2007-08-28

Plasma Volume and Albumin mRNA Expression in Exercise Trained Rats

Nathan Alex Bexfield
Brigham Young University - Provo

PLASMA VOLUME AND ALBUMIN mRNA EXPRESSION IN EXERCISE TRAINED RATS

by

Nathan A. Bexfield

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Exercise Sciences

Brigham Young University

December 2007

BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Nathan A. Bexfield

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 C. Parcell
Date	Pat R. Vehrs

BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Nathan A. Bexfield 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.

_____ _____

Date Gary W. Mack
Chair, Graduate Committee

Accepted for the Department

_____

Larry T. Hall
Chair, Department of Exercise Sciences

Accepted for the College

_____

Gordon B. Lindsay, Associate Dean
College of Health and Human Performance

ABSTRACT

PLASMA VOLUME AND ALBUMIN mRNA EXPRESSION IN EXERCISE TRAINED RATS

Nathan A. Bexfield

Department of Exercise Sciences

Master of Science

Introduction- Exercise-induced plasma volume (PV) expansion is typically associated with an increase in plasma albumin content. Increased hepatic albumin synthesis, a transcriptionally regulated process, is thought to contribute to the increase in albumin content. ***Objective-*** We tested the hypothesis that exercise training induces an increase in albumin gene expression in relationship to the increase in PV. ***Methods and Results-*** 40 adult male Sprague-Dawley rats weighing between 245-350 grams were randomly assigned to one of four groups: cage control (CC); sham exercise 10 min/day at 48% VO_2max (NE); continuous exercise training, 60 min /day at 72% VO_2max (LI); and high intensity, intermittent exercise training, 8 bouts of 4 min at 98% VO_2max followed by 5 min at 48% VO_2max (HI). The training period lasted for two weeks with 12 training sessions with equalized training volumes in the exercise groups. 24 hours after the last training session the rats were anesthetized and a jugular catheter was placed for collecting

blood samples during PV determination by a dilution of a labeled-albumin molecule (Texas Red albumin). The liver and red quadriceps (RQ) muscle tissue was then removed, flash frozen, and stored for later analysis. The training protocol produced a significant increase in RQ citrate synthase activity ($p < 0.05$). PV increased in proportion to the exercise intensity ($p < 0.05$) averaging 23.6 ± 2.7 ml•kg^{-1} body weight in the CC group and 26.6 ± 1.3 ml•kg^{-1} body weight in the HI group. Albumin mRNA expression determined by real time polymerase chain reaction (PCR) increased 2.2 ± 0.1 and 2.9 ± 0.2 fold following LI and HI exercise training, respectively. ***Conclusion-*** These data support the hypothesis that, during exercise-induced PV expansion, albumin gene expression is increased and contributes to an increase in plasma albumin content and PV.

ACKNOWLEDGMENTS

I would like to thank Dr. Gary W. Mack for making this project happen for me. He went above and beyond his responsibility as my advisor and mentor to see that this project was completed, as well as done properly. He often provided patience and kindness when things became difficult. He showed me how research should be done properly. He also made this project fun and interesting and gave me guidance only a friend could give. In addition, I would like to thank Brad Nelson, Kris Foote, Rory Mitchell, and Allen Parcell for their willingness and efforts to help me complete this project by assisting me in the lab. They made this project go smoothly and effectively.

This project was supported with financial assistance by the Mary Lou Fulton Endowment Chair for Health and Human Performance and National Heart, Lung, and Blood Institute Grant HL-20634.

Table of Contents

List of Tables

Table Page

List of Figures

Plasma volume and albumin mRNA expression in exercise trained rats.

Nathan A. Bexfield, Allen C. Parcell, Pat R. Vehrs, W. Bradley Nelson, Kris M. Foote, Rory C. Mitchell, and Gary W. Mack, Department of Exercise Sciences, Brigham Young University, Provo, UT, 84604

Correspondence: Gary W. Mack, 120F Richards Building, Provo, UT, 84602

(801) 422-6651

Email: Gary_mack@byu.edu

Abstract

Introduction- Exercise-induced plasma volume (PV) expansion is typically associated with an increase in plasma albumin content. Increased hepatic albumin synthesis, a transcriptionally regulated process, is thought to contribute to the increase in albumin content. ***Objective-*** We tested the hypothesis that exercise training induces an increase in albumin gene expression in relationship to the increase in PV. ***Methods and Results-*** 40 adult male Sprague-Dawley rats weighing between 245-350 grams were randomly assigned to one of four groups: cage control (CC); sham exercise 10 min/day at 48% VO_2max (NE); continuous exercise training, 60 min /day at 72% VO_2max (LI); and high intensity, intermittent exercise training, 8 bouts of 4 min at 98% VO_2max followed by 5 min at 48% VO_2max (HI). The training period lasted for two weeks with 12 training sessions with equalized training volumes in the exercise groups. 24 hours after the last training session the rats were anesthetized and a jugular catheter was placed for collecting blood samples during PV determination by a dilution of a labeled-albumin molecule (Texas Red albumin). The liver and red quadriceps (RQ) muscle tissue was then removed, flash frozen, and stored for later analysis. The training protocol produced a significant increase in RQ citrate synthase activity ($p < 0.05$). PV increased in proportion to the exercise intensity ($p < 0.05$) averaging 23.6 ± 2.7 ml•kg^{-1} body weight in the CC group and 26.6 ± 1.3 ml•kg^{-1} body weight in the HI group. Albumin mRNA expression determined by real time polymerase chain reaction (PCR) increased 2.2 ± 0.1 and 2.9 ± 0.2 fold following LI and HI exercise training, respectively. ***Conclusion-*** These data support the hypothesis that, during exercise-induced PV expansion, albumin gene expression is increased and contributes to an increase in plasma albumin content and PV.

Introduction

Exercise-induced hypervolemia refers to increased blood volume (BV) as a result of exercise training (7-9, 24, 30). Both plasma volume (PV) and red blood cell volume increase with endurance training. The expansion of PV can occur within 24 hours following a high intensity intermittent exercise protocol without a significant change in red blood cell volume (22). In humans, exercise-induced plasma volume expansion is associated with an increase in plasma albumin content (21). The increase in plasma albumin content is due to several physiological responses including 1) a redistribution of albumin from the interstitial fluid (ISF) to the plasma compartment (10, 22, 23, 31), 2) a reduction in the rate of albumin escape rate from the plasma compartment (10, 15), and 3) an increase in hepatic albumin synthesis following exercise (21, 37).

To investigate the cellular and molecular signally pathways involved in turning on albumin synthesis during exercise requires an appropriate animal model. Exercise-induced PV expansion occurs in several animals, including the rabbit (4), dog (16) and horse (17). Although not clearly documented, antidotal evidence indicates a similar response occurs in the rat. Stahl et al. has demonstrated in the rodent that PV expansion and plasma albumin dynamics in response to hemorrhage (31) is similar to that seen in humans following high-intensity intermittent exercise (10, 15, 22). One purpose of this study was to develop a rodent exercise protocol that mimics the PV expansion seen in human studies (7-9, 24, 30). A 12-day training schedule using a continuous (60 min) moderate exercise intensity (70-80% $\dot{V}O_2max$) was chosen in an attempt to duplicate PV adaptations seen in human studies (3, 28). Because high intensity intermittent exercise elicits plasma volume expansion and increased albumin synthesis in humans (21) we

also evaluated a high intensity intermittent protocol for the rodent for comparison to the continuous training protocol.

Because albumin synthesis is transcriptionally regulated (27), we expect that exercise training will enhance albumin gene expression (6, 18, 21, 22, 27, 31, 35). Thus, a second focus of this study was to test the hypothesis that aerobic exercise training stimulates an increase in liver albumin mRNA expression.

Methods and Materials

Forty adult male Sprague-Dawley rats weighing between 245-350 grams were randomly assigned to one of four groups: cage control (CC, n=13), sham training (NE, n=10), continuous exercise training (LI, n=10), and high intensity, intermittent exercise training (HI, n=10). All of the rats (except CC) were handled for 14 days before treatment was applied. Rats in the NE group walked on a motorized treadmill for 10 minutes per day at 10 m/min for the entire 14-day acclimatization period. Rats in the LI and HI groups participated in a pre-training program that increased in speed and duration.

The respective treatments for each group were applied for two weeks including 12 training sessions with 2 days of rest interspersed. The rats ran on a standard multi-lane animal treadmill (Quinton®, Bothell, WA). Exercise intensities for each exercise group were based upon an established $\dot{V}O_2$ and speed relationships at 10% grade (1, 20, 25, 36) and an estimated $\dot{V}O_2max$ for the male Sprague-Dawley rat of 100 $ml \bullet kg^{-1} \bullet min^{-1}$ in the literature (1, 20, 25, 36).

The NE group walked on the treadmill for 10 min per day at 46 ± 2% of estimated aerobic capacity every training bout. The LI group ran continuously on the treadmill at 78 ± 3% of estimated aerobic capacity for 60 min. The HI group performed a high

intensity intermittent exercise protocol that consisted of running at 98 ± 4% of estimated aerobic capacity for 4 min followed by a 5-min recovery period of running at 48 ± 2% aerobic capacity. The rats in the HI group repeated this exercise pattern 8 times per session (72 min total time). The CC group was left in the cage for 14 days and sacrificed after that period. We assumed the resting metabolic rate of the rodent at about 21 ± 1% of $\dot{V}O_2max$ (14).

Approximately 24 hrs after the last training session, the rats were anesthetized with a ketamine/xylazine cocktail. The right jugular vein was cannulated and a control blood sample of 0.45 ml was taken. A 0.25 ml Texas Red albumin solution was injected into the cannulated jugular vein. After a 5-min period, a second blood sample of 0.5 ml was taken in order to determine blood volume using a dye dilution method described by Gillen et al. (6). The liver was first exposed through a midline incision and then the animal was euthanized with approximately 0.23 ml of pentobarbital (200 $mg \bullet ml^{-1}$). The liver was rapidly removed and back-flushed with ice cold 0.9% saline to remove blood cells. The liver was frozen in liquid nitrogen and stored at -80° C until RNA was isolated. Muscle tissue from the quadriceps was harvested in order to analyze citrate synthase enzyme activity using a citrate synthase colorimetric assay (Sigma-Aldrich, kit #CS0720). The heart was also excised and weighed.

Muscle tissue was crushed into a fine powder on liquid nitrogen and dry ice. Homogenate was made using Tris buffer and 40-75 mg of muscle tissue. Frozen liver was crushed into a fine powder on dry ice and liquid nitrogen and RNA was isolated from 100-150 mg of liver powder using a guanidine thyocyanate method. cDNA was synthesized from 2 µg of total RNA with Oligo (dT) and Stratascript RT (Stratagene®, La

Jolla, CA) using an MJ Research PTC-200 Peltier® Thermal Cycler (Global Medical Instruments®, Ramsey, MN). cDNA amount was quantified using picogreen reagent (Invitrogen-Molecular Probes®, Carlsbad, CA) based upon a standard curve derived from a serial dilution of a known concentration of double stranded DNA (dsDNA). Real-time polymerase chain reaction (PCR) was performed on 100 ng of cDNA using a BioRad® I-Cycler (Hercules, CA) with amplication detection using SyberGreen® for both albumin and a common housekeeping gene (GAPDH). The primers (Invitrogen-Molecular Probes®, Carlsbad, CA) used for albumin were:

Sense:

5'-GGCAACAGACGTTACCAAAATCA-3'

Antisense:

5'-TCATCCGCGATTCCAA-3'

Rat GAPDH primers were supplied as a set from SuperArray Bioscience Corportation (Fredrick, MD). PCR was quantified using a standard curve produced using serial dilution of cDNA derived from pooled liver samples for the target gene albumin and the endogenous reference (GAPDH). The efficiencies of the PCR reaction for albumin and GAPDH were determined from duplicate standard curves and averaged 89% and 92%, respectively.

Based upon our randomized block design we used factorial ANOVA to compare albumin mRNA expression, plasma volume, blood volume and other variables. Least squares linear regression was used to identify significant relationships between variables. When a significant F value was obtained we used a least significant difference post-hoc test to compare each group to each other. Significance levels were set at $p<0.05$.

Results

Training Stimulus. Treadmill speed for the NE, LI and HI exercise groups were estimated based upon the linear relationship between treadmill speed and oxygen consumption from published data on male Sprague-Dawley rats running on a treadmill at a 10% grade ($\dot{V}O_2$, $ml \bullet min^{-1} \bullet kg^{-1}BW = 1.61 \bullet speed\ (m \bullet min^{-1}) + 29.8$) (1, 20, 25, 36). We assumed an average $\dot{V}O_2max$ for a male Sprague-Dawley rat of 100 ml $O_2 \bullet min^{-1} \bullet kg^{-1}$ body weight (1, 20, 25, 36). The rats in each group were able to complete the 12 days of assigned training with the exception of 3 rats in the LI group that were unable to complete the entire 60 min workout during the earlier sessions (sessions 1-4) but were able to work up to the required amount of work in the later sessions. The 12-day training stimulus produced increases in citrate synthase activity in the red quadriceps (RQ) in proportion to the exercise training intensity ($p < 0.05$, $r^2 = 0.96$). Citrate synthase activity was 70% higher in the HI group compared to CC ($p < 0.05$, Figure 1). Heart weight-to-body weight ratio, which was not expected to change over this short training period, was similar for all treatment groups. The training did reduce body weight in the exercise groups over the training period. At the time of sacrifice, rats in the CC (294 ± 9 g) and NE (298 ± 7 g) groups were significantly ($p < 0.05$) heavier than rats in the LI (269 ± 4 g) and HI (274 ± 5 g) groups.

Hematological responses. Hematocrit increased ($p < 0.05$) following exercise training from 46.8 ± 0.5 % for CC to 47.4 ± 0.7 and 50.4 ± 0.4 % for LI and HI groups, respectively. The NE group's Hematocrit averaged 48.9 ± 0.4 %, which was also higher than CC ($p < 0.05$) but lower than HI ($p < 0.05$). Plasma volume and blood volume averaged 7.3 ± 0.6 and 13.9 ± 1.3 ml in the CC group, respectively. Mean PV and BV

tended to increase in proportion to the estimated exercise-training stimulus ($p < 0.05$, r^2 = 0.87 and $r^2 = 0.98$, respectively). However, ANOVA did not detect a significant difference between the four groups (Table 1). Similarly, mean plasma albumin content increased in proportion to the estimated exercise-training stimulus ($p < 0.05$, $r^2 = 0.92$). Plasma albumin content is shown for each treatment group in Table 1. Plasma volume and plasma albumin content were significantly correlated (Figure 2, $p < 0.05$, $r^2 = 0.84$) with a slope of 23 ml of plasma volume per gram of plasma albumin.

Albumin mRNA expression increased ($p < 0.05$) above CC levels for NE, LI, and HI groups (Figure 3). In addition, the increase in hepatic albumin mRNA expression was proportional to the estimated exercise-training stimulus ($p < 0.05$, $r^2 = 0.74$, Figure 4).

Discussion

The objective of this study was to develop a rodent model for exercise-induced plasma volume expansion that could be used to investigate molecular signaling pathways in the liver related to albumin synthesis. In general, the12-day training protocol produced adaptations in muscle enzyme activity, PV, plasma albumin content and albumin mRNA expression in proportion to the exercise stimulus intensity. The significant finding of this study was that 12 days of high intensity intermittent training in the rat produced an increase in RQ citrate synthase activity and an increase in hepatic albumin mRNA expression (Figure 3). In addition, we noted a consistent relationship between plasma albumin content and plasma volume.

Citrate synthase activity in the RQ was measured as a marker of aerobic adaptations in skeletal muscle following training. In this study, the HI group produced a significant increase in citrate synthase activity in the RQ compared to the CC group,

indicating significant aerobic adaptations after only 12 days of training. The citrate synthase levels in the LI group were similar to CC or NE. This observation is consistent with the literature such that significant skeletal muscle adaptations to moderate intensity continuous exercise in the rodent requires more than 8 weeks to establish (11). The HI group performed slightly more work (2152 meters per workout) than the LI group (1822 meters per workout). However, regression analysis demonstrated a significant relationship between RQ citrate synthase activity and exercise intensity (treadmill speed). This relationship indicates that exercise intensity may represent the primary signal in the citrate synthase response to the exercise stimulus rather than exercise volume (duration x intensity).

Our rodent exercise training protocol differs significantly from previous models with longer periods of training. Our exercise program involved only 12 days of training. Yet our protocol produced increases in citrate synthase activity in the red quadriceps muscle similar to exercise training programs lasting much longer (5, 12, 19, 32). Our rodent exercise protocol did not affect the heart weight-to-body weight ratio. Eight weeks of aerobic training does not produce an increase in heart weight-to-body weight ratio in the rodent (26, 33). However, training programs greater than 4 months will produce an increase in heart weight to body weight ratio (13).

We noted an increase in hematocrit in all the groups compared to the cage control animals, however, the increase in hematocrit in the HI group was larger than the NE or LI group. Increased hematocrit can be caused by an increase in red blood cell volume or a decrease in PV. Since PV either remained the same or increased slightly in the NE, LI and HI groups, the increase in hematocrit was most likely due to an increase in red blood

cell volume. Aerobic exercise training for 12 weeks does not typically induce an increase in rodent hematocrit or hemoglobin concentration (34). We noted an increase in hematocrit in the sham exercise group. It is unclear why hematocrit was increased in the sham exercise rats, however the increase in hematocrit may be attributed to the combination of altitude and the slight exercise stimulus they received. Our animal care facilities are located at an elevation of about 4500 ft. This altitude in combination with light activity appeared sufficient to raise red blood cell volume in the NE group. The increase in hematocrit in the LI group was similar to that seen with NE. The combination of slight altitude with intense exercise produced the greatest increase in hematocrit. The hematocrit data are in agreement with our citrate synthase data and indicated that the high intensity intermittent training protocol produces marked aerobic adaptations in the rodent in a mere 12 days.

Regression analysis indicated a positive relationship between PV and exercise training intensity. Despite the ability of our exercise training protocols to promote adaptations that promote enhanced aerobic capacity we did not identify (by ANOVA) a clear increase in PV or BV in 12 days. One interpretation of these data is that the adaptation in body fluid compartments with training may require more time to develop than other physiological adaptations to exercise. This seems unlikely since it has been clearly demonstrated that PV expansion occurs within 24 h after a high intensity intermittent training protocol in humans (3, 24). A more likely explanation is a limitation in the measurement technique to resolve small (≈12%) changes in PV with training within the confines of cross section study design. This limitation could be overcome by

implementing a longitudinal study design using chronically instrumented rodents. Alternatively, we could extend the training period an additional week or two.

Hepatic albumin mRNA expression increased 2 fold in the NE group and 3 fold in HI rodents. Hepatic albumin mRNA expression in chronically instrumented rodents can be downregulated by 50% following albumin infusion (27). Larger effects on albumin mRNA expression (2.3-2.5 fold increase) have been seen in cultured hepatocytes exposed to hydrocortisone or cAMP derivatives (2). Following high intensity intermittent exercise training we noted a large three-fold increase in hepatic albumin mRNA. We considered these changes to be reasonable and a valid index of the impact of exercise on liver gene expression for several reasons. First, the mRNA expression of our housekeeping gene (GAPDH) was similar in all groups. Second, the efficiency of our PCR reactions was similar for GAPDH and albumin averaged 92% and 89% respectively. Since albumin synthesis is transcriptionally regulated (35) the three-fold increase of albumin mRNA expression should have also been reflected by an increase in plasma albumin content. We did note that the increase in plasma albumin content was proportional to exercise intensity. In addition, the slope of the relationship between PV and plasma albumin content was equal to 23 $ml \bullet g^{-1}$ albumin. This value corresponds with the expected ability of albumin to attract water into the vascular space (18 ml water per gram albumin) as described by Scatchard et al (29). These data support the hypothesis that exercise training stimulated albumin expression (Figure 3), which was reflected in a proportional change in plasma albumin content and plasma volume (Figure 2).

Limitations. Regression analysis indicated a significant relationship between PV and exercise intensity. Plasma volume increased ≈12% in the HI group compared to the

CC group (Table 1). This level of expansion in PV is similar to those reported for human studies however (7-9, 24, 30). However, variability in the PV measurement and the cross sectional design of the experiment limited our ability to detect group differences in PV and plasma albumin content by ANOVA. Our measurement of PV in the anesthetized rodent was 18-35% lower than that reported for chronically instrumented, awake rodents using a similar Texas Red albumin dilution technique (6, 31). The contribution of both acute trauma and anesthesia may have impacted cardiac output distribution and ultimately the size of the albumin distribution space leading to reductions in absolute PV determinations. Since all animals were treated the same, this error should not have impacted the outcome of the analysis, but could explain the difference in absolute PV between awake and anesthetized rats. In the future, a longitudinal approach monitoring PV before and after training in chronically instrumented rodents, may allow sufficient resolution to detect smaller increases in PV and plasma albumin content with this training model.

In conclusion, our exercise protocol produced increased enzyme activity in skeletal muscle (increased citrate synthase activity in RQ) and albumin mRNA expression in the liver. Plasma volume and plasma albumin content increased in proportion to the exercise intensity. These latter two responses provide general support for the hypothesis that an exercise stimulus that induces PV expansion also stimulates expression of albumin mRNA. One interpretation of these data is that exercise can induce molecular signaling within the hepatocytes that should lead to increased albumin gene expression. Our study design produced promising trends, however, a longitudinal

design would allow for improved resolution of small changes in PV associated with this 12-day training program.

References

1. **Brooks GA and White TP.** Determination of metabolic and heart rate responses of rats to treadmill exercise. *J Appl Physiol* 45: 1009-1015, 1978.
2. **Brown PC and Papaconstantinou J.** Coordinated modulation of albumin synthesis and mRNA levels in cultured hepatoma cells by hydrocortisone and cyclic AMP analogs. *J Biol Chem* 254: 9379-9384, 1979.
3. **Convertino VA, Brock PJ, Keil LC, Bernauer EM, and Greenleaf JE.** Exercise training-induced hypervolemia: role of plasma albumin, renin, and vasopressin. *J Appl Physiol* 48: 665-669, 1980.
4. **DiCarlo SE and Bishop VS.** Exercise training attenuates baroreflex regulation of nerve activity in rabbits. *Am J Physiol* 255: H974-979, 1988.
5. **Durante PE, Mustard KJ, Park SH, Winder WW, and Hardie DG.** Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. *Am J Physiol Endocrinol Metab* 283: E178-186, 2002.
6. **Gillen CM, Takamata A, Mack GW, and Nadel ER.** Measurement of plasma volume in rats with use of fluorescent-labeled albumin molecules. *J Appl Physiol* 76: 485-489, 1994.
7. **Green HJ, Carter S, Grant S, Tupling R, Coates G, and Ali M.** Vascular volumes and hematology in male and female runners and cyclists. *Eur J Appl Physiol Occup Physiol* 79: 244-250, 1999.
8. **Green HJ, Sutton JR, Coates G, Ali M, and Jones S.** Response of red cell and plasma volume to prolonged training in humans. *J Appl Physiol* 70: 1810-1815, 1991.

9. **Green HJ, Thomson JA, Ball ME, Hughson RL, Houston ME, and Sharratt MT.** Alterations in blood volume following short-term supramaximal exercise. *J Appl Physiol* 56: 145-149, 1984.

10. **Haskell A, Nadel ER, Stachenfeld NS, Nagashima K, and Mack GW.** Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 83: 407-413, 1997.

11. **Hickson RC, Heusner WW, and Van Huss WD.** Skeletal muscle enzyme alterations after sprint and endurance training. *J Appl Physiol* 40: 868-871, 1976.

12. **Holloszy JO.** Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 242: 2278-2282, 1967.

13. **Li Y, Lincoln T, Mendelowitz D, Grossman W, and Wei JY.** Age-related differences in effect of exercise training on cardiac muscle function in rats. *Am J Physiol* 251: H12-18, 1986.

14. **Mack GW and Lin YC.** Hypothermia impairs but hyperthermia does not promote inert gas elimination in the rat. *Undersea Biomed Res* 13: 133-145, 1986.

15. **Mack GW, Yang R, Hargens AR, Nagashima K, and Haskell A.** Influence of hydrostatic pressure gradients on regulation of plasma volume after exercise. *J Appl Physiol* 85: 667-675, 1998.

16. **McKeever KH, Schurg WA, and Convertino VA.** Exercise training-induced hypervolemia in greyhounds: role of water intake and renal mechanisms. *Am J Physiol* 248: R422-425, 1985.

17. **McKeever KH, Schurg WA, Jarrett SH, and Convertino VA.** Exercise training-induced hypervolemia in the horse. *Med Sci Sports Exerc* 19: 21-27, 1987.

18. **Mendel DB and Crabtree GR.** HNF-1, a member of a novel class of dimerizing homeodomain proteins. *J Biol Chem* 266: 677-680, 1991.

19. **Mole PA, Baldwin KM, Terjung RL, and Holloszy JO.** Enzymatic pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise. *Am J Physiol* 224: 50-54, 1973.

20. **Moraska A, Deak T, Spencer RL, Roth D, and Fleshner M.** Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol* 279: R1321-1329, 2000.

21. **Nagashima K, Cline GW, Mack GW, Shulman GI, and Nadel ER.** Intense exercise stimulates albumin synthesis in the upright posture. *J Appl Physiol* 88: 41-46, 2000.

22. **Nagashima K, Mack GW, Haskell A, Nishiyasu T, and Nadel ER.** Mechanism for the posture-specific plasma volume increase after a single intense exercise protocol. *J Appl Physiol* 86: 867-873, 1999.

23. **Okazaki K, Kamijo Y, Takeno Y, Okumoto T, Masuki S, and Nose H.** Effects of exercise training on thermoregulatory responses and blood volume in older men. *J Appl Physiol* 93: 1630-1637, 2002.

24. **Oscai LB, Williams BT, and Hertig BA.** Effect of exercise on blood volume. *J Appl Physiol* 24: 622-624, 1968.

25. **Pattengale PK and Holloszy JO.** Augmentation of skeletal muscle myoglobin by a program of treadmill running. *Am J Physiol* 213: 783-785, 1967.

26. **Paulson DJ, Kopp SJ, Peace DG, and Tow JP.** Myocardial adaptation to endurance exercise training in diabetic rats. *Am J Physiol* 252: R1073-1081, 1987.

27. **Pietrangelo A, Panduro A, Chowdhury JR, and Shafritz DA.** Albumin gene expression is down-regulated by albumin or macromolecule infusion in the rat. *J Clin Invest* 89: 1755-1760, 1992.

28. **Roberts MF, Wenger CB, Stolwijk JA, and Nadel ER.** Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol* 43: 133-137, 1977.

29. **Scatchard G, Gibson ST, Woodruff LM, Batchelder AC, and Brown A.** Chemical, Clinical, And Immunological Studies On The Products Of Human Plasma Fractionation. Iv. A Study Of The Thermal Stability Of Human Serum Albumin. *J Clin Invest* 23: 445-453, 1944.

30. **Shoemaker JK, Green HJ, Coates J, Ali M, and Grant S.** Failure of prolonged exercise training to increase red cell mass in humans. *Am J Physiol* 270: H121-126, 1996.

31. **Stahl AM, Gillen CM, Takamata A, Nadel ER, and Mack GW.** Reduced blood-to-tissue albumin movement after plasmapheresis. *Shock* 19: 440-447, 2003.

32. **Taylor EB, Lamb JD, Hurst RW, Chesser DG, Ellingson WJ, Greenwood LJ, Porter BB, Herway ST, and Winder WW.** Endurance training increases skeletal

muscle LKB1 and PGC-1alpha protein abundance: effects of time and intensity. *Am J Physiol Endocrinol Metab* 289: E960-968, 2005.

33. **Tibbits G, Koziol BJ, Roberts NK, Baldwin KM, and Barnard RJ.** Adaptation of the rat myocardium to endurance training. *J Appl Physiol* 44: 85-89, 1978.

34. **Tobin BW and Beard JL.** Interactions of iron deficiency and exercise training in male Sprague-Dawley rats: ferrokinetics and hematology. *J Nutr* 119: 1340-1347, 1989.

35. **Tronche F, Rollier A, Herbomel P, Bach I, Cereghini S, Weiss M, and Yaniv M.** Anatomy of the rat albumin promoter. *Mol Biol Med* 7: 173-185, 1990.

36. **Woodman CR, Monnin KA, Sebastian LA, and Tipton CM.** Influence of simulated microgravity on the VO2 max of nontrained and trained rats. *J Appl Physiol* 74: 1941-1947, 1993.

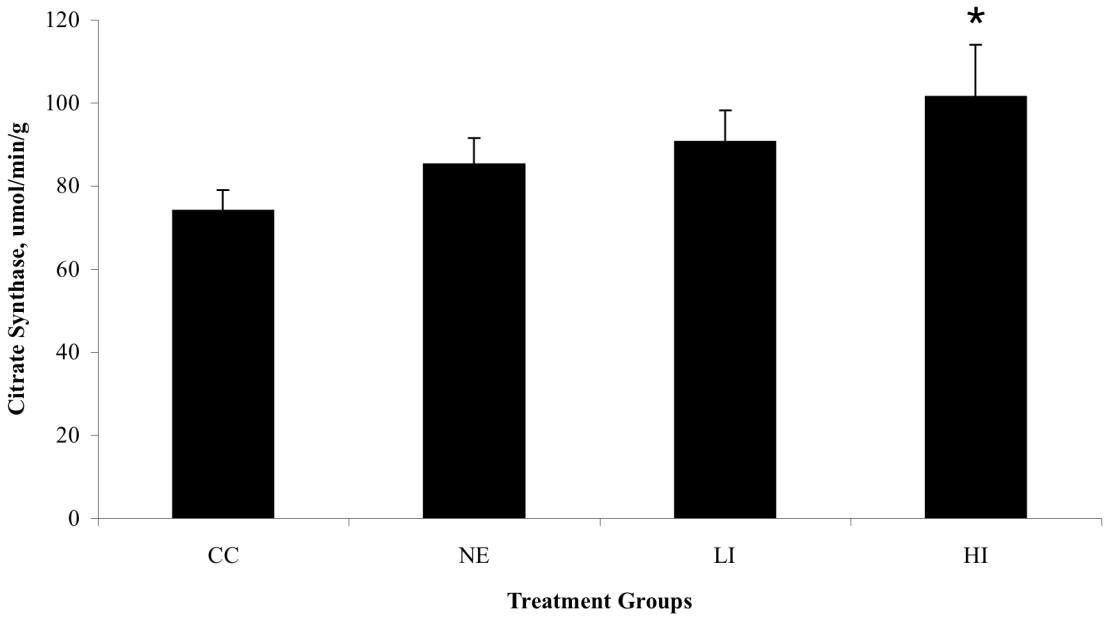
37. **Yang RC, Mack GW, Wolfe RR, and Nadel ER.** Albumin synthesis after intense intermittent exercise in human subjects. *J Appl Physiol* 84: 584-592, 1998.

Table 1. Hematological changes following 12 days of training

	TREATMENT GROUP			
Variable	CC	NE	LI	HI
HCT, %	46.8 ± 0.6	48.9 ± 0.3*	47.4 ± 0.7*	50.4 ± 0.4*†#
PV, ml/kg	23.6 ± 2.7	23.4 ± 2.8	27.1 ± 3.1	26.4 ± 1.2
BV, ml/kg	44.2 ± 5.4	46.2 ± 5.5	51.6 ± 5.8	53.1 ± 2.5
$C_{Albumin}$, g/kg	0.70 ± 0.09	0.67 ± 0.13	0.83 ± 0.11	0.84 ± 0.07

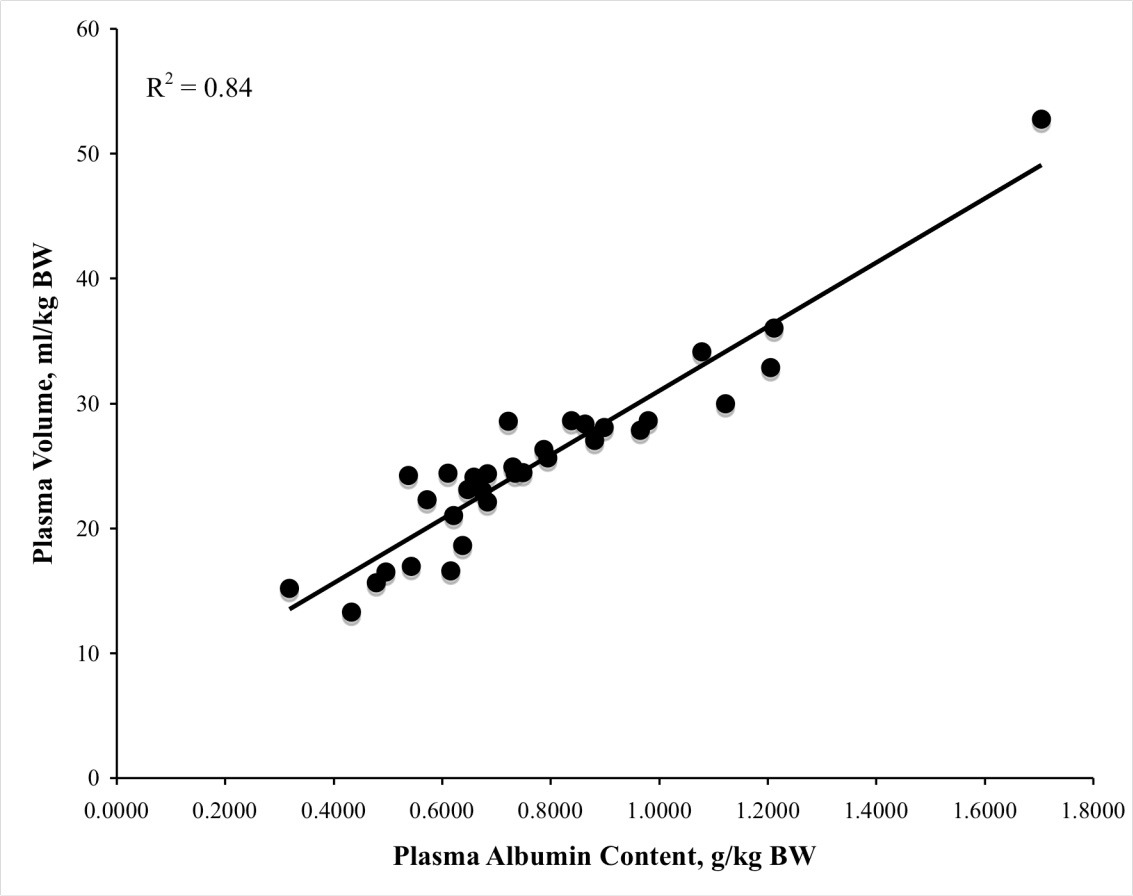
Where CC = cage control; NE = sham exercise; LI = continuous exercise; HI = high intensity intermittent exercise; HCT = hematocrit; PV = plasma volume; BV = blood volume; $C_{albumin}$ = plasma albumin content. Values represent mean ± 1 SE. * $p < 0.05$ different from CC, † $p < 0.05$ different from NE, # $p < 0.05$ different from LI

Figure 1



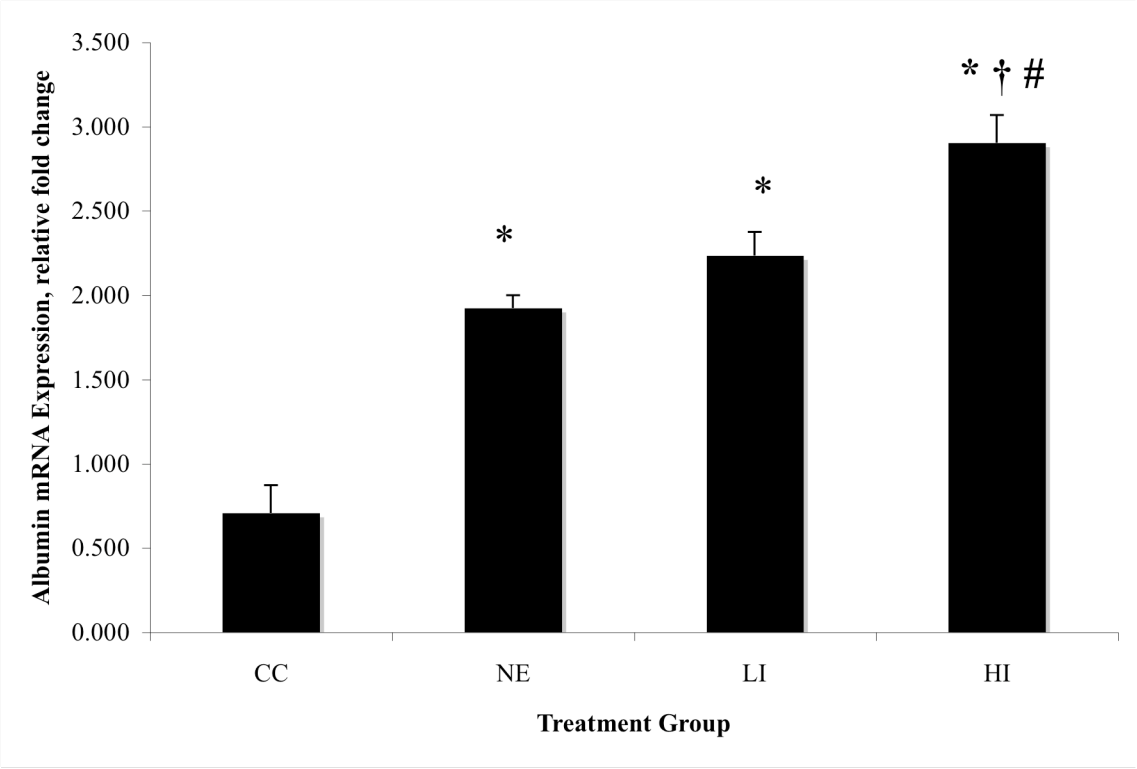
Citrate synthase activity in the red quadriceps in each treatment group after the 12-day training period. Where CC = cage control; NE = sham exercise; LI = continuous exercise; HI = high intensity intermittent exercise. * $p < 0.05$ different from CC. ($N = 43$)

Figure 2



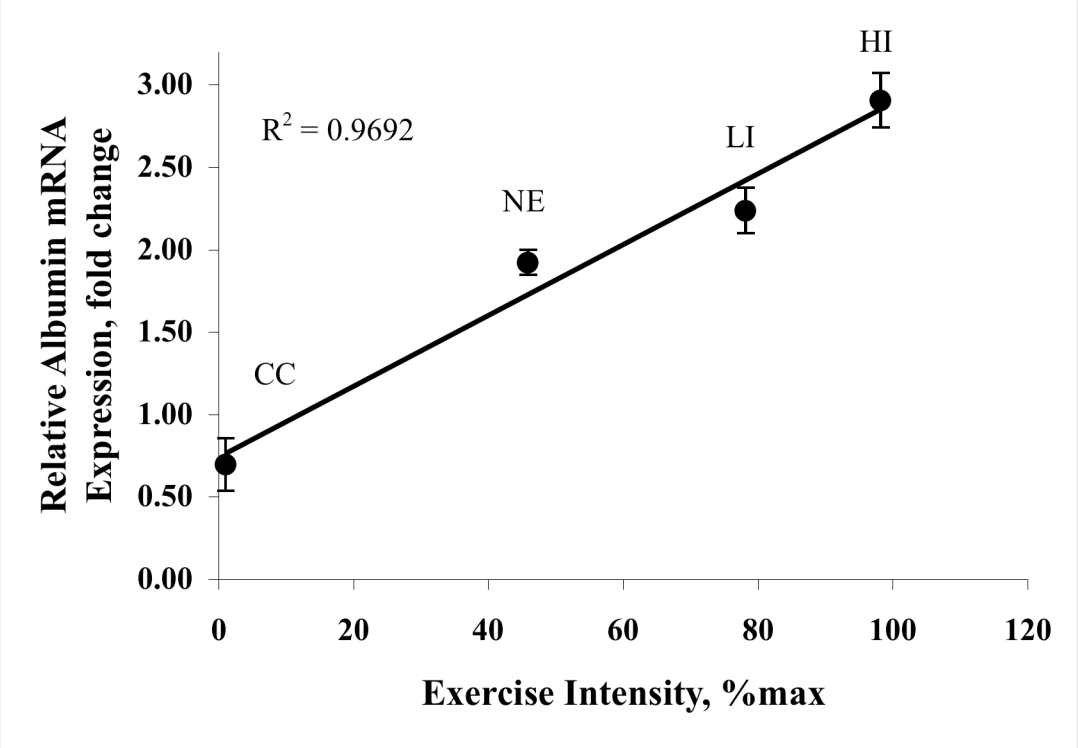
Correlation between individual relative PV and individual relative plasma albumin content in each subject ($N = 43$) following the 12- day training period. R^2 =0.84 represents a proportional increase in plasma albumin content with the PV increase.

Figure 3



Relative fold change in Albumin mRNA expression following the 12- day training period in each group. Where CC = cage control; NE = sham exercise; LI = continuous exercise; HI = high intensity intermittent exercise. * $p < 0.05$ different from CC, † $p < 0.05$ different from NE, # $p < 0.05$ different from LI.

Figure 4



Fold change in relative albumin mRNA expression compared to exercise intensity in each group following 12- day training period. Where CC = cage control; NE = sham exercise; LI = continuous exercise; HI = high intensity intermittent exercise. Positive correlation (r^2 = 0.9692) indicates a proportional increase in albumin mRNA expression with increased exercise intensity.

Appendix A

Prospectus

Chapter 1

Introduction

The physiological mechanisms involved in the increase in plasma volume during exercise training are not clearly identified or understood. Elevated plasma volume provides improved cardiovascular function, which includes increased venous return. An increase in venous return leads to increased stroke volume (SV) (7, 38). Cardiac output (Q) is calculated by multiplying SV by heart rate (15). An increase in SV will increase Q. According to the Fick equation, VO_2 is calculated by multiplying Q by the difference between arterial oxygen content and venous oxygen content (a-v O_2 diff) (15). Plasma volume expansion increases VO_2 and oxygen supply to contracting muscles by increasing Q (11, 12, 13, 18). Augmented plasma volume also increases blood flow to the skin during exercise thereby allowing increased cooling capacity of the body via convection (31,32).

In general, it is clear that plasma volume expansion improves athletic performance (31). Understanding how specific mechanisms act to stimulate plasma volume expansion could help in the development of procedures or training techniques that might optimize this process in athletes. Most of what is known about exercise-induced plasma volume expansion has come from human experimentation (3). However, little is known concerning the cellular and/or molecular mechanisms that regulate these adaptations. The use of an animal model for exercise-induced plasma volume expansion would allow for a more in depth study of these cellular and molecular mechanisms.

This study emphasizes the control in the rate of albumin synthesis in determining total plasma albumin content and overall plasma volume expansion. Plasma albumin is

responsible for 75% of the colloid osmotic pressure of plasma and is critical to plasma volume regulation (15). During exercise-induced plasma volume expansion a hallmark response is an increase in plasma albumin content. This increase in plasma albumin content is due, in part, to an increase in the hepatic rate of albumin synthesis (26). The preceding observations have been made in humans during plasma volume expansion induced by a high intensity intermittent exercise protocol. While it is well documented that hepatic albumin synthesis increases during exercise-induced plasma volume expansion, the molecular signals that govern this response are unknown. It is assumed that increased albumin synthesis is regulated, primarily, at the level of DNA transcription (37). The purpose of this study is two-fold. First, to develop a rodent exercise model that will mimic the plasma volume expansion seen in human studies. Second, to directly measure albumin mRNA expression in response to exercise, thereby providing insight into the molecular control of albumin synthesis.

Hypothesis

Exercise training, in the rodent will induce an increase in plasma albumin content and plasma volume.

Exercise training will cause an increase in albumin mRNA expression as defined by quantitative rtPCR.

Null Hypothesis

Exercise training will have no effect on plasma albumin content and plasma volume.

Exercise training will have no effect on albumin mRNA expression.

Assumptions

1. The *in vitro rtPCR* data will accurately reflect the *in-vivo* status of mRNA within the hepatocyte.
2. The measurement of albumin mRNA expression by rtPCR will accurately reflect the level of albumin gene expression.

Limitations

This study is limited by several factors. First, generalization to humans will be limited because of the possible differences in the response to exercise between species. This difference may pertain to the fact that rats are quadrupeds and humans are bipeds. Another limitation with the rodent model is accurately quantifying rodent aerobic capacity. The accuracy of determining maximal aerobic capacity in the rodent has direct impact of defining the appropriate exercise that should elicit a plasma volume expansion in rodents. The results obtained from this study will only identify possible mechanisms associated with our exercise protocol and may not be generalized to all exercise protocols. However, in human studies exercise induced plasma volume expansion has been demonstrated under a variety of exercise protocols. As such, we might expect similar results in the rodent model.

Delimitations

The subjects for this study are adult, male Sprague-Dawley rats that weigh 245-350 grams each. They will be purchased from the same company and co-habit a standard rat cage with one to two other rats. They will all receive the same standard rat diet.

Significance

This study will develop a rodent exercise model that will induce plasma volume expansion and allow us to identify the contribution of increased albumin mRNA expression to the increase in plasma albumin content. Future studies can then focus on the cellular signal pathways that may be activated that would promote increased albumin gene expression and eventually increased plasma albumin content and plasma volume expansion.

Chapter 2

Review of Literature

Total Body Water and its Distribution

Total body water (TBW) is the combination of intracellular fluid (ICF) compartments and extracellular fluid (ECF) compartments. The ECF represents about 60% of the TBW, while the ICF represents about 40% of TBW. The distribution fluctuates based on osmotic factors (1). The ECF can be further separated into smaller compartments: plasma volume (PV) or intravascular and the interstitial fluid (ISF) volume. ECF volume is determined by sodium content and PV is about 20% of ECF and influenced by the plasma protein content, which is determined primarily by the albumin content. Values for the aforementioned compartments in trained and untrained individuals are included in Table 1 below (18):

Table 1. Total Body Water Compartments

	TBW	ICF	ECF	ISF	PV
Untrained (ml/kg)	600	340	260	220	40
Trained (ml/kg)	671	424	247	189	58

Distribution of ECF between the ISF and Plasma

Fluid distribution between the ISF and plasma compartments is regulated by hydrostatic and osmotic forces as described by the Starling-Landis equation (2, 17, 23):

$$J_v = L_p * S \{(P_c - P_i) - \sigma (\pi_c - \pi_i)\}$$

Where: J_v= the total water flow across the capillary (indicate filtration), L_p = hydraulic conductivity of the capillary membrane, S = surface area available for fluid movement, P_c = capillary hydrostatic pressure, P_i = interstitial space pressure, σ = reflection coefficient of capillary membrane for plasma proteins, π_c = protein osmotic pressure (oncotic pressure), π_i = oncotic pressure of ISF.

The hydrostatic pressure gradient is defined by the difference between capillary (P_c) and interstitial space (P_i) pressure (36). Nevertheless, normal values of capillary hydrostatic pressure are found to be around 35 mmHg at the arterial portion of the capillary bed and 10 mmHg at the venous portion of the capillary bed. Factors that effect P_c are the arterial blood pressure, venous blood pressure, arteriolar resistance, and tone of precapillary sphincters. The pressure difference between arteries and capillaries is greater than the pressure difference between capillaries and veins. Due to these differences in pressure, a change in venous pressure has a far greater effect on capillary hydrostatic pressure than changes in arterial pressure. Factors that affect venous pressure are total blood volume, venous capacity, and hydrostatic pressure gradients caused by gravity or artificial g-forces (i.e. centrifugation).

Interstitial hydrostatic pressure (P_i) is defined as the pressure within the interstitial space of the tissue. Factors that affect P_i include interstitial compliance, rate of fluid filtration from capillaries, and rate of lymph flow. Normal values for P_i in subcutaneous tissue and muscle are between 1-4 mmHg (2, 14) and 2.8 mmHg (16), respectively.

Plasma oncotic pressure (π_c) is determined by the concentration of protein concentration in the plasma. Normal values for plasma proteins and plasma albumin

concentration are 7.3 g/dl and 4.5 g/dl, respectively (15). Normal values for π_c range from 25-30 mmHg (2). Increases in plasma π_c will lead to increases in plasma volume.

Interstitial oncotic pressure (π_i) is determined by the concentration of proteins in the interstitial fluid. Normal values of ISF protein concentration range from 2.5 to 3 g/dl (15, 16). π_i is determined by the rate of filtration of water and protein into the interstitium and lymphatic removal of water and proteins. π_i can be determined from a sample of interstitial or lymphatic fluid. As with P_i, the values of π_i vary between diverse tissues. Normal values of π_i are between 6 and 15 mmHg in subcutaneous tissue (2, 15).

The surface area (S) available for fluid exchange is modulated by the following factors: arterial, venous, and interstitial pressures, humoral factors, and the sympathetic nervous system (17). The reflection coefficient (σ) is the ratio of measured osmotic pressure by the predicted value of osmotic pressure. This value depends on the capillary permeability to plasma proteins and is affected by the following factors: capillary hydrostatic pressure and humoral factors that modify capillary permeability. Capillary permeability varies throughout the body. Most capillaries are impermeable to plasma proteins and have a σ value between 0.8 and 1 (2). Some capillaries (those in the liver and spleen), however, are permeable to plasma proteins and have σ values as low as 0.2 (17). Hydraulic conductivity (L_p) is the rate of water flow across a capillary membrane at a given pressure gradient. Kidney and intestinal tissue have high values of L_p, while muscle and skin have low L_p values (2,23).

The Starling equation is very effective at providing an instantaneous measurement of fluid movement across a capillary wall. However, applying the Starling force equation to whole body fluid movement under physiological stress can be misleading. The vast

variability in body tissue characteristics presents one problem to a whole body application of the Starling equation. Another problem is the fact that all the variables included in the equation are interdependent. A change in one variable could have differing effects on other variables in the equation. Despite these issues, careful application of the Starling equation can provide valuable comprehension of the dynamics of fluid shift between PV and ISF.

Albumin Content of Plasma

Albumin is a protein synthesized in the liver. Albumin is not stored in the liver but is immediately secreted into the plasma. An increase in the synthesis of albumin is therefore reflected in an increase in albumin in the plasma. Albumin accounts for 50% of total plasma protein by weight as well as 75% of oncotic pressure of plasma (14). Due to its large abundance, albumin plays a significant role in determining π_c and fluid distribution between the plasma and ISF. Albumin is a 65-70 kDa protein that is synthesized at a rate of 200 mg/ kg body weights per day and has a half life of 3 weeks (3, 9, 30). Normal values of plasma albumin content are 1.86 g/ kg body weight (16). The extravascular content of albumin is roughly equal to intravascular albumin content due to the larger volume of ISF. Plasma albumin filters across the capillary at a slow rate (5-7%/ hour) and albumin in the ISF is returned to circulation via the lymphatic vessels.

Exercise-induced hypervolemia

Human studies show that exercise training produces an increase in plasma albumin content (26) and an increase in plasma volume (25). We also know from human studies that the increase in plasma albumin content is due to several physiological

adaptations including 1) a redistribution of albumin from the ISF to the plasma compartment (35), 2) a reduction in the rate of albumin escape rate from the plasma compartment (35), and 3) an increase in hepatic albumin synthesis following exercise (26). Exercise-induced hypervolemia refers to increased blood volume (BV) as a result of exercise training (11, 12, 13, 27, 34). Both PV and red blood cell volume increase with endurance training. The response to high intensity intermittent exercise on PV expansion occurs within 24 hours without a significant change in red blood cell volume (25). With prolonged training red blood cell volume also increases and accounts for about 40% of the increase in BV.

The PV expansion, which is influenced by the changes in plasma albumin content, is the only factor with which we will be concerned. Observing the mechanisms that occur during this transient expansion phase of BV will provide insights on the role of albumin in the process. The increase in BV is almost directly proportional to increases in PV. Numerous studies have shown that a linear relationship exists between maximum oxygen uptake (VO_2max) and blood volume following exercise-induced hypervolemia (6, 11, 16, 19, 33) demonstrated that plasma proteins played a significant role in BV expansion. In support of these findings, Freund et al. (8) reported that plasma protein content in blood increased significantly after bouts of maximal exercise, thereby showing evidence that increase in plasma protein concentration plays a major role in BV expansion. Convertino et al. (5) determined that 86% of plasma protein increase can be attributed to albumin content. Because albumin synthesis is transcriptionally regulated (29), changes in albumin synthesis rate should be reflected in changes of albumin gene expression (10, 22, 25, 26, 29, 35, 37). Albumin gene expression can be monitored by

measurement of albumin mRNA within the hepatocytes. Using rtPCR to amplify the mRNA signal, we should be able to demonstrate a change in albumin gene expression.

While the relationship exists between albumin and PV expansion, the mechanism is not well understood. The increase in albumin concentration may be attributed to increased albumin synthesis or a decrease in albumin degradation. This study focuses on the establishment of a mechanism by which exercise-induced plasma volume expansion in the rat is associated with a simultaneous increase in liver albumin mRNA expression.

Chapter 3

Methods and Materials

Subjects

The subjects are 40 adult male Sprague-Dawley rats weighing between 245-350 grams. They will be purchased from Charles River Laboratories, Inc. (251 Ballardvale Street, Wilmington, MA 01887-1000) and co-habit a cage with one to two other rats. They will eat a standard rat diet provided by the animal care facilities at Brigham Young University.

Procedure

The rats will be randomly assigned, with 10 in each group, to one of four groups: cage control (CC), sham exercise (NE), continuous exercise training (LI), and high intensity, intermittent exercise training (HI). All of the rats will be handled for 14 days before treatment will be applied. The handling process will include actual human contact. Rats in the sham exercise or exercise-training groups will walk on the treadmill for 10 minutes per day at 10 m/min for the entire acclimatization period.

We will assume a maximum oxygen capacity of 100 ml/kg/min for the rodent based upon the literature for male Sprague-Dawley rats (4, 24, 28, 39). Based upon the established VO_2 and speed relationships determined on the treadmill, we will assign treadmill speeds (at 10%grade) that elicit 98.2%, 78.1%, 45.9%, and 29.8% of VO_2 max (4, 24, 28, 39).

The training period will last for two weeks. The respective treatments for each group will be applied for two weeks with six consecutive days of training followed by one day of rest. There will be a total of twelve training days. The rats will run on a

standard multi-lane animal treadmill (Quinton). The NE group will walk on the treadmill for 10 minutes at 45.9% aerobic capacity every training bout. The LI group will run on the treadmill at 78.1% aerobic capacity for 60 continuous minutes per session. The HI group will run at 98.2% aerobic capacity for 4 minutes and then at 48% aerobic capacity for 5 minutes. This group will perform 8 bouts per session (72 min total time). The LI group and HI group will consume approximately equal volumes of VO_2 (ml/g/min) over time. The CC group will be left in the cage for 14 days and sacrificed after that period.

Approximately 24 hours after the last training session, the rats will be anesthetized using a dosage (ml/g) of ketamine/xylazine cocktail based upon the weight of the rat. An incision will be made superior to the heart. The right jugular vein will be cannulated and a control blood sample of 0.45 ml will be taken. A 0.25 ml Texas red solution will be injected into the cannulated jugular vein. After a five-minute period, a second blood sample of 0.5 ml will be taken in order to determine blood volume using a dye dilution method described by Gillen (10). The animal will be euthanized with approximately 0.23 ml of pentobarbital (200mg/ml). The liver will be exposed through a midline incision and the liver will be rapidly removed and backed-flushed with ice cold 0.9% saline to remove blood cells. The liver will be frozen in liquid nitrogen and stored at -80° C until the RNA isolation process. Muscle tissue from the quadriceps will be harvested in order to analyze citrate synthase enzyme activity using a citrate synthase colorimetric assay (Sigma-Aldrich, kit #CS0720). The heart will also be excised and weighed.

In the RNA isolation process, the frozen liver will be crushed into a fine powder on dry ice and liquid nitrogen. RNA from 100-150 mg of liver powder will be isolated

using a guanidine thyocyanate method. cDNA will be synthesized with 2 µg of total RNA with Oligo (dT) and Stratascript RT using an MJ Research PTC-200 Peltier Thermal Cycler. cDNA amount will be quantified using a standard curve derived from a known concentration of double stranded DNA (dsDNA) and picogreen. Picogreen is a reagent that binds to dsDNA. This binding triggers a chemical reaction that emits fluorescence. Therefore, the strength of the fluorescent signal is dependent on the amount of dsDNA present in the sample. Real-Time reverse transcriptase PCR (rt-PCR) will be performed using a BioRad I-Cycler with amplication detection using CyberGreen. Cybergreen is a fluorescent used to detect an increase in product specific DNA using TAQ enzyme. The primers used for albumin are

Sense:

5'-GGCAACAGACGTTACCAAAATCA-3'

Antisense:

5'-TCATCCGCGATTCCAA-3'

PCR will be quantified using the BioRad MyiQ Single Color Real-time PCR Detection System (BioRad). PCR analysis will employ a standard curve produced using serial dilution of cDNA derived from pooled liver samples for the target gene albumin and the endogenous reference (GAPDH).

Statistical Analysis

This study uses a randomized block design and will compare albumin mRNA expression, plasma volume, and blood volume. Factorial ANOVA will be employed to determine significant difference. A Tukey minimum significant difference test will also

be employed as a multiple post-hoc test to compare each group to each other.

Significance levels are set at $p < .05$.

References

1. Aronson, P.S. Distribution of sodium and chloride across cell membranes. In: The regulation of sodium and chloride balance, edited by D.W. Seldin and G. Giebisch, New York: Raven Press, Chapt. 1, 1990.
2. Aukland, K. and R.K. Reed. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Reviews* 73(1): 1-78, 1993.
3. Ballmer, P.E., M.A. McNurlan, E. Milen, S.D. Heys, V. Buchan, A.G. Calder, and P.J. Garlick. Measurement of albumin synthesis in humans: a new approach employing stable isotopes. *Am J Physiol* 259: E797-E803, 1990.
4. Brooks, G.A. and T.P. White. Determination of metabolic and heart rate responses of rats to treadmill exercise. *J Appl Physiol* 45(6): 1009-1015, 1978.
5. Convertino, V.A., P.J. Brock, L.C. Keil, E.M. Bernauer, and J.E. Greenleaf. Exercise training-induced hypervolemia: role of plasma albumin, rennin, and vasopressin. *J Appl Physiol* 48: 665-669, 1980a.
6. Convertino, V.A., J.E. Greenleaf, and E.M. Bernauer. Role of thermal and exercise factors in the mechanism of hypervolemia. *J Appl Physiol* 48: 657-664, 1980b.
7. Convertino, V.A., G.W. Mack, and E.R. Nadel. Elevated central venous pressure: a consequence of exercise training-induced hypervolemia? *Am J Physiol* ?: R273-R277, 1991.
8. Freund, B.J., J.R. Claybaugh, M.S. Dice, and G.M. Hashiro. Hormonal and vascular fluid responses to maximal exercise in trained and untrained males. *J Appl Physiol* 63: 675-699, 1987.

9. Gersovitz, M., H.N. Munro, J. Udall, and V. R. Young. Albumin synthesis in young and elderly subjects using a new stable isotope methodology: response to level of protein intake. *Metabolism* 29(11): 1075-1086, 1980.
10. Gillen, C.M., A. Takamata, G.W. Mack, and E.R. Nadel. Measurement of plasma volume in rats with use of fluorescent-labeled albumin molecules. *J Appl Physiol* 76 (1): 485-489, 1994.
11. Green, H.J., J.A. Thomson, M.E. Ball, R.L. Hughson, M.E. Houston, and M.T. Sharratt. Alterations in blood volume following short-term supramaximal exercise. *J Appl Physiol* 56: 145-149, 1984.
12. Green, H.J., J.R. Sutton, G. Coates, M. Ali, and S. Jones. Response of red cell and plasma volume to prolonged training in humans. *J Appl Physiol* 70(4): 1810-1815, 1991.
13. Green, H.J., S. Carter, S. Grant, R. Tupling, G. Coates, and M. Ali. Vascular volumes and hematology in male and female runners and cyclists. *Eur J Appl Physiol Occup Physiol* 79(3): 244-250, 1999.
14. Guyton, A.C. Textbook of Medical Physiology. W.B. Saunders, Philadelphia, 1986.
15. Guyton, A.C. Textbook of Medical Physiology. W.B. Saunders, Philadelphia, 1996.
16. Haskell, A., E.R. Nadel, N.S. Stachenfeld, K. Nagashima, and G.W. Mack. Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J Appl Physiol* 83(2): 407-413, 1997.

17. Lassiter, W.E. Regulation of sodium chloride distribution within the extracellular fluid space. In: The regulation of sodium and chloride balance, edited by D.W. Seldin and G. Giebisch, New York: Raven Press, Chapt. 2, 1990.
18. Mack, Gary W. "The Body Fluid and Hemopoietic Systems." ACSM's Advanced Exercise Physiology. Baltimore: Lippincott, Williams, and Wilkins, 2006. 503.
19. Mack, G.W., X. Shi, H. Nose, A. Tripathi, and E.R. Nadel. Diminished baroreflex control of forearm vascular resistance in physically fit humans. *J Appl Physiol* 63: 105-110, 1987.
20. Mack, G.W., B.M. Quigley, T. Nishiyasu, X. Shi, and E.R. Nadel. Cardiopulmonary baroreflex control of forearm vascular resistance after acute blood volume expansion. *Aviat Space Environ Med* 62: 938-943, 1991.
21. Maw, G.J.; I.L. Mackenzie; D.A.M. Comer. Whole-body hyperhydration in endurance-trained males determined using radionuclide dilution. *Med Sci Sport Exer* 28: 1038-44, August 1996.
22. Mendel, D.B. and G.R. Crabtree. HNF-1, a member of a novel class of dimerizing homeodomain proteins. *J Biol Chem* 266: 677-680, 1991.
23. Michel, C.C. Fluid movement through capillary walls. Handbook of Physiology. The cardiovascular system. Microcirculation. Bethesda, MD: Am Physiol Soc., sect. 2 vol. 4, Chapt. 9, 1984.
24. Moraska, A., T. Deak, R.L. Spencer, D. Roth, and M. Fleshner. Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R1321-R1329, 2000.

25. Nagashima, K., G.W. Mack, A. Haskell, T. Nishiyasu, and E.R. Nadel. Mechanism for the posture-specific plasma volume increase after a single intense exercise protocol. *J Appl Physiol* 86(3): 867-873, 1999.

26. Nagashima, K., G.W. Cline, G.W. Mack, G.I. Shulman, and E.R. Nadel. Intense exercise stimulates albumin synthesis in the upright position. *J Appl Physiol* 88: 41-46, 2000.

27. Oscai, L.B., B.T. Williams, and B.A. Hertig. Effect of exercise on blood volume. *J Appl Physiol* 24(5): 622-624, 1968.

28. Pattengale, P.K. and J.O. Holloszy. Augmentation of skeletal muscle myoglobin by a program of treadmill running. *Am J Physiol* 213(3): 783-785, 1967.

29. Pietrangelo, A., A. Panduro, J.R. Chowdhury, and D.A. Shafritz. Albumin gene expression is downregulated by albumin or macromolecule infusion in the rat. *J Clin Invest* 89: 1755-1760, 1992.

30. Rothschild, M.A., M. Oratz, and S.S. Schreiber. Serum Albumin. *Hepatology* 8(2): 385-401, 1988.

31. Sawka, M.N. and E.F. Coyle. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exer Sport Sci Rev* 27: 167-218, 1999.

32. Sawka, M.N., V.A. Convertino, E.R. Eichner, et al. Blood volume: importance and adaptations to exercise training, environmental stress, and trauma/sickness. *Med Sci Sports Exerc* 32: 332-348, 2000.

33. Senay, L.C., D. Mitchell, and C.H. Wyndham. Acclimatization in a hot, humid environment: Body fluid adjustments. *J Appl Physiol* 40: 786-796, 1976.

34. Shoemaker, J.K., H.J. Green, J. Coates, M. Ali, and S. Grant. Failure of prolonged exercise training to increase red cell mass in humans. *Am J Physiol* 270(1 Pt 2): H121-H126, 1996.

35. Stahl, A.M., C.M. Gillen, A. Takamata, E.R. Nadel, and G.W. Mack. Reduced blood-to-tissue albumin movement after plasmapheresis. *Shock* 19(5): 440-447, 2003.

36. Starling, E.H. On the absorption of fluids from the connective tissue spaces. *J Physiol* 19:312-326, 1896.

37. Tronche, F., A. Rollier, P. Herbomel, I. Bach, S. Cereghini, M. Weiss, and M. Yaniv. Anatomy of rat albumin promoter. *Mol Biol Med* 7: 173-185, 1990.

38. Vroman, N.B., J.A. Healy, and R. Kertzer. Cardiovascular response to lower body negative pressure (LBNP) following endurance training. *Aviat Space Environ Med* 59: 330-334, 1988.

39. Woodman, C.R., K.A. Monnin, L.A. Sebastian, and C.M. Tipton. Influence of simulated microgravity on the VO_2 max of nontrained and trained rats. *J Appl Physiol* 74(4): 1941-1947, 1993.