INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.
Williams, Donna Ann

RELATIONSHIP BETWEEN PLASMA VOLUME AND BLOOD LACTATE DURING EXERCISE FOLLOWING SIMULATED WEIGHTLESSNESS

The University of Arizona

M.S. 1985

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106

Copyright 1985 by Williams, Donna Ann

All Rights Reserved
RELATIONSHIP BETWEEN PLASMA VOLUME AND BLOOD LACTATE 
DURING EXERCISE FOLLOWING SIMULATED WEIGHTLESSNESS 

by 
Donna Ann Williams 

A Thesis Submitted to the Faculty of the 
COMMITTEE ON ANIMAL PHYSIOLOGY (GRADUATE) 
In partial Fulfillment of the Requirements 
For the Degree of 
MASTER OF SCIENCE 
WITH A CONCENTRATION IN EXERCISE PHYSIOLOGY 

In the Graduate College 
THE UNIVERSITY OF ARIZONA 

1 9 8 5 

Copyright 1985 Donna Ann Williams
STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

SIGNED: Donna J. Williams

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Victor A. Convertino
V. A. Convertino
Associate Professor of Physical Education
DEDICATION

To my mother who lives love, my father who lives persistence and humility, my brother and sister-in-law who live simultaneous togetherness and independence, and my grandparents who live courage. All these things, they teach me each day, and to them I am indebted.
ACKNOWLEDGMENTS

Each of us has at least one special gift to pass on to others and by giving, we find ourselves personally fulfilled. Some of us must search for our gift and the journey provides opportunity to interact with very special people. Victor A. Convertino knows what he has to give and the world is fortunate he has dedicated his time to science and the pursuit of truth in physiology. I would like to thank Dr. Convertino for allowing our paths to cross at a timely moment in my quest. He patiently instructed laboratory and scientific writing skills as he molded a student and planted the seed of inquiry. During the thesis process, I found Dr. Convertino inspiring in his tireless attention to details, exceptional as a scientist role model, and a gifted communicator. I hope the educational process we shared and product we created brought him satisfaction, because he allowed me to come closer to discovering the special gift I may have to give.

Those individuals receiving no formal recognition for this thesis deserve the most applause. Each of these individuals selflessly gave of their time, patience, and understanding. Silently standing in the wings watching a process heavily steeped in tradition, they offered tireless support.
I am especially grateful to Margaret Wade for her never-ending patience and gentle prodding. Her comments, criticisms, proof reading, and moral support were indispensible and, although receiving no academic credit for this thesis, I know she took much pride in the finished product. Selfless giving was Margaret's special quality and I greatly appreciated her kindness in assisting me to complete this project.

Ten subjects and the staff at NASA-Ames Research Center, captained by Dee O'Hara, must not be forgotten. Their devotion and pride in a job well done was a lesson in professionalism that I will always treasure.

To Bess Maxwell and Tom Sather a heart-felt thanks for kind words along the way. As Ph.D. students working in the world of science, Bess and Tom were exceptional role models, striving for excellence, but careful not to let status nor ego mar their interactions with those around them. An additional special thank you to Bess, who shared unselfishly of herself and her talents and assisted me at critical points through the writing process. I am grateful for the time spent, advice given, and listening ear.

I am also grateful to Nancy Tkacz for caring support and wisdom that essentially guided me through my Master's Degree. From the state of Maine, Nancy counseled, spread humor, and kept my perspective clear and I greatly valued her understanding.
A special thanks to Chris Kirby, Rhonda Bean, Barbara Convertino, Marie Pease, and Scott Kinser who each played a different, but critical role in this thesis. I am thankful for their friendship and marvel at the special gifts they shared with me.

Finally, thank you to Joseph Pechinski, Ph.D. for pointing the way to the University of Arizona. His influence in my life has been considerable and I have appreciated his guidance.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>Physiological Adaptations to Simulated Weightlessness</td>
<td>5</td>
</tr>
<tr>
<td>Exercise Responses</td>
<td>5</td>
</tr>
<tr>
<td>Muscular Adaptation</td>
<td>6</td>
</tr>
<tr>
<td>Blood Lactate</td>
<td>8</td>
</tr>
<tr>
<td>Exercise Responses</td>
<td>8</td>
</tr>
<tr>
<td>Indirect Measurement of Blood Lactate via Gas Exchange</td>
<td>10</td>
</tr>
<tr>
<td>Direct Measurement of Blood Lactate via Blood Analysis</td>
<td>13</td>
</tr>
<tr>
<td>Body Fluid Adaptation to Simulated Weightlessness</td>
<td>14</td>
</tr>
<tr>
<td>Summary and Approach</td>
<td>17</td>
</tr>
<tr>
<td>Hypothesis</td>
<td>17</td>
</tr>
<tr>
<td>Null Hypothesis</td>
<td>17</td>
</tr>
<tr>
<td>3. METHODS</td>
<td>19</td>
</tr>
<tr>
<td>Subject Characteristics</td>
<td>19</td>
</tr>
<tr>
<td>Restrictions and Controls</td>
<td>19</td>
</tr>
<tr>
<td>Treatment</td>
<td>20</td>
</tr>
<tr>
<td>Exercise Test Protocols</td>
<td>21</td>
</tr>
<tr>
<td>Maximal Exercise Test</td>
<td>21</td>
</tr>
<tr>
<td>Submaximal Exercise Test</td>
<td>22</td>
</tr>
<tr>
<td>Body Composition</td>
<td>23</td>
</tr>
<tr>
<td>Calculations of Body Density and Percent Body Fat</td>
<td>23</td>
</tr>
<tr>
<td>Measurement of Respiratory Gas Exchange</td>
<td>23</td>
</tr>
<tr>
<td>Estimate of Blood Lactate Onset</td>
<td>24</td>
</tr>
<tr>
<td>Plasma Volume Determination</td>
<td>25</td>
</tr>
<tr>
<td>Plasma Volume Analysis</td>
<td>25</td>
</tr>
<tr>
<td>Plasma, Blood, and Red Cell Volumes Calculations</td>
<td>28</td>
</tr>
<tr>
<td>Blood Sample Collection</td>
<td>29</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS--Continued

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Lactate Analysis</td>
<td>30</td>
</tr>
<tr>
<td>Blood Lactate Concentration and Content Calculations</td>
<td>32</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>33</td>
</tr>
<tr>
<td>4. MANUSCRIPT</td>
<td>34</td>
</tr>
<tr>
<td>Abstract</td>
<td>34</td>
</tr>
<tr>
<td>Introduction</td>
<td>36</td>
</tr>
<tr>
<td>Methods</td>
<td>37</td>
</tr>
<tr>
<td>Results</td>
<td>42</td>
</tr>
<tr>
<td>Maximal Exercise Test</td>
<td>42</td>
</tr>
<tr>
<td>Submaximal Exercise Test</td>
<td>43</td>
</tr>
<tr>
<td>Body Composition</td>
<td>43</td>
</tr>
<tr>
<td>Plasma Volume</td>
<td>44</td>
</tr>
<tr>
<td>Blood Lactate Concentration and Content</td>
<td>44</td>
</tr>
<tr>
<td>Discussion</td>
<td>50</td>
</tr>
<tr>
<td>LIST OF REFERENCES</td>
<td>57</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blood Lactate Concentration at Rest and During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest</td>
<td>48</td>
</tr>
<tr>
<td>2.</td>
<td>Blood Lactate Content at Rest and During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest</td>
<td>49</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Changes in Plasma, Blood, Red Cell Volume and Maximal Work Capacity During Supine Cycling Exercise Pre- and Post-Bedrest</td>
<td>46</td>
</tr>
<tr>
<td>2. Changes in Respiratory Gas Exchange and Heart Rate During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest</td>
<td>47</td>
</tr>
</tbody>
</table>
ABSTRACT

Since a previously reported decrease in the ventilatory threshold following bedrest-induced deconditioning was related to a reduction in plasma volume (PV), this study was undertaken to determine the relationship between changes in PV and blood lactate (BL) during exercise following simulated weightlessness. Ten healthy men (35-49 yr) performed a submaximal upright cycle ergometer test consisting of pedaling at 60 rpm for 15 min at a workrate of 630 ± 30 kgm/min (57 ± 1.6% peak \( \dot{V}O_2 \)) before (pre) and after (post) 10 days of continuous -6° headdown bedrest (BR). During exercise, oxygen uptake (\( \dot{V}O_2 \)) was measured to assure equivalent energy expenditure for the pre- and post-BR workrate. Antecubital venous blood samples were collected at rest and during the last 30 sec of exercise and analyzed for BL concentration and hematocrit. Resting PV was measured pre- and post-BR with Evans blue dye and total circulating BL content was calculated as the product of PV and BL concentration. Submaximal \( \dot{V}O_2 \) was unchanged following BR. Post-BR exercise BL concentration of 2.9 mmol/l was greater (p<0.05) than the pre-BR BL concentration of 2.5 mmol/l. However, as a result of a 17% reduction (p<0.05) in resting PV, total circulating BL content during exercise at the same absolute workrate was
unchanged following BR. These data demonstrate that the increase in BL concentration during exercise following the deconditioning effects of simulated weightlessness cannot be completely explained by changes in lactate metabolism but are dependent on the reduction in PV.
CHAPTER 1

INTRODUCTION

Human physiological, morphological, and biochemical adaptations to the stress of hypokinesia have been addressed in the literature using many experimental models and perturbations. Antiorthostatic (-6° headdown) bedrest has been used frequently as an effective simulation of chronic cardiovascular adjustment to weightlessness, while exercise performed before and after bedrest has been used to investigate specific physiologic adaptations. The intent of the present study was to determine the effect of plasma volume change following simulated weightlessness in relation to the specific blood lactate response to acute exercise.

Increased blood lactate levels may occur when performing exercise of moderate to heavy intensity and accumulation of blood lactate is specifically related to the rate at which the working muscle can oxidize pyruvate. Appearance of blood lactate is dependent on oxygen supply, fiber recruitment, enzyme activity and lactate clearance capacity of the muscles involved. Recent literature has suggested that bedrest deconditioning may result in adaptation of many of these mechanisms. A decrease in oxygen uptake has also been observed before compared to
after bedrest-induced deconditioning when subjects performed at equal workrates during a maximal exercise test. This relationship between decreased oxygen supply and appearance of blood lactate provides evidence of a possible increased dependence on anaerobic metabolism for energy supply to the working muscle following simulated weightlessness.

Elevated blood lactate levels are an index of a shift from aerobic to more anaerobic energy exchange during muscular work. During exercise direct measurement of blood lactate onset has been correlated with gas exchange indices such as the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) and respiratory exchange ratio ($R$). Using non-invasive indicators of blood lactate accumulation, acute exercise respiratory gas exchange responses are increased before compared to after prolonged bedrest suggesting an earlier onset of blood lactate accumulation and subsequently increased anaerobic metabolism in the working muscle.

Prolonged bedrest-deconditioning causes dehydration in human subjects. This observed fluid loss results in decreased plasma volume and subsequent decline in blood volume. Experiments involving simulated weightlessness (-6° headdown position) have demonstrated a diuresis associated with this plasma volume reduction. The fluid loss may alter cardiovascular responses to exercise by decreasing stroke volume and may have a direct effect on the interpretation of
changes in substrate and gas exchange responses to exercise following simulated weightlessness.

Data from a recent study of simulated weightlessness indicated that the ventilatory threshold occurred at a lower workrate following bedrest. Since this respiratory gas exchange breakpoint is an index of blood lactate accumulation, an earlier blood lactate onset and greater increase in blood lactate concentration during exercise at equal workrate following simulated weightlessness may occur. However, the magnitude of the reduced ventilatory threshold correlated with the plasma volume decline.

Indirect respiratory gas exchange indicators of blood lactate accumulation are inadequate for determination of substrate kinetics during exercise. Acid-base changes are largely regulated by elevated respiration rate and increased carbon dioxide output while lactate may accumulate in the working muscle, be oxidized, and/or diffuse into the blood thus elevating circulating lactate levels. Thus, direct measurement of blood lactate during exercise is essential to quantify unoxidized lactate diffusing from the working muscle and verify its possible link to plasma volume decline following bedrest-deconditioning. Only one group of investigators have reported direct measures of blood lactate during exercise following simulated weightlessness. The data indicating blood lactate concentration increase, however, were not corrected for the fluid loss that occurred
during prolonged bedrest and gave no indication of total circulating lactate. Therefore, the present study was designed to measure blood lactate during exercise before and after bedrest-induced deconditioning and determine how much of the elevated blood lactate concentration may result from decreased plasma volume following simulated weightlessness.
CHAPTER 2

LITERATURE REVIEW

Physiological Adaptations to Simulated Weightlessness

Exercise Responses

The decrease in maximal oxygen uptake ($\dot{V}O_2^{\text{max}}$) and work capacity during maximal effort is well documented (6, 9, 15, 19, 35, 42). Much attention has been given to a greater decrease in $\dot{V}O_2^{\text{max}}$ during upright versus supine work. Hung et al. (25) reported no change in supine peak $\dot{V}O_2$ in contrast to a 15% decrease in the same subjects as a result of upright exercise following bedrest. These results suggested a dichotomy in the cardiovascular deconditioning response to exercise. "A decrease in physical working capacity in the supine position reflects central (cardiac) and peripheral (circulatory and musculoskeletal) deconditioning effects of bedrest, whereas a further decrease in physical working capacity in the upright position reflects the additional influence of orthostatic stress" (25). Increased peak heart rate (9, 25, 42) and decreased stroke volume (15, 25), also indices of a deconditioned subject, reflected cardiac underfilling which may result from lowered plasma volume. These observations
illustrate particular responses indicative of physiological adaptation in subjects exposed to simulated weightlessness. Decreased $\dot{V}O_2\text{max}$ and stroke volume, and increased peak heart rate, provide a framework of parameters used to assess alterations in subjects exposed to simulated weightlessness. Methodological controls must also be considered when testing individuals before and after bedrest deconditioning, i.e., elimination of orthostatic factors associated with upright cycling using supine maximal exercise testing.

In contrast to decreased $\dot{V}O_2\text{max}$, oxygen uptake during submaximal work (submax $\dot{V}O_2$) remained constant following bedrest (6). The increased heart rate observed during a post-bedrest submaximal effort (1,4,6) appeared to adequately compensate for decreased stroke volume in order to supply enough blood and oxygen to the working muscles during exercise. These observations reflected cardiac deconditioning effects of bedrest but provided little insight into possible muscular adaptations to bedrest-induced deconditioning.

Muscular Adaptation

The effects of disuse on the structure of skeletal muscle include decreased muscle mass and cross-sectional area of both fast and slow-twitch fibers (2,30). Coupled with the observed decrease in lean body weight, limb circumference (6), and leg volume (36), these observations
implicated skeletal muscle atrophy with hypokinesia. Strength also declined in casted limbs of humans (36) and astronauts exposed to zero gravity (2). The loss of size and strength in disused muscle translated into structural observations at the cellular level. Number of mitochondria decreased in atrophic rat muscle following hindlimb immobilization (34) and the remaining mitochondria exhibited a marked loss of respiratory capacity (31). This mitochondrial decline had implications for energy sources in working muscle. Enzymatic analyses of disused muscles have revealed decreased mitochondrial enzymes (2, 29). Chi et al. (5) worked with muscle biopsies from runners and cyclists after 10-24 months of intensive training followed by 12 weeks of detraining. These investigators reported decreases in the oxidative enzymes citrate synthase, succinate dehydrogenase, malate dehydrogenase, and hexokinase. Increases in the glycolytic enzymes lactate dehydrogenase and glycogen phosphorylase were also observed. These studies suggested greater capacity for anaerobic metabolism and less aerobic enzyme activity occurring as a result of deconditioning in endurance-trained individuals.

Edes et al. (13) compared m. soleus in rabbit, which is composed of mainly slow oxidative fibers and regarded as muscle with increased aerobic capacity, with m. gastrocnemius, a fast-glycolytic muscle with anaerobic characteristics. With increasing time of immobilization,
the data revealed decreases in the total activities of all enzymes investigated in both muscles. The activity of the m. soleus oxidative enzymes isocitrate dehydrogenase and malic dehydrogenase, however, showed a greater decrease than the rate of glycolytic enzymes in the same muscle. Conversely, the extent of decreased activities of lactic dehydrogenase and aldolase, both glycolytic enzymes, exceeded that of the rates of oxidative enzymes in m. gastrocnemius. The experimental conclusion served to highlight the specificity involved in muscular deterioration, i.e., slow oxidative muscles tend to lose slow oxidative enzyme activity. Therefore, the specific nature of cellular adaptation to muscular inactivity appears to involve greater anaerobic enzyme capacity and subsequently increased anaerobiosis and elevated muscle lactate levels during exercise following bedrest-induced deconditioning. Increased anaerobic glycolysis coupled with decreased aerobic enzyme activity would result in greater net lactate accumulation and elevated blood lactate content during equal intensity exercise following simulated weightlessness.

**Blood Lactate**

Exercise Responses

Much literature has dealt with the accumulation of lactate in the blood during acute exercise. Elevated blood
lactate was suggested to reflect a shift from predominantly aerobic to more anaerobic metabolism (40). During steady-state exercise, this accumulation may be determined by the combination of oxygen supply to the working muscle, fiber recruitment, and the enzymatic potential of each fiber to produce, as well as, clear lactate (22,32,43).

Elevations of blood lactate in exercising muscle were proportional to work intensity (46) and equivalent to 4 times basal metabolism during exercise of heavy intensity (47). The blood lactate accumulation appeared to be due to the inability of muscle to oxidize pyruvate at the same rate as its production. In resting and exercising dogs, Issekutz et al. (26) demonstrated a tripled rate of lactate production with exercise, but the fraction of lactate oxidized stayed the same as that at rest. Finally, human studies by Mazzeo et al. (32) showed this same dependence of the muscle on oxidative mechanisms to clear lactate. After 2 hr of steady-state exercise, appearance of 86.2% of 13C, injected as 13C-lactate and expired as 13CO₂, clearly indicated oxidation of lactate in working muscle. Muscular dependence on oxidation to clear lactate (32) and lower oxidative enzyme activity in detrained muscle (5) may indicate lactate level increases during exercise in subjects following exposure to simulated weightlessness. The working muscle may be incapable of oxidizing pyruvate at the same rate as it is produced and may demonstrate an inability to
clear lactate during exercise. The increased muscle lactate and lack of oxidative clearance may result in elevated circulating lactate indicative of net increased anaerobic metabolism for energy supply during exercise following simulated weightlessness.

Indirect Measurement of Blood Lactate via Gas Exchange

Although much attention has been given to blood lactate onset before and after training, only one study has addressed the issue of deconditioning. Ready et al. (33) found alterations in the ventilatory threshold as the result of endurance training and detraining. Cycle ergometer training at 80% VO2max, 4 times/week for 30 min for 9 weeks showed a 70.4% increase in the ventilatory threshold expressed as absolute VO2 (1/min). The increased ventilatory threshold due to training decreased significantly after 6 and 9 weeks of detraining (3.63 to 2.92 1/min VO2). These observations suggested a detraining response to inactivity. Decreased ventilatory threshold, used as an indirect measurement of blood lactate onset (3), may have indicated an earlier blood lactate accumulation resulting from increased anaerobic energy production during exercise. Direct measurement of blood lactate was not made in the Ready study allowing only speculation concerning possible peripheral metabolic deconditioning responses.
Convertino et al. (6) have proposed an increased anaerobic component in work performed following bedrest-deconditioning. Increases in $V_E$, $\dot{V}CO_2$, and $R$ together with a submax $\dot{V}O_2$ that did not change during exercise following hypokinesia, suggested that increased energy demand and possible low oxidative fiber recruitment, could be explained by a greater proportion of anaerobic metabolism. The effect of bedrest deconditioning on exercise-induced anaerobic threshold was to lower the gas exchange breakpoint and may indicate earlier onset of blood lactate accumulation (8). Direct measures of blood lactate accumulation were not taken to substantiate the proposed increased anaerobic glycolysis.

Investigations by Stegemann et al. (41) and Convertino et al. (8) of $\dot{V}O_2$ kinetics following bedrest-induced deconditioning have demonstrated a reduction of total oxygen transport/utilization capacity during the transient phase of upright exercise. Convertino associated delayed $\dot{V}O_2$ kinetics during submaximal constant-load exercise following simulated weightlessness with orthostatic factors inherent in upright cycling exercise. This explanation was highlighted by no delay in $\dot{V}O_2$ kinetics observed during supine cycling efforts performed by the same subjects.

Stegemann et al. (41) suggested an alternative explanation for impaired $\dot{V}O_2$ kinetics following 7 days of
antiorthostatic (-6° headdown) bedrest. The observed alteration in \( \dot{V}O_2 \) kinetics occurred at frequencies which appeared to be low in terms of blood pressure control or circulatory adjustment. Furthermore, results did not support impairment of venous return as cause for delayed oxygen delivery/utilization and, finally, heart rate kinetics and \( \dot{V}O_2 \) kinetics were not closely connected. It was concluded that the observed delayed \( \dot{V}O_2 \) kinetics may be mainly related to muscular nonhemodynamic factors.

Lactate kinetics during exercise following bedrest deconditioning have not been investigated, however, one can postulate, on the basis of the above studies, an increased rate of lactate production during the transient phase of upright cycling exercise. The studies by Ready et al. (33) and Convertino et al. (6,8) support the hypothesis of increased anaerobic energy supply during exercise following hypokinesia. Stegemann et al. (41) and Convertino et al. (8) reported delayed \( \dot{V}O_2 \) kinetics under the same conditions. Since total energy expenditure during submaximal exercise was unchanged following bedrest-induced deconditioning, as evidenced by equal steady-rate \( \dot{V}O_2 \) (6), more energy, in the form of ATP, must be supplied during the transient phase via increased anaerobiosis; subsequently, lactate kinetics would be expected to be more rapid during exercise following bedrest deconditioning perhaps remaining elevated throughout the constant-load work bout. The respiratory gas exchange
in these studies (6,41) provided indirect evidence of elevated anaerobic metabolism during exercise following simulated weightlessness, but direct measurement of blood lactate accumulation was not performed to substantiate the proposal.

Direct Measurement of Blood Lactate via Blood Analysis

Unlike the well-documented trained-state response of blood lactate, muscular responses to exercise following detraining are less understood. Only one study has examined blood lactate levels during submaximal exercise before and after bedrest. Elevated blood lactate concentrations during supine cycling at 600 kpm/min were reported for 5 subjects. The mean values were 3.7 mmol/l before compared to 6.5 mmol/l after bedrest deconditioning (35). Houston et al. (24) also measured blood lactate accumulation following deconditioning, but only examined post-exercise levels after a maximal effort on the treadmill. Blood samples taken from 6 distance runners immediately after the maximal exercise test indicated a mean blood lactate concentration of approximately 11.0 mmol/l at the peak of their training and 20% lower lactate concentration (approximately 8.0 mmol/l) after 15 days of detraining. Finally, Witzmann et al. (49) immobilized the rat soleus (slow twitch) and extensor digitorum longus (fast twitch) muscles for six weeks and reported decreased muscle ATP with greater dependence on
anaerobic metabolism as indicated by increased glycogen depletion and lactate production. Each of these experimental findings supported Saltin's earlier work and the hypothesis that there is decreased ability to generate ATP via aerobic mechanisms due to biochemical changes resulting from reduction in contractile activity (23).

**Body Fluid Adaptation to Simulated Weightlessness**

In the above studies, fluid loss following simulated weightlessness could have possibly contributed to increased blood lactate concentration. All blood lactate accumulation data was reported in concentration for comparison of measurements taken pre- and post-bedrest. It was difficult to determine whether observed changes were due to increased blood lactate content or reduced plasma volume associated with simulated weightlessness.

Prolonged bedrest induces dehydration (16). This fluid loss has been observed in man after 10 days of recumbency (45), in monkeys following horizontal body casting (12), and in astronauts post-spaceflight (14). Head-down tilt-table experiments used to simulate acute cardiovascular responses to weightlessness have shown an initial shift of venous blood toward the head and thorax followed by increased diuresis occurring within hours of exposure to hypogravity (20). The long-term dynamics of fluid loss were described by Greenleaf et al. (17) by
monitoring fluid and electrolyte shifts during 14-days of bedrest-induced deconditioning. There was equal distribution of fluid loss between the interstitial and plasma volumes within the first few days of bedrest. After 2 weeks, however, the extracellular fluid volume had returned to normal by increased interstitial volume which rose to above pre-bedrest levels compensating for the still depressed level of plasma volume. This decrease in plasma volume was accompanied by increased hematocrit and plasma osmolality, both indices of fluid loss specific to the cardiovascular system. It was concluded that extracellular fluid volume was regulated at the expense of a plasma volume reduction to maintain a constant internal environment.

The decrease in plasma volume and subsequent decrease in blood volume is of particular importance when interpreting solute concentration data obtained from analyses of serum or plasma as well as tissue samples. Blood lactate concentration during exercise after bedrest deconditioning requires correction for fluid loss to determine whether observed changes are due to increased solute or decreased solvent, i.e., elevation of circulating lactate indicating increased anaerobiosis. Correction for changes in plasma volume following bedrest allows accurate comparisons of actual changes in blood lactate content from pre- and post-bedrest measurements and subsequent predictions as to energy supply mechanisms.
Investigations quantifying cardiovascular deconditioning responses have incriminated plasma volume decrease as a primary limiting factor to work capacity following prolonged bedrest. It would logically follow that changes at the biochemical level may, too, be related to the plasma volume decrease. Convertino et al. (8) have reported a +0.80 correlation coefficient between decreased exercise-induced ventilatory threshold from mean (± SE) 1.26 ± 0.09 to 0.95 ± 0.05 l/min and 398-ml reduction in resting plasma volume (-11%) before compared to after bedrest. Since the ventilatory threshold is considered a sensitive on-line indicator for onset of blood lactate accumulation (48), an earlier blood lactate concentration increase during graded exercise may have occurred in these subjects following 10 days of bedrest as a result of similar total lactate content diluted in a smaller plasma volume. However, since blood lactate was not measured in this study, an investigation designed for direct measurement of blood lactate during exercise following simulated weightlessness should provide evidence to substantiate the ventilatory threshold/plasma volume correlation.
Summary and Approach

Resting plasma volume reduction and blood lactate concentration elevation occur during acute exercise following bedrest-induced deconditioning. Whether exercise increases blood lactate content, i.e., net lactate accumulation, following simulated weightlessness remains unclear since it was uncertain how much of the change in exercise lactate concentration was due to the change in plasma volume resulting from chronic bedrest adaptation. Therefore, the purpose of this thesis was to determine the relationship between changes in plasma volume and blood lactate accumulation during submaximal exercise following simulated weightlessness.

Hypothesis

Greater elevations in blood lactate concentration during constant-load submaximal exercise following simulated weightlessness are dependent on plasma volume reductions associated with bedrest-induced deconditioning and not elevated blood lactate content, i.e., decreased solvent and not increased solute.

Null Hypothesis

Greater elevations in blood lactate concentration during submaximal exercise following simulated
weightlessness are independent of plasma volume reductions associated with bedrest-induced deconditioning.
CHAPTER 3

METHODS

Subject Characteristics

Ten healthy men, with a mean ± SE age of 39 ± 2 yr (range 35-49), a mean height of 178 ± 2 cm, a mean weight of 74.2 ± 2.5 kg (range 64.0-92.9), and a mean relative body fat of 17.2 ± 1.5% (range 9.8-24.9) were selected to participate in this study. A treadmill test, mean peak $\dot{V}O_2$ of 47.3 ± 1.9 ml/kg/min, and physical examination verified the healthy, aerobically fit state of each individual.

After selection, the subjects signed a written consent form describing the purpose of the study, nature of all tests, foreseeable inconvenience, discomforts, risks, restrictions and controls.

Restrictions and Controls

The subjects were required to live at the Human Research Facility at NASA-Ames Research Center for 19 days in relative confinement. For the duration of the study, lights were automatically turned on at 7:00 AM and went off at 11:00 PM each day. Meals were served three times daily. No visitors were allowed into the Human Research Facility as long as test subjects were present and working on the research project.
During the study, subjects were provided with a normal diet, laundry service, suitable clothes (jumpsuits and shorts) to wear during the tests, shower facilities, stereo, television, books and games. Each individual was required to abstain from the use of all drugs (including vitamins and aspirin), coffee, alcoholic beverages, tobacco, snacks, candy and food that was not provided for them. Drugs specifically prescribed by an attending physician were allowed. No one was permitted to diet during the study and each individual was expected to eat the meals that were served. No physical activity was permitted during the 19 days of relative confinement. In addition, the subjects were not allowed to eat for at least two hours prior to any exercise test.

**Treatment**

By performing two trial exercise tests during a 5-day orientation period, the subjects were familiarized with the test protocol and mechanics of cycle ergometry in the upright and supine positions. These pre-study familiarization tests were necessary to minimize the possible learning curve responses of the subjects.

The overall experimental treatment consisted of a 7-day ambulatory control period followed by 10 days of continuous bedrest in the antiorthostatic (-6° headdown) position and 2
days of ambulatory recovery. The subjects were then discharged from the Human Research Facility.

**Exercise Test Protocols**

**Maximal Exercise Test**

On day 7 of the pre-bedrest control period, each subject performed a maximal exercise tolerance test in the supine position on a Collins electronic cycle ergometer. The ergometer was rotated backwards 90° and attached to an aluminum frame covered with nylon webbing. This allowed nearly maximal skin exposure and evaporative heat loss in the supine position. To determine peak oxygen uptake, the maximal exercise test began with a 4-min warm-up period of zero-resistance pedaling and increased 15 watts each minute until volitional exhaustion. The supine position was chosen to minimize the orthostatic factor which contributes to the reduction in work tolerance following bedrest-induced deconditioning (7). This maximal exercise test was repeated on day 1 of the post-bedrest recovery period prior to the subjects resuming ambulation.

Oxygen uptake and heart rate were measured during each 30 sec of the exercise test. Heart rate was recorded continuously and counted from an electrocardiogram record. Systolic and diastolic blood pressures were measured manually during the last 15 sec of each minute of the test with a sphygmomanometer and stethoscope.
Submaximal Exercise Test

Two to three hours after the maximal exercise test and after resuming ambulation and a stabilized upright blood pressure, each subject performed a submaximal exercise test on the cycle ergometer in the upright position. The test commenced with a 30-min sitting period followed by a 5-min resting control on the ergometer and 15 min of exercise. The exercise test consisted of pedaling at 60 rpm for 15 min at a workrate of $630 \pm 30$ kgm/min ($57 \pm 1.6\%$ peak $\dot{V}O_2$) and was performed by each subject before (pre) and after (post) 10 days of continuous $-6^\circ$ headdown bedrest. During exercise, $\dot{V}O_2$ was measured to assure equivalent energy demand for the pre- and post-bedrest workrate. Antecubital venous blood samples were collected at rest one minute before the onset of exercise, and during the last 30 sec of exercise and analyzed for blood lactate concentration and hematocrit. During the first 5-10 min of this submaximal effort, expired air samples were collected and analyzed. Heart rate and blood pressure measurements were also taken at this time. The submaximal exercise workrate was determined individually for each subject based on the lactate accumulation break point demonstrated in the pre-bedrest supine maximal exercise test using ventilatory and gas exchange indices. The same absolute workrate was used for pre- and post-bedrest submaximal tests in order to determine changes in blood lactate concentration and content.
Body Composition

Body composition was assessed on day 7 of pre-bedrest and day 1 of the post-bedrest recovery period. Percent body fat was calculated using body density values obtained from hydrostatic weighing and residual volume (28).

Calculations of Body Density and Percent Body Fat

Calculations of body density (BD) and percent body fat (%BF) were performed using the following equations. Symbols are defined below the equation.

\[ BD = \frac{Ma}{Ma - Mw - Vr/Dw} \]

\[ Ma = \text{Mass in air (kg)} \]
\[ Mw = \text{Net mass in water (kg)} \]
\[ Dw = \text{Density of water} \]
\[ Vr = \text{Residual volume} \]

\[ %BF = \frac{4.570 - 4.142}{BD} \]

Measurement of Respiratory Gas Exchange

The subjects used an Otis-McKerrow respiratory valve and the volume of expired gas was measured by a Parkinson-Cowan high-velocity, low-resistance meter. A potentiometer at the gas meter dial transmitted electrical outputs to a two-channel recorder (MRE Model M22). In this way, expired minute ventilation volume (\(\dot{V}_E\)) and respiratory frequency (f) could be recorded. The expired gas was
continuously extracted via a Dynapump (Scientific Products) from a 5-liter mixing chamber (R-Pel) which was placed between the subject and the gas meter to a 2-liter gas bag (Ohio Medical) attached to a Costill-Wilmore valve. The ventilatory flow volume was corrected by adding the Dynapump withdrawal volume rate of 2.5 l/min to the recorded volume of expired air. The composition of expired gas was analyzed for the fraction of mixed expired oxygen ($F_{EO_2}$) and carbon dioxide ($F_{ECO_2}$) using mass spectrometry (Perkin-Elmer). The gas analyzers were calibrated with known oxygen and carbon dioxide concentrations. The Haldane transformation for calculation of oxygen uptake ($\dot{V}O_2$) and volume of carbon dioxide expired ($\dot{V}CO_2$) was performed by computer program. Respiratory exchange ratio (R) was calculated by $\dot{V}CO_2$ divided by $\dot{V}O_2$.

**Estimate of Blood Lactate Onset**

The lactate accumulation break point for pre- and post-bedrest supine maximal exercise tests was determined by the ventilatory and gas indices $\dot{V}O_2$, $\dot{V}E$, $\dot{V}CO_2$, R, and the ventilatory equivalents for oxygen and carbon dioxide ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$, respectively). The $\dot{V}O_2$ corresponding to the lowest value of $\dot{V}E/\dot{V}O_2$ without a subsequent increase in $\dot{V}E/\dot{V}CO_2$ was used as the estimate of blood lactate onset (3).
Plasma Volume Determination

Thirty minutes after the subjects assumed a supine position they were injected intravenously with 0.3 mg Standard Evans Blue Dye T-1824/kg body weight as a 0.1% solution in isotonic saline (maximum dose, 20 mg). A pre- and post-injection syringe weight was taken to determine the exact volume of dye injected. A heparinized blood sample yeilding 1 ml of plasma was drawn immediately before dye injection and 10 min after, and then placed in a vacutainer. A microcapillary tube was prepared from the vacutainer containing the pre-dye blood sample for determination of hematocrit (Hct). Within 2 hours all samples were spun in a refrigerated centrifuge for 10 min. A plasma sample was drawn from the separated contents in the tube, and immediately frozen (-40° C). Hematocrit samples were also centrifuged at this time and read immediately. This procedure was followed on day 1 of the control period, and day 5 during bedrest.

Plasma Volume Analysis

The following six reagents were required for this analysis:

1. Disodium Hydrogen Phosphate, Na₂HPO₄ (anhydrous), 2% (gm/100 ml).

2. Teepol-phosphate: Add 2 gm Na₂HPO₄ to 3 ml Teepol. Make up to 100 ml with distilled water (Teepol 610 concentrate, Shell Co.).
3. Potassium Phosphate, Monobasic, \(KH_2PO_4\) (anhydrous), 8%.

4. 1:1 acetone-water solution.

5. Solka Floc SW-40A (Brown Company, Boston, Mass.). Suspend approximately 1 gm Solka Floc in 100 ml of 2% \(Na_2HP0_4\) (reagent 1).

6. Normal plasma

The first step in the plasma volume analysis procedure was preparation of the column. One column was prepared for each standard and one for each plasma sample. Columns were approximately 1 cm in diameter and conveniently made from broken 50 ml burettes. A 22-gauge needle was fitted to the base of each column with an adapter to limit the rate of flow. Approximately 100 mg of glass wool was inserted in each column, the tube was washed down with distilled water, and the glass wool packed firmly above the small constriction with the aid of a large stirring rod. The height of the glass wool was about 0.5 cm. Using a 10-ml pipette (upside down), about 12 ml of the Solka-Floc suspension was dispensed into the column so the height of the Solka-Floc was 3-5 cm. Each column was washed with approximately 10 ml of 2% \(Na_2HP0_4\) to pack the column. A circular motion was used to wash down the sides of the column. The drip frequency of the column was approximately 1/sec.

The plasma samples were prepared by pipetting 1 ml TEST plasma into a 50-ml Erlenmeyer flask. If the Evans Blue Dye (T-1824) blood volume procedure was performed
within the last 10 days, as was the case in this study, 1 ml of the subject's plasma obtained prior to dye injection was pipetted into a 50-ml Erlenmeyer flask. This second sample became the blank against which the TEST sample was compared. Teepol-phosphate (15 ml) was added to each flask, to wash down any remaining plasma from the sides, and mixed thoroughly by gently swirling for about 15 sec.

After preparing the column and plasma samples, extraction and elution of the dye from the plasma ensued. The drip column was started and the contents of each flask were gently transferred onto the pulp column with a Pasteur pipette avoiding disturbance of the surface. The flask was rinsed with 5 ml Teepol-phosphate and this added to the column. When the level of the solution had reached that of the Solka-Floc, at least 10 ml 2% Na₂HPO₄ was added to the column. It was essential that this wash sufficiently remove all interfering substances to prevent cloudiness or an appreciable artifactual color in the final eluate. The wash was allowed to pass through the column until the fluid level was just above the Solka-Floc.

Elution was proceeded with immediately by pipetting 0.5 ml 8% KH₂PO₄ into a 10-ml volumetric flask, the flask placed under the delivery needle, and the eluate collected. This, with the Na₂HPO₄ from the column, buffered the pH of the eluate to 7.0. Approximately 5 ml acetone-water was gently transferred to the column and allowed to pass down
until the blue front dripped through the column to the flask. The columns then stood 15 min before completion of elution. Additional acetone-water (4-5 ml) was next pipetted into the column and elution continued until the eluate nearly filled the flask to the 10 ml mark. By this time the column had lost all blue coloration. The volume was brought to the 10 ml mark with acetone-water and contents of the flask mixed by inversion. The solutions were read and recorded on a Beckman spectrophotometer in 1 cm cuvettes at 615 nm. (18)

Plasma, Blood, and Red Cell Volumes Calculations

Resting plasma volume (PV) was calculated using the following equation. Symbols are defined below the equation.

\[
PV \ (\text{ml}) = \frac{V \times D \times St \times v}{T \times 1.03}
\]

\( V = \) Volume 1-1824 injected (ml)
\( D = \) Dilution of STANDARD (0.2 ml of 1:50; therefore dilution equals 1:250)
\( St = \) Absorbance of STANDARD
\( v = \) volume of sample extracted (1 ml)
\( T = \) Absorbance of test: subtract plasma blank if determined
\( 1.03 = \) Factor introduced to correct for slow dye uptake by the tissues

Total blood volume was calculated by multiplying resting plasma volume times \([100/(100-Hct)]\). Red cell volume was obtained by subtracting plasma volume from blood volume. Calculation of percent change in plasma volume
(\% \Delta PV) during acute submaximal exercise was performed using the following equation (44):

\[
\% \Delta PV = \frac{100}{100 - Hctb} \times 100 \times \frac{Hctb - Hcta}{Hcta}
\]

Hctb = pre-exercise hematocrit
Hcta = post-exercise hematocrit

**Blood Sample Collection**

Blood samples (5 ml) were taken from the antecubital vein pre- and during submaximal exercise testing sessions. Two 100 microliter pipettes were immediately filled with fresh whole blood from this venous sample and each blown into 200 microliters of an 8% cold, dilute perchloric acid solution (7 ml of 70% perchloric acid to 100 ml water) to yield duplicate 100/200, blood/perchloric acid solutions. These blood/perchloric acid solution mixtures were covered, kept on ice, centrifuged for 7 min in a refrigerated centrifuge within 15 min of collection, and kept cold (0-5°C) until analysis 24 hours later. The remaining blood sample in the syringe was placed in a vacutainer from which 2 hematocrit capillary tubes were prepared. These tubes were centrifuged and read within 3 hours of the exercise tests.
Blood Lactate Analysis

The enzyme, lactate dehydrogenase catalyzes the following reversible reaction:

\[
\text{LDH} \quad \text{Pyruvic Acid} + \text{NADH} \quad \text{Lactic Acid} + \text{NAD} \\
(\text{High} \quad A_{340}^\text{High}) \quad (\text{Low} \quad A_{340}^\text{Low})
\]

To measure lactate, the reaction was carried out from right to left with excess NAD. To force the reaction to completion in this direction, it was necessary to trap formed Pyruvic Acid with hydrazine. The increased absorbance at 340 nm due to NADH formation became a measure of the lactate originally present.

Five reagents were required for this assay.

Provided by the Sigma Co., they were as follows:

1. Lactic Dehydrogenase, Stock No. 826-6
   Contains LDH suspension isolated from beef heart in ammonium sulfate. Approximately 1000 units/ml when prepared. Store in refrigerator at 0-5°C. Invert until suspension is uniform before removing an aliquot.

2. Glycine Buffer, Stock No. 826-3
   Contains glycine and hydrazine, pH 9.2. Chloroform added as preservative. Store in refrigerator at 0-5°C.

3. NAD Preweighed Vial, Stock No. 260-110
   Vial contains nicotinamide adenine dinucleotide, Grade III, 10 mg. Store below 0°C in desiccator box provided.

4. Lactic Acid Standard Solution, Stock No. 826-10
   Contains L(+)lactic acid, 0.40 mg/ml (4.44 mmol/liter). Preservative added. Store in refrigerator at 0-5°C.
5. Lactic Acid diluted Standard, 0.08 mg/ml
Dilute 1.0 ml of Lactic Acid Standard Solution, Stock No. 826-10, to 5.0 ml with water. Discard solution after one day.

During each of the four collection sessions, five pre-exercise and five post-exercise blood samples were drawn. Duplicate tests were run on each, thus a total of 20 assays were prepared the following day when the actual blood lactate analyses were performed. To detect faulty reagents, pipetting or dilution errors, and to assure a properly functioning spectrophotometer, duplicate 40 mg% and 80 mg% standard lactate dilutions were also prepared each day. The mean (± SE) difference between blood lactate concentration samples was .086 ± .035 mmol/1 with a 4% error of method.

Into each of the 13 NAD Vials, 2.0 ml Glycine Buffer, 4.0 ml distilled water, and 0.1 ml Lactic Dehydrogenase were added. Vials for 20 samples, 1 blank and 4 Standard dilutions were inverted several times to dissolve the NAD then all 13 separate solutions were combined into one larger flask and mixed well. Into each appropriately labeled tube was pipetted 2.8 ml of the above mixture. To the BLANK 0.2 ml of 8% perchloric acid was added. The 40 mg% and 80 mg% STANDARD tubes received 0.2 ml and 0.1 ml, respectively, of 8% perchloric acid plus 0.1 ml and 0.2 ml, respectively, of Lactic Acid Diluted Standard. Added to the TEST tubes was 0.2 ml of the protein-free solution prepared as described under the "Blood Sample Collection" section. Each of these tubes were covered, mixed on a Vortex machine,
and incubated approximately 30 min at 37°C. After incubation each sample absorbance was read and recorded on a Beckman narrow-bandwidth spectrophotometer using a 1-cm cuvette at 340 nm versus BLANK as reference.

**Blood Lactate Concentration and Content Calculations**

Blood lactate (BL) concentration was calculated using the following equation. Symbols are defined below the equation.

\[
\text{BL (mmol/liter)} = \frac{A_{340} \times 3.0}{6.22 \times 0.0667 \times 1} = 7.23 \times A
\]

- \(A_{340}\) = final maximum absorbance at 340 nm
- 3.0 = reaction volume (ml)
- 6.22 = millimolar extinction coefficient of NADH at 340 nm
- 0.0667 = volume (ml) of blood sample in cuvette
- 1 = light path (cm)

Blood lactate concentration data were corrected for percent change in plasma volume during acute submaximal exercise as well as change in resting plasma volume associated with simulated weightlessness. Multiplication of blood lactate concentration times the appropriate plasma volume changes for each work bout yielded blood lactate content, i.e., circulating lactate. Thus, solute content equaled blood lactate concentration times volume.
**Statistical Analysis**

The data were analyzed by paired t-test statistics. The 0.05 level of significance was chosen prior to the experiment for rejection of the null hypothesis (p<0.05).
CHAPTER 4

MANUSCRIPT

Abstract

Since a previously reported decrease in the ventilatory threshold following bedrest-induced deconditioning was related to a reduction in plasma volume (PV), this study was undertaken to determine the relationship between changes in PV and blood lactate (BL) during exercise following simulated weightlessness. Ten healthy men (35-49 yr) performed a submaximal upright cycle ergometer test consisting of pedaling at 60 rpm for 15 min at a workrate of 630 ± 30 kgm/min (57 ± 1.6% peak $\dot{V}O_2$) before (pre) and after (post) 10 days of continuous -6° headdown bedrest (BR). During exercise, oxygen uptake ($\dot{V}O_2$) was measured to assure equivalent energy expenditure for the pre- and post-BR workrate. Antecubital venous blood samples were collected at rest and during the last 30 sec of exercise and analyzed for BL concentration and hematocrit. Resting PV was measured pre- and post-BR with Evans blue dye and total circulating BL content was calculated as the product of PV and BL concentration. Submaximal $\dot{V}O_2$ was unchanged following BR. Post-BR exercise BL concentration of 2.9 mmol/l was greater (p<0.05) than the pre-BR BL concentration of 2.5 mmol/l. However, as a result of a 17%
reduction (p<0.05) in resting PV, total circulating BL content during exercise at the same absolute workrate was unchanged following BR. These data demonstrate that the increase in BL concentration during exercise following the deconditioning effects of simulated weightlessness cannot be completely explained by changes in lactate metabolism but are dependent on the reduction in PV.

Key Words: blood lactate; plasma volume; blood volume; bedrest deconditioning; anaerobic threshold
Introduction

Much literature has dealt with the accumulation of lactate in the blood during acute exercise. Elevated blood lactate was suggested to reflect a shift from predominantly aerobic to more anaerobic metabolism (40). During steady-state exercise, this accumulation may be determined by the combination of oxygen supply to the working muscle, fiber recruitment, and the enzymatic potential of various fibers to produce, as well as, clear lactate (22,32,43). Elevations of blood lactate in exercising muscle were proportionate to work intensity (46) and equivalent to 4 times basal metabolism during exercise of heavy intensity (47). The blood lactate accumulation appeared to be due to the inability of muscle to oxidize pyruvate at the same rate as its production. Muscular dependence on oxidation to clear lactate (32) and lower oxidative enzyme activity in detrained muscle (5) may have accounted for elevated blood lactate concentration during exercise in subjects following exposure to simulated weightlessness (35). However, since blood lactate accumulation data were reported in concentration, i.e., mmol/l, for comparison of pre- and post-bedrest measurements, it was difficult to determine whether observed changes were due to increased blood lactate content or reduced plasma volume associated with simulated weightlessness (16).
The decrease in plasma volume associated with simulated weightlessness is of particular importance when interpreting solute concentration data obtained from plasma as well as tissue samples. Investigations quantifying cardiovascular deconditioning responses have incriminated plasma volume reduction as a primary limiting factor to exercise performance following prolonged bedrest (15,25). It would follow that biochemical changes at the cellular level may, too, be affected by fluid volume changes which in turn could contribute to the observed reduction in maximal oxygen uptake and work capacity. Therefore, the purpose of this investigation was to determine the relationship between changes in plasma volume and blood lactate accumulation during submaximal exercise following simulated weightlessness.

Methods

Ten healthy men, with a mean ± SE age of 39 ± 2 yr (range 35-49), a mean height of 178 ± 2 cm, a mean weight of 74.2 ± 2.5 kg (range 64.0-92.9), and a mean relative body fat of 17.2 ± 1.5% (range 9.8-24.9) were selected to participate in this study. A treadmill test, mean peak $\dot{V}O_2$ of 47.3 ± 1.9 ml/kg/min, and physical examination verified the healthy, aerobically fit state of each individual.
After selection, the subjects signed a written consent form describing the purpose of the study, nature of all tests, foreseeable inconvenience, discomforts, risks, restrictions and controls.

By performing two trial exercise tests during a 5-day orientation period, the subjects were familiarized with the test protocol and mechanics of cycle ergometry in the upright and supine positions. These pre-study familiarization tests were necessary to minimize the possible learning curve responses of the subjects.

The overall experimental treatment consisted of a 7-day ambulatory control period followed by 10 days of continuous bedrest (BR) in the antiorthostatic (-6° headdown) position and 2 days of ambulatory recovery. During BR, the subjects remained in the headdown or horizontal position continuously for all activities including excretory functions, showering, and eating. During this 19-day experimental period, the subjects were housed 24 hr/day in the Human Research Facility at NASA-Ames Research Center.

On day 7 of the pre-BR control period, each subject performed a maximal exercise tolerance test in the supine position on a Collins electronic cycle ergometer. The ergometer was rotated backwards 90° and attached to an aluminum frame covered with nylon webbing. This allowed
nearly maximal skin exposure and evaporative heat loss in the supine position. To determine peak oxygen uptake (peak $\dot{V}O_2$), the maximal exercise test began with a 4-min warm-up period of no resistance pedaling and increased 15 watts each minute until volitional exhaustion. The supine position was chosen to minimize the orthostatic factor which contributes to the reduction in work tolerance following bedrest-induced deconditioning (7). Oxygen uptake ($\dot{V}O_2$) and heart rate (HR) were measured during each 30 sec of the exercise test. HR was recorded continuously and counted from an electrocardiogram record. Systolic and diastolic blood pressures were measured manually during the last 15 sec of each minute of the test with a sphygmomanometer and stethoscope.

Two to three hours after the maximal exercise test and after resuming ambulation and a stabilized upright blood pressure, each subject performed a submaximal exercise test on the cycle ergometer in the upright position. The test commenced with a 30-min sitting period followed by a 5-min resting control on the ergometer and 15 min of exercise. The exercise test consisted of pedaling at 60 rpm for 15 min at a workrate of $630 \pm 30$ kgm/min ($57 \pm 1.6\%$ peak $\dot{V}O_2$) and was performed by each subject pre- and post-BR. During exercise, $\dot{V}O_2$ was measured to assure equivalent energy demand for the pre- and post-BR workrate. Antecubital venous blood samples were collected during rest at one
minute before the onset of exercise, and during the last 30 sec of exercise and analyzed for blood lactate concentration and hematocrit. During the first 5-10 min of this submaximal effort, expired air samples were collected and analyzed. HR and blood pressure measurements were also taken at this time. The submaximal exercise workrate was determined individually for each subject based on the lactate accumulation break point demonstrated in the pre-BR supine maximal exercise test. The break point was determined by the ventilatory and gas indices \( \dot{V}O_2 \), expired minute ventilation volume (\( \dot{V}E \)), volume of carbon dioxide expired (\( \dot{V}CO_2 \)), the ventilatory equivalents for oxygen and carbon dioxide (\( \dot{V}E/\dot{V}O_2 \) and \( \dot{V}E/\dot{V}CO_2 \), respectively), and respiratory exchange ratio (R). The \( \dot{V}O_2 \) corresponding to the lowest value of \( \dot{V}E/\dot{V}O_2 \) without a subsequent increase in \( \dot{V}E/\dot{V}CO_2 \) was used as the estimate of blood lactate accumulation onset (3). The same absolute workrate was used for pre- and post-BR submaximal tests in order to determine changes in blood lactate concentration and content.

The subjects used an Otis-McKerrow respiratory valve and the volume of expired gas was measured by a Parkinson-Cowan high-velocity, low-resistance meter. A potentiometer at the gas meter dial transmitted electrical outputs to a two-channel recorder (MRE Model M22). In this way, expired \( \dot{V}E \) and respiratory frequency (f) could be recorded. The expired gas was continuously extracted via a
Dynapump (Scientific Products) from a 5-liter mixing chamber (R-Pel) which was placed between the subject and the gas meter to a 2-liter gas bag (Ohio Medical) attached to a Costill-Wilmore valve. The ventilatory flow volume was corrected by adding the Dynapump withdrawal volume rate of 2.5 l/min to the recorded volume of expired air. The composition of expired gas was analyzed for the fraction of mixed expired oxygen ($F_{E-O_2}$) and carbon dioxide ($F_{E-CO_2}$) using mass spectrometry (Perkin-Elmer). The gas analyzers were calibrated with known oxygen and carbon dioxide concentrations. The Haldane transformation for calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ was performed by computer program and $R$ was calculated by $\dot{V}CO_2$ divided by $\dot{V}O_2$.

Plasma volume (PV) was measured before and after BR with a modified Evans blue dye dilution method (18). The post-injection dye, recovered from the plasma through a chromatographic column, was compared with a standard dye solution at 615 nm with a Beckman spectrophotometer. Blood for duplicate micro-hematocrit (Hct) determinations was drawn into capillary tubes and spun for 10 min. Total circulating blood and red cell volumes were calculated from the PV and Hct.

Blood samples (5 ml) were taken from the antecubital vein pre- and during submaximal exercise testing sessions. Two 100-microliter pipettes were immediately filled with whole blood and each blown into 200 microliters of an 8%
cold, dilute perchloric acid solution (7 ml of 70% perchloric acid to 100 ml water) to yield duplicate 100/200, blood/perchloric acid solutions. These blood/perchloric acid solution mixtures were covered, kept on ice, centrifuged for 7 min in a refrigerated centrifuge within 15 min of collection, and kept cold (0-5°C) until analysis 24 hours later. The remaining blood sample in the syringe was placed in a vacutainer from which 2 hematocrit capillary tubes were prepared. These tubes were centrifuged and read within 3 hours of the exercise tests. Blood lactate concentration was determined by enzymatic analysis using assay materials from Sigma Co. and calculated from absorbance values read on a Beckman narrow-bandwidth spectrophotometer using a 1-cm cuvette at 340 nm versus BLANK as reference (38).

The data were analyzed by paired t-test statistics. The 0.05 level of significance was chosen prior to the experiment for rejection of the null hypothesis (p<0.05).

**Results**

Maximal Exercise Test

Changes in maximal work capacity during supine cycling before and after -6° headdown antiorthostatic deconditioning are listed in Table 1. Mean absolute peak
\( \dot{V}O_2 \) (l/min) declined from 2.84 to 2.40 (-15%, p<0.05). However, peak workrate and exercise duration were not changed by BR. Despite the unaltered peak workrate, mean peak HR significantly increased by 4% following bedrest-induced deconditioning (p<0.05).

Submaximal Exercise Test

The mean values in Table 2 represent respiratory gas exchange and HR measurements during constant-load cycling exercise before and after BR. Submax \( \dot{V}O_2 \) (l/min) remained unchanged after deconditioning when compared to pre-treatment values. Mean \( \dot{V}E/\dot{V}O_2 \) and R, increased by 8% and 4%, respectively (p<0.05). For the same submax \( \dot{V}O_2 \) workrate, mean HR showed a significant 8% increase during the pre- to post-exercise bout from 130 to 140 bpm.

Body Composition

Mean (+ SE) total body weight was 74.85 ± 2.48 kg pre-BR and 74.77 ± 2.34 kg post-BR (NS). Relative body fat was 17.3 ± 1.4% and 17.8 ± 1.3% before and after BR, with fat weight being 12.98 ± 1.14 kg and 13.30 ± 1.07 kg, respectively (NS). Lean body weight was 82.7 ± 1.4% (61.87 ± 2.34 kg) before BR compared to 82.2 ± 1.3% (61.47 ± 2.24 kg) after BR (NS).
Plasma Volume

Plasma volume (PV) was reduced from 3407 ± 126 to 2826 ± 103 ml (-17%, p<0.05) following exposure to simulated weightlessness. PV decreased by 5% (p<0.05) during both pre- and post-BR constant-load exercise bouts. Mean (± SE) pre-BR PV decreased during exercise from 3406 ± 120 to 3252 ± 131 ml and post-BR PV was reduced from 2826 ± 97 to 2677 ± 197 ml for the same workrate.

Blood Lactate Concentration and Content

Figure 1 illustrates BL concentration ([HLa]) before and after bedrest. During acute exercise, [HLa] increased from 1.42 ± .19 mmol/l to 2.52 ± .18 mmol/l (+77%, p<0.05) before BR and from 1.54 ± .17 mmol/l to 2.88 ± .26 mmol/l (+87%, p<0.05) after BR. Furthermore, [HLa] during exercise before compared to after BR increased from 2.52 ± .18 mmol/l to 2.88 ± .26 mmol/l (p<0.05). Resting [HLa] values were 1.42 ± .19 mmol/l before BR and 1.54 ± .17 mmol/l after BR (NS).

Figure 2 illustrates total circulating lactate before and after BR corrected for the significant chronic (BR-induced) and acute (exercise-induced) reductions in PV. During acute exercise, lactate significantly increased from 4.82 ± .65 mmol to 8.18 ± .61 mmol before BR (+70%, p<0.05) and from 4.38 ± .55 mmol to 7.69 ± .73 mmol
(+75%, p<0.05) following BR. There was no significant change in total circulating blood lactate content during exercise before compared to after simulated weightlessness.
Table 1. Changes in Plasma, Blood, Red Cell Volume and Maximal Work Capacity During Supine Cycling Exercise Pre- and Post-Bedrest

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Pre-Bedrest</th>
<th>Post-Bedrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Volume, ml</td>
<td>3407 (126)</td>
<td>2826 (103)*</td>
</tr>
<tr>
<td>Blood Volume, ml</td>
<td>5646 (207)</td>
<td>4847 (186)*</td>
</tr>
<tr>
<td>Red Blood Cell Volume, ml</td>
<td>2240 (97)</td>
<td>2024 (92)</td>
</tr>
<tr>
<td>Peak Heart Rate, bpm</td>
<td>171 (4)</td>
<td>179 (4)*</td>
</tr>
<tr>
<td>Cycle Ergometer Dura., min</td>
<td>19.1 (0.8)</td>
<td>18.0 (0.7)</td>
</tr>
<tr>
<td>Peak Work Rate, watts</td>
<td>227 (13)</td>
<td>212 (11)</td>
</tr>
<tr>
<td>Peak VO$_2$, l/min</td>
<td>2.84 (0.18)</td>
<td>2.40 (0.14)*</td>
</tr>
<tr>
<td>Peak VO$_2$, ml/kg/min</td>
<td>38.17 (2.40)</td>
<td>32.38 (2.08)*</td>
</tr>
</tbody>
</table>

*p<0.05 vs. Pre-Bedrest value
Values are mean (± SE)
Table 2. Changes in Respiratory Gas Exchange and Heart Rate During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Pre-Bedrest</th>
<th>Post-Bedrest</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}O_2$, 1/min</td>
<td>1.61 (0.08)</td>
<td>1.61 (0.09)</td>
</tr>
<tr>
<td>$R$</td>
<td>.96 (.01)</td>
<td>1.00 (.01)*</td>
</tr>
<tr>
<td>$\dot{V}E/\dot{V}O_2$</td>
<td>25.9 (0.97)</td>
<td>28.0 (1.09)*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>130 (4)</td>
<td>141 (4)*</td>
</tr>
</tbody>
</table>

*p<0.05 vs. Pre-Bedrest value
Values are mean (+ SE)
Figure 1. Blood Lactate Concentration at Rest and During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest

*Rest and submaximal exercise comparisons, p<0.05
#Submaximal exercise comparison pre- and post-bedrest, p<0.05
Figure 2. Blood Lactate Content at Rest and During Submaximal Upright Cycling Exercise Pre- and Post-Bedrest

*Rest and submaximal exercise comparisons, p<0.05
Discussion

The 17% decrease in plasma volume observed in the present study was similar in magnitude to that previously reported for middle-aged men following 10 days of bedrest (16). Associated with the decline in circulating fluid were decreased peak $\dot{V}O_2$ and elevated peak heart rate indicative of adaptation following prolonged bedrest. We hypothesized that plasma and blood volume reduction could account for increased blood lactate concentration during exercise following simulated weightlessness. Despite post-bedrest blood lactate concentration rising 10% higher than pre-bedrest values, there was no change in total circulating lactate accumulation due to a 17% reduction in resting plasma volume. Therefore, the most important finding of this study was that greater exercise-induced elevation in blood lactate concentration following simulated weightlessness was not due to an increased lactate accumulation, but can be explained by reduced plasma volume.

In our study, the increased blood lactate concentration during submaximal exercise following bedrest-induced deconditioning supported data reported by Saltin et al. (35). The unchanged blood lactate content observed in the present study, however, was inconsistent with the possible explanation of greater anaerobiosis during exercise following simulated weightlessness. Chi et al. (5) reported significant decreases in oxidative enzymes and
increases in enzymes of anaerobic glycolysis following 12 weeks of detraining in 3 cyclists and 4 runners. Furthermore, decreased numbers of mitochondria and loss of respiratory capacity in remaining aerobic energy-producing organelles of atrophic rat muscle following hindlimb immobilization have been reported (31). These specific metabolic and structural observations could suggest the necessity for increased anaerobic metabolism during exercise following hypokinesia. Based on the data of the present study, however, increased utilization of carbohydrate with subsequent increased anaerobic glycolysis and decreased aerobiosis appeared unlikely since our data suggested no net increased anaerobic metabolism in these subjects during exercise following simulated weightlessness. The lack of increased circulating lactate in our study substantiated the fact that at equal submaximal workrates during pre- and post-bedrest exercise, energy was not supplied via increased anaerobic glycolysis. Moreover, our data indicated that predicting the specific energy pathway utilized in the working muscles from indirect metabolic and gas exchange values following simulated weightlessness may be inappropriate without the direct substrate and fluid volume measurements to substantiate the conclusion.

Decreased oxygen supply to the exercising muscle may be considered a plausible cause of increased lactate during exercise following prolonged bedrest. Exercise at high
altitudes and under pathological conditions have illustrated instances when increased lactate was presumed to occur because of decreased oxygen supply and delivery (11, 21). In the present study, oxygen delivery did not appear to be a limiting factor during exercise, as evidenced by equal submaximal $\dot{V}_{\text{O}_2}$ values for pre- and post-bedrest. Furthermore, our data indicated unaltered levels of total circulating lactate during muscular work providing additional evidence of equal energy expenditure before compared to after simulated weightlessness.

Onset of blood lactate accumulation occurs during exercise at approximately 50% $\dot{V}_{\text{O}_2}\text{max}$ (27). Caiozzo et al. (3) demonstrated a high correlation between increased blood lactate and the ventilatory equivalent for oxygen ($\dot{V}_{\text{E}}/\dot{V}_{\text{O}_2}$) during incremental work. Respiratory exchange ratio (R) has also been considered an indicator of greater anaerobic metabolism during exercise (48). These changes in the gas exchange values are regarded as sensitive on-line indicators of blood lactate accumulation onset, thus bypassing the need for measuring blood lactate or acid-base parameters to indicate anaerobiosis. Our data indicate, however, significant 8% and 4% increases in $\dot{V}_{\text{E}}/\dot{V}_{\text{O}_2}$ and R, respectively, during exercise following bedrest with no change in blood lactate content. This illustrates the potential inadequacy inherent in the use of indirect respiratory gas exchange methods for determination of
metabolic substrate kinetics. The ventilatory threshold increased in the present study during graded exercise following bedrest compared to pre-bedrest and blood lactate accumulation did not occur. It would appear that the gas-exchange ventilatory threshold can be dissociated from the blood lactate content accumulation threshold during submaximal exercise following bedrest deconditioning by reducing plasma volume and subsequently elevating [HLa] under similar metabolic conditions. An increase in lactate concentration at the same absolute workrate following simulated weightlessness may result in a proportionate increase in H⁺ and lower pH (acidosis). Although pH was not measured in the present study, increased \( \dot{V}_E \), \( \dot{V}CO_2 \), and R during exercise after bedrest is consistent with greater acidosis. A factor which may further complicate lactate and associated hydrogen ion concentration effects on respiratory drive during exercise would be the isotonic plasma volume decrease during prolonged bedrest which may be associated with a proportionate reduction in total circulating bicarbonate. Therefore, if bicarbonate concentration remains relatively constant at 25 mmol/l following bedrest, a 17% reduction in plasma volume would produce a decrease in total circulating bicarbonate from 85 mmol to 71 mmol, thus reducing the buffering capacity of the blood by a proportionate 17%. Therefore, the additive effects of higher lactate and hydrogen concentration and lower
circulating bicarbonate due directly to deconditioning-induced hypovolemia may cause the increased respiratory drive at the same workrate observed after bedrest in the present study.

Convertino et al. (8) reported significantly high correlation coefficients between changes in respiratory gas exchange indices of blood lactate and changes in blood and plasma volumes following bedrest. This correlation with fluid loss was cited as the primary factor contributing to the lower gas exchange breakpoint and apparent earlier onset of blood lactate accumulation. With direct measures showing unchanged blood lactate content in our study, the significantly elevated blood lactate concentration can only be explained by the bedrest-induced deconditioning decline in plasma volume. Although the muscle lactate kinetics have yet to be investigated, the rate of lactate appearance in the blood during exercise may remain unaltered following bedrest and the fluid lost during bedrest may have caused a hemoconcentration of this lactate during exercise. This elevated concentration of blood lactate may be further accentuated by muscular uptake of fluid during acute exercise (39). Finally, Sejersted et al. (37) reported a linear relationship between lactate concentration and hematocrit during reuptake of water by the circulating blood following exercise, providing further support for this plasma volume/blood lactate relationship.
Finally, this investigation provided evidence that production of equal energy during submaximal exercise before and after bedrest did not appear to be a result of net increased anaerobic glycolysis. This does not, however, necessarily exclude increased anaerobiosis in all conditions. In a study of longitudinal design Saltin et al. (35) observed the response of five male subjects during submaximal exercise following bedrest. Blood lactate concentration data was not corrected for 8% decreased plasma volume which occurred during 20 days of bedrest. Adjustment of blood lactate concentration data for fluid loss due to chronic adaptation to prolonged bedrest actually reveals greater increased circulating lactate content following bedrest. By simply multiplying resting plasma volume before and after bedrest times blood lactate concentration values measured under the corresponding conditions in the Saltin study, blood lactate content (mmol) could be reported as a 62% greater increase during supine cycling as opposed to 57% increase as calculated from blood lactate concentration (mmol/l) changes before compared to after bedrest. These data did, indeed, illustrate net increased anaerobiosis following bedrest which is in direct contrast to the present study and further emphasize the importance of plasma volume corrections when reporting accurate percent changes in circulating solute. Under some experimental conditions, prediction of energy pathways from data influenced by, but
not corrected for fluid shifts, can lead to inaccurate assumptions as illustrated by the conclusion of no apparent net increased anaerobiosis in the present study contrasting with Saltin's data indicating greater increase anaerobiosis under the same environmental perturbation.

In conclusion, the data of the present study demonstrated that blood lactate concentration increase during acute exercise following simulated weightlessness was a function of reduction in plasma volume. This study further illustrated the caution necessary when interpreting data showing concentration changes measured in blood. Training and detraining studies designed to assess chronic metabolic adaptation must account for occurrence of plasma and blood volume alterations before data analyses and interpretation. Furthermore, absence of fluid volume data may lead to misinterpretation of clinical data when assessing patients confined to prolonged bedrest. Most importantly, the present study served to clearly illustrate the necessity for taking fluid changes into consideration when interpreting solute data collected during exercise following bedrest deconditioning. Procedures which enhance retention of body fluid during prolonged bedrest could act as countermeasures to the elevated blood lactate concentration and provide a more constant internal environment within the body when perturbed by the stress of acute exercise following simulated weightlessness.
LIST OF REFERENCES


