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# Plasma Lactate Accumulation During Running with Body Weight Unloading by LBPP

Nicole Nevitt Rasmussen
*Brigham Young University - Provo*

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Nicole Nevitt Rasmussen

A thesis submitted to the faculty of
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Master of Science

Gary W. Mack, Chair
Allen Parcell
Iain Hunter

Department of Exercise Sciences

Brigham Young University

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# ABSTRACT

Plasma Lactate Accumulation During Running with Body Weight Unloading by LBPP



Nicole Nevitt Rasmussen
Department of Exercise Sciences, BYU
Master of Science



At any given running speed, weight support with a lower body positive pressure (LBPP) device (i.e. Alter-G treadmill) reduces $\dot{V}O_2$. However, it is unknown how application of LBPP during running impacts lactate metabolism, specifically lactate threshold. Purpose: To determine if body weight unloading with the Alter-G treadmill alters lactate threshold. Methods. Maximal aerobic capacity ($\dot{V}O_2$max) and lactate threshold (LT) was determined in 8 male subjects on an Alter-G treadmill at 100% and 80% body weight loading at 0% grade in a randomized crossover design. $\dot{V}O_2$max tests started at 7 mile $h^{-1}$ and increase speed by 1 mile $h^{-1}$ every 2 min till voluntary exhaustion and were separated by a minimum of 7 days. LT tests started at 5 mile $h^{-1}$ and increased speed to 6, 7, 7.5, 8.0, 8.5, 9.0 (additional stages increase speed by 0.5 mile $h^{-1}$) every 3 min until the subject reached ≈85% of $\dot{V}O_2$max. LT tests were separated by a minimum 3 days. $\dot{V}O_2$, heart rate (HR), mean arterial blood pressure (MAP) and changes in Hct, [Hb], and total protein ([TP]) were determined on separate days in a randomized crossover design. Plasma lactate concentrations were determined from venous blood samples (4 ml) obtained at rest and during the last minute of each exercise stage. Lactate threshold was determined from a log-log plot of lactate concentration (mM) and relative $\dot{V}O_2$ (ml $O_2$ $min^{-1}$ $kg^{-1}$ BM). Results. $\dot{V}O_2$max determined during running at 100% and 80% loading were similar (52.3 ± 0.9 and 52.7 ± 0.7 ml $O_2$ $min^{-1}$ $kg^{-1}$ BM, respectively). The energy cost of running at 9 mile $h^{-1}$ (all subjects completed stages between 5 and 9 mile $h^{-1}$) was reduced by 12% at 80% body weight (37.2 ± 2.9 ml $O_2$ $min^{-1}$ $kg^{-1}$ BM) compared to running at 100% body weight (42.3 ± 1.7 ml $O_2$ $min^{-1}$ $kg^{-1}$ BM, $p<0.05$). However, plasma lactate at 9 mile $h^{-1}$ was similar during 80% and 100% body weight running (3.4 ± 0.4 and 3.1 ± 0.7 mM, respectively). Plasma lactate at a given $\dot{V}O_2$ was higher ($p < 0.05$) while running at 80% body weight compared to 100% body weight running. Calculated LT at 100% BW loading (36.3 ± 1.3 ml $O_2$ $min^{-1}$ $kg^{-1}$ BM) was higher than 80% BW loading (32.2 ± 1.8 ml $O_2$ $min^{-1}$ $kg^{-1}$ BM, $p<0.05$). During running at 80% BW HR was reduced compared to 100% BW running ($p<0.05$) however the MAP response was similar. During exercise the reduction in PV, at any given $\dot{V}O_2$ was larger at 80% BW compared to 100% BW running ($p<0.001$). Conclusion. During running, BW unloading with LBPP decreased the energy cost of exercise but not lactate levels. Body weight unloading caused a lowering of the LT. The reduction in whole body energy cost was not associated with a reduction in the lactate production since plasma lactate accumulation at a given speed was similar with and without LBPP.



Keywords: alter gravity treadmill, lower body positive pressure, metabolism, plasma lactate, lactate threshold, $\dot{V}O_2$max, heart rate, plasma volume

# ACKNOWLEDGMENTS

Several individuals contributed valuable assistance in the preparation and completion of this study. First I would like to thank my thesis committee for their expertise and knowledge. Thank you to Dr. Gary Mack for guiding me through the entire process. Thank you to my wonderful parents for teaching me the importance of education and how to work hard. Thanks also to Grandma Rasmussen for watching baby Tate for countless hours while I worked in the lab on data collection and analysis. Last but not least, I express my appreciation to my loving husband, Erik, for always encouraging and assisting me throughout my studies.

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## Introduction

Exercising on a lower body positive pressure (LBPP) treadmill is a relatively new exercise mode for rehabilitation of injured runners. This device allows unloading of the lower extremities during exercise in a pressurized treadmill chamber. This treadmill is capable of unweighing across a variety of body types, and is able to reduce ground reaction force for walking and running in proportion to the amount of unweighing (20). The presumed properties associated with LBPP treadmills, such as decreased ground reaction forces while maintaining metabolic demand, make it an appealing exercise medium for rehabilitation of individuals with lower extremity injuries associated with running (11, 20).

Methods other than LBPP have been used previously to support body weight (BW) during running, including harness suspension systems and water immersion. Harness suspension systems are beneficial because a purely vertical force can be applied to the person, and the independent effects of supporting BW can be addressed (19, 21). However, harness suspension systems may not be applicable for extended rehabilitation and training use because they can cause discomfort and may impede circulation. Water immersion is also commonly used as a rehabilitation tool (11). However, the viscous drag forces associated with water exercise act in opposition to movement and cause significant changes in movement velocity, gait timing, joint kinematics, joint kinetics, and muscle activity compared with over ground movement. (9, 30)

LBPP devices do not apply meaningful drag forces to the legs and may be advantageous because they allow kinematic gait patterns similar to normal weight over-ground running, a normative range of running velocities, and a decrease in the forces transmitted by the legs (11). Surface EMG activity while running on a LBPP treadmill shows that muscle firing patterns and gait mechanics are maintained for all levels of weight support and speeds (25). For any given

running speed, weight support with the LBPP device reduces $\dot{V}O_2$ (20). Running at faster velocities with weight support requires the same metabolic power as running at slower velocities with normal weight. Additionally, LBPP devices are comfortable and can be used over extended periods. Therefore, LBPP devices could be very effective for maintaining cardiovascular fitness during rehabilitation involving running. However, it is unknown if positive pressure alters muscle blood flow or muscle metabolism, specifically related to lactate accumulation or lactate threshold. Specifically, the impact of LBPP treadmill running on the lactate threshold is unknown. As such, we tested the hypothesis that lactate threshold would occur at a higher treadmill speed during body weight unloading treadmill running compared to full body weight treadmill running but that the lactate threshold expressed as a percentage of $\dot{V}O_2$max would be similar determined during treadmill running with body weight unloading and full body weight running.

**Methods**

Eight active college aged (18-33 years old) active males participated in this study. Subjects provided written informed consent for the study that was approved by the Brigham Young University Institutional Review Board. Maximal aerobic capacity ($\dot{V}O_2$max) and lactate threshold was determined for each subject while running at 100% body weight and 80% body weight on an Alter-G Pro Version 1.20 (Alter-G Inc., Fremont, CA) treadmill in a randomized cross-over design.

Each subjects $\dot{V}O_2$max was determined at 100% body weight and 80% body weight using graded exercise tests on the Alter-G treadmill with oxygen consumption monitored every 15 seconds using a Parvo Medics True One (Parvo Medics, Inc., Sandy, UT) metabolic cart. The

graded exercise test consisted of a 5 min warm-up at 0% grade during which time the subject ran at a self-selected speed that approximated a typical running velocity. The speed was then set to 7 mile $h^{-1}$ and speed was increased by increments of 1 mile $h^{-1}$ every 2 minutes until the point of volitional fatigue. The two tests were separated by a minimum of seven days.

Lactate thresholds were also determined on the Alter-G treadmill at 100% body weight and 80% body weight. For each lactate threshold test, an 18 gauge needle was placed in a large vein of the subjects forearm. The subject was fitted with a 3 lead EKG, blood pressure cuff, and a headpiece holding a one way breathing valve to allow measurement of heart rate, blood pressure, and oxygen uptake, respectively. The subject stood for 30 min before a resting blood sample (5 ml) was collected. The lactate threshold protocol was performed entirely at 0% grade on the treadmill. An seconds resting blood sample was collected 1 minute following application of LBPP to produce an 80% body weight condition. The first stage of the test protocol began at a treadmill speed of 5 mile $h^{-1}$. This speed was maintained for 3 min and a blood was drawn between min 2 and 3 of this (and every) stage. After each blood sample a new stage was initiated by an increase in speed of 0.5 mile $h^{-1}$. The test was terminated once the subject's oxygen consumption met or exceeded 90% of their measured $\dot{V}O_2$max. The two tests were separated by a minimum of 48 hours.

Blood samples were immediately placed in pre-cooled EDTA-vacutainers, mixed by gentle inversion, and stored on ice until the end of the LT trial. After the LT test the blood samples were mixed and a small amount of whole blood was used to determine hematocrit and hemoglobin concentration in triplicate. The remainder of the blood was centrifuged at 1500 x g for 15 min at 4°C. The plasma was immediately separated from the red cells and a small amount of plasma (40 µl) was used to determine plasma protein concentration using a handheld

refractometer in duplicate. If the plasma was not analyzed immediately for lactate it was stored frozen at -20°C until analysis. Plasma lactate was determined in duplicate using an YSI 2300 lactate analyzer.

Hematocrit and hemoglobin measurements were used to calculate fluid shifts out of the vascular space (the change in plasma volume) using the Dill-Costill equation (13). To account for the fluid shift out of the vascular system, plasma lactate concentration was normalized for changes in plasma water and expressed as mM kg $H_2O^{-1}$ plasma water. Plasma water was determined from a regression equation relating total plasma proteins to total solids (33).

Lactate threshold was determined as the abrupt transition in the rate of increase of blood lactate with increasing $\dot{V}O_2$. The transition point in plasma lactate accumulation was determined using a log-log transform plot (2). Error effects were minimized by using least squares curve-fitting procedures.

The experimental design to assess changes in lactate threshold was a simple two-way balanced ANOVA. The independent variable was full weight bearing (100% BW) or BW unloading (80% BW) and the dependent variable was oxygen uptake (absolute or relative to maximal aerobic capacity) at which time the lactate threshold occurred. A simple balanced ANOVA for repeated measures was also used to examine differences in the time course of responses of the following variables: oxygen uptake, cardiovascular function (heart rate, systolic, diastolic, and mean arterial blood pressure), and lactate concentration (mM and mM kg $H_2O^{-1}$ plasma water). We collected a complete set of data for these variables at rest, 5, 6, 7, 7.5, 8, 8.5, and 9 mile $h^{-1}$. All eight subjects were able to complete the 100% and 80% body weight LT tests. The statistical analysis was performed using SAS general linear models with the level of significance set at of $p < 0.05$.

**Results**

During $\dot{V}O_2$max testing subjects were able to perform longer and reached faster speeds when running at 80% body weight compared to 100% BW. However, $\dot{V}O_2$max was similar regardless of body weight loading (Table 1).

During the lactate threshold testing, the energy cost of running at any given speed between 5 and 9 mile $h^{-1}$ at 80% body weight was significantly lower ($p<0.05$) than full body weight running (Figure 1). The cardiovascular response to 100 and 80% BW treadmill exercise are listed in Table 2. Heart rate at rest and during running was significantly higher at any given speed at full body weight than during 80% BW running (Table 2). At any given oxygen uptake, HR while running at 80% BW was elevated compared to 100% BW running ($p<0.05$, Figure 2). In addition, the slope of the linear relationship between HR and oxygen uptake was steeper for 80% BW running compared to 100% BW running (Figure 2). Body weight unloading did not alter the blood pressure response to treadmill running (Table 2).

Table 3 shows the changes in hematocrit, hemoglobin, plasma protein, and plasma volume during 100 and 80% BW treadmill running. The reduction in plasma volume at any given speed was similar for 100 and 80% BW loading. However, during 80% BW treadmill running the reduction in plasma volume at any given oxygen uptake was greater than 100% BW running ($p<0.05$, Figure 3).

Table 1 lists individual lactate thresholds for all eight subjects. The lactate threshold determined while running at 80% body weight (61.5 ± 1.5% $\dot{V}O_2$max) was lower than that determined while running at full body weight. (69.7 ± 1.5 % $\dot{V}O_2$max). Figure 4 illustrates the leftward shift in lactate threshold while running at 80% body weight in a plot of the mean plasma lactate concentration as a function of oxygen uptake.

In an effort to account for fluid shifts during treadmill running at 100 and 80% body weight, plasma lactate concentrations were also expressed in mM per kg water. This normalization, however, had no impact on the leftward shift in lactate threshold during 80% BW running.

**Discussion**

In this study we measured cardiovascular and metabolic responses to treadmill running with and without mild LBPP. Our data support recent observations that the energy cost of exercise at any given speed is significantly reduced compared to 100% BW running. In addition, we demonstrated that it is possible to reach maximal aerobic capacity while running at 80% BW on the LBPP treadmill and that this maximal value is not different than that obtained during 100% BW running. In addition, there are several significant new findings from this study. First, plasma lactate concentrations were similar while running at 80% BW compared to 100% BW at all running speeds examined, despite of the reduction in energy requirement. As such, we observed a significant leftward shift, or decrease in the lactate threshold during 80% BW running. Another novel observation was that the fluid shift out of the vascular space during treadmill running was larger during 80% BW running than 100% BW running.

Our data supports previous studies that also showed that for any given running speed, weight support with the LBPP device reduced the metabolic demand by the individual (20). Running at faster velocities with weight support requires the same metabolic power as running at slower velocities with normal weight. Liebenberg et al (25) investigated how lower extremity muscles are influenced by body weight support through LBPP. They showed that reducing body weight through LBPP leads to a reduction in the amplitude of all muscle EMG activity, even though the timing of the muscle firing patterns and gait mechanics are maintained for all levels

of weight support and speeds. This reduction in muscle activation probably explains the decrease in metabolic cost of a given exercise intensity.

The heart rate response to 80% body weight running at all speeds was significantly lower than 100% body weight running. This is to be expected given the reduced effort required to move in the LBPP environment. However this observation may also be linked to a positive effect of lower body positive pressure on venous return with a consequent increase in stroke volume. Both Cutuk et al (11) and Hoffman and Donaghe (22) observed a reduction in heart rate with increasing amounts of pressure on a LBPP treadmill. However both of their studies were done using much higher levels of pressure (25 to 50 mmHg) than we observed in our study. Pressure changes to unload subjects in our study did not exceed 15mmHg. In addition, the larger reduction in PV during exercise at 80% BW running should have caused a small reduction in stroke volume and an increase in HR. Overall, the small amount of LBPP required to decreased heart rate was more likely a reflection of the decreased metabolic load than any mechanism involved in altering stoke volume and venous return.

Plasma lactate levels were similar at all speeds regardless of whether or not body weight was supported. This similar plasma lactate level at any given speed occurred despite the reduction in energy cost and heart rate. One interpretation of these data is that running with LBPP either promotes greater lactate production or limits lactate clearance. Our data do not directly assess how LBPP might increase anaerobic stress. It is well established that the application of LBPP during cycling in the supine and semi-recumbent positions reduces blood flow to working muscles (32, 37, 39). This change in blood flow to the lower extremities produced by the application of LBPP reduces the $O_2$ saturation in the femoral venous blood and increases accumulation of metabolic by-products such as lactate (37). It has also been observed

that underwater running elicits higher lactate levels than on ground running (17), presumably due to the impact of hydrostatic pressure. If tissue pressure during LBPP running is enough to decrease muscle blood, we suspect that LBPP running may decrease muscle perfusion and lead to a state of increased anaerobic stress.

Lactate threshold while running at 80% body weight was lower (occurred at a lower energy cost) than running at 100% body weight. We are not aware of any data describing similar findings in LBPP treadmill running. Williamson et al (40) designed a study to determine if LBPP could alter the ventilatory threshold during cycling. They found that the ventilatory threshold was progressively decreased with increased levels of LBPP (0, 15, 30, 45mmHg). However, measured blood lactate was only slightly elevated above control, while pH and plasma $[K^+]$ were unchanged. Because is it thought that LBPP can enhance "trapping" of metabolites in working muscle (37), the increase in metabolite buildup in contracting muscles could stimulate increased ventilation as a result of impeded blood flow. If application of LBPP reduces blood flow in the leg muscles during treadmill running we might expect an increase in lactate release from active skeletal muscle.

An intriguing observation was the larger reduction in PV at any given energy cost during 80% BW running. At the onset of exercise, blood plasma leaves the vascular system and enters the interstitial space. This reduction in plasma volume results in increased hemoconcentration in the blood and potentially a decrease in stroke volume. The cardiovascular system responds with concomitant increase in heart rate in order to maintain cardiac output (35). The larger reduction in PV at 80% body weight running should have contributed to the higher HR at 80% body weight running via a reduction in stroke volume. However, we noted a reduced HR response and a reduced slope of the $\dot{V}O_2$-HR relationship.

Another finding of our study that has application for rehabilitation in athletic populations was that maximal aerobic capacity at 80% body weight was similar to running at 100% body weight. This supports reports by Gojanovic et al (18) that maximal aerobic capacity was similar across all body weight loading conditions (100 to 80% BW) Subjects in our study were able to obtain faster speeds when running at 80% during the graded exercise test than when running at 100% body weight. Thus, the LBPP treadmill has the potential to be used for high speed training in athletes as proposed by Gojanovic et al (18).

In summary, the LBPP treadmill promises to be a useful tool in rehabilitation for injured runners or for improving performance through training. The results of our study show that the energy cost and heart rate response to running during LBPP is reduced compared to full body weight running but with a concomitant increase in plasma lactate. This could potentially be seen as a training advantage if high intensity lactate tolerance work could be accomplished at lower metabolic costs. However there may be some disadvantages to training using this mode. The duration of training runs and possibly overall training stimulus during LBPP treadmill running may be limited by an early accumulation of blood lactate. Specifically, sustained high intensity training at or above lactate threshold may be limited.

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Table 1. Maximal aerobic capacity and lactate thresholds

| | 100% BW | | | 80% BW | | |
|---|---|---|---|---|---|---|
| SUBJECT | $\dot{V}O_2$max ml/min/kg BM | Speed mile $h^{-1}$ | LT % $\dot{V}O_2$max | $\dot{V}O_2$max ml/min/kg BM | Speed mile $h^{-1}$ | LT % $\dot{V}O_2$max |
| 1 | 61.5 | 10 | 69.5 | 54.1 | 12 | 68.5 |
| 2 | 50.3 | 11 | 68.8 | 50.5 | 11 | 65.5 |
| 3 | 49.5 | 13 | 68.3 | 50.5 | 14 | 57.4 |
| 4 | 52.1 | 12 | 72.6 | 54.0 | 13 | 64.0 |
| 5 | 53.7 | 12 | 76.8 | 52.7 | 13 | 60.7 |
| 6 | 54.7 | 14 | 68.3 | 52.4 | 15 | 62.5 |
| 7 | 54.7 | 14 | 61.7 | 55.3 | 14 | 56.2 |
| 8 | 56.7 | 11 | 71.6 | 55.9 | 12 | 57.5 |
| MEAN ± SEM | 54.2 ± 1.3 | 12.1 ± 0.5 | 69.7 ± 1.5 | 53.2 ± 0.7 | 13.0 ± 0.5 | 61.5 ± 1.5* |

Values represent mean ± 1 SEM for 8 subjects. BM, body mass; mile $h^{-1}$, miles per hour; LT, lactate threshold.
* $p < 0.05$ different from 100% Body Weight

Table 2. Cardiovascular parameters during lactate threshold assessment

| | 100% Body Weight | | | | | 80% Body Weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Speed (mile $h^{-1}$) | HR, bpm | SBP, mmHg | DBP, mmHg | MAP, mmHg | LBPP, mmHg | HR, bpm | SBP, mmHg | DBP, mmHg | MAP, mmHg | LBPP, mmHg |
| 0 | 66 ± 4 | 127 ± 3 | 71 ± 4 | 89 ± 3 | 0 | 66 ± 4 | 130 ± 5 | 72 ± 4 | 88 ± 4 | 0 |
| 0 | | | | | | 66 ± 4 | 130 ± 5 | 72 ± 4 | 91 ± 4 | 13.4 ± 0.3 |
| 5.0 | 114 ± 4 | 146 ± 8 | 57 ± 4 | 87 ± 4 | 5.9 ± 0.2 | 108 ± 4* | 141 ± 8.4 | 64 ± 2 | 90 ± 4 | 13.2 ± 0.5 |
| 6.0 | 129 ± 3 | 160 ± 5 | 62 ± 7 | 95 ± 6 | 6.1 ± 0.2 | 119 ± 5* | 140 ± 5 | 59 ± 5 | 86 ± 5 | 13.7 ± 0.5 |
| 7.0 | 141 ± 5 | 160 ± 4 | 65 ± 7 | 97 ± 5 | 6.3 ± 0.2 | 130 ± 6* | 141 ± 7 | 61 ± 4 | 88 ± 2 | 14.1 ± 0.3 |
| 7.5 | 148 ± 6 | 152 ± 6 | 62 ± 4 | 92 ± 4 | 6.8 ± 0.2 | 138 ± 6* | 155 ± 6 | 57 ± 5 | 90 ± 4 | 13.8 ± 0.4 |
| 8.0 | 156 ± 6 | 151 ± 8 | 60 ± 4 | 90 ± 5 | 6.0 ± 0.2 | 141 ± 7* | 153 ± 8 | 58 ± 4 | 90 ± 3 | 13.8 ± 0.3 |
| 8.5 | 162 ± 5 | 144 ± 5 | 61 ± 5 | 89 ± 4 | 6.2 ± 0.2 | 149 ± 7* | 148 ± 8 | 55 ± 3 | 86 ± 3 | 13.5 ± 0.5 |
| 9.0 | 168 ± 6 | 163 ± 4 | 63 ± 5 | 96 ± 4 | 6.3 ± 0.2 | 156 ± 7* | 151 ± 7 | 65 ± 7 | 94 ± 6 | 13.9 ± 0.5 |

Values represent mean ± 1 SEM for 8 subjects. HR, heart rate; bpm, beats per min; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, Mean arterial blood pressure; LBPP, lower body positive pressure. * $p < 0.05$ different from 100% Body Weight

Table 3. Blood parameters during lactate threshold assessment

| Speed, mile $h^{-1}$ | 100% Body Weight | | | | 80% Body Weight | | | |
|---|---|---|---|---|---|---|---|---|
| | Hct,% | [Hb, g •$dl^{-1}$ | [PP], g•$dl^{-1}$ | Δ PV, % | Hct,% | [Hb, g •$dl^{-1}$ | [PP], g•$dl^{-1}$ | Δ PV, % |
| 0 | 45.0 ± 0.7 | 16.8 ± 0.6 | 6.6 ± 0.2 | 0 | 42.5 ± 1.7 | 15.7 ± 0.5 | 6.0 ± 0.2 | 0 |
| 0 | | | | | 44.3 ± 1.7 | 16.5 ± 0.7 | 6.5 ± 0.2 | -7.5 ± 1.9 |
| 5 | 47.2 ± 0.7 | 17.6 ± 0.4 | 6.6 ± 0.1 | -8.4 ± 1.3 | 45.2 ± 1.6 | 16.7 ± 0.7 | 6.6 ± 0.1 | -8.8 ± 1.7 |
| 6 | 46.4 ± 1.0 | 17.2 ± 0.5 | 6.7 ± 0.2 | -5.0 ± 2.3 | 45.2 ± 1.5 | 16.6 ± 0.6 | 6.7 ± 0.2 | -7.6 ± 2.7 |
| 7 | 46.3 ± 0.7 | 17.8 ± 0.5 | 6.8 ± 0.2 | -8.1 ± 1.9 | 45.7 ± 1.5 | 17.4 ± 0.6 | 6.8 ± 0.2 | -13.4 ± 1.1 |
| 7.5 | 46.8 ± 1.0 | 18.0 ± 0.5 | 6.8 ± 0.2 | -9.9 ± 2.2 | 46.2 ± 1.3 | 17.6 ± 0.8 | 6.8 ± 0.2 | -13.1 ± 1.7 |
| 8 | 47.2 ± 1.0 | 18.3 ± 0.5 | 6.9 ± 0.2 | 12.1 ± 2.6 | 45.7 ± 1.3 | 18.1 ± 0.7 | 6.9 ± 0.2 | -15.0 ± 1.8 |
| 8.5 | 46.9 ± 1.0 | 18.5 ± 0.5 | 6.9 ± 0.2 | -12.4 ± 1.9 | 45.9 ± 1.4 | 18.2 ± 0.7 | 6.9 ± 0.2 | -16.4 ± 1.6 |
| 9 | 47.1 ± 0.9 | 18.3 ± 0.5 | 7.2 ± 0.2 | -11.8 ± 2.0 | 46.4 ± 1.4 | 18.3 ± 0.9 | 7.2 ± 0.2 | -15.7 ± 2.1 |

Values represent mean ± 1 SEM for 8 subjects. mile $h^{-1}$, miles per hour; Hct, hematocrit; Hb, hemoglobin; TP, total plasma protein; PV, plasma volume. * $p < 0.05$ different from 100% Body Weight

Figure Legends

*Figure 1*: Energy cost of running on a treadmill at 100% body weight and 80% body weight as a function of speed (5.0 to 9.5 mile $h^{-1}$). Values represent mean ±SEM. N = 8. * $p<0.05$ 100% body weight different from 80% body weight.

*Figure 2*: Relationship between Heart Rate and $\dot{V}O_2$ (ml $O_2$ $min^{-1}$ $kg^{-1}$ BM) during treadmill running at 100% body weight and 80% body weight. Values represent mean ±SEM. N = 8.

*Figure 3*: Mean change in plasma volume as a function of average energy cost of running on a treadmill at 100% body weight and 80% body weight. Values represent mean ±SEM. N = 8.

*Figure 4*: Mean plasma lactate concentration as a function of average energy cost of running on a treadmill at 100% body weight and 80% body weight. Values represent mean ±SEM. N = 8.

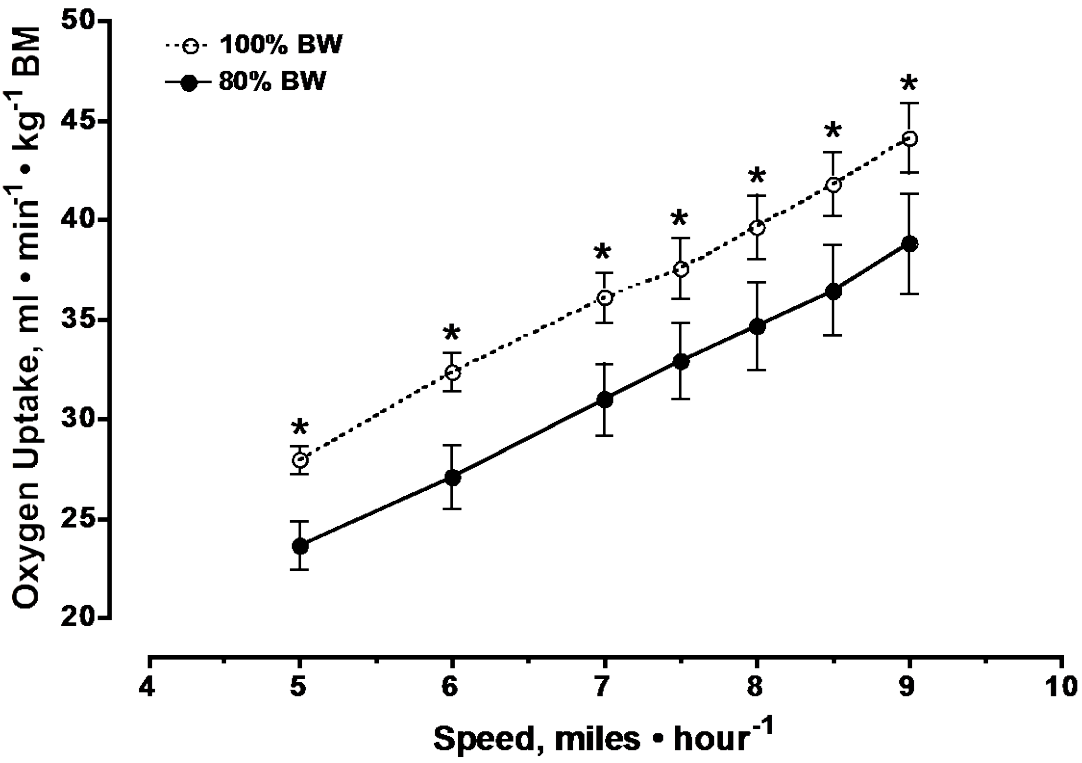


Figure 1. Energy cost of running

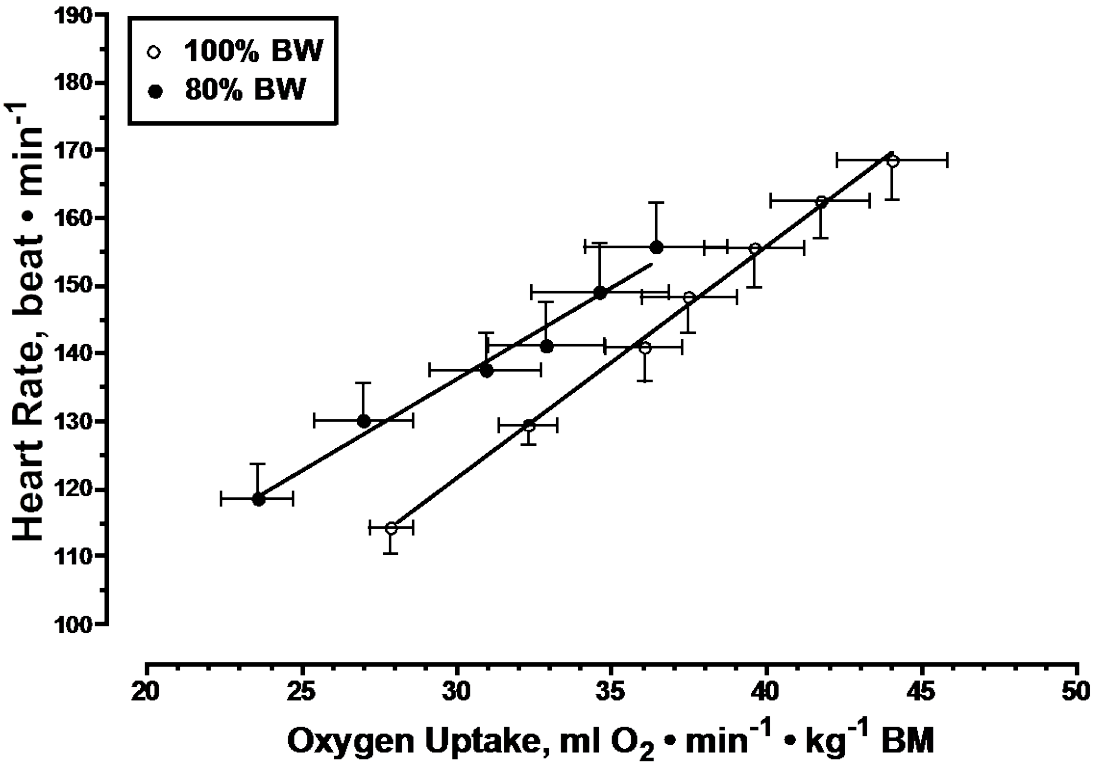


Figure 2. Relationship between heart rate and $\dot{V}O_2$

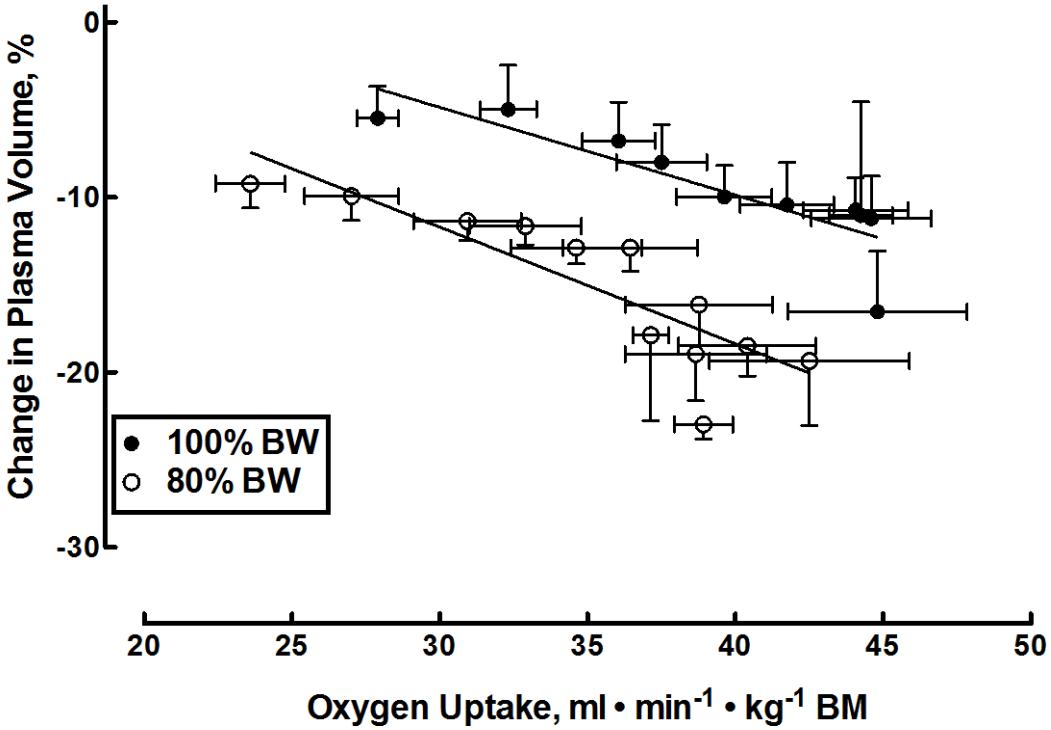


Figure 3. Mean change in plasma volume

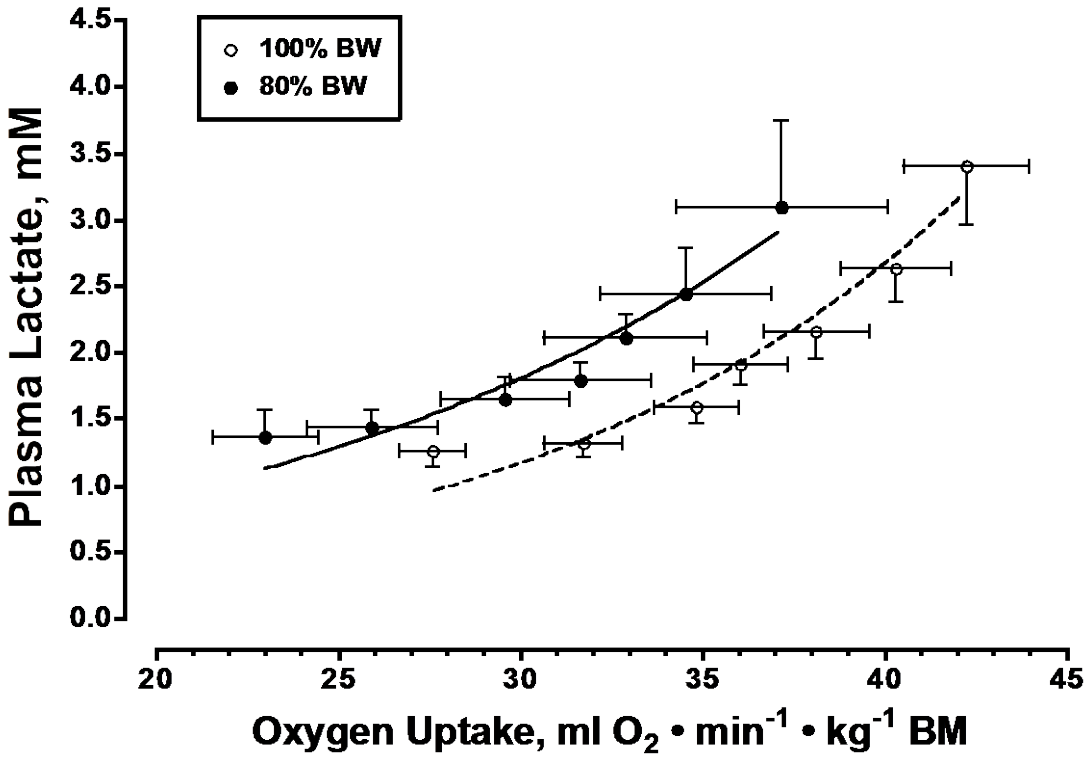


Figure 4. Mean plasma lactate concentration

# APPENDIX

Prospectus

# Chapter 1

## Introduction

The running motion is utilized in most sports and it is well established that injuries frequently occur due to the constant impact on the lower extremities. For example, it has been estimated that up to 70% of recreational and competitive runners experience an overuse injury in any one year period (25). The treatment for such injuries commonly involves a period of rest for 4 to 6 weeks (15). However, this period of inactivity typically leads to significant loss of muscular and cardiovascular fitness (10). Many therapists, coaches, and athletes integrate cardiovascular fitness into their therapeutic programs to minimize detraining effects. To maintain fitness during rehabilitation, therapists and coaches use cross training modes of exercise such as cycling, swimming, elliptical running, and underwater running to maintain fitness during rehabilitation. While these exercise modalities are designed to maintain cardiopulmonary fitness, each has practical limitations. Many of their adaptations are not specific to running.

Exercising on a lower body positive pressure (LBPP) treadmill is a relatively new exercise mode for rehabilitation of injured runners. This device allows unloading of the lower extremities during exercise in a pressurized treadmill chamber. This treadmill is capable of accurate and precise unweighting across a variety of body types, and is able to reduce ground reaction force for walking and running in proportion to the amount of unweighting (20). This benefits athletes in their high demand rehabilitation, allowing a running workout without the negative effects of increased lower extremity loading (11). The presumed properties associated with LBPP treadmills, such as decreased ground reaction forces while maintaining metabolic demand, make it an appealing exercise medium for rehabilitation of individuals with lower extremity injuries associated with running (20).

Methods other than LBPP have been used previously to support body weight (BW) during running, including harness suspension systems and water immersion. Harness suspension systems are beneficial because a purely vertical force can be applied to the person, and the independent effects of supporting BW can be addressed (19, 21). However, harness suspension systems may not be applicable for extended rehabilitation and training use because they can cause discomfort and impede circulation. Water immersion is also commonly used as a rehabilitation tool (11). However, the viscous drag forces associated with water exercise act in opposition to movement and cause significant changes in movement velocity, gait timing, joint kinematics, joint kinetics, and muscle activity compared with over ground movement. (9, 30)

LBPP devices do not apply meaningful drag forces to the legs and may be advantageous because they allow kinematic gait patterns similar to normal weight over-ground running, a normative range of running velocities, and a decrease in the forces transmitted by the legs (11). Surface EMG electrode activity while running on a LBPP treadmill shows that the timing of muscle firing patterns and gait mechanics are maintained for all levels of weight support and speeds (25). For any given running speed, weight support with the LBPP device reduces $\dot{V}O_2$ and metabolic demand by the individual (20). Running at faster velocities with weight support requires the same metabolic power as running at slower velocities with normal weight. Additionally, LBPP devices are comfortable and can be used over extended periods. Therefore, LBPP devices could be very effective for maintaining cardiovascular fitness during rehabilitation involving running.

To provide the best possible training stimulus during LBPP treadmill running in the rehabilitative athlete a better understanding of the physiological response to LBPP running is needed. Specifically, it is unknown if positive pressure alters muscle blood flow or muscle

metabolism, specifically lactate accumulation. These are key factors in optimizing adaptations to exercise training. One essential adaptation to aerobic training is an increase in lactate threshold. This increase allows athletes to work at higher workloads without a significant accumulation of lactate. Recently, LBPP treadmills have been introduced to training and rehabilitation facilities. There has been a limited amount of research done on the physiologic responses to LBPP treadmill running and the impact of LBPP treadmill running on the lactate threshold is unknown.

*Hypotheses*

1. The lactate threshold will occur at a higher treadmill speed during body weight unloading treadmill running compared to full body weight treadmill running.
2. There is no significant difference between lactate threshold expressed as a percentage of $\dot{V}O_2$max determined during treadmill running with body weight unloading and full body weight running.

*Null hypotheses*

1. There is no significant difference between lactate threshold treadmill speed determined during treadmill running with body weight unloading and full body weight running.
2. The lactate threshold, expressed as a percentage of $\dot{V}O_2$max, will occur at a higher $\dot{V}O_2$ % during body weight unloading treadmill running compared to full body weight treadmill running.

*Limitations*

The results are only applicable to the subject population: active male college students.

*Significance of this study*

Results from this study will explain the lactate response to LBPP treadmill running compared to running on a land treadmill. Weighted impact on the legs generated from running on the ground can produce injuries in the lower extremities in many athletes. Therefore, the purpose of this study is to show that LBPP treadmill running provides a therapeutic and rehabilitative alternative. A better understanding of the lactate threshold response to LBPP treadmill running will help athletes and trainers know the intensity needed to exercise at to elicit the same physiologic response they would if using a land treadmill. Not only will this help with therapeutic rehabilitation for injured athletes, this study will also further the understanding of the body's response to LBPP treadmill running.

# Chapter 2

## Review of the Literature

### *Lactate and H+ Production*

Lactate is the final product of anaerobic glycolysis, and is an active metabolite playing many important roles in muscle glycogen production and fatigue. During muscle contraction, the energy molecule adenosine triphosphate (ATP) is used by myosin adenosine triphosphatase (ATPase) to allow cross-bridge cycling between actin and myosin filaments, resulting in force production. However, stored ATP concentrations are normally so low that with repeated contractions the phosphocreatine (PCr) stores in muscle are used to resynthesize and maintain ATP concentrations. With an increasing number of contractions, the PCr concentration declines, resulting in a need to utilize other fuels. Consequently muscle glycogen is called upon with glycogenolysis being activated, leading to the formation of pyruvate and ATP. During intense dynamic exercise this pathway must be used to produce the necessary ATP to meet the demand of the exercise. (8)

With lower intensity exercise, pyruvate is destined for oxidation by aerobic metabolism in the mitochondria. Unfortunately, the mitochondria are unable to oxidize all of the pyruvate produced during intense exercise, leading to its conversion to lactate in the myoplasm (5). Lactate is produced through the reduction of pyruvate with the concomitant oxidation of NADH to NAD+, a reaction catalyzed by lactate dehydrogenase (LDH). While skeletal muscle is contracting and glycolysis is active, lactate is always being produced. When lactate production exceeds lactate removal, lactate accumulates in the muscle and blood (23).

Once produced lactate is moved from lactate-producing to lactate-consuming sites. Lactate is transported across membranes via monocarboxylate transporters (MCTs). Transport

through these shuttles occurs by facilitated exchange down the lactate concentration and H+ ion gradients (6). Many cells and tissues, such as liver, cardiac tissue, active type I muscle fibers, inactive skeletal muscle, and neurons can utilize lactate. In the liver it is either converted into glucose via gluconeogenesis or it is converted to glycogen through glyconeogenesis (7). Cardiac tissue, type I muscle fibers, and inactive skeletal muscle metabolize lactate by converting it to pyruvate through LDH and then using pyruvate for energy production.

Blood lactate concentration is a useful marker of exercise intensity and adaptation to training. The presence of lactate is also an indicator of the balance between glycolytic and mitochondrial activity. Increased lactate production is usually a result of high intensity short duration activities that require the recruitment of glycolytic fast-twitch muscle fibers. Lactate is also produced during lower intensity activities utilizing aerobic slow twitch fibers, however lactate can move into the mitochondria via MCT's and be oxidized. Roughly 75% of lactate produced during anaerobic glycolysis is removed by mitochondrial oxidation in active slow twitch fibers via the intracellular lactate shuttle (6).

As exercise increases the turnover of lactate oxidation increases proportionately up to an exercise intensity of approximately 75% of $\dot{V}O_2$max (28). There is a direct linear relationship between the rate of blood lactate disposal and the metabolic rate ($\dot{V}O_2$). Linnarsson, Karlsson, Fagraeus and Saltin (26) demonstrated that tissue hypoxia exaggerates, but does not dictate the formation of lactic acid.

*Lactate Threshold*

During modest increases in exercise intensity, lactate will continue to increase, but will stabilize, indicating a balance between rate of production and utilization (5). This demonstrates

that the body is able to provide enough ATP for energy, primarily through aerobic metabolism. Eventually, an exercise intensity will be reached at which lactate increases abruptly, or in a nonlinear fashion. This represents the intensity level at which lactate production exceeds the body's maximum capability to utilize, or clear lactate. Lactate will continue to accumulate at a rapid rate above this level. This is partially due to the recruitment of fast twitch muscle fibers that are specified for anaerobic metabolism, and have a higher dependency on glycolysis for ATP production and thereby for lactate production.

The level at which lactate continues to progressively accumulate designates a transition from primarily aerobic metabolism to greater involvement of anaerobic bioenergetic pathways (1). This critical intensity at which a nonlinear and progressive increase in lactate occurs has been identified as the lactate threshold (1). Lactate accumulation at this point is attributed, in part, to the failure of the cardiovascular system to supply the oxygen to the working muscle. This lactate threshold has also been termed the anaerobic threshold by Wasserman, Whipp, Koyl and Beaver (38) and is identified by an exercise intensity of workload ($\dot{V}O_2$) above which lactate levels rise and minute ventilation increases disproportionately in relation to oxygen consumption. The rise in minute ventilation is due in part to an increase in carbon dioxide production resulting from the buffering of the lactic acid.

After periods of endurance training, adaptations occur that result in markers of lactate accumulation shifting to a higher relative workload. Donovan and Brooks (14) used $C^{13}$ and $H^3$ tracers to show lower lactate levels during both easy and hard exercises in trained animals compared to untrained. Demarle, Heugas, Slawinski, Tricot, Koralsztein and Billat (12) suggests that improvements in lactate levels may be related to elevated capacity for aerobic synthesis of ATP molecules due to 1) an increase in mitochondria size and number and 2) an increase in the

activity of oxidative enzymes. Additionally, an increase in the enzymes for lipid oxidation will decrease the dependence on glycolysis for energy and may improve lactate threshold as well. Enhancement of intra- and extracellular buffering mechanisms of pH regulation and lactate/proton transport may have an effect on human work capacity. Attenuating the onset of pH decline and lactate accumulation is thought to provide improvements in exercise performance (29). Endurance training doesn't alter the production; it increases the body's capacity for lactate clearance (27). Increased capacity for oxidation (4) and gluconeogenesis (3) in trained endurance athletes explains this increased clearance rate.

Greater oxygen delivery also results in decreased lactate concentrations in the blood and working muscle. Katz and Sahlin (24) demonstrated that lactate accumulation occurs during submaximal exercise, even while oxygen is present; however, when oxygen is limited the rate of lactate production is amplified. The reduced availability of oxygen leads to an acceleration in glycolysis and increased lactate production. Therefore, the adaptations associated with endurance training that increase oxygen delivery contribute to a delay in lactate accumulation.

*Lower Body Positive Pressure Physiology*

While research on the physiologic and metabolic responses to lower body positive pressure (LBPP) treadmill running is limited, the effects of LBPP application during supine and recumbent cycling exercise have been explored. LBPP application typically elicits physiologic changes in mean arterial pressure (16, 31, 32, 34, 36, 39), a redistribution of blood from peripheral tissues to central regions of the body (19, 28), and metabolite accumulation in the active skeletal muscles (32). These changes are thought to be brought on by activation of both mechanical (mechanoreceptors) and chemical (metaboreceptors) sensitive nerve endings located

within the muscle. LBPP promotes accumulation of metabolic by-products that stimulate muscle type III & IV afferents (metaboreceptors) that increase sympathetic nervous system activity and drive the increase in mean arterial pressure (MAP). The increased tissue pressure associated with LBPP stimulates mechanoreceptors, thereby increasing SNS and drive the increase in MAP. LBPP also increases tissue pressure that reduces blood vessel diameter and mechanically increases total vascular resistance and therefore increases MAP (MAP = Q x TPR).

A typical response to application of LBPP to the lower extremities during exercise is an increase in tissue pressure which reduces the transmural pressure gradient across the vasculature leading to increases in both central venous pressure and mean arterial pressure (MAP) (16, 31, 32, 34, 36, 39). Elevation of tissue pressures in lower extremities generated by LBPP also slightly increases venous return (34). The extent of the blood pressure changes are dependent on factors including the amount of pressure applied and the exercise posture.

During supine and recumbent cycling, the greater the LBPP applied, the greater the blood pressure response (34, 36, 39). Shi, Potts, Foresman and Raven (36) documented that MAP increases 3-6 mmHg at 20-30 mmHg LBPP and by 4-15mmHg at 40-50mmHg LBPP in supine subjects. This supports previously documented evidence that changes in pressure around the limb are fully transmitted from the skin to the muscle (34).

Nishiyasu, Nagashima, Nadel and Mack (31) conducted a study to determine the effect that LBPP would have on cardiovascular responses during cycling exercise in the supine and upright positions. They found that LBPP resulted in an increased MAP in both positions; with a greater effect seen in the supine group. They also saw an increase in stroke volume (SV) in the upright posture that was not seen in the supine. The response in subjects is dependent on the intravascular fluid shift from lower extremities to torso and the body position. Cutuk, Groppo,

Quigley, White, Pedowitz and Hargens (11) performed the first study with subjects standing and ambulating fully upright on an exercise treadmill under the influence of LBPP. Their study confirmed that MAP, diastolic blood pressure (DBP), and systolic blood pressure (SBP), although trending upward, did not significantly increase in standing and treadmill ambulating subjects exposed to 0-50mmHg of LBPP. Hoffman and Donaghe (22) later supported this evidence by showing an increase in SBP by 10 mmHg at rest while standing in LBPP, but once exercise began, there was no change in MAP. The findings from Nishiasu, Cutuk, and Hoffman demonstrate that although LBPP is highly correlated with an increase in MAP, the effects decrease when subjects are in the upright position and minimized when upright and running on a treadmill.

Application of LBPP involves a shift in blood volume from peripheral to more central regions of the body. This shift in blood volume is typically associated with a greater stroke volume due to increased preload from venous return. However, LBPP is capable of inducing a pressor response during rest and exercise without producing increases in stroke volume (SV), heart rate (HR), $\dot{V}O_2$ or cardiac output (Q) even with an increased MAP (39). Because there was no increase in Q, blood pressure was elevated through increases in total peripheral resistance. This suggests input from exercising muscles dictated blood pressure responses, while Q is determined by the actual activity performed as expressed by the oxygen uptake.

The metabolic responses to large muscle mass dynamic exercise differ on land compared to being surrounded by a LBPP device. The application of LBPP in the supine and semi-recumbent positions reduces blood flow to working muscles (32, 37, 39). This change in blood flow to the lower extremities produced by the application of LBPP reduces the $O_2$ saturation in

the femoral venous blood and increases accumulation of metabolic by-products such as lactate (37).

While effects of LBPP on lactate threshold have not been explored, Williamson, Raven, Foresman and Whipp (40) designed a study to determine if LBPP could alter the occurrence of the ventilatory threshold. They found that the ventilatory threshold was progressively decreased with increased levels of LBPP (0, 15, 30, 45mmHg). However, measured blood lactate was only slightly elevated above control, while pH and $[K^+]$ were unchanged. Because is it known that LBPP can enhance "trapping" or accumulation of metabolites in working muscle (37), they concluded that the increase in metabolite buildup in contracting muscles can stimulate ventilation as a result of impeded blood flow from applied pressure.

LBPP can "trap" or further enhance accumulation of lactate at working muscles in the supine and recumbent positions, however, the build-up in the muscles is much greater than levels seen in blood samples (40, 41). The increase in ventilation (VE) and respiratory exchange ratio (RER) during exercise in the recumbent position supports this notion (32). It is assumed that application of similar LBPP could reduce blood flow in the leg muscles with a consequent release of lactate in the muscle during exercise in the upright position. However, VE and RER did not change when LBPP was applied to subjects exercising in the upright position (32). It is unknown how LBPP applied to the upright position while running on a treadmill effects muscle or blood lactate levels.

Another metabolic difference between LBPP running and over-ground running is a decrease in metabolic demand. For any given running speed, weight support with the LBPP device reduced $\dot{V}O_2$ and metabolic demand by the individual (20). Running at faster velocities with weight support requires the same metabolic power as running at slower velocities with

normal weight. Liebenberg, Scharf, Forrest, Dufek, Masumoto and Mercer (25) investigated how lower extremity muscles are influenced by body weight support through LBPP. They showed that reducing body weight through LBPP leads to a reduction in the amplitude of all muscle activity, even though the timing of the muscle firing patterns and gait mechanics are maintained for all levels of weight support and speeds. This reduction in muscle activation explains the decrease in metabolic power needed to maintain a given intensity.

*Lower Body Positive Pressure and Lactate Threshold*

Although the effects of LBPP on blood lactate levels during treadmill running are unknown, there are several factors that may contribute to an increase in lactate threshold. It has been established that weight support during LBPP running decreases metabolic demand ($\dot{V}O_2$), decreases muscle activation, and increases the fluid shift from the legs to the abdomen and thorax. The decreased metabolic demand may allow aerobic systems to support energy production at lower intensities, thus increasing oxidation and reduce the production and /or accumulation of lactate. All these in turn might lead to an increased lactate threshold while performing LBPP running exercise.

# Chapter 3

# Methods

*Subjects*

Ten college-age (18-33 years old) male athlete subjects will be recruited for this project. Subjects will qualify if they meet all of the following requirements:

1. A $\dot{V}O_2$max of $\geq 55$ ml $O_2$ $min^{-1}$ $kg^{-1}$ BM
2. Active, engaging in 30 min of moderate aerobic activity at least 3 days/week
3. No lower extremity injuries in the last 4 months
4. Not taking medication

Subjects will be asked not to change their current exercise program during the course of the study.

*Experimental Design*

The proposed project consists of four days of testing.

1. Day 1 and Day 2 (30 min each) consists of the determination of:
    a. $\dot{V}O_2$max on an Alter-G treadmill with 0% unloading
    b. $\dot{V}O_2$max on a Alter-G treadmill with 20% body weight unloading
2. Days 3 and 4 (90 minutes each day) consist of determination of:
    a. Lactate threshold (LT) on a Alter-G treadmill with 0% unloading
    b. Lactate Threshold (LT) on a Alter-G treadmill with 20% body weight unloading

Day 1 and 2: *Maximal Aerobic Capacity*.

Two tests to determine the aerobic capacity of the subjects will be given. One test will be performed on a land treadmill and the other performed with 20% body weight unloading on the Alter-G Pro Version 1.20 (Alter-G Inc., Fremont, CA) treadmill. The two exercise tests will be completed in a randomized cross over design. The two tests will be separated by a minimum of 7 days to allow adequate recovery and accuracy of measurement. Subjects will be taken to the Alter-G treadmill to be familiarized with running on an LBPP treadmill. As part of this familiarization process subjects will be fitted with a heart rate monitor, fitted to their appropriate size neoprene shorts, and treadmill size settings will be determined.

Each exercise test will consist of a 5-min warm-up at 0% grade during which time the subjects self-select a treadmill speed that approximates a running velocity they would select if they were going to choose a comfortable speed for 30 min. The speed will then be set to 7 mile $h^{-1}$ for 2 minutes. Then the speed will increase by increments of 1 mile $h^{-1}$ every 2 minutes. The subjects will continue to exercise until the point of volitional fatigue. The test will be considered a true max if two of the following variables are met: 1) an increase in speed resulting in a failure of $\dot{V}O_2$ to also rise, 2) a respiratory exchange ratio of >1.05, and 3) a heart within 80% of the age predicted max heart rate.

During the exercise test, oxygen consumption will be monitored every 15 sec using a Parvo Medics True One (Parvo Medics, Inc., Sandy, UT) metabolic cart. Immediately prior to the initiation of the exercise test the subjects will be instrumented with a headpiece holding a one-way breathing valve to allow collection of expired gases. Calibration of the portable metabolic cart will be performed before each exercise and verified immediately following each test. Flow meter calibration involves a 5-stroke sequence of a 3-liter syringe at three difference

flow rates (<80 L/min, ≈ 200 L/min, and ≈ 400 L/min). The oxygen and carbon dioxide analyzers will be calibrated prior to each exercise test using samples of room air and a medical grade calibration gas.

Days 2 and 3: *Lactate threshold testing*.

The evening before each trial the subject will be asked to ingest 8ml/kg of water. All tests will be performed during the same time period between 8:00 and 11:00 am. Upon arrival, subjects will sign an informed consent that was previously approved by the IRB before testing is started. Subjects will void their bladder and a small urine sample will be taken to monitor urine specific gravity (USG) to insure adequate hydration. A USG of less than 1.015 will be considered adequately hydrated. Those subjects not meeting this requirement will be asked to ingest an additional 5 ml/kg of water and USG reexamined 60 min post-ingestion. Following verification of proper hydration the subjects' height and weight will be measured and the subjects will be instrumented with a three lead EKG and a venous catheter will be placed in a large forearm vein. Each subject's lactate threshold will be determined during treadmill running at full body weight and at an unloading of 20%. The two exercise tests will also be performed in a randomized cross over design. The two tests will be separated by at least 48 hours.

The subject will then stand in the upright posture for 30 min to allow equilibration of body water compartments before a resting blood sample is collected. During this equilibration period, just prior to the initiation of the exercise test, subject will be instrumented with a blood pressure cuff (left arm) to allow measurement of blood pressure. The subject will also be instrumented with a headpiece holding a one-way breathing valve to allow collection of expired

gases and the measurement of oxygen consumption by the Parvo Medics True One (Parvo Medics, Inc., Sandy, UT) metabolic system.

The lactate threshold protocol will be performed entirely at 0% grade on the treadmill. The subject will start by walking at 3.5 mile $h^{-1}$ for 5 min as a warm-up (see Table 2). After a 3-ml blood sample is collected during the final min of stage 1 the speed will be increased to 5.0 mile $h^{-1}$. This speed will be maintained for 3 min and a blood sample will be drawn between min 2 and 3 of this (and every) stage. After each blood sample a new stage will be initiated by an increase of speed (0.5 mile $h^{-1}$ /stage). When the subject's oxygen consumption meets or exceeds 90% of their measured $\dot{V}O_2$ max the test will be terminated and treadmill speed will be reduced to 3.0 mile $h^{-1}$ to allow the subject to recover and the pressure will be released from the chamber. One final blood sample will be taken five minutes post exercise.

During the lactate threshold trial blood samples (4 ml) will be immediately placed in pre-cooled EDTA-vacutainers, mixed by gentle inversion, and stored on ice until the end of the LT trail. After the LT test the blood samples will be well mixed and a small amount of whole blood will used for determine hematocrit and hemoglobin concentration. The remainder of the blood will be centrifuged at 1500 xg for 15 min at 4°C. The plasma will be immediately separated from the red cells. A small amount of plasma (40 µl) will be used to determine plasma protein concentration using a handheld refractometer. The plasma will be analyzed for lactate concentrations using an YSI 2300 lactate analyzer. If the plasma is not analyzed immediately after separation it will be stored frozen at -20°C until analysis. Analysis of lactate threshold will be assessed from the samples drawn during the study. During incremental exercise an abrupt transition occurs in the rate of increase of blood lactate with increasing $\dot{V}O_2$. A mathematical model that fits the data to a line of best fit can be used to detect lactate threshold

with more precision than using visual detection techniques. The log-log transform model easily defines the transition point in plasma lactate accumulation. Error effects are minimized using this method by using the least squares curve-fitting procedure (2).

We will use hematocrit and hemoglobin samples at each stage to measure the reduction in plasma volume that occurs during exercise. To account for the fluid shift out of the vascular system, we will express lactate concentrations in mM kg $H_2O^{-1}$ with plasma water determined from the shift in plasma volume and changes in protein concentration. Analysis of plasma lactate levels at specific treadmill speeds and at specific percentages of $\dot{V}O_2$max will be examined.

*Data Analysis*

The experimental design is two simple balanced ANOVA tests to analyze the point at which lactate threshold occurs. The independent variable being the running condition (20% BW unloading or 0% unloading), and the dependent variables being the speed at which threshold occurs and the $\dot{V}O_2$max percentage at which threshold occurs. A simple balanced ANOVA for repeated measures will be also used to examine the following variables: $\dot{V}O_2$, heart rate, blood pressure, and lactate concentration. The analysis will be performed using SAS general linear model analysis with the level of significance set at a p-value of $p<0.05$.

List of Tables

Table 1. $\dot{V}O_2$max test protocol

| Stage | Time | Mile $h^{-1}$ | Grade |
|---|---|---|---|
| 1 | 0-5 min | 3.5 to 7* | 0 |
| 2 | 5-7 min | 7 | 0 |
| 3 | 7-9 min | 8 | 0 |
| 4 | 9-11 min | 9 | 0 |
| 5 | 11-13 min | 10 | 0 |
| 6 | 13-15 min | 11 | 0 |
| 7 | 15-17 min | 12 | 0 |
| 8 | 17-19 min | 13 | 0 |
| 9 | 19-21 min | 14 | 0 |

* self-selected speed during warm up

**Additional stages will follow same protocol if necessary

Table 2. Lactate threshold test 0% BW unloading protocol

| Stage | Time | Mile $h^{-1}$ | Sample # |
|---|---|---|---|
| 1 | Resting | Standing | 1 |
| 2 | 1-5 min | 3.5 | 2 |
| 3 | 5-8 min | 5.0 | 3 |
| 4 | 8-11 min | 5.5 | 4 |
| 5 | 11-14 min | 6.0 | 5 |
| 6 | 14-17min | 6.5 | 6 |
| 7 | 17-20 min | 7.0 | 7 |
| 8 | 20-23 min | 7.5 | 8 |
| 9 | 23-26 min | 8.0 | 9 |
| 10 | 26-29 min | 8.5 | 10 |
| 11 | 29-32 min | 9.0 | 11 |
| 12 | 32-35 min | 9.5 | 12 |
| 13 | 35-38 min | 10.0 | 13 |
| 14 | 38-41 min | 10.5 | 14 |
| 15 | 5 min Post | 3.0 | 15 |

*Additional stages will follow same protocol if necessary

Table 3. Lactate threshold test 20% BW unloading protocol

| Stage | Time | Mile $h^{-1}$ | % Unloading | Sample # |
|---|---|---|---|---|
| 1 | Resting | Standing | 20% | 1 |
| 2 | 1-5 min | 3.5 | 20% | 2 |
| 3 | 5-8 min | 5.0 | 20% | 3 |
| 4 | 8-11 min | 5.5 | 20% | 4 |
| 5 | 11-14 min | 6.0 | 20% | 5 |
| 6 | 14-17min | 6.5 | 20% | 6 |
| 7 | 17-20 min | 7.0 | 20% | 7 |
| 8 | 20-23 min | 7.5 | 20% | 8 |
| 9 | 23-26 min | 8.0 | 20% | 9 |
| 10 | 26-29 min | 8.5 | 20% | 10 |
| 11 | 29-32 min | 9.0 | 20% | 11 |
| 12 | 32-35 min | 9.5 | 20% | 12 |
| 13 | 35-38 min | 10.0 | 20% | 13 |
| 14 | 38-41 min | 10.5 | 20% | 14 |
| 15 | 5 min Post | 3.0 | 0% | 15 |

*Additional stages will follow same protocol if necessary

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