has been shown that ambient and muscle temperature also may affect the rate of glycogen synthesis (66, 81, 82). Therefore, these variables need to be consistent and controlled in order to achieve reliable data.

To date no research has evaluated the effects of either compression garments or massage on the rate of glycogen synthesis during recovery in athletes. As Barnett comments in a recent review of recovery modalities, important factors associated with recovery such as the rate of post-exercise glycogen synthesis need to be considered in future research (4).

Blood Glucose and Insulin

Despite periods of feeding and fasting, plasma glucose remains in a narrow range between 4-7mM in healthy individuals (78). This tight control is governed by the balance of glucose absorption from the small intestine, production by the liver, and uptake in muscle and fat tissue. Insulin also inhibits hepatic glucose production thus serving as the primary regulator of blood glucose concentration. Finally, insulin promotes the storage of muscle glycogen (50) as well as storage of other substrates that are beyond the scope of this review.

After orally ingesting CHO blood glucose elevates to higher values than seen during normal resting. This elevated blood glucose triggers the release of insulin from the β -cells of the pancreas. Insulin then acts to aid in the translocation of the GLUT-4 transporter protein to the muscle cell membrane. GLUT-4 then carries the glucose into the muscle cell where the glucose

is either utilized or undergoes a series of transformations that results in the production of muscle glycogen. Additionally, recent studies have also shown that acute exercise enhances GLUT-4 translocation in addition to insulin (10).

After CHO ingestion the typical time period for elevated blood glucose and insulin can last for several hours in a normal, healthy individual. However, peak insulin and glucose generally occurs in the first 60 minutes post CHO feeding. During this time period a significant amount of glucose is taken into the cell via the translocation of GLUT-4 to the cell wall as a result of elevated insulin values. This increased glucose uptake into the muscle cell lends to the potential for increased glycogen resynthesis. However, if this process were to be enhanced or diminished then an increased or decreased glycogen resynthesis rate would respectively occur.

Summary

While anecdotal information from coaches, athletic trainers, and athletes suggest that various types of recovery modalities may have a positive effect on returning the body's internal environment to homeostatic levels at an accelerated rate these claims have yet to be fully substantiated in scientific literature. Therefore, the role of how each of these recovery modalities affects the recovery process is largely unknown or inconclusive. Therefore, the purpose of this study is to evaluate the effects of a mechanical pneumatic compression/peristaltic pulsing applied by a commercially available portable compression device (PCD) on muscle glycogen recovery, blood glucose, insulin, lactate and heart rate after exercise and oral glucose feedings.

METHODS

Experimental Approach to the Problem

In the present study, a randomized crossover experimental design was used to determine the effects of wearing a non-invasive pneumatic compression device (PCD) vs. a non-compression passive recovery (PR) control condition. Muscle glycogen, blood glucose, insulin, and lactate were monitored with heart rate during the recovery period.

Subjects

The investigation was approved by the University of Montana's Institutional Review Board for use of human subjects in research. Each subject had the experimental risks and the study explained to them and subsequently provided written informed consent to participate. Prior to data collection each subject completed a Physical Activity Readiness Questionnaire (PAR-Q). Ten active male participants (n=10) completed the study. All study participants were healthy, injury free, and familiar with moderate-to-high intensity exercise (Table 1).

Procedures

Preliminary testing

Timing. All preliminary testing for each subject was performed no less than 48 hours prior to any experimental testing and after a minimum of a three hour fast.

Body composition. Body composition was measured using hydrodensitometry. Briefly, an electronic strain-gauge scale (Exertech, Dreshbach, MN) measured underwater weight (33).

Under water weight was then used to calculate body density and using estimated residual lung volume and converted to body composition using the Siri equation (80).

VO₂ peak testing. A maximal exercise test was completed on a laboratory cycle ergometer (Velotron, RacerMate Inc. , Seattle WA) to determine peak oxygen uptake (VO_{2peak}) and maximal power output (W_{max}). Subjects completed a graded exercise protocol, starting at 95 watts and increasing by 35 watts every 3 minutes until volitional fatigue. During the test, expired gases were continuously collected and analyzed every 15 seconds in a mixing chamber using a calibrated metabolic cart (Parvomedics, Inc., Sandy, UT). Peak oxygen uptake was determined as the highest achieved oxygen uptake during the test while maximum power output was calculated as the last completed stage in watts plus the fraction of time that was completed in partial stages multiplied by 35 watts.

Experimental testing (Figure 1a and 1b)

Dietary recall. For 24 hours prior to the subject's first exercise/recovery trial, a detailed food log was maintained. For the second trial the subjects were asked to consume the same food and quantity of those foods as they did for the first trial. The subjects were also asked to refrain from physical activity in the 24 hour period prior to each trial.

Glycogen depletion ride. Participants were scheduled for two exercise trials which were separated by no less than one week. Before each exercise trial the subjects were asked to fast

for 12 hours prior to arrival. Upon arrival to the laboratory the subjects cycled in a thermo-neutral environment for a total of 90 minutes on the same Velotron ergometer. Each 90 minute cycling session included 10 minutes at the power output corresponding to 55% watt max. Thereafter a series of 10 intervals (including two minutes at 80% watt max followed by four minutes at 50% watt max) were completed. After the series of 10 intervals the subjects completed 12 minutes at 60% watt max followed by 10 minutes at 50% watt max. During the exercise trials subjects were allowed to consume water ad libitum, which was then standardized for the second trial.

Blood Lactate. Eight finger stick blood samples were taken during each trial to measure blood lactate concentration. The samples were collected starting after the completion of the last (10th) interval during the depletion ride. Additional samples were collected at 10 and 20 minutes after completion of the last interval. Sampling was continued after the collection of the post-exercise muscle biopsy at 0, 5, 10, 15, 25 minutes into the recovery period. Prior to collection, the site was cleaned with alcohol before obtaining the blood sample. Once the 25 μ l blood sample was obtained it was placed in 50 μ l of a cell lysing agent and frozen to -30 °C until subsequent analysis using a YSI 1500 (Yellow Spring Instruments, Inc., Yellow Springs, OH).

Muscle Biopsies. Muscle biopsies of the vastus lateralis muscle were taken immediately and 4 hours after the exercise bout using the percutaneous biopsy needle technique with the aid of suction (26). The 4-hour post exercise biopsies were taken from a site approximately 2 cm proximal to the previous biopsy location. Biopsies for the second trial were taken from the

opposite leg from the first trial. Excess blood, fat, and connective tissue were immediately removed, and tissue samples were frozen in liquid nitrogen and stored in a freezer at -80°C for the later analysis of muscle glycogen.

Blood sampling. Blood samples to examine plasma glucose and insulin concentration were drawn immediately after exercise, then at 30, 60, 120, 150, 180, and 240 minutes after exercise. Samples were obtained from the antecubital vein using a venopuncture technique using heparin as an anticoagulant. Samples were spun at 4000 rpm for 15 minutes in a refrigerated centrifuge (4°C). The subsequent plasma was aliquoted into multiple micro-centrifuge tubes and stored at -30°C for later analysis of glucose and insulin concentrations.

Post Ride Recovery. Following each post-ride biopsy the subjects recovered in a thermo-neutral environment ($\sim 20^{\circ}\text{C}$) for four hours. During this time the subjects remained lying down but were permitted to read a book, watch TV, listen to music, etc. , but were not allowed to exercise. The experimental condition utilized a noninvasive pneumatic compression device (PCD) (NormaTec pneumatic compression device, Newton Center, MA) for two 1-hour treatments starting at the beginning (0-60 minutes) of the recovery period and again 2 hours into the recovery period (120-180 minutes). The PCD contains 5 air filled chambers that create pressure by filling and emptying that according to the manufacturer create pressures ranging from 30-90mmHg. During each hour long treatment the noninvasive PCD cycled through each of the five chambers from the ankles, at chamber 1, to the upper quadriceps, at chamber 5. Each of the five chambers was set to inflate and pulse at 70 mmHg for 30 second intervals

before moving to the next successive chamber. After the inflation of chambers 1, 2, and 3 the pressure from chamber 1 was released as chamber 4 began inflation. After completion of the 30 second pulse time on chamber 4 the pressure in chamber 2 was released. Meanwhile chamber 5 begins pulsing at the same time that pressure in chamber 3 was released. Upon completion of the pulsing in the 5th and final chamber, all of the remaining chambers depressurized for 30 seconds before repeating the next cycle. These cycles repeated until one hour was reached. For the passive recovery condition (PR) the subjects were outfitted with the pneumatic compression device but were not operational, therefore chamber pressures did not change. (Image 1)

Carbohydrate feeding. Subjects received two oral carbohydrate feedings that consisted of 1.8 g·kg⁻¹ BW of an oral dextrose solution (Azer Scientific glucose tolerance test beverage, 100g: 296 ml⁻¹, Azer Scientific, Morgantown, PA). The first feeding was provided immediately after the initial muscle and blood sample was obtained post-ride and the second after the 120-minute sample collections. The same beverage schedule was applied to both trials.

Heart rate. Heart rate monitors were worn by all subjects throughout each trial. The monitors (Polar RS800CX, Polar Electro, Kempele, Finland) collected the subjects' heart rate during each minute and the data was downloaded to a computer using the supplied software (Polar ProTrainer 5.0, Polar Electro, Kempele, Finland). This data was then analyzed during the entire recovery period and averaged for 0-60 minutes and 120-180 minutes for each trial.

Blood and Tissue Analysis. Muscle samples were analyzed in triplicate to determine muscle glycogen concentrations using an enzymatic spectrophotometric method (77). Samples were weighed and placed in 0.5 ml of a 2 N HCL solution. The sample solutions were weighed, incubated in an oven for two hours at 100 °C, and re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 M NaOH was added. A volume of this muscle extract (100 µl) was added to 1 ml of infinity glucose reagent (ThermoTrace Ltd., Middletown, VA) and read on a spectrophotometer at 340 nm. Muscle glycogen concentration was calculated using the extinction coefficient of NADH and expressed in $\text{mmol}\cdot\text{kg}^{-1}\text{ wet wt}\cdot\text{hr}^{-1}$ of muscle.

Blood samples were analyzed for glucose in triplicate using infinity glucose reagent (ThermoTrace Ltd., Middletown, VA) and read on a spectrophotometer at 340 nm. Blood glucose concentrations were calculated using the extinction coefficient of NADH and expressed in $\text{mmol}\cdot\text{L}^{-1}$. Samples were analyzed for insulin in triplicate using an enzymatic spectrophotometric ELISA method (EIA-2935, DRG International, Marburg Germany) and expressed in $\mu\text{IU}\cdot\text{mL}^{-1}$. Total area under the curve (AUC) was calculated for blood glucose and insulin concentrations using the trapezoidal method.

Statistical Analysis

A two-tailed paired t-test was used to compare insulin and glucose AUC and rate of glycogen resynthesis, using Excel software (Microsoft Corp., Redmond, WA). Muscle glycogen, blood glucose, insulin, lactate, and heart rate were analyzed using a two-way repeated measure

ANOVA (trial x time) with SPSS software (SPSS Inc., Chicago, IL). A probability of type 1 error less than 5% was considered significant ($p < 0.05$). All descriptive data is reported as mean \pm SD and experimental data is reported as mean \pm SEM.

MANUSCRIPT**EFFECT OF LOWER LIMB COMPRESSION (NORMATEC) ON GLYCOGEN RESYNTHESIS**

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EFFECT OF LOWER LIMB COMPRESSION (NORMATEC) ON GLYCOGEN RESYNTHESIS

ABSTRACT

The purpose of this study was to investigate the effects of pneumatic compression pants on post-exercise glycogen resynthesis. Active male subjects (n=10) completed two trials consisting of a 90-minute glycogen depleting ride, followed by 4 hours of recovery with either a pneumatic compression device (PCD) or passive recovery (PR) in a random counterbalanced order. A carbohydrate beverage (1.8 g·kg⁻¹ bodyweight) was provided at 0 and 2 hours post exercise. Muscle biopsies (vastus lateralis) were obtained immediately and 4 hours post exercise for glycogen analyses. Blood samples were collected throughout recovery to measure glucose and insulin. Eight finger stick blood samples for lactate were collected in the last 20 minutes of the exercise period and during the initial portion of the recovery period. Heart rate was monitored throughout the entire trial. During the PCD trial subjects recovered using a commercially available recovery device (NormaTec PCD, Newton Center, MA) operational at 0-60 and 120-180 min into recovery period. The same PCD was worn during the passive recovery trial but was not turned on to create pulsatile pressures. Muscle glycogen increased similarly over the recovery period for both trials (6.9 ± 0.8 and 6.9 ± 0.5 mmol·kg⁻¹ wet wt.·hr⁻¹ for the PR and PCD trials, respectively) additionally, blood glucose, insulin, and lactate concentrations changed in respect to time but were not different between trials (p>0.05). The use of PCD did not alter the rate of muscle glycogen resynthesis, blood lactate and the blood glucose and insulin concentrations associated with a post exercise oral glucose load.

Key Words

massage, insulin, glucose, carbohydrate, recovery

INTRODUCTION

Recovery post exercise is a key component to repeat performances. After exercise the internal milieu of the body is disrupted (60). This disruption may result from creatine phosphate and adenosine triphosphate depletion, a decrease in muscle and blood pH, blood glucose concentration, and muscle glycogen levels (30, 37, 63), as well as increased inflammation (54) and fluid shifts from vasculature to the muscle (30). Consequently, any recovery modality that returns the disrupted internal environment back to homeostatic levels should lead to the ability to perform with maximal effort in subsequent exercise sessions (60). Therefore many recovery modalities have become common. These include, among others, massage (14, 60, 92, 95), compression garments (95), contrast bathing (29), and active recovery (24, 60). However, evidence as to whether these enhance between-training recovery is equivocal (4).

If muscle glycogen recovery is compromised, it may reduce an athlete's ability to train at the desired intensity (39, 89). Several factors have been shown to influence the rate of glycogen resynthesis following depletion. These include the amount (48), timing (47), and carbohydrate (CHO) composition ingested post exercise (46, 52, 53, 77). Additionally, it has been shown that ambient and muscle temperature also may affect the rate of glycogen synthesis (66, 81, 82). It is unclear whether this is because of an effect of blood flow, or temperature regulation of metabolism. Despite this, it remains unknown how massage/compression may affect glycogen resynthesis.

There appears to be a lay acceptance that compression garments aid in post exercise recovery (4). Compression garments are considered to provide an external pressure gradient that is

promoted to reduce the space available for swelling, hemorrhage, or hematoma formation (68). To date, the evidence as to whether the recovery modality of compression is effective at enhancing recovery between exercise bouts remains to be fully elucidated (4). Additionally, the limited research that has been published has produced conflicting results regarding the potential benefits of wearing compression garments during recovery from exercise (8, 59). These conflicting results are evidenced by two similar studies conducted by Berry et al. where subjects either cycled (8) or ran (7) on an ergometer for 3 minutes at 110% VO_2 peak. These results showed that the subjects who ran had no difference in lactate concentration when compared to a control group, while the cyclists had lower lactate concentrations when compared to a control group. Although evidence may be conflicting, what remains apparent through anecdotal reports is that post exercise interventions such as compression garments and massage are commonly used among performance athletes in hopes of enhancing muscle recovery (29).

The purpose of this study was to evaluate the effect of a mechanical pneumatic compression/peristaltic pulsing applied by a commercially available portable compression device (PCD) on muscle glycogen recovery, blood glucose, insulin, lactate and heart rate after exercise and oral glucose feedings.

METHODS

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Body composition. Body composition was measured using hydrodensitometry. Briefly, an electronic strain-gauge scale (Exertech, Dreshbach, MN) measured underwater weight (33).

Under water weight was then used to calculate body density and using estimated residual lung volume and converted to body composition using the Siri equation (80).

VO₂ peak testing. A maximal exercise test was completed on a laboratory cycle ergometer (Velotron, RacerMate Inc. , Seattle WA) to determine peak oxygen uptake (VO_{2peak}) and maximal power output (W_{max}). Subjects completed a graded exercise protocol, starting at 95 watts and increasing by 35 watts every 3 minutes until volitional fatigue. During the test, expired gases were continuously collected and analyzed every 15 seconds in a mixing chamber using a calibrated metabolic cart (Parvomedics, Inc., Sandy, UT). Peak oxygen uptake was determined as the highest achieved oxygen uptake during the test while maximum power output was calculated as the last completed stage in watts plus the fraction of time that was completed in partial stages multiplied by 35 watts.

Experimental testing (Figure 1a and 1b)

Dietary recall. For 24 hours prior to the subject's first exercise/recovery trial, a detailed food log was maintained. For the second trial the subjects were asked to consume the same food and quantity of those foods as they did for the first trial. The subjects were also asked to refrain from physical activity in the 24 hour period prior to each trial.

Glycogen depletion ride. Participants were scheduled for two exercise trials which were separated by no less than one week. Before each exercise trial the subjects were asked to fast

for 12 hours prior to arrival. Upon arrival to the laboratory the subjects cycled in a thermo-neutral environment for a total of 90 minutes on the same Velotron ergometer. Each 90 minute cycling session included 10 minutes at the power output corresponding to 55% watt max. Thereafter a series of 10 intervals (including two minutes at 80% watt max followed by four minutes at 50% watt max) were completed. After the series of 10 intervals the subjects completed 12 minutes at 60% watt max followed by 10 minutes at 50% watt max. During the exercise trials subjects were allowed to consume water ad libitum, which was then standardized for the second trial.

Blood Lactate. Eight finger stick blood samples were taken during each trial to measure blood lactate concentration. The samples were collected starting after the completion of the last (10th) interval during the depletion ride. Additional samples were collected at 10 and 20 minutes after completion of the last interval. Sampling was continued after the collection of the post-exercise muscle biopsy at 0, 5, 10, 15, 25 minutes into the recovery period. Prior to collection, the site was cleaned with alcohol before obtaining the blood sample. Once the 25 μ l blood sample was obtained it was placed in 50 μ l of a cell lysing agent and frozen to -30 °C until subsequent analysis using a YSI 1500 (Yellow Spring Instruments, Inc., Yellow Springs, OH).

Muscle Biopsies. Muscle biopsies of the vastus lateralis muscle were taken immediately and 4 hours after the exercise bout using the percutaneous biopsy needle technique with the aid of suction (26). The 4-hour post exercise biopsies were taken from a site approximately 2 cm proximal to the previous biopsy location. Biopsies for the second trial were taken from the

opposite leg from the first trial. Excess blood, fat, and connective tissue were immediately removed, and tissue samples were frozen in liquid nitrogen and stored in a freezer at -80°C for the later analysis of muscle glycogen.

Blood sampling. Blood samples to examine plasma glucose and insulin concentration were drawn immediately after exercise, then at 30, 60, 120, 150, 180, and 240 minutes after exercise. Samples were obtained from the antecubital vein using a venopuncture technique using heparin as an anticoagulant. Samples were spun at 4000 rpm for 15 minutes in a refrigerated centrifuge (4°C). The subsequent plasma was aliquoted into multiple micro-centrifuge tubes and stored at -30°C for later analysis of glucose and insulin concentrations.

Post Ride Recovery. Following each post-ride biopsy the subjects recovered in a thermo-neutral environment ($\sim 20^{\circ}\text{C}$) for four hours. During this time the subjects remained lying down but were permitted to read a book, watch TV, listen to music, etc. , but were not allowed to exercise. The experimental condition utilized a noninvasive pneumatic compression device (PCD) (NormaTec pneumatic compression device, Newton Center, MA) for two 1-hour treatments starting at the beginning (0-60 minutes) of the recovery period and again 2 hours into the recovery period (120-180 minutes). The PCD contains 5 air filled chambers that create pressure by filling and emptying that according to the manufacturer create pressures ranging from 30-90mmHg. During each hour long treatment the noninvasive PCD cycled through each of the five chambers from the ankles, at chamber 1, to the upper quadriceps, at chamber 5. Each of the five chambers was set to inflate and pulse at 70 mmHg for 30 second intervals

before moving to the next successive chamber. After the inflation of chambers 1, 2, and 3 the pressure from chamber 1 was released as chamber 4 began inflation. After completion of the 30 second pulse time on chamber 4 the pressure in chamber 2 was released. Meanwhile chamber 5 begins pulsing at the same time that pressure in chamber 3 was released. Upon completion of the pulsing in the 5th and final chamber, all of the remaining chambers depressurized for 30 seconds before repeating the next cycle. These cycles repeated until one hour was reached. For the passive recovery condition (PR) the subjects were outfitted with the pneumatic compression device but were not operational, therefore chamber pressures did not change. (Image 1)

Carbohydrate feeding. Subjects received two oral carbohydrate feedings that consisted of 1.8 g·kg⁻¹ BW of an oral dextrose solution (Azer Scientific glucose tolerance test beverage, 100g: 296 ml⁻¹, Azer Scientific, Morgantown, PA). The first feeding was provided immediately after the initial muscle and blood sample was obtained post-ride and the second after the 120-minute sample collections. The same beverage schedule was applied to both trials.

Heart rate. Heart rate monitors were worn by all subjects throughout each trial. The monitors (Polar RS800CX, Polar Electro, Kempele, Finland) collected the subjects' heart rate during each minute and the data was downloaded to a computer using the supplied software (Polar ProTrainer 5.0, Polar Electro, Kempele, Finland). This data was then analyzed during the entire recovery period and averaged for 0-60 minutes and 120-180 minutes for each trial.

Blood and Tissue Analysis. Muscle samples were analyzed in triplicate to determine muscle glycogen concentrations using an enzymatic spectrophotometric method (77). Samples were weighed and placed in 0.5 ml of a 2 N HCL solution. The sample solutions were weighed, incubated in an oven for two hours at 100 °C, and re-weighed and re-constituted to their original weight using distilled water. To normalize pH, 1.5 ml of 0.67 M NaOH was added. A volume of this muscle extract (100 µl) was added to 1 ml of infinity glucose reagent (ThermoTrace Ltd., Middletown, VA) and read on a spectrophotometer at 340 nm. Muscle glycogen concentration was calculated using the extinction coefficient of NADH and expressed in $\text{mmol}\cdot\text{kg}^{-1}\text{ wet wt}\cdot\text{hr}^{-1}$ of muscle.

Blood samples were analyzed for glucose in triplicate using infinity glucose reagent (ThermoTrace Ltd., Middletown, VA) and read on a spectrophotometer at 340 nm. Blood glucose concentrations were calculated using the extinction coefficient of NADH and expressed in $\text{mmol}\cdot\text{L}^{-1}$. Samples were analyzed for insulin in triplicate using an enzymatic spectrophotometric ELISA method (EIA-2935, DRG International, Marburg Germany) and expressed in $\mu\text{IU}\cdot\text{mL}^{-1}$. Total area under the curve (AUC) was calculated for blood glucose and insulin concentrations using the trapezoidal method.

Statistical Analysis

A two-tailed paired t-test was used to compare insulin and glucose AUC and rate of glycogen resynthesis, using Excel software (Microsoft Corp., Redmond, WA). Muscle glycogen, blood glucose, insulin, lactate, and heart rate were analyzed using a two-way repeated measure

ANOVA (trial x time) with SPSS software (SPSS Inc., Chicago, IL). A probability of type 1 error less than 5% was considered significant ($p < 0.05$). All descriptive data is reported as mean \pm SD and experimental data is reported as mean \pm SEM.

RESULTS

Muscle Glycogen. Post-exercise muscle glycogen concentrations were similar for both the pneumatic compression device condition (PCD) and for the passive recovery condition (PR). The main effect for time was significant ($p < 0.05$) indicating an increase in muscle glycogen after 4-hours of recovery. The rate of glycogen resynthesis was similar between both conditions 6.9 ± 0.8 vs. 6.9 ± 0.5 $\text{mmol}\cdot\text{kg}^{-1}$ wet wt. $\cdot\text{hr}^{-1}$ for the PCD and PR trials, respectively. However, there were no differences between treatments. (Figure 3)

Plasma Glucose. Plasma glucose concentrations were not different between PCD and PR trials at all time points. The main effect for time was significant ($p < 0.05$) demonstrating that the blood glucose concentration was elevated above post-exercise (time 0) for 150 minutes following the initial carbohydrate feeding, and dropped below baseline at the end of the recovery period (240 min). (Figure 4). Glucose AUC was also similar between trials ($p > 0.05$). (Figure 2a)

Plasma Insulin. Plasma insulin concentrations were not different between PCD and PR trials at all time points. The main effect for time was significant ($p < 0.05$) demonstrating that the blood insulin concentration was elevated above post-exercise (time 0) baseline at all time points

during the recovery period following carbohydrate feeding. (Figure 5). Insulin AUC was also similar between trials ($p>0.05$). (Figure 2b)

Lactate. Blood lactate concentrations were not different between PCD and PR trials at all time points. The main effect for time was significant ($p<0.05$) demonstrating that the blood lactate concentration was decreased below the post (10th) interval blood lactate concentration (time 0) at all time points during the recovery period. (Figure 6)

Heart rate. Average heart rates were similar between PCD and PR trials during 0 to 60 minutes during the recovery period (73 ± 3 vs. 76 ± 3 BPM, respectively) and 120 to 180 minutes (69 ± 3 vs. 69 ± 3 BPM respectively). However, the average heart rate was significantly lower during the 120-180 min measurement period

DISCUSSION

The aim of this study was to determine the effects of a commercially available pneumatic compression device (PCD) on glycogen resynthesis after a glycogen depleting bout of cycling exercise. The primary findings indicated that the two 60 min pneumatic compression sessions did not alter rates of glycogen resynthesis compared to passive recovery over a 4 hour recovery period.

The feeding protocol used in the current study adheres to previous recommendations on carbohydrate feeding strategies (47, 48, 70) to enhance short-term glycogen synthesis (≤ 4 hr).

Carbohydrate feedings were given immediately after exercise which has been shown to result in rates of glycogen synthesis 45% greater than if delayed, while the addition of a 2 hour feeding has been demonstrated to further increase muscle glycogen synthesis (47). The recovery protocol in the present study was further optimized by using carbohydrate feedings of $1.8 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ every two hours, which is in accordance with the recommendation for optimal glycogen synthesis of at least $1.5 \text{ g} \cdot \text{kg}^{-1} \text{ BW}$ every two hours (48).

While there is a lack of data regarding the impact of compression or manual massage on glycogen resynthesis, our results parallel those found by Crane et al. who found that massage has no effect on muscle glycogen resynthesis after cycling to exhaustion (23). Muscle glycogen synthesis is most likely influenced by a combination of carbohydrate intake, intestinal glucose absorption, glucose delivery via the blood stream, glucose extraction by other tissues, and the muscles glucose-transport capacity (51), and more recently temperature of the ambient (66) and muscle environments (81). Because recovery modalities typically target blood flow, they may theoretically alter glucose availability to muscle during recovery and therefore alter the process of glycogen synthesis (4). However, it is unclear how massage and/or pneumatic compression alter blood flow to muscle during recovery. If indeed muscle blood flow is altered by PCD, then extraction of glucose from the blood may be changed, in which case glycogen replenishment, and blood glucose and insulin may be affected. In the current study the significant increase in plasma blood glucose and insulin ($p < 0.05$) post exercise shows that our feeding strategy was effective in presenting substantial glucose to the muscle for metabolism. Furthermore the rate of muscle glycogen resynthesis was similar to those found in other

investigations (47, 51, 66, 77, 81) at 6.9 ± 0.8 vs. 6.9 ± 0.5 $\text{mmol}\cdot\text{kg}^{-1}$ wet wt. $\cdot\text{hr}^{-1}$ for the PCD and PR trials, respectively. Hemmings et al. also showed no difference in blood glucose following a 20 minute massage treatment after twenty minutes of boxing when compared to a passive recovery control group (38). Therefore, it is unlikely that the glucose bolus feeding strategy was limiting to glycogen synthesis post exercise.

Although some earlier studies seemed to support the effects of massage on performance enhancements (reviewed by Goats (32) and Weerapong et al. (92)), recent investigations of arterial inflow measured with Doppler ultrasound velocimetry during rest found that massage had no effect on blood flow (79, 88). Hinds et al. also found no effect of massage on blood flow immediately after leg exercise (41). Furthermore, Wiltshire et al. noted a decrease in blood flow during massage of the forearm (98). In the current study no changes in HR and blood lactate glucose or insulin were noted ($p < 0.05$) which would indicate that the PCD had little to no effect on blood flow. However, blood flow was not directly measured in our study. Similarly, other studies have also noted no changes in lactate concentrations post massage (38, 62). Collectively this suggests that compression/massage has any effect on muscle blood flow post exercise.

A potential limitation of commercially manufactured lycra and nylon compression tights is whether or not they can exert enough pressure to be effective (24). Further, it is doubtful that standard sizes of compression tights would be effective given the widespread differences in leg dimensions and tissue structure within a given population (24). However with the use of

pneumatic compression devices, leg girth does not affect the amount of compressive forces experienced by the user. Additionally, the pressure applied using a pneumatic compression system is much greater than that of lycra and nylon compression tights. This is evident in other studies done on healthy subjects where the compression using various lycra and nylon garments ranged from 18-22mm Hg (2, 8). In contrast the PCD used in this study were set to apply intermittent compression equivalent to 70mmHg. The effect of this increased pressure in relation to recovery in healthy subjects still remains unknown.

Anecdotal information from coaches, athletic trainers, and athletes suggest that various types of recovery modalities including the use of massage (14, 60, 92, 95) and compression garments (95) are commonly used in an effort to restore homeostatic environments within the body. However, based upon our findings in addition to other recent studies, there appears to be limited evidence for the use of compression/massage as an effective modality to increase rates of muscle glycogen synthesis. However, recent evidence has shown that massage attenuates inflammatory signaling after exercise induced muscle damage (23). Therefore, further research in this area is needed to elicit the effects of compression on inflammation and how this influences the recovery process of the muscle. It is also unclear how compression may alter aspects of muscle recovery after trauma or eccentric damage has been incurred.

PRACTICAL APPLICATIONS

This is the first study that compares recovery outcomes after the use of a non-invasive pneumatic compression device (PCD) to a non compression passive recovery (PR) control group

in healthy moderately trained male cyclists. Wearing PCD had no effect on glycogen resynthesis rates or blood lactate post exercise. Our results suggest that this may be due to similar responses in blood glucose and insulin levels after feedings in the PR and PCD groups. Therefore, using a PCD to increase muscle glycogen post exercise in an effort to increase energetic recovery does not appear effective. However, our findings do not mean that other mechanisms of recovery such as inflammation reduction, DOMS, muscle damage. Etc. may or may not be affected by the use of a PCD.

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TABLES

	Mean \pm SD
Age (years)	28.1 \pm 7.3
Height (cm)	181.6 \pm 4.0
Weight (kg)	77.4 \pm 9.1
Body composition (% fat)	15.3 \pm 8.2
VO_{2peak} (L·min⁻¹)	4.5 \pm .4
W_{max} (watts)	328.7 \pm 34.4

Table 1. Descriptive data of subjects (n=10)

IMAGES

Image 1. NormaTec Pneumatic Compression Device

FIGURES

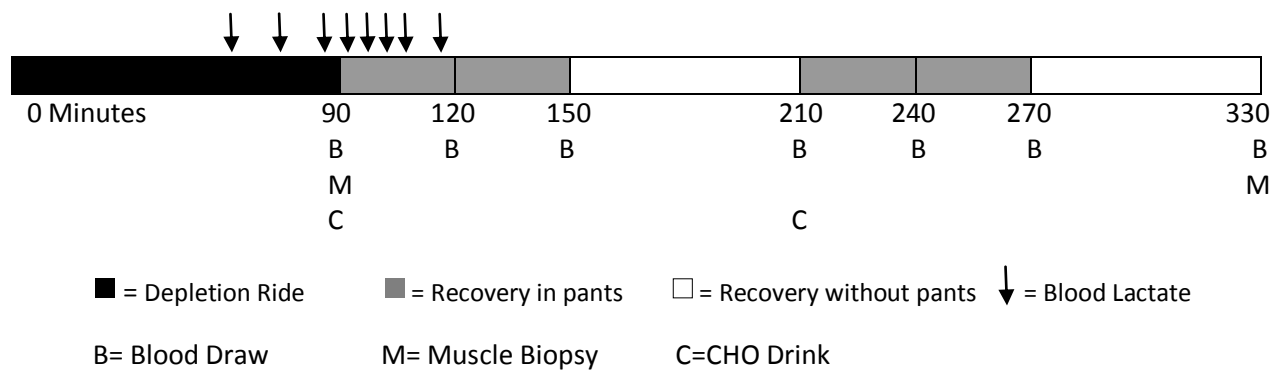


Figure 1a. Pneumatic Compression Device Trial Timeline

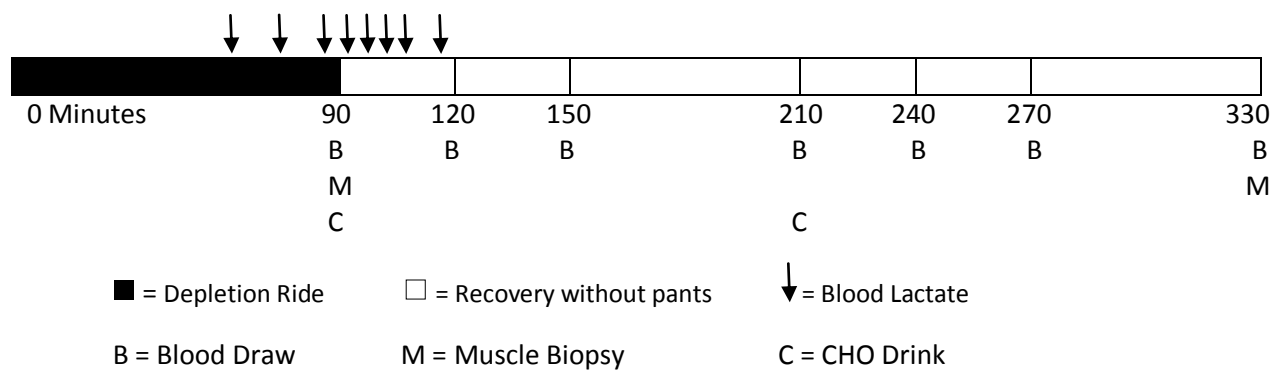


Figure 1b. Passive Recovery Trial Timeline

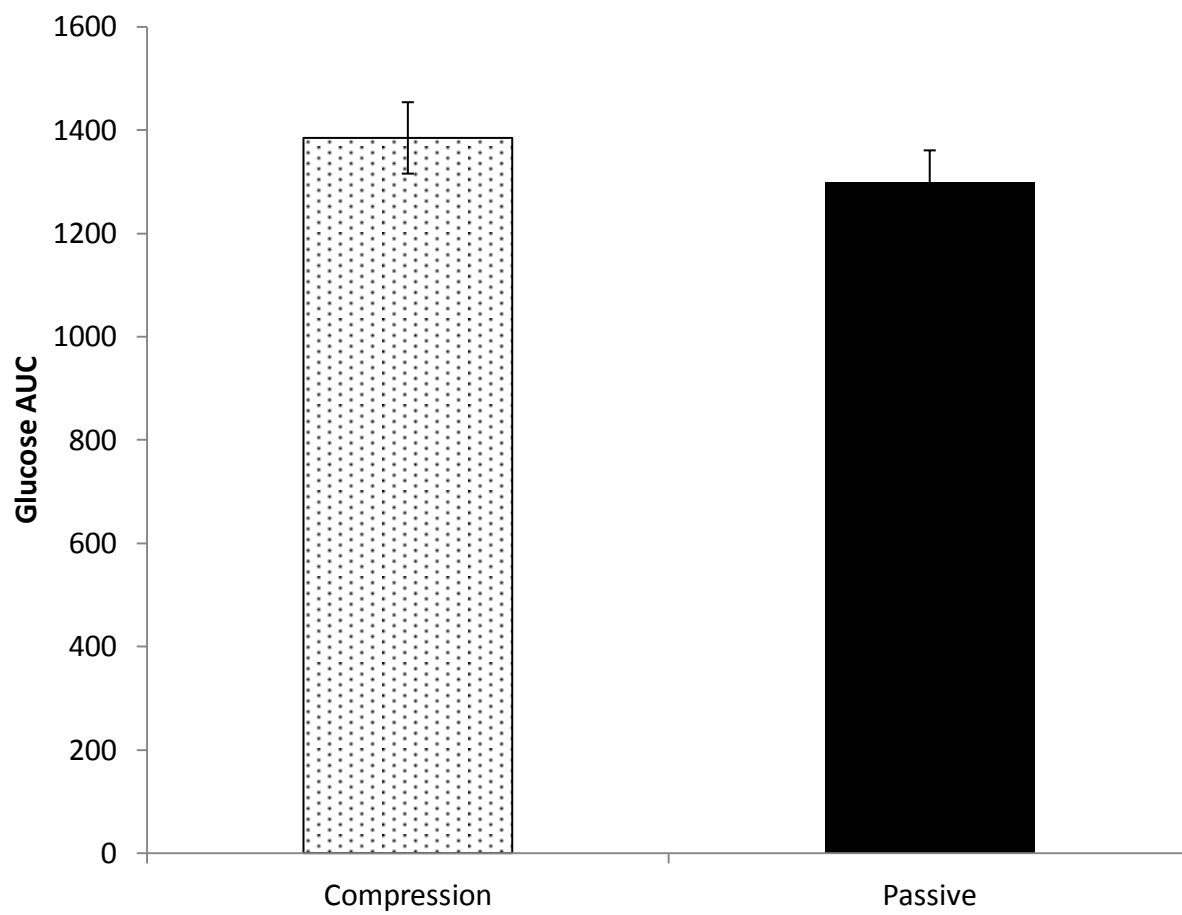


Figure 2a. Total AUC for blood glucose.

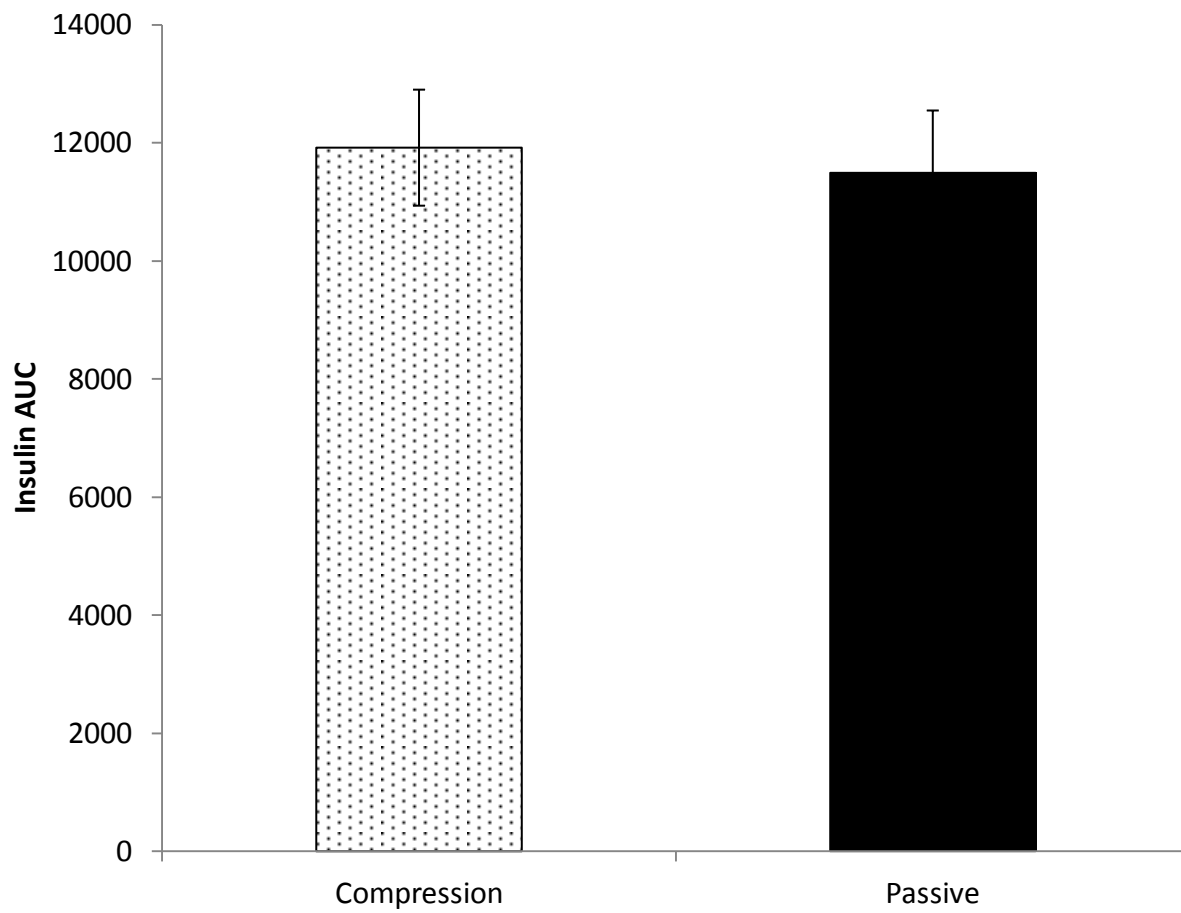


Figure 2b. Total AUC for blood insulin.

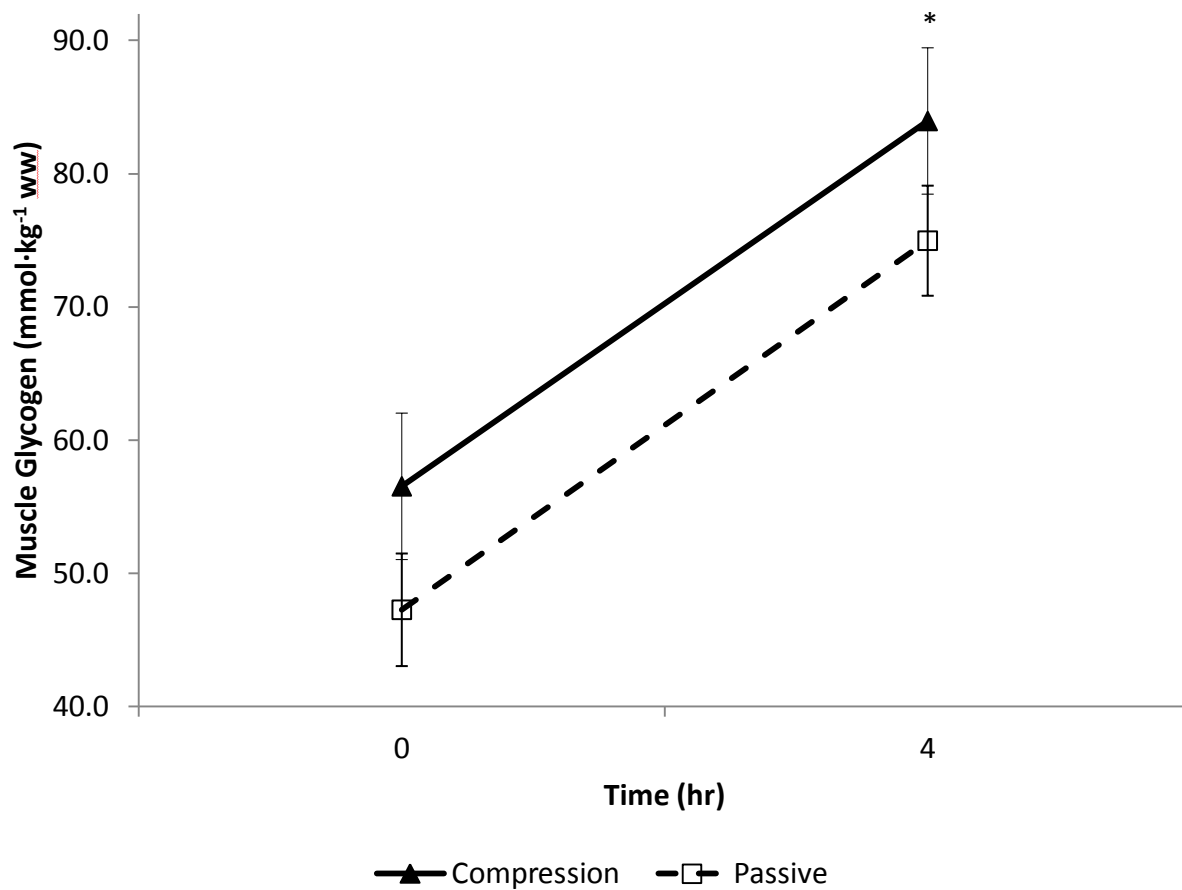


Figure 3. Changes in muscle glycogen concentration during the four hour post exercise period.
* $p < 0.05$ vs. time point 0 (main effect of time).

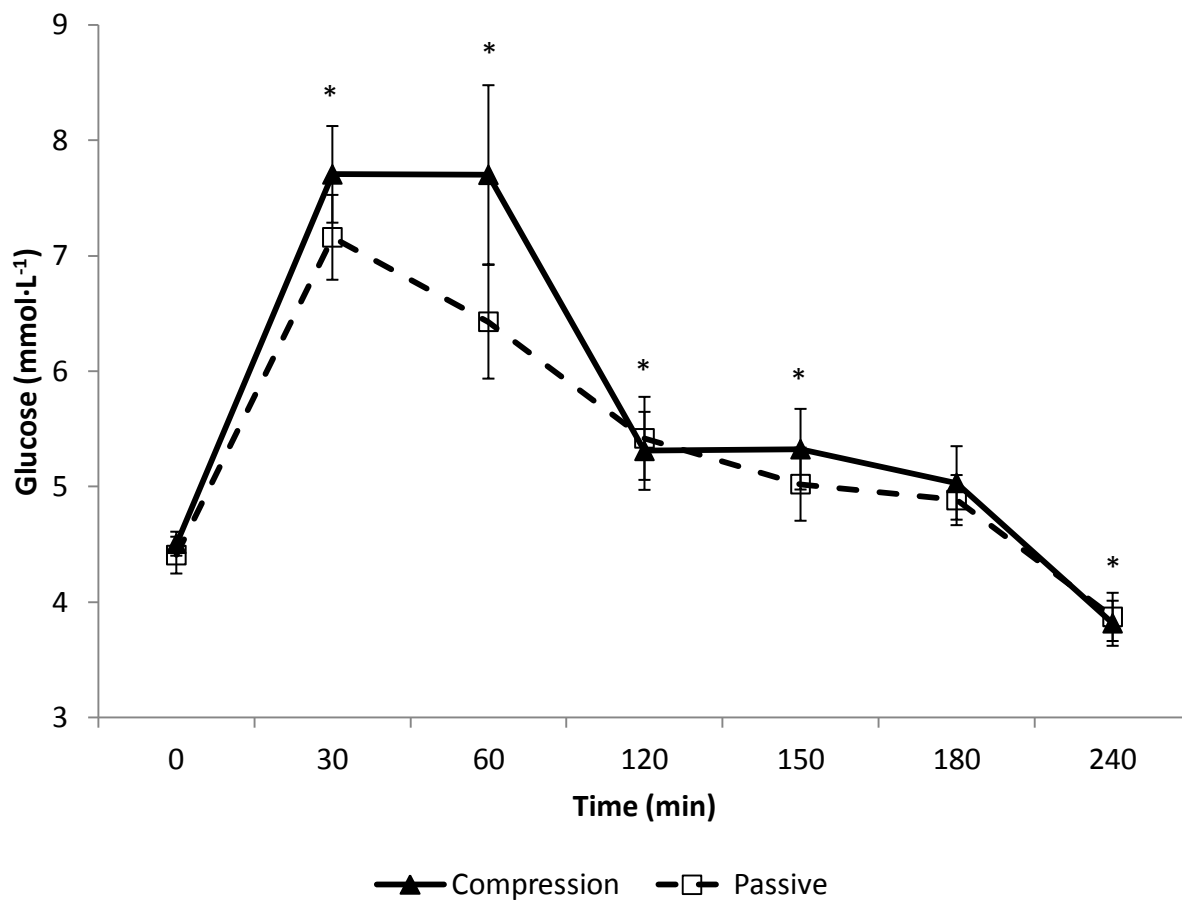


Figure 4. Changes in blood glucose concentration during the four hour post exercise period. * p<0.05 vs. time point 0 (main effect of time).

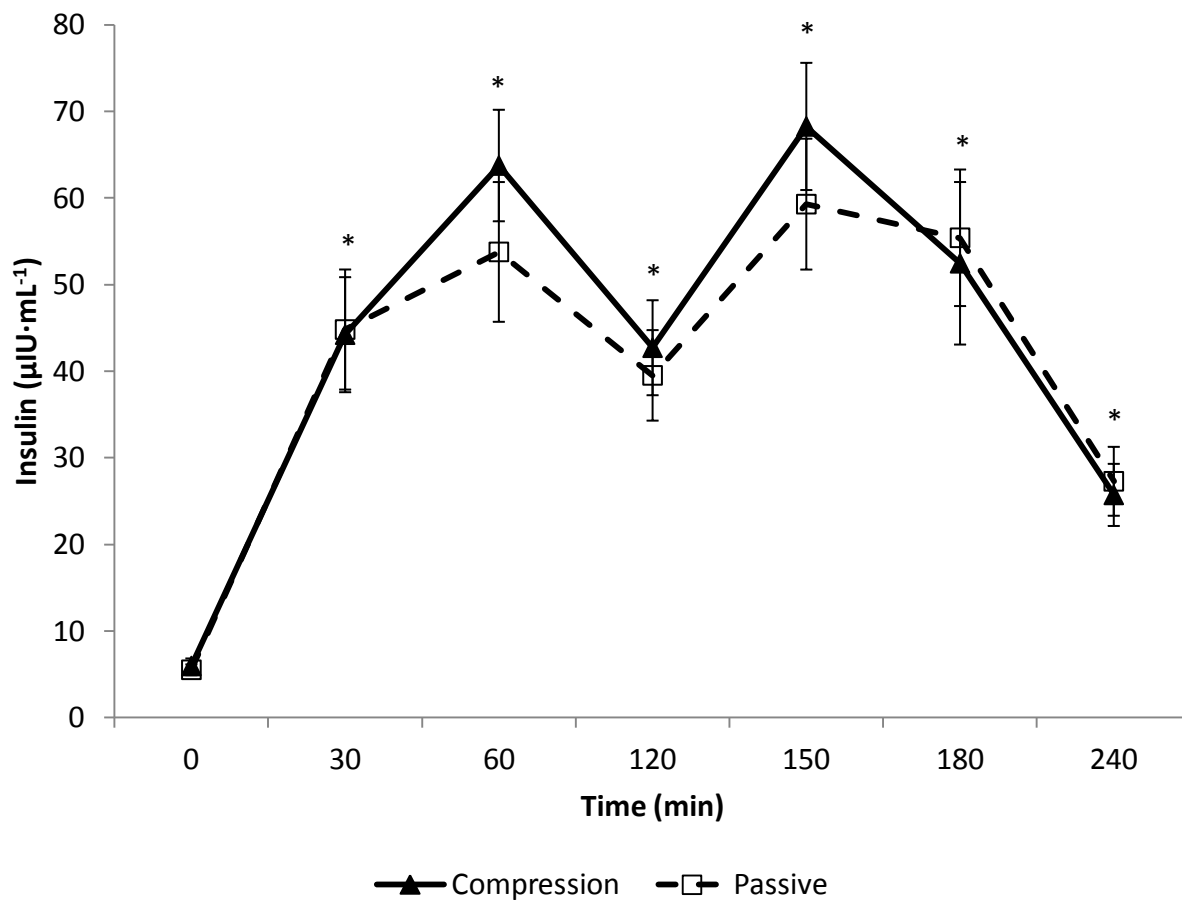


Figure 5. Changes in blood insulin concentration during the four hour post exercise period. * $p < 0.05$ vs. time point 0 (main effect of time).

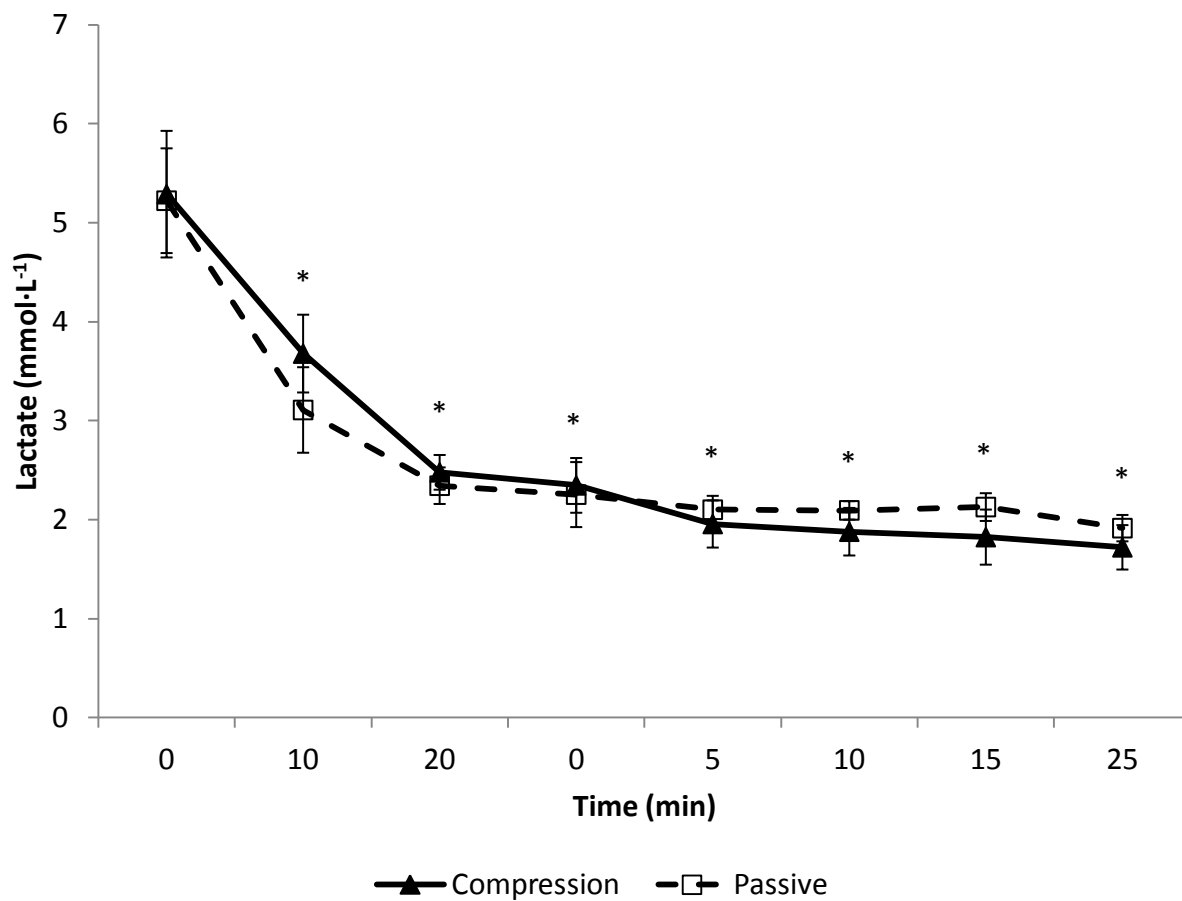


Figure 6. Changes in blood lactate concentration post exercise. * $p < 0.05$ vs. time point 0 (main effect of time).

Appendix A



Montana Center for Work Physiology and Exercise Metabolism

SUBJECT INFORMATION AND CONSENT FORM

PROJECT IN BRIEF: Effect of lower limb compression (NormaTec) post-exercise on glycogen resynthesis and glucose kinetics.

RESEARCHERS: Dr. Brent Ruby (406) 243-2117
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(406) 243 – 2117 (Dr. Brent Ruby)

Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.

REQUIREMENTS

This research study requires that you meet the following criteria:

- Participants must be males between the ages of 18 and 40.

PURPOSE OF THE STUDY

The purpose of this research is to determine the effect of noninvasive pneumatic lower limb compression post-exercise on muscle glycogen (muscle sugar) after acute exercise in a laboratory setting. The effect of using pneumatic compression post exercise on glycogen resynthesis remains unknown.

TEST PROCEDURES

Participants in the study will be asked to complete the following assessments:

1. A pre-screening assessment which involves a health/exercise questionnaire (Par-Q)
 - a. Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease. If any of these questions on the PAR-Q are answered with a "yes" the you will be eliminated from the study
2. A measure of percent body fat obtained using underwater weighing
 - a. This test session will require that you do not eat for a minimum of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighing tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. A nose clip will be provided upon request. This test will take approximately 30 minutes.
3. A maximal cycle ergometer test to measure aerobic fitness
 - a. This test will consist of riding on a laboratory exercise cycle ergometer to volitional fatigue. The resistance of the cycle will increase every three minutes and will progress to fatigue. You will be encouraged to continue to ride until volitional fatigue. During this test you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 30 - 45 minutes. You will be asked to fast for approximately 3 hours prior to this test.
4. Two, one-hour exercise sessions followed by four hours of recovery in a thermo-neutral environment
 - a. You will report to the laboratory after a 12-hour fast. You will then exercise (cycle) in a thermo-neutral environment for 10-minute warm up at 55% peak VO₂. Thereafter, you will complete a series of ten intervals, which include two minutes at approximately 80% peak VO₂ followed by four minutes at approximately 50% peak VO₂. After the series of 10 intervals, you will complete 12 minutes at 60% peak VO₂ followed by 10 minutes at 50% peak VO₂. During the exercise trials, you will be allowed to consume water ad libitum. During both trials you will recover in a thermo-neutral environment (approximately 68°F) for four hours. You are permitted to read a book, watch TV, listen to music, etc., but cannot exercise. You must remain in a lying down or sitting up position.
 - b. During this time you will either use a noninvasive pneumatic compression device for a total of two (2) hours or recover in the thermo-neutral environment while wearing the noninvasive pneumatic compression device in an inactivated state. During both trials you must wear shorts and a t-shirt. You are free to terminate the exercise at any time if you feel fatigued. Before the first trial, you will be randomly selected to either use the noninvasive pneumatic compression device or use the noninvasive pneumatic compression device in an inactivated state. Then, during the second trial, you will recover with the opposite treatment of what you received during the first trial.

5. Consumption of liquid carbohydrate after exercise
 - a. Immediately following and two-hours after exercise, you will be provided a standardized high-carbohydrate drink. You will be asked to consume this drink as quickly as possible without upsetting your stomach. The amount of carbohydrate consumed will be 1.8 g/kg.
6. Venous blood samples collected from an arm vein for measuring blood glucose and insulin
 - a. A total of fourteen blood samples (Seven per trial) will be collected using a venopuncture technique. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. These samples will be collected to measure blood glucose and insulin. All of the blood samples will be obtained under the direction of Dr. Brent Ruby or Dr. Charles Dumke. Blood samples will be taken after exercise, and then at intervals of 30, 60, 120, 150, 180, and 240 min after exercise. ~3 ml will be drawn each time for a total of ~21 ml per trial or 42 ml total.
7. Finger stick blood draws to measure blood lactate
 - a. A total of sixteen finger stick blood draws (eight per trial) will be collected using a sterile lancet. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. These samples will be collected to measure blood lactate. All of the blood samples will be obtained under the direction of Dr. Brent Ruby or Dr. Charles Dumke.
8. Muscle biopsies obtained immediately after ride, and four hours after ride
 - a. A total of four (two per trial x two trials) muscle biopsies (two from each leg) will be obtained from the front of your thigh muscle (vastus lateralis, approximately 6 inches up from the kneecap on the lateral side of your thigh). The muscle biopsy procedure requires that the site be sterilized. After the site is cleaned, a small amount of lidocaine will be injected just under the skin surface. Additional small amounts of lidocaine will be injected around a small 1-inch area around the site on the leg. After the area is treated with the lidocaine (approximately 5 ml, 1% lidocaine), a small incision (approximately 1/4 inch long) will be made through the skin and the outer covering (fascia) of your muscle to a depth of approximately 3/4-1.5 inches. The biopsy needle will then be inserted through the incision and the sample obtained. After the sample is obtained, the site will be cleaned and closed with steri-strips and/or a single stitch and bandaid and wrapped with a compression bandage. The biopsy samples will be obtained a) immediately after the exercise session, and b) four hours after the exercise session (biopsies for each trial will be taken on the same leg, proximal to the initial or previous sample). This will be repeated for the second trial using the opposite leg. The muscle biopsies will be used to evaluate alterations in muscle carbohydrate and fat utilization kinetics in response to physical activity. Latex free bandages will be provided if subjects have a known allergy to latex. All of the muscle biopsies will be conducted by Dr. Brent Ruby or Dr. Charles Dumke.
9. A 24-hour dietary recall to be repeated during the subsequent trial
 - a. For 24-hours before your first exercise trial you will be asked to record the foods and quantity that you consume. For the second trial, you will be asked to consume the same foods and quantity of those foods that you did for the first trial.
10. A 24-hour exercise recall to be repeated during the subsequent trial

- a. For 24-hours before your first exercise trial you will be asked to record all physical activity and quantity that of that activity you do. For the second trial, you will be asked to repeat the same activity and duration of activity that you did for the first trial.
11. Nude body weight will be taken before, after, and during each trial
 - a. Nude body weight will be measured in private on a calibrated scale. Weights will be taken before, during, and after each trial.
 12. Urine volume will be measured during each trial.
 - a. You will be asked to void your bladder before each trial. After the initial void, urine will be collected in a disposable plastic container and urine volume will be measured for the duration of each trial.

RISKS AND DISCOMFORTS

1. Mild discomfort may result during and after the exercise. These discomforts include shortness of breath, tired and/or sore legs, nausea and possibility of vomiting.
2. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.
3. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. The laboratory has standard emergency procedures should any potential problems arise.
4. Mild symptoms of dehydration such as headache and general fatigue may result during and after the exercise.
5. You will be informed of any new findings that may affect your decision to remain in the study.
6. The muscle biopsy and blood sampling techniques may cause some local and temporary discomfort. It is normal to have the sensation of a deep tissue bruise around the site of the muscle biopsy. This pain should be manageable and not above the pain associated with a "charlie horse" type bruise.
7. There is a minor risk of infection associated with blood sampling and the muscle biopsy. Should you notice unusual redness, swelling or drainage at the biopsy incision site or at the sites of the blood sampling you should seek medical attention and then notify Brent Ruby, study director.
8. There are minimal risks associated with the use of lidocaine (the local anesthetic). The risk of a reaction to the lidocaine is extremely low (approximately 1/1,000,000). However, to minimize this risk, no more than 5-9 ml of a 1% lidocaine solution will be used per biopsy. You will be excluded from participation if you have a known history of allergic reactions to local anesthetics.
9. During any of the exercise tests, should symptoms such as chest discomfort, unusual shortness of breath or other abnormal findings develop the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.

PAYMENT FOR PARTICIPATION

For the preliminary tests (body fat, and cycle ergometer max test), you will be paid \$50. You will be paid \$125 for each exercise/recovery session. Therefore, upon completion of the entire study, you will be paid a total of \$300. If you decide to withdraw at any time, you will be compensated for the test sessions you have initiated.

BENEFITS OF PARTICIPATION

1. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport, but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.
2. There is no promise that you will receive any benefit outside of the financial payment as a result of taking part in this study.
3. The scientific benefit includes elucidating the effects of recovery while using noninvasive pneumatic compression on muscle glycogen resynthesis.

CONFIDENTIALITY

1. Your records will be kept private and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept confidential.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an anonymous ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data.

COMPENSATION FOR INJURY

Although we believe that the risk of taking part in this study is minimal, the following liability statement is required in all University of Montana consent forms. *In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by negligence of the University or any of its employees, you may be entitled to reimbursement pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University's Claim representative or University Legal Counsel.*

VOLUNTARY PARTICIPATION AND WITHDRAWAL

It is important that you realize that you are free to withdraw from the study at any time. As mentioned above, even if you decide to drop out of the study, you will receive full compensation for all the test sessions you have initiated. A signed copy of this consent form will be provided for you. In addition, the data collected during this study will be done at no cost to you.

QUESTIONS

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Brent C. Ruby at (406) 243-2117 (office) or (406) 396-4382. If you have any questions regarding your rights as a subject, you may contact the chair of the IRB through the University of Montana Research Office at (406) 243-6670.

STATEMENT OF CONSENT

I have read the above statements and understand the risks involved with this study. I authorize Brent C. Ruby and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Brent C. Ruby at home (406) 542-2513, cell (406) 396-4382 or at the WPEM Laboratory (406) 243-2117.

Participant (print)

Signature

Date

STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names will not be associated with them.

Signature

Date
