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Lactate Threshold: The Comparison of Running on a Land Treadmill Versus Head-Out Water Immersion Treadmill Running

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LACTATE THRESHOLD: THE COMPARISON OF RUNNING ON A LAND TREADMILL VERSUS HEAD-OUT WATER IMMERSION TREADMILL RUNNING

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Stephanie Jones Zobell

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

Date	Gary W. Mack, Chair
Date	Allen Parcell
Date	Pat Vehrs

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As chair of the candidate's graduate committee, I have read the thesis of Stephanie Jones Zobell in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

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ABSTRACT

Introduction. Exercise and head-out water immersion (HOI) have consistently reported an increase in central blood volume associated with the cephalad shift in blood volume. This causes an increase in left ventricular end diastolic volume and greater stroke volume during exercise compared to exercise in air at similar metabolic costs. In contrast, the metabolic response, specifically, blood lactate accumulation during exercise combined with HOI has yielded varying results depending on the mode of exercise. At present it appears that during exercise at similar metabolic costs, cycle ergometry exercise augments plasma lactate over treadmill running while HOI reduces the plasma lactate response to cycle ergometry exercise. The interaction between treadmill running and HOI appears less certain. Thus, we tested the hypothesis that running on a treadmill on

land would result in a lesser accumulation of lactate than during HOI treadmill running. **Methods.** Eleven subjects' lactate thresholds were determined while running at a 0% grade at increasing speeds on a treadmill on land or during HOI on an underwater treadmill in a randomized cross-over design. Exercise tests were separated by a minimum of 3 days. Lactate concentrations were expressed in $mM \bullet kg^{-1}$ H_2O after correcting for plasma solid concentration. During exercise changes in plasma volume were calculated from changes in hematocrit and hemoglobin. Lactate threshold was estimated from a log-log plot of lactate concentration ($mM \bullet kg^{-1}$ H_2O) as a function of relative oxygen consumption (ml $O_2 \bullet min^{-1} \bullet kg^{-1}$ BW). **Results.** The energy cost and heart rate response to running at speeds between 5.5 and 7.5 mph was similar for land and HOI. During treadmill running on land, plasma volume decreased by 6.4 ± 4.0% at a speed of 7.5 mph. The decrease in plasma volume was significantly greater during HOI and averaged 18.7 ± 1.7% (p <0.05) at 7.5 mph. Plasma lactate was higher at any given treadmill speed $\geq$ 5.5 mph during HOI compared to land (p <0.05). Lactate threshold during HOI running (21.8 $\pm$ 1.6 $mM \bullet kg^{-1}$ H_2O) was lower (p <0.05) than during running on the land treadmill (27.0 $\pm$ 1.6 $mM \bullet kg^{-1}$ H_2O). **Discussion.** HOI running resulted in a consistent shift to the left (rise in plasma lactate occurred at a lower $\dot{V}O_2$) in the lactate threshold and elevated plasma lactate concentration at speeds between 5.5-7.5 mph despite similar metabolic and HR responses to the exercise.

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Lactate threshold: The comparison of running on a land treadmill versus head-out water immersion treadmill running

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ABSTRACT

Introduction. Exercise and head-out water immersion (HOI) have consistently reported an increase in central blood volume associated with the cephalad shift in blood volume. This causes an increase in left ventricular end diastolic volume and greater stroke volume during exercise compared to exercise in air at similar metabolic costs. In contrast, the metabolic response, specifically, blood lactate accumulation during exercise combined with HOI has yielded varying results depending on the mode of exercise. At present it appears that during exercise at similar metabolic costs, cycle ergometry exercise augments plasma lactate over treadmill running while HOI reduces the plasma lactate response to cycle ergometry exercise. The interaction between treadmill running and HOI appears less certain. Thus, we tested the hypothesis that running on a treadmill on land would result in a lesser accumulation of lactate than during HOI treadmill running. *Methods.* Eleven subjects' lactate thresholds were determined while running at a 0% grade at increasing speeds on a treadmill on land or during HOI on an underwater treadmill in a randomized cross-over design. Exercise tests were separated by a minimum of 3 days. Lactate concentrations were expressed in mM• kg^{-1} H_2O after correcting for plasma solid concentration. During exercise changes in plasma volume were calculated from changes in hematocrit and hemoglobin. Lactate threshold was estimated from a log-log plot of lactate concentration (mM• kg^{-1} H_2O) as a function of relative oxygen consumption (ml O_2•min^{-1}•kg^{-1} BW). *Results.* The energy cost and heart rate response to running at speeds between 5.5 and 7.5 mph was similar for land and HOI. During treadmill running on land, plasma volume decreased by 6.4 ± 4.0% at a

speed of 7.5 mph. The decrease in plasma volume was significantly greater during HOI and averaged 18.7 ± 1.7% (p <0.05) at 7.5 mph. Plasma lactate was higher at any given treadmill speed $\geq$ 5.5 mph during HOI compared to land (p <0.05). Lactate threshold during HOI running (21.8 $\pm$ 1.6 mM• kg^{-1} H_2O) was lower (p <0.05) than during running on the land treadmill (27.0 $\pm$ 1.6 mM• kg^{-1} H_2O). *Discussion.* HOI running resulted in a consistent shift to the left (rise in plasma lactate occurred at a lower $\dot{V}O_2$) in the lactate threshold and elevated plasma lactate concentration at speeds between 5.5-7.5 mph despite similar metabolic and HR responses to the exercise.

INTRODUCTION

Exercise and head-out water immersion (HOI) have been combined to evaluate the role of pre-load in the cardiac response to exercise (13) , simulate cardiac responses to exercise in microgravity (13), and evaluate the cardiovascular adaptations to training during simulated microgravity (2, 3, 13). These studies have consistently reported that the increase in central blood volume associated with the cephalad shift in blood volume during HOI causes an increase in left ventricular end diastolic volume and greater stroke volume during exercise compared to exercise on land at similar metabolic costs (2, 12, 13). In contrast, the metabolic response, specifically, blood lactate accumulation during exercise combined with HOI has yielded varying results depending on the mode of exercise.

Connelly et al. (3) found plasma lactate concentration was significantly reduced at maximal effort during combined HOI and cycle ergometry exercise compared to land. They concluded that the observed decrease in plasma lactate concentration could be due to increased aerobic metabolism and improved lactic acid clearance due to increased skeletal muscle blood flow (14). Other studies incorporating cycling and HOI have shown decreased plasma lactate concentrations in HOI exercise compared to exercising on land (2, 3, 13). However, simulated running in water (deep or shallow) usually results in similar or elevated plasma lactate concentrations. Svendenhag and Seger (14) reported higher levels of plasma lactate while subjects performed deep water running compared to running on land. The blood lactate curves were shifted to the left in the deep water running compared to running on a treadmill on land, demonstrating higher blood lactate

levels at any percent of $\dot{V}O_{2max}$ during deep water running (14). Nagashima et al. (10) compared subjects at treadmill speed that elicited 32, 49, 65, and 78% of their $\dot{V}O_2$ peak on land and during underwater treadmill exercise. Nagashima et al. (10) reported slightly higher concentrations of plasma lactate during running on the underwater treadmill, but these differences were not significant (10). Finally, Fragnolias and Rhodes (5) reported no differences in lactate concentrations 30 seconds after the onset of maximal exercise and 5 minutes post exercise in non weight bearing water immersion running compared to land.

Earlier studies comparing treadmill running and cycle ergometry exercise on land demonstrate a clear difference in lactate response with plasma lactate levels being higher during cycle ergometry exercise at the same oxygen uptake (2, 3, 12, 13). Thus, exercise mode at identical metabolic costs can impact plasma lactate levels. When HOI is introduced during cycle ergometry exercise, plasma lactate levels are reduced compared to land. The interaction between treadmill running and HOI appears less certain but certainly the studies indicate either no change or a slight increase in plasma lactate. As such, we tested the hypothesis that during HOI running on a treadmill would result in higher plasma lactate levels compared to treadmill running on land at an identical metabolic cost. Based upon this hypothesis we would also predict that the lactate threshold would shift leftward with the rise in plasma lactate occurring at a lower $\dot{V}O_2$ than during treadmill running on land.

METHODS

Eleven college-aged (18-33 yrs 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. Lactate threshold was determined for each subject while running at a 0% grade at increasing speeds on a treadmill on land or during HOI on an underwater treadmill (HydroWorx, Middletown, PA) in a randomized cross-over design. Water temperature of the underwater treadmill averaged ≈ 30.8 ± .2°C and the water level was set at the level of the zyphoid process ± 5 cm. The upper limit of speed for the underwater treadmill was 7.5 mph.

Aerobic capacity ($\dot{V}O_2peak$) of each of the subjects was determined using a graded exercise test on a land treadmill with oxygen consumption monitored every 15 sec using a ParvoMedics TrueOne (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 self-selected a treadmill speed that approximated their normal running velocity. Then the grade was increased 2.5% every 2 min until the subject reached the treadmill's maximal grade of 15%. If needed, additional stages consisted of increasing treadmill speed by 0.5 mph per stage.

Each subject's lactate threshold was determined while running at 0% grade on a treadmill on land and in water. The two exercise tests were spaced a minimum of three days apart. For each lactate threshold exercise test, an 18 gauge catheter was placed in a large vein of the subject's forearm. The subject was fitted with a heart rate monitor and a headpiece holding a one-way breathing valve to allow measurement of heart rate and the

measurement of oxygen consumption by the metabolic cart. The subject then stood on the treadmill (in air or during HOI) for 30 min to allow equilibration of body water compartments before a resting blood sample was collected. The lactate threshold protocol consisted of walking at a level grade for 4 min with a blood sample drawn during the last minute of the stage. Subsequent stages were 3 minutes long with blood samples drawn in the last minute. Some subjects took several stages to get to a jogging speed due to varied fitness levels. This resulted in a different time course for each subject. All subjects walked at a speed of 3.5 mph. Jogging consisted of 3 minute stages of running at a speed expected to elicit an oxygen consumption approximately equal to 40% of their previously determined land $\dot{V}O_2peak$. Thereafter the treadmill speed was increased by 0.5 mph every 3 minutes until the subject met or exceeded 90% of their measured land $\dot{V}O_2peak$. A blood sample was drawn during the last minute of each stage.

Blood samples (5 ml) were immediately placed in pre-cooled EDTA-vacutainors and placed on ice. After the exercise test the blood sample was mixed and a small amount of whole blood was used to determine hematocrit (microhematocrit) and hemoglobin concentration (cyanomethemoglobin method). The remainder of the blood was centrifuged at 1500 xg for 15 min at 4°C. The plasma was immediately separated from the red cells and analyzed for lactate concentrations using a YSI 2300 lactate analyzer. Plasma protein concentration (refractometry in triplicate) was measured after plasma was refrigerated or put on ice. Lactate concentrations were expressed in $mM \bullet kg^{-1}H_2O$ after correcting for plasma solid concentration. Plasma solids were

determined with a regression equation of plasma protein concentration (refractometry) and plasma solid concentration (dry weight method) (11). During exercise, fluid shifts were determined from changes in plasma volume calculated from changes in hematocrit and hemoglobin (4) during the lactate threshold run.

During incremental exercise, an abrupt transition occurs in the rate of increase of blood lactate with increasing $\dot{V}O_2$. The transition point, identified as the lactate threshold, was determined using a log-log plotting of lactate concentrations ($mM \bullet kg^{-1}$ H_2O) and oxygen consumption (ml $O_2 \bullet min^{-1} \bullet kg^{-1}$ BW) (1). Least squares linear regression was used to define the linear relationship between plasma lactate and $\dot{V}O_2$ before and following the rapidly rising phase of plasma lactate. The intersection of these two linear lines was used to estimate lactate threshold.

The statistical analysis for the experimental design represented an ANOVA for repeated measures with main effects of treatment (land treadmill versus underwater treadmill running) and treadmill speed as repeated measures. We used SAS general linear models to determine main effects (treatment or speed) and any speed x treatment interactions. Post-hoc analysis consisted on a Tukey minimum significant difference test comparing treatment variables at similar treadmill speeds or $\dot{V}O_2$. Significance set at a *p* value of $p < 0.05$. Power calculations indicate that to detect a 10% shift in lactate threshold with a standard deviation of ≈ 7.5% would require 10-11 subjects.

RESULTS

The energy cost of running at speeds between 5.5 and 7.5 mph was similar for land and underwater treadmills (Figure 1). However, the energy cost of walking at 3.5

mph on the underwater treadmill was significantly higher (p <0.05) than on land. In addition, the heart rate response was similar for land and underwater treadmills at speeds between 5.5 and 7.5 mph. Heart rate at rest and during walking was higher on land than during HOI (Figure 1). HOI did not significantly alter the HR-$\dot{V}O_2$ relationship (Figure 2). Figure 1 also shows the percent change in plasma volume that occurs during exercise on land and HOI. During treadmill running on land, plasma volume decreased by 6.4 ± 4.0% at a speed of 7.5 mph. The decrease in plasma volume was significantly greater during HOI and averaged 18.7 ± 1.7% (p <0.05) at 7.5 mph.

Figure 3 illustrates the ventilatory response ($\dot{V}e$) and respiratory exchange ratio (RER) during land and HOI treadmill exercise. The $\dot{V}e$ response to graded exercise were very similar for land and HOI running except when at a running speed of 7.0 mph where $\dot{V}e$ was slightly elevated during HOI running (p <0.05). The RER was significantly greater (p <0.05) during HOI compared to land at speeds 3.5, 5.5, 6.0, 6.5, 7.0, 7.5 mph. The RER exceeded 0.95 for HOI running at speeds of 6-7.5 mph indicating a carbohydrate utilization of about 83%. In contrast, the RER during land treadmill running averaged only 0.91 indicating only about 69% carbohydrate utilization.

Table 1 compares hematocrit, hemoglobin concentration, and plasma protein concentration at rest and during various treadmill speeds for both land and water. To account for fluid shifts during treadmill running on land and in water, plasma lactate concentrations were expressed in mM per kg water. Resting plasma lactate while standing on the treadmill was similar on land (1.25 $\pm$ .10 mM• kg^{-1} H_2O) and water (1.10 $\pm$ .08 mM• kg^{-1} H_2O). Figure 3 illustrates the plasma lactate (mM• kg^{-1} H_2O) response to

increasing intensity on both land and underwater treadmills for six representative subjects. During running, plasma lactate increased ($p<0.05$) above resting when treadmill speed reached 5.5 mph for both land and water treadmill trials (Figure 4). The plasma lactate response to HOI running was elevated by 49 $\pm$ 29% at 5.5 mph compared to the land treadmill (Figure 4). Figure 3 shows an upward shift of plasma lactate (mM• kg^{-1} H_2O) at any given treadmill speed with significant differences between land and water treadmill running at speeds $\geq$ 5.5 mph ($p<0.05$). Table 2 lists individual lactate thresholds for all 11 subjects. The lactate threshold determined during running on the underwater treadmill (21.8 $\pm$ 1.6 mM• kg^{-1} H_2O) was lower ($p<0.05$) than that determined while running on the land treadmill (27.0 $\pm$ 1.6 mM• kg^{-1} H_2O). Figure 5 plots the mean plasma lactate concentration as a function of mean $\dot{V}O_2$ values and illustrates the leftward shift in the lactate threshold with running on the underwater treadmill.

DISCUSSION

The significant findings of this study are that plasma lactate concentration and RER were elevated during HOI running compared to running on a land treadmill at speeds between 5.5-7.5 mph. Furthermore, in every subject lactate threshold was shifted to the left (rise in plasma lactate occurred at a lower $\dot{V}O_2$) during HOI running compared to running on land. These differences in the lactate responses occurred in spite of our finding that the $\dot{V}O_2$ and heart rate response to HOI running at speeds between 5.5 and 7.5 mph was similar to that on land. These observations contrast those obtained during

HOI cycle ergometry exercise where plasma lactate concentration were lower during HOI cycling compared to land cycling (3).

During HOI cycling at maximal effort Connelly et al. (3) reported lower plasma lactate concentrations compared to land. The lower levels of lactate in the blood during HOI cycling were attributed to reduced lactate production presumably due to reduced plasma epinephrine and norepinephrine response to HOI cycling compared to land (3). The reduced catecholamine response during HOI cycling may have contributed to a decreased rate of glycogenolysis in the active skeletal muscles and thereby reduced lactate production. The cephalad shift in central blood volume during HOI would act to reduce the sympathetic nervous system response to graded exercise and limit the release of catecholamines from the adrenal medulla. The shift in fluid out of the vascular compartment during dynamic exercise will elicit homeostatic reflexes that will act to stabilize plasma volume and will include the sympathetic nervous system.

In our study, the shift of fluid out of the vascular compartment was limited (only 6.4 ± 4.0%) during treadmill running. In contrast, the ΔPV during HOI running peaked at 18.7± 1.7%. The change in plasma volume seen in our study is consistent with a study performed by Nagashima et al. (10). While there was a greater shift of fluid out of the vascular compartment during HOI compared to land the Hct, [Hb], and [PP] at 7.5 mph were similar for both HOI and land treadmill running. This is explained by the significant hemodilution that occurred during HOI prior to exercise. Nagashima et al. showed that the catecholamine response to HOI running was similar to that observed on

land (10). It appears that the difference in fluid shifts during HOI and land treadmill running are not critical in determining the catecholamine response to running.

To correct for the fluid shifts, we expressed the lactate concentrations in mM• kg^{-1} H_2O. Nagashima et al. (10) monitored plasma lactate concentrations during HOI running on a treadmill. At exercise intensities of 32, 49, 65, and 78% of their $\dot{V}O_2$ peak they reported a slightly higher plasma lactate during HOI treadmill running compared to land, however these differences were not statistically significant. In our study, the metabolic cost ($\dot{V}O_2$) and the HR response to HOI running was the same as running on land at speeds between 5.5-7.5 mph. However, the lactate threshold occurred at a lower $\dot{V}O_2$ during HOI running. A higher metabolic cost of exercise cannot account for the increase in plasma lactate seen during HOI running. We did not determine maximal aerobic capacity during HOI running because the underwater treadmill had an upper limit of 7.5 mph. However, similar peak $\dot{V}O_2$ measurements have been obtained during HOI and land cycling (6) . Thus, it is unlikely that a reduction in maximal aerobic capacity during HOI running could explain the leftward shift in lactate threshold. The increase in lactate production during HOI running may have resulted from an altered muscle use during HOI running. Gait analysis walking and running has identified several key differences in muscle use during HOI. Specifically, there is an increase use of hip flexor muscles and of the tibialis anterior during HOI while activity of the gastrocnemius and soleus muscles is reduced (7-9). Anecdotally, our subjects consistently reported fatigue of the tibialis anterior muscle group following running on the underwater treadmill but never following running on the land treadmill. The increase in RER seen during HOI

running compared to land (Figure 3) infers a shift toward more CHO utilization during exercise. While the metabolic cost ($\dot{V}O_2$) is the same during HOI and land treadmill running, it may be that few muscles are working at a higher intensity (higher RER) and thereby greater lactate formation.

In summary, $\dot{V}O_2$ and heart rate response to HOI running is similar to land but with a concomitant elevation in plasma lactate. As a tool for maintaining cardiovascular fitness or improving fitness, HOI running appears to provide a similar metabolic and cardiovascular stimulus without excessive weight-bearing. However, HOI produces a lower lactate threshold and higher concentration of plasma lactate at any given $\dot{V}O_2$. As such, HOI running may limit the duration of HOI training runs and possibly overall training stimulus.

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Table 1. Blood constituents during lactate threshold determination

	LAND TREADMILL			WATER TREADMILL		
Speed, mph	Hct%	[Hb]($g \cdot dl^{-1}$)	[PP]($g \cdot dl^{-1}$)	Hct%	[Hb]($g \cdot dl^{-1}$)	[PP]($g \cdot dl^{-1}$)
0	48.4 ± 0.9	16.5 ± 0.3	7.30 ± 0.2	45.3 ± 0.7*	15.5 ± 0.3*	6.40 ± 0.2*
3.5	48.6 ± 0.8	16.8 ± 0.3	7.30 ± 0.2	45.9 ± 0.7*	15.8 ± 0.3*	6.60 ± 0.2*
5.5	48.4 ± 0.6	16.5 ± 0.2	7.30 ± 0.1	47.9 ± 0.6†	16.4 ± 0.2†	7.00 ± 0.2†
6.0	49.0 ± 0.6	16.7 ± 0.3	7.40 ± 0.1	48.2 ± 0.7†	16.8 ± 0.3†	7.20 ± 0.1†
6.5	49.3 ± 0.7	16.9 ± 0.3	7.50 ± 0.1	48.9 ± 0.7†	17.1 ± 0.3†	7.40 ± 0.2†
7.0	49.5 ± 0.6	16.8 ± 0.3	7.60 ± 0.1	49.5 ± 0.6†	17.2 ± 0.4†	7.50 ± 0.2†
7.5	49.8 ± 0.5	16.9 ± 0.3	7.50 ± 0.2	50.2 ± 1.0†	17.6 ± 0.4†	7.60 ± 0.2†

Hct, hematocrit; [Hb], hemoglobin concentration; [PP], plasma protein concentration.

Values repesent Mean ± 1 SEM. N = 11 for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph.

* = $p < 0.05$ different from LAND TREADMILL. † $p < 0.05$ different from 0 mph

Table 2. Individual $\dot{V}O_2$ peak and lactate thresholds

Subject	$\dot{V}O_2$ max	LAND LT	WATER LT
1	57.4	27.5	15.1
2	60.2	30.2	24.0
3	60.0	30.9	26.3
4	56.9	31.6	27.5
5	52.6	28.8	24.0
6	56.0	27.5	24.3
7	54.4	19.3	16.6
8	46.7	24.0	16.6
9	45.7	27.9	24.0
10	43.9	15.8	13.8
11	60.0	33.0	27.5
N	11	11	11
MEAN	54.0	27.0	21.8*
SE	1.8	1.6	1.6

LAND LT = lactate threshold during land treadmill running.

WATER LT = lactate threshold during water treadmill running

Lactate thresholds estimated from the plasma lactate concentation in mM • kg^{-1} H_2O and expressed as $\dot{V}O_2$ in ml•min^{-1}• kg^{-1} Body Weight.

* p <0.05 different from LAND.

Figure Legends

Figure 1: Energy cost of walking (3.5 mph) and running (5.5 to 7.5 mph) on a treadmill on land and underwater (top). Changes in plasma volume (middle) and heart rate (bottom) as a function of treadmill speed Values represent Mean ± 1 SEM. N = 11 for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph. * p <0.05 water different from land. † p <0.05 different from 0 mph.

Figure 2: Relationship between heart rate and $\dot{V}O_2$ ($ml \bullet min^{-1} \bullet kg^{-1}$ B.W.) during treadmill running on land and during head-out water immersion. Values represent Mean ± 1 SEM. n = 11 for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph.

Figure 3: Relationship between rate of ventilation ($\dot{V}e$) and speed (top) and respiratory exchange ratio and speed (bottom) during treadmill running on land and during head-out water immersion (top). Values represent Mean ± 1 SEM. n = 11 for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph. * p <0.05 water different from land.

Figure 4: Representative data from six subjects illustrating the relationship between plasma lactate concentration ($mM \cdot kg\ H_2O^{-1}$) during walking (3.5 mph) and running on a treadmill on land and underwater.

Figure 5: Plasma lactate concentration ($mM \cdot kg\ H_2O^{-1}$) during walking (3.5 mph) and running on a treadmill on land and underwater. Values represent Mean ± 1 SEM. n = 11

for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph * p <0.05 water different from land. † p <0.05 different from 0 mph.

Figure 6: Mean plasma lactate concentration as a function average energy cost of walking (3.5 mph) and running on a treadmill on land and underwater. Values represent Mean ± 1 SEM. n = 11 for 0 to 6.0 mph, n =10 for 6.5 mph, n = 8 for 7.0 mph, and n =7 for 7.5 mph. Arrows represent average lactate threshold for each treatment group.

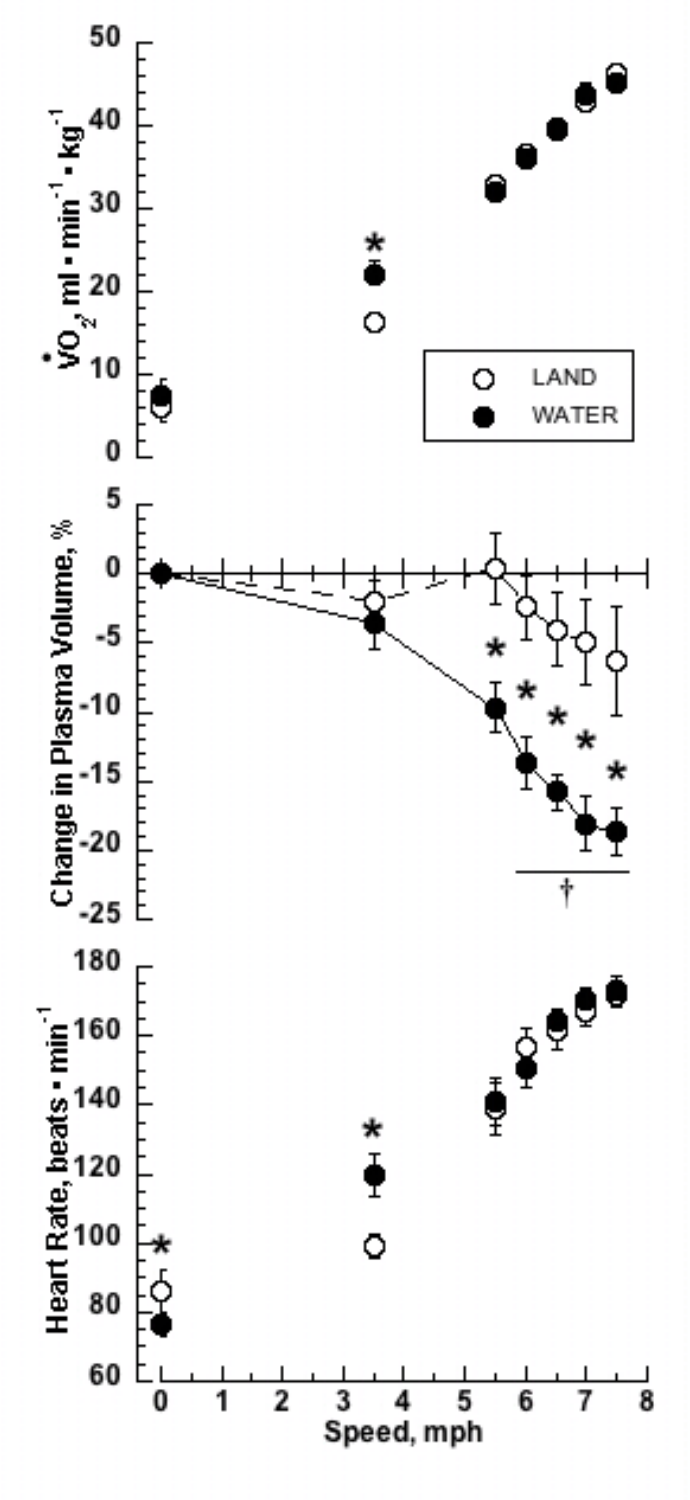
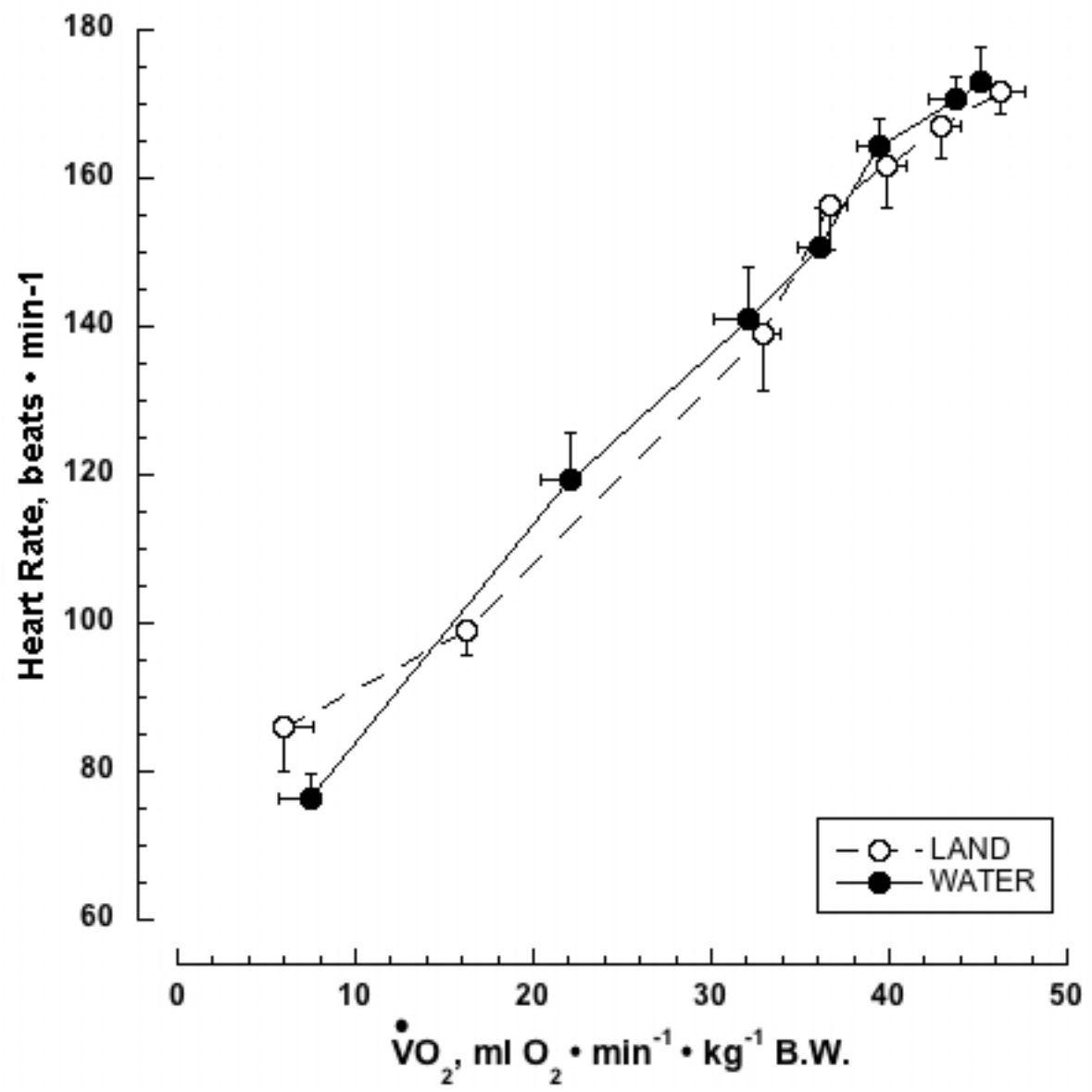


Figure 1.



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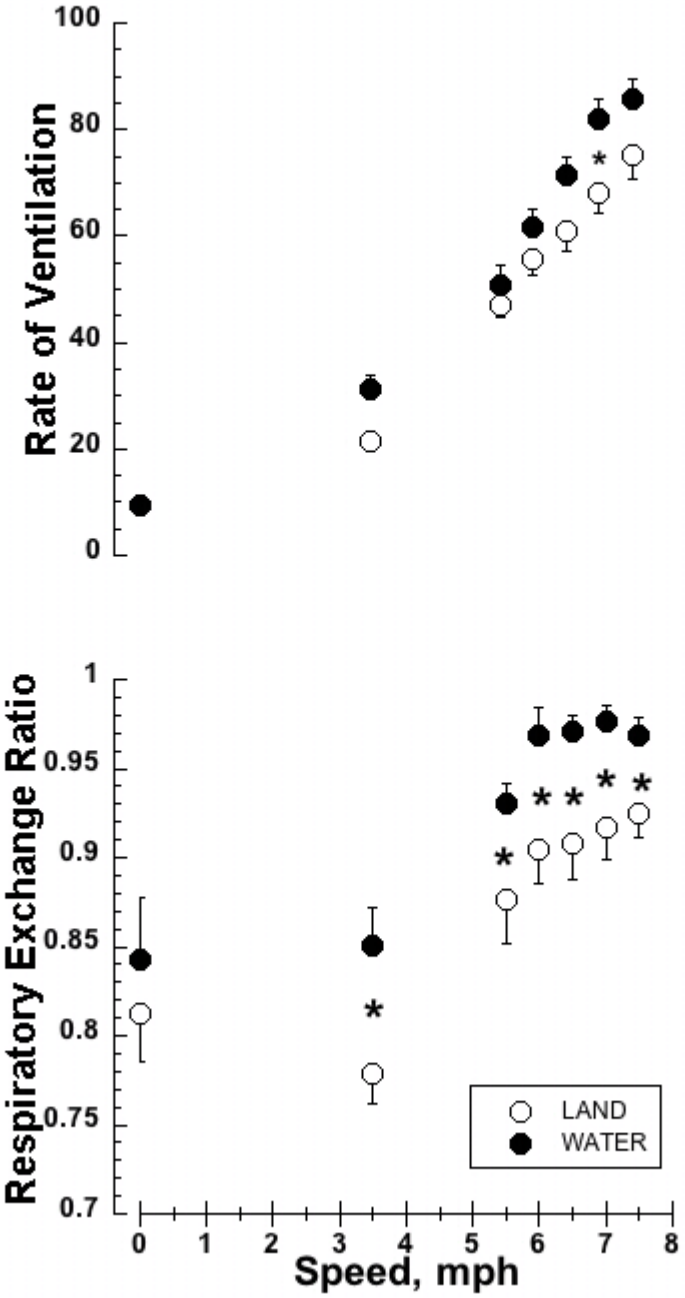

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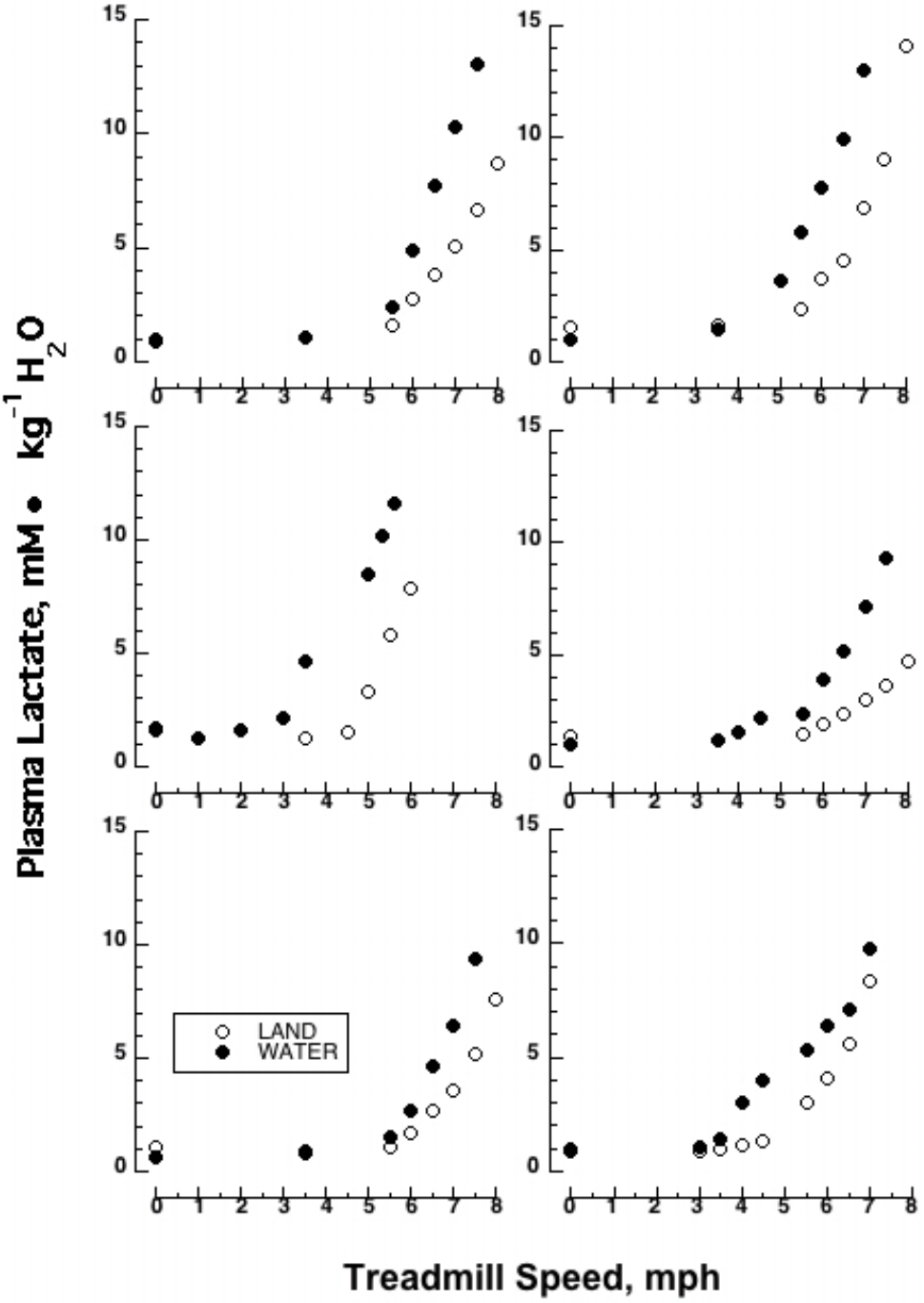

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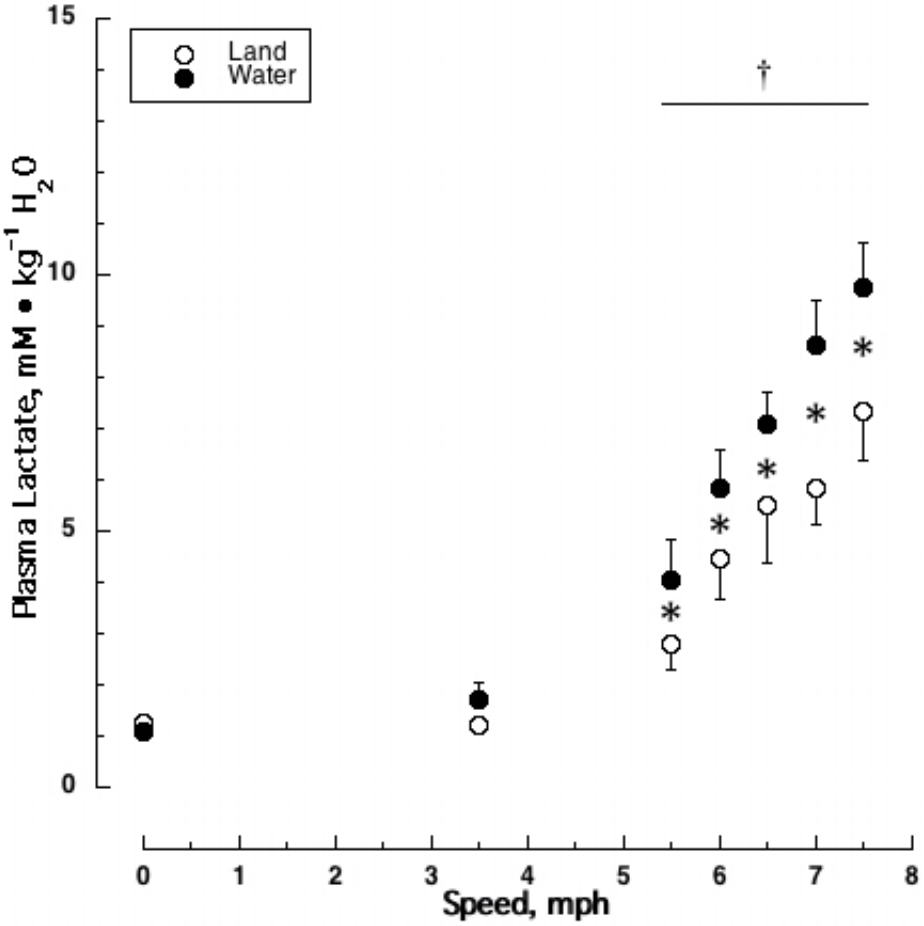

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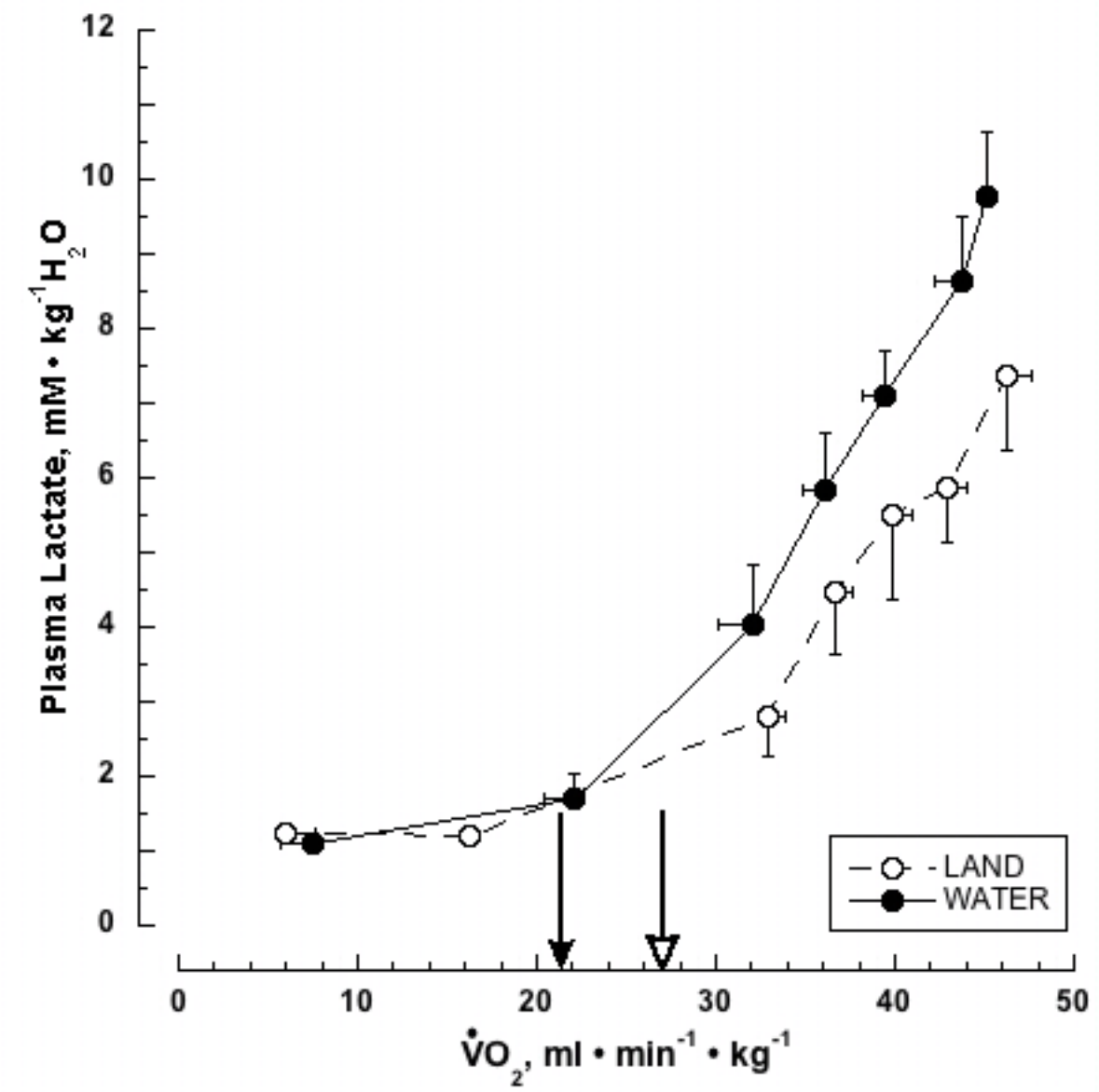

Figure 6.

Appendix A

Prospectus

Chapter 1

Introduction

Weighted impact on the legs generated from running on the ground can produce lower extremity injuries in areas such as the knee, ankle, and foot in long distance runners. The conventional mode of treatment for such injuries is discontinuance of training for 4 to 6 weeks (15). This period of inactivity can lead to significant loss of cardiovascular fitness (12) as well as muscular tone. Physiotherapists incorporate cardiovascular training elements into their therapeutic programs to combat these detraining effects on athletes (19). To maintain a $\dot{V}O_{2\,max}$ during rehabilitation, physiotherapists use modes of exercise such as cycling, swimming, walking, and cross-country skiing as cross-training exercises. The most widely used therapeutic alternative to running is cycling (15); however, while these supplemental modalities are designed to maintain cardiopulmonary fitness, many of their adaptations are not specific to the muscles used in running.

Exercising in water is a relatively new exercise mode for rehabilitation of injured runners. The properties associated with water, such as buoyancy and viscosity, make it an appealing exercise medium for rehabilitation of individuals with lower extremity injuries associated with running (37). More importantly, the low impact nature of running in water has increased interest in this exercise modality as a cross-training option for injured running athletes (17).

Eyestone et al. (15) compared the ability of deep water running, cycling, and land treadmill running to maintain $\dot{V}O_{2\,max}$ and 2-mile run performance over a 6-week

training period. The objective of this study was to determine if deep-water running would help minimize the decrease in $\dot{V}O_{2max}$ normally seen in athletes when exercise is stopped after a lower extremity injury. Runners were able to prevent the typical 16% decrement in $\dot{V}O_{2max}$ over 6 weeks of inactivity by deep water running (15). The ability of deep water running to prevent decrements in $\dot{V}O_{2max}$ was similar to that seen with cycling exercise. Eyestone et al. (15) concluded runners who cannot run because of injury can maintain $\dot{V}O_{2max}$ and 2-mile run performance with a rehabilitative intervention that includes either cycling or deep water running.

Recently, rehabilitation of lower extremity injuries has involved two distinct modes of water running: deep water running and shallow water running. Deep water running attempts to mimic the motion of running on the ground. The subject runs in motion in the deep end of the pool while wearing a floatation jacket. No contact with the bottom of the pool occurs at any time during deep-water running. Shallow water running allows foot contact on the ground while running in a pool with water at the level of the xyphoid process (36). Town and Bradley (33) compared the oxygen consumption differences ($\dot{V}O_{2max}$) of trained runners in shallow and deep water running to that of land treadmill running. $\dot{V}O_{2max}$ values measured during shallow water running and deep water running were 90.3% and 73.5%, respectively of their $\dot{V}O_{2max}$ measured during land treadmill running (33). One explanation for the 90.3% shared variance between shallow water and land treadmill running is the similarity of motion the two

different mediums entail. The "push-off phase" during shallow water and land treadmill running require similar movements that deep water running does not incorporate (33).

To provide the best possible training stimulus during shallow water running in the rehabilitative athlete we need a better understanding of the physiologic response to water running. Specifically, it is unknown how water immersion alters oxygen uptake kinetics, lactate threshold, and fuel utilization. These are key factors in optimizing adaptations to exercise training. One key adaptation with aerobic training is obtaining a rightward shift or increase in lactate threshold. This enables athletes to work at a higher percentage of their $\dot{V}O_{2max}$ without the accumulation of lactate. Recently, underwater treadmills have been introduced to training and rehabilitation facilities to incorporate underwater running in a smaller pool of water. To the best of our knowledge, no research has been published on the physiologic responses to underwater treadmill running. More specifically, the impact of treadmill running in water on the lactate threshold is unknown.

Assumptions

The relationship between treadmill speed and $\dot{V}O_2$ will be similar for treadmill running in water and treadmill running on land.

Hypothesis

The lactate threshold will occur at a higher $\dot{V}O_2$ % during treadmill running in water compared to treadmill running on land.

Null hypothesis

There is no significant difference between lactate threshold determined during treadmill running in water and on land.

Delimitations

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

Limitations

Due to speed limitations of the underwater treadmill, we will not be able to evaluate individuals with a maximal aerobic capacity of > 60 ml • min^{-1} • kg^{-1} body mass.

Significance of this study

Results from this study will clarify the lactate response to underwater treadmill running compared to running on a land treadmill. The running motion is utilized in most sports and injuries occur a lot due to the constant impact on the joints. Therefore, the purpose of this study is to show that water treadmill running provides a therapeutic and rehabilitative alternative to land treadmill running. Previous studies have been performed in deep and shallow water pools. No studies have reported the physiologic responses to underwater treadmill running. A better understanding of the lactate threshold response to underwater treadmill running will help athletes and trainers know the intensity needed to exercise to elicit the same 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 underwater treadmill running.

Chapter 2

Review of Literature

Water Immersion Physiology

The cardiovascular and metabolic responses to large muscle mass dynamic exercise differ on land compared to being immersed in water. While immersed, the body is subjected to hydrostatic pressure which increases venous return to the heart (28). There is a shift of blood volume from peripheral to more central regions of the body that increases intrathoracic pressure and decreases the venous capacitance in the body (9, 28, 38). Water makes the submerged part of the body weightless which eliminates the gravitational influence on blood distribution and decreases the impact on the joints when running. The cephalad redistribution of blood volume in the immersed body elicits changes in cardiovascular and metabolic parameters at rest and during dynamic exercise.

Compared to land tests, cardiac output ($\dot{Q}$ = SV x HR) at rest and during dynamic exercise is higher during head-out water immersion (HOI) (9, 28, 29). HOI produces a slightly lower resting heart rate compared to resting on land suggesting the increase in cardiac output at rest is due mostly to the increase in stroke volume (9, 10, 28, 29). The higher stroke volume (SV) during HOI is due to the greater preload from increased venous return (9, 29). Yun et al. (38) found a marked (50%) increase in cardiac output when comparing HOI at rest to resting on land. In their study they compared female divers and non divers' cardiovascular response while performing leg cycle exercise of moderated intensity in water. At rest, cardiac output was increased in all their subjects during HOI due to an increase in SV, with no significant change in heart rate or arterial

blood pressure compared to rest on land. During HOI exercise increased cardiac output is mainly due to a rise in heart rate (38).

Stroke volume is determined by three factors: preload, afterload, and cardiac contractility. At rest it is apparent that an increase in ventricular diastolic filling is facilitated by the shift of blood from peripheral vessels to the central regions of the body during HOI. The increase in cardiac preload increases the stroke volume by the Frank-Starling mechanism (9, 28). Christie et al. (9) performed upright cycling exercise on land and in water at work loads corresponding to 40, 60, 80, and 100% maximal oxygen consumption. Right atrial pressure, pulmonary arterial pressure, and cardiac index were measured and left ventricular end diastolic and systolic volume indexes were assessed. They concluded that water immersion alters cardiac performance at rest and during graded dynamic exercise and preload remains elevated during graded exercise to maximal effort.

Cardiac afterload does not have a significant effect on the change of stroke volume during HOI (1, 2, 13, 28). Contractility of the heart also does not seem to play a role in the increase in stroke volume at rest during HOI. Connelly et al. (10) showed that increased central blood volume during HOI reduced plasma catecholamine levels. An increase in central blood volume stimulates baroreceptors which act to decrease sympathetic nerve activity. Plasma norepinephrine concentrations were significantly reduced in high and maximal intensity exercise during HOI compared to land treadmill running. Plasma epinephrine levels were significantly reduced during maximal intensity exercise. Connelly et al. (10) concluded that water immersion alters the sympathoadrenal

response to graded leg cycle exercise. Considering the major factors affecting cardiac performance, the increased preload resulting from cephalic shift of circulating blood is mainly responsible for the increased stroke volume during rest and exercise during HOI.

Connelly et al. (10) found that plasma lactate concentration was also significantly reduced during maximal effort during HOI. They concluded that the observed decrease in plasma lactate concentration could be due to increased aerobic metabolism and improved lactic acid clearance due to increased skeletal muscle blood flow (32). However, Fragnolias and Rhodes (16) reported no differences in lactate concentrations 30 seconds after the onset of maximal exercise and 5 minutes post exercise. In contrast, Svendenhag and Seger (32) reported higher levels of plasma lactate in individuals exercising in water. Submaximal and maximal deep water and land treadmill tests were performed first and then subjects ran at four different specified submaximal loads while oxygen uptake, heart rates, perceived exertion, and blood lactate were measured. The water running tests in their study were performed wearing a buoyant vest in deep water and no contact on the floor of the pool was allowed. The blood lactate curves were shifted to the left in the deep water running compared to running on a treadmill on land, implying higher blood lactate levels expressed relative to $\dot{V}O_{2\,max}$ and to a percent $\dot{V}O_2$ at submaximal and maximal exercise intensities (32). Discrepancies in these studies may be attributed to lack of familiarity with water running and variability of types of underwater test such as deep versus HOI water immersion tests.

Metabolite: Lactic Acid

The circulatory system transports oxygen to skeletal muscle and removes waste products via the blood. If the local circulation and oxygen delivery to active skeletal muscle are adequate, then the energy requirement of skeletal muscle may be met by aerobic metabolism. During exercise when the metabolic demand for adenosine triphosphate (ATP) exceeds the rate at which ATP can be provided by oxidative metabolism, ATP production by anaerobic glycolysis is enhanced and consequently lactic acid is produced at a higher rate (6, 35).

Lactic acid is produced in muscle cells when the NADH+ H^+ formed after glycolysis is oxidized to NAD^+ by a transfer of the hydrogen ions to pyruvic acid. Pyruvic acid in turn is reduced to lactic acid by the enzyme lactate dehydrogenase (6). When production exceeds removal, lactate accumulates in the blood. While skeletal muscle is contracting, lactate is always being produced. Because the concentration of lactate does not change appreciably at low work rates, the rate of lactate degradation is also assumed to exceed production (6, 21, 26). Once produced, lactate is moved from lactate-producing to lactate-consuming sites by means of intra- and extracellular lactate shuttles in the blood. Transport via these shuttles occurs by facilitated exchange down concentration and H^+ ion gradients utilizing monocarboxylate transporters (7). Lactate in the bloodstream can be transported to the liver where it is either converted into glucose via gluconeogenesis or converted to glycogen through glyconeogenesis (8). Other ways lactate can be removed from the bloodstream is through oxidation in cardiac tissue, type I skeletal muscle fibers and in inactive skeletal muscle. Lactate produced as a result of

anaerobic glycolysis is mostly removed by oxidation (about 75%) mainly in active slow-twitch muscle fibers (7). Mitochondrial oxidation of lactate via the intracellular lactate shuttle in the cell is favored probably because of the proximity of lactate to the Tricarboxylic Acid Cycle (TCA) (7).

During high intensity, short duration activities, there is an increased recruitment of fast-twitch muscle fibers which usually results in increased lactate production. Aerobic, slow-twitch muscle fibers are also capable of producing lactate, but it is usually oxidized within the mitochondria during lower intensity activities. The presence of lactate reflects the use of the anaerobic glycolytic pathway for ATP production and the balance between glycolytic and mitochondrial activity.

Lactate produced by skeletal muscles can be utilized by other muscles by means of the extracellular lactate shuttle, as well as tissues and organs via oxidative metabolism for their energetic needs. Lactic acidosis in muscle has been noted to facilitate oxygen-hemoglobin dissociation and therefore increase oxygen extraction while preserving the oxygen pressure gradient from capillary to mitochondria (18, 31). A study performed by Grassi et al. (18) measured the relationship between blood lactate accumulation and muscle deoxygenation during incremental exercise. Near-infrared spectroscopy (NIRS) was used to monitor the saturation of hemoglobin and myoglobin during exercise. There was a significant correlation between the onset of blood lactate accumulation and the onset of muscle deoxygenation. Muscle deoxygenation could be attributed to an accelerated fall of capillary-venular Po_2, occurring in association with the appearance of lactate in blood (18).

Lactic acid formation at any given work intensity is exaggerated, but not dictated, by tissue hypoxia (25). A direct linear relationship exists between the rate of blood lactate disposal and the metabolic rate ($\dot{V}O_2$). As exercise intensity increases, the turnover rate of lactate oxidation increases proportionally up to an exercise intensity of 75% the $\dot{V}O_{2\,max}$ (26).

Lactate Threshold

Beaver et al. (3) reported that lactate accumulates in the blood as a threshold phenomenon when working at high intensities. This lactate threshold has also been coined as the anaerobic threshold studied by Wasserman et al. (35) which implies the onset of increased anaerobic glycolysis. The anaerobic threshold is identified by a specific exercise intensity of workload or $\dot{V}O_{2\,max}$ above which blood lactate levels rise and minute ventilation increases disproportionately in relation to oxygen consumption (35). Lactate accumulation is attributed to the failure of the cardiovascular system to supply the oxygen required to the muscle tissue. The rise in minute ventilation is ascribed to the increase in carbon dioxide resulting from the buffering of the lactic acid (34). Exercise above the anaerobic threshold results in altered oxygen uptake kinetics, with a delay in the oxygen uptake steady state time and an increase in the oxygen deficit and debt (35).

Endurance training has an effect on glucose and lactate turnover during rest and exercise. By using ^{14}C and ^{3}H tracers Donovan and Brooks (14) saw lower lactate levels during both easy and hard exercises in trained animals compared to untrained. Training doesn't alter the production or turnover rate of lactate; it increases the clearance rate from

the blood. Mazzeo et al. (27) demonstrated that training reduces blood lactate during given levels of submaximal exercise by improving the body's capacity for lactate clearance. Increased capacity of oxidation (5) and gluconeogenesis (4) in trained endurance athletes explains the increased clearance rates seen by Brooks et al. (8). Intense endurance exercise training induces adaptations that result in a lower blood lactate level during exercise requiring the same percentage of $\dot{V}O_{2\,max}$. Athlete's $\dot{V}O_{2\,max}$ increases during endurance training as a result of increased stroke volume as described by the Fick equation:

$$\dot{V}O_{2\,max} = HR_{max} \bullet SV_{max} \bullet (Ca - C\bar{v})O_2 diff_{max}$$

Where, Ca is the content of arterial oxygen and $C\bar{v}$ is the content of venous oxygen. As a consequence, the same work rate represents lower relative exercise intensity and a lower blood lactate concentration after training. Hurley et al. (22) suspect the smaller increase in lactate concentration at the same absolute work rate after training could be entirely due to the adaptations responsible for the increase in $\dot{V}O_{2\,max}$.

Increasing the fraction of inspired oxygen results in decreased lactate concentrations in blood and working muscle. Katz and Sahlin (24) demonstrated that lactate accumulation during submaximal exercise will occur while oxygen is present; however, when oxygen is limited the rate of lactate production is augmented. While the absence of oxygen will cause acceleration in glycolysis and increased lactate production, it does not mean lactate formation only occurs in anoxic cells. Lactate production occurs

in fully oxygenated contracting muscles with mitochondrial electron transport unrestricted by lack of oxygen (11, 23, 30).

The central shift in venous blood flow during HOI increases cardiac preload (19, 20, 28, 29). These increases might account for the assumed increase in lactate threshold during underwater running. The increased cardiac output may increase blood flow to skeletal muscle and result in an increase of oxygen deliver, which would reduce lactate accumulation within the cell. This in turn might lead to an increased lactate threshold while performing HOI running exercise.

Chapter 3

Methods

Subjects

Ten college-aged (18-33 yrs old) males 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 between 40 and 60 ml $O_2 \bullet min^{-1} \bullet kg^{-1}$ body mass
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
5. Body fat not to exceed 25%

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

Subject Preparation

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 following a 60 min post-ingestion period. Following verification of proper hydration the subjects' height and weight will be measured and the subjects will be instrumented with a polar heart rate monitor.

Experimental Design

The proposed project consists of three days of testing.

1. Day 1 (30 min) consists of the determination of $\dot{V}O_{2\,max}$. If the subject's $\dot{V}O_{2\,max}$ exceeds 61 ml O_2 • min^{-1} • kg^{-1} body mass no further test will be administered and the individual will be excluded from the study. The exclusion criterion is dictated by the maximal speed of the underwater treadmill. If the subjects $\dot{V}O_{2\,max}$ exceeds this defined criteria it is likely that we will not be able to define the lactate threshold while running in water.
2. Days 2 and 3 (90 minutes each day) consist of determination of lactate threshold while running on a land treadmill and on an underwater treadmill.

Day 1: *Maximal Aerobic Capacity.*

Subjects will first have a USG sample of their urine to validate hydration. Body fat will be measured using a 3-site skinfold measurement. A $\dot{V}O_{2\,max}$ test will be initially performed on a land treadmill to determine the aerobic capacity of each of the subjects. The 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 their normal running velocity. Then the grade will be increased 2.5% every 2 min until the treadmill's maximal grade of 15% is reached. If needed, additional stages will be accomplished by increasing treadmill speed by 0.5 mph per stage (see Table 1). We anticipate that the selected speed will range between 6.5 and 8 miles per hour (an expected oxygen cost of 32-40 ml O_2 • min^{-1} • kg^{-1} body mass). The subjects will continue to exercise until the point of

volitional fatigue. During the exercise test, oxygen consumption will be monitored every 15 sec using a ParvoMedics TrueOne (ParVo Medics, Inc., Sandy, UT) metabolic cart. Just 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 metabolic cart will be performed before each exercise and verified immediately following each test. Flowmeter 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.

If the subjects meet the criteria with a $\dot{V}O_{2\,max}$ between 40 and 60 ml $O_2 \bullet min^{-1} \bullet kg^{-1}$, they will be taken to the underwater treadmill where they will be familiarized with running on a treadmill underwater. When on the underwater treadmill, subjects will be fitted with running shoes designed specifically for running underwater.

Days 2 and 3: *Lactate threshold testing.*

Each subject's lactate threshold will be determined during treadmill running on land and in water. The two exercise tests will be completed in a random cross over design.

An 18 gauge catheter will be placed in a large vein of the forearm of the subject by a well-trained technician. The subject will then stand on the treadmill 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, the subject will 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 metabolic cart.

The lactate threshold protocol will be performed entirely at 0% grade on the treadmill. The subject will start by walking at 3.5 mph 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 elicit an oxygen consumption approximately equal to 40% of their previously determined $\dot{V}O_{2\,max}$. This selected 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 mph/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 3.5 mph to allow the subject to recover.

During the lactate threshold trial the catheter will be flushed with sterile saline solution frequently to prevent clotting. Blood samples (3-5 ml) will be immediately placed in cooled EDTA-vacutainors. After the exercise test the blood sample will be well mixed and a small amount of whole blood will be used to 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 and analyzed for lactate concentrations using a YSI 2300 lactate analyzer. This protocol will be similar during exercise tests performed on land and underwater treadmills.

Water temperature where the underwater treadmill is found is 30.6 degrees Celsius. Placement of the water on the subject when immersed will be around their

xyphoid process. When running on the treadmill, the subject's arms will be able to be in the water and swing in the same movement as if running on a land treadmill. Because the maximal speed of the water treadmill is 7.5 mph, once the subject reaches its max, jets will be turned on at 20% of maximal flow for the next stage (see Table 3).

Analysis of lactate threshold will be assessed from the samples drawn during the study. Lactate increases with increasing oxygen uptake ($\dot{V}O_{2\,max}$) during incremental exercise. 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. During incremental exercise, an abrupt transition occurs in the rate of increase of blood lactate with increasing $\dot{V}O_{2\,max}$. The transition is defined in the log-log model via a mathematical model that can define the location of this transition. Error effects are minimized using this method by using the least squares curve-fitting procedure. The functional relationship can be expressed mathematically as the power law:

$$[La^-] = [La^-]_0 \text{ x } \left(\frac{\dot{V}O_2}{\dot{V}O_2 o} \right)^b$$

where $[La^-]$ is the lactate concentration at any time t, $[La^-]_0$ is the lactate concentration at the threshold, $\dot{V}O_2$ is the oxygen uptake, $\dot{V}O_{2\,0}$ is the $\dot{V}O_2$ at the threshold, and ^{b} is the slope of the regression line (3). We will use hematocrit and hemoglobin samples at each stage to measure the change in plasma volume that occurs in the blood. To account for the fluid shift in the blood, we will measure lactate concentrations in mM·kg H_2O^-1. Plasma protein concentrations will be measured with a refractometer. Analysis of lactate

data will include comparison of speed to plasma lactate concentration in mM·kg H_2O^{-1}, and % $\dot{V}O_2$ based on the land treadmill speed versus lactate concentration.

Data Analysis

The experimental design is a simple balanced ANOVA for repeated measures with main effects of treatment (land treadmill versus underwater treadmill running) as repeated measures. This design allows each subject to act as their own control. Major variables measured and used for analysis will include: $\dot{V}O_2$, heart rate, rate of perceived exertion, lactate concentration, and LT expressed as a percent of maximal oxygen consumption. Variables will be measured for each day of the study and analyzed to form conclusions. The analysis will be performed using SAS general linear model analysis with the level of significance set at a p-value of $p<0.05$.

Power calculation

The number of subjects required to detect a 10% change in the lactate threshold was estimated from the following equation assuming two treatment conditions (a=2) and a standard deviation for the measurement of lactate threshold of 7.5%.

$$n \geq 2\left(\frac{\sigma}{\delta}\right)^2 \left\{ t_{\alpha[\upsilon]} + t_{2(1-P)[\upsilon]} \right\}^2$$

$$a = 2$$

$$n = 10$$

$$\upsilon = a(n-1) = 2(10-1) = 18$$

$$\sigma = \text{standard deviation of the measurement, \%}$$

$$\delta = 10\%$$

$$\alpha = 0.05$$

$$P = 0.8$$

By substitution:

$$n \geq 2(\frac{\sigma}{\delta})^2 \left\{ t_{\alpha[\upsilon]} + t_{2(1-P)[\upsilon]} \right\}^2$$

$$n \geq 2(\frac{\sigma}{\delta})^2 \left\{ t_{.05[18]} + t_{.4[18]} \right\}^2$$

$$n \geq 2(\frac{7.5}{10})^2 \{2.101 + .862\}^2$$

$$n \geq 9.9$$

Based upon this equation we would expect to measure a 10% shift in lactate threshold with 10 subjects.

Table 1. *Test protocol example with self-selected speed of 7 mph*

Stage	Time	MPH	Grade
1	Resting	standing	0
2	0-5 min	3.5 to 7*	0
3	5-7 min	7	2.5
4	7-9 min	7	5.0
5	9-11 min	7	7.5
6	11-13min	7	10
7	13-15min	7	12.5
8	15-17min	7	15.0
9	17-19min	7.5	15.0
10	19-21min	8.0	15.0

* self selected speed during warmup

Table 2. *Lactate Threshold Test Land Treadmill Protocol:*

Stage	Time	MPH	Sample#
1	Resting	standing	1
2	1-5 min	3.5	2
3	3-6 min	5.5	3
4	6-9 min	6.0	4
5	9-12min	6.5	5
6	12-15min	7.0	6
7	15-18min	7.5	7
8	18-21min	8.0	8
9	21-24min	8.5	9
10	24-27min	9.0	10
11	27-30min	9.5	11

Table 3. *Lactate Threshold Test Protocol for Underwater Treadmill:*

Stage	Time	MPH	Jet	Sample#
1	Resting	standing	0%	1
2	1-5 min	3.5	0%	2
3	3-6 min	5.5	0%	3
4	6-9 min	6.0	0%	4
5	9-12min	6.5	0%	5
6	12-15min	7.0	0%	6
7	15-18min	7.5	0%	7
8	18-21min	7.5	20%	8
9	21-24min	7.5	30%	9
10	24-27min	7.5	40%	10
11	27-30min	7.5	50%	11

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