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McKeever, Kenneth Harrington

EXERCISE TRAINING-INDUCED HYPERVOLEMIA: THE PHYSIOLOGICAL MECHANISMS IN THE GREYHOUND DOG AND THE HORSE

The University of Arizona

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Ph.D. 1984
EXERCISE TRAINING-INDUCED HYPERVOLEMIA: THE PHYSIOLOGICAL
MECHANISMS IN THE GREYHOUND DOG AND THE HORSE

by
Kenneth Harrington McKeever

A Dissertation Submitted to the Faculty of the
COMMITTEE ON ANIMAL PHYSIOLOGY (GRADUATE)
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1984
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Kenneth Harrington McKeever entitled EXERCISE TRAINING-INDUCED HYPERVOLEMIA: THE PHYSIOLOGICAL MECHANISMS IN THE GREYHOUND DOG AND THE HORSE and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

September 28, 1984

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

September 28, 1984
STATEMENT BY AUTHOR

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SIGNED: Kenneth Harrington McKee
ACKNOWLEDGMENTS

I would like to dedicate this dissertation to my Mother and Father and my Wife who have given me the unlimited support and the inspiration that I’ve needed to attain my academic goals. I would also like to thank the following people for their help with this project: Drs. Schurg and Convertino for the use of their laboratory facilities, for their technical advice, and for their editorial review of my dissertation; Harrell Jarrett Jr., Sally Jarrett, David Christiansen, Helen Tout and Lewis Keller for their help with the collection of samples and their help with the exercising of the horses; and lastly, the many Professors from the Department of Animal Sciences whose laboratory equipment I borrowed during the experiment (all of which I hopefully returned to the proper labs). To all of these people, I sincerely say thank you for your help.
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ABSTRACT

Four Greyhound dogs and six horses were utilized to study the physiological mechanisms associated with the development of an exercise training-induced hypervolemia. The animals were used in two separate experiments and were trained for 14 days on a treadmill ergometer and the data were used to formulate conclusions regarding the physiological and practical implications related to the phenomenon. The data reported in this dissertation indicate that exercise training will cause an expansion of the plasma volume in the Greyhound dog (+27%, P<0.05) and the horse (+29.1%, P<0.05). Physiologically the result is similar in man, the dog, and the horse, however, the mechanisms by which this adaptation is reached appears to differ in each of the species. In the dog, water intake (+33%, P<0.05) appears to be the primary mechanism for the increase in fluid volume. In the horse, renal control mechanisms (24-hr urine output -24.5%, P<0.05) appear to be the primary mechanism with those that control the retention of solutes other than sodium predominating over those that control the reabsorption of sodium and water. Based upon the literature, it appears that in man, renal mechanisms predominate the hypervolemic response and mechanisms which control the conservation of sodium appear to be most active in the defense of the
tonicity and volume of the vascular compartment. These species differences are important to the understanding of the physiology behind the onset of the training-induced hypervolemia and they provide pertinent information upon which decisions regarding the choice of animal models for future research.
CHAPTER 1

INTRODUCTION

Exercise training produces an expansion of blood volume in humans and horses, primarily through an expansion of the plasma volume (15, 36). This response to endurance exercise training has been shown to be correlated to an improved aerobic exercise capacity in trained individuals (17, 37). Furthermore, it has been suggested that there are species specific physiological mechanisms associated with the onset of this response to exercise training (32). With the increased use of the horse and the Greyhound dog in athletic activities comes the need for an increase in the understanding of the physiological controls which regulate the adaptations that occur in response to exercise training.

The objectives of the present study were: 1) to demonstrate that exercise training produces a hypervolemic adaptation in the Greyhound dog and the horse; and 2) to identify the physiological mechanisms which contribute to this response. Using these data, scientific and practical conclusions will be formulated to derive a better understanding of the physiological differences which may exist between human, canine, and equine athletes and their roles as models for future research.
CHAPTER 2

LITERATURE REVIEW

Exercise training induced-hypervolemia is by definition the expansion of the blood volume in response to chronic exercise training. In humans, aerobic (endurance) exercise training has been shown to produce an increase in the total blood volume, primarily through an expansion of the plasma volume (14, 15, 28) and a great deal of information has been published (13, 14, 15, 19, 28, 29) about the possible physiological mechanisms that are responsible for this adaptation to chronic exercise in man. However, very little is known about the physiological processes that are responsible for the exercise training-induced blood volume changes that are seen in the Greyhound dog and the horse.

**Documentation of Training-induced Hypervolemia in Man, Dogs, and Horses**

In man the effects of exercise training include a significant expansion of plasma volume primarily through an increase in total body water (14, 15, 17). It is well documented that in the human, trained individuals have a larger plasma and total blood volume than untrained individuals (16, 28, 29).
Courtice (18) reported that exercise trained Greyhound dogs had a significantly larger blood volume than untrained mongrels and suggested that this difference may have been due to the exercise training. There have been no data published that demonstrate that exercise training produces an expansion of blood volume in the Greyhound dog. In addition, a study by Porter and Canaday (39) strongly suggests that the hematological and blood chemistry profile of the Greyhound is significantly different from the profile of the mongrel. However, data have been published on the effects of exercise on the physiological control of fluid and electrolyte balance in the dog (22) and the general physiological responses appear to be the same regardless of the particular breed utilized as a model.

Information on the effect of training upon the blood volume of the horse is limited in both the number of studies present in the literature and the usefulness of information for the formulation of conclusions about the physiological mechanisms which might be involved with the overall adaptive process. Several studies have established a data base of values for the average blood and plasma volumes of normal horses (7, 18, 30, 36, 37). However, the first indication that training might produce an increase in blood volume was recorded in a study by Marcilese et al. (31) which noted that Thoroughbreds had a significantly larger plasma and blood volume than saddle horses and Percherons. Since no
mention of the physical condition was made except that the trained Thoroughbreds were off the track at the time of the study, it is difficult to draw any conclusions as to the residual training effect that might have been present in their horses.

Persson (36) conducted two studies to examine the possibility that training has a significant effect upon blood volume. One experiment compared trained and untrained two-year-old Standardbred trotters and the other followed the effects of training over a regimented training period. In both experiments it was concluded that training produces a significant expansion of the blood and plasma volume of the horse.

The Physiological Implication of the Hypervolemic Response to Exercise Performance

Fluid and electrolyte levels are regulated within narrow limits to maintain a normal internal environment for the physiological processes of the body. As quoted in Persson (36) and as stated by the physiologist Claude Bernard, "All the vital mechanisms varied as they may be have but one objective, that of preserving constant the conditions of life in the internal environment". To maintain the internal environment, physiological controls are present to respond to acute stresses and disturbances. When these disturbances become chronic, such as with
exercise training, adaptive effects in response to the conditioning occur. The increase in blood volume seen in response to endurance exercise training is an example of such an adaptation. This adaptive hypervolemia occurs in response to elevated environmental temperature (19, 38, 43, 49) and exercise. Interestingly, exercise training has been used as a method to preacclimate individuals to areas with high environmental temperatures (38, 43, 49, 51).

Cardiovascular adaptations which may be derived from an exercise training-induced hypervolemia include a decreased heart rate and an increased stroke volume at rest and at levels of maximal cardiac output (7, 16, 28, 36).

In man, the suggested benefits to thermoregulatory processes, that have been attributed to an enlarged plasma volume include an increased sweat rate, a decreased level of heat storage, and a decrease in core temperature at any given absolute work intensity (16, 33).

More specifically, Convertino (16) has reported that exercise training-induced hypervolemia was associated with proportional increases in sweat rate and decreases in submaximal and maximal heart rate. He hypothesized that "the mechanisms involved may include a greater volume for peripheral circulation and sweating, which would enhance heat transfer to maintain rectal temperatures at a higher absolute exercise intensity". He also suggested that an enlarged plasma volume may contribute to the maintenance of
venous return and stroke volume, accounting for the decrease in submaximal and maximal heart rates seen in his subjects with training.

A great deal of information has been published on the acute fluid and electrolyte changes that occur in endurance trained horses (10, 11, 12, 26, 30, 48). The primary objective of these studies was to establish a data base of physiological parameters which would best indicate when a horse is no longer fit to continue competing in an endurance activity. In most cases, where horses were removed from competition, the decision was based on the individual's inability to return to pre-competition physiological levels for heart rate, respiration rate, or rectal temperature. The primary factor correlated to these insufficient and disqualifying thermoregulatory and cardiovascular responses was the inability to regulate core body temperature (11, 26). These cardiovascular and thermoregulatory insufficiencies may be due to lowered plasma fluid levels resulting from exercise-induced dehydration and fluid shifts. Excessive fluid losses during exercise can cause increases in heart rate, respiratory rate, plasma electrolyte concentration and blood viscosity; a decreased sweat rate; and an increased core temperature (10, 11, 12). It has been suggested that in the exercising horse, the failure
to maintain the levels of these at a steady state is related to the resting plasma volume (10, 11, 26).

The relationship between the total blood volume and the aerobic exercise capacity of an individual is similar in man and the horse (36). Persson (36) has reported on the relationship between total blood volume and the working capacity (ie., work load required to produce a heart rate of 150 beats per minute) of the horse. He found that training produced an expansion of plasma volume in trained two-year-old trotters. When compared to untrained animals, body weight was found to be increased by 2%, total blood volume increased 19%, and total hemoglobin increased 34%. In man (15), blood volume expansion in response to training is due to proportional increases in plasma volume and body weight, indicating a net increase in total body water. The work by Persson (36) does not correlate the body weight gain with the increase in blood volume, however, his reported values do suggest that a net gain in total body water may have occurred.

In a follow up study (37), it was found that the aerobic capacity of the horse increased with the training associated expansion of blood volume and that total blood volume was significantly correlated to harness racing (2100-2200 meters) performance in Standardbred trotters. These conclusions were based upon the observation that the horses with the larger plasma volumes required a greater work load
to reach a heart rate of 150 beats per minute. Persson speculated that the increased plasma volume aided in the maintenance of venous return which, when accompanied by an increase in stroke volume and a decrease in peripheral resistance, would account for the observed improvement in cardiovascular performance.

Based upon the literature, it can be concluded that chronic exercise training produces an expansion of blood volume and that this increase may have important physiological benefits. Little information has been reported on the physiological mechanisms which may be responsible for this physiological adaptation in the dog or the horse. With this in mind, the major objectives of this dissertation were to determine if repeated chronic exercise produces a hypervolemic response in the dog and the horse and to identify the physiological mechanisms that contribute to this response.
CHAPTER 3

EXERCISE TRAINING INDUCED HYPOVOLUME IN GREYHOUND DOGS

Summary

The purpose of this study was to determine if the chronic hypervolemia that accompanies endurance exercise training is due only to an increase in the rate of water intake or if there were contributions from renal mechanisms. Four Greyhound dogs, previously sedentary for three years, were utilized for this study. During the 28-day experiment, each dog was trained on a treadmill ergometer for 14 consecutive days at 65% of its pre-training maximum work intensity. Following training, plasma volume increased by 472 ml (27.5%, P<0.05). The rate of water intake increased by 328 ml/day (33%, P<0.05) while urine output increased by 87 ml/day (20.8%, P<0.05). The mean resting 24-hr values for clearance of sodium increased by 0.29 ml/min (90.3%, P<0.05) and clearance of potassium decreased by 1.51 ml/min (16.1%, NS). Glomerular filtration rate, free water clearance, and osmotic clearance were not significantly altered. These data suggest that the primary mechanism for the exercise training-induced hypervolemia in dogs is a net positive water balance via increased water consumption without
significant contribution from an increase in renal water reabsorption.

**Introduction**

Chronic exercise training produces an expansion of plasma volume (14, 35). This adaptation to training may provide an increased vascular volume to meet the increased cardiovascular and thermoregulatory needs of an individual during acute exercise (14, 16, 33, 35). In man, suggested physiological mechanisms responsible for this hypervolemic response include repeated elevations in renin and vasopressin activity implicating the role of chronic enhancement of renal sodium and water retention (16). However, it has been demonstrated that the rate of water intake increases significantly with exercise training and heat acclimation, suggesting that drinking may be a significant contributing mechanism associated with the defense of total body water and extracellular volume (24).

The purpose of the present study was to determine if the increase in plasma volume that accompanies exercise training in dogs is due to an increase in the rate of fluid intake and/or if there may be additional contributions from renal control mechanisms.
Materials and Methods

Four Greyhound dogs, previously sedentary for three years, were housed in metabolism cages for the 28-day experimental period. An outline of the experiment can be found in Figure 1. The 28 days were divided into 4 phases: a 5-day metabolism cage adaptation period, a 5-day pre-training collection period, a 4-day treadmill adaptation period, and a 14-day training period. Daily water intake and urine output values for the 5-day pre-training period and the last 5 days of training were averaged for each animal to eliminate day-to-day variation. Resting plasma and urine samples were obtained on the third day of the pre-training period and the first day of the post-training period. Heparinized blood samples were withdrawn following 24-hours of rest (14 ml) via vacutainer needle (Vacutainer Systems) from either the jugular or cephalic veins without stasis. The dogs were removed from their cages and blood was taken while they were sitting and totally inactive. Pre-training maximum work capacities for the four dogs were determined on the last day of the treadmill adaptation period. During the experiment, the composition of the diet and the amount of food consumed by the dogs remained constant therefore, sodium intake remained constant however, the dogs were allowed to drink ad libitum. The water was at room temperature and was measured into the metabolism cage bowls with a graduated cylinder. The amount of water consumed was recorded 3 times daily at
## Experimental Protocol

<table>
<thead>
<tr>
<th>CAGE ADAPTATION</th>
<th>CONTROL</th>
<th>TREADMILL ADAPTATION</th>
<th>EXERCISE TRAINING</th>
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<tr>
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<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 +1</td>
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<td>U U U U U U</td>
<td>W W W W W W</td>
<td>U U U U U U</td>
</tr>
<tr>
<td>BU PV</td>
<td>BU PV</td>
<td>BU PV</td>
<td>BU PV</td>
</tr>
</tbody>
</table>

**W = 24-HOUR WATER VOLUME**

**BU = BLOOD AND URINE SAMPLE**

**U = URINE VOLUME**

**PV = PLASMA VOLUME**

Figure 1. Experimental Protocol - Greyhounds
7:00 AM, 12:00 PM, and 6:00 PM and the values were totalled to determine the 24-hr volume.

Environmental conditions varied insignificantly from day-to-day with an average dry bulb temperature of $20.3 \pm 3.1^\circ C$ and a relative humidity of $15.9 \pm 2.1\%$ during the experimental period. The same static thermoneutral time of day was used for each training session.

The exercise training was performed on a treadmill ergometer (Talbot Carlson Inc.). In man (15), the exercise intensity required for the onset of a training-induced hypervolemia has been reported to be between 50 and 65% of an individual's $\dot{V}O_2$ max therefore, each dog was trained for 20 minutes per day at 65% of its individual pre-training maximum work capacity. The average treadmill speed was 12.6 km/hr. Treadmill grade remained at 4.2% for the entire training period.

Resting plasma volume was determined by a modified Evans blue dye dilution method (23). Total blood volume and red cell volume were calculated from the plasma volume and the hematocrit. The venous hematocrit was corrected for whole body hematocrit by multiplication with the factor 0.91 (13). Hemoglobin concentration was determined by using standard cyanmethemoglobin procedures (Sigma Kit #525, Sigma Chemical Company) and the results were used to calculate
hemoglobin content and mean corpuscular hemoglobin concentration (MCHbCn).

Plasma and urine creatinine concentrations were determined by standard analytical procedures (45). Endogenous creatinine clearances were calculated and the results were used as an estimate of glomerular filtration rate. Plasma osmolality and urine osmolality were determined via freezing point depression osmometry (Osmette Model A, Precision Systems). The data obtained were used to calculate osmotic clearance and free water clearance. Plasma and urine sodium and potassium concentrations were measured by a sodium/potassium ion-sensitive electrode analyzer (Nova 1, Nova Biomedical) and the results were used to calculate clearances for both substances.

Total plasma protein concentration was determined using a modified Lowry procedure (34). Plasma albumin concentration was determined using standard procedures (21). Total plasma globulin concentration was calculated by subtracting the albumin concentration from the concentration of total protein.

Resting plasma osmotic, protein and electrolyte contents were calculated by multiplying their concentration by the plasma volume. In addition, total urine osmotic and
electrolyte contents were calculated by multiplying their concentrations by the urine volume.

Pre-training and post-training values were analyzed for training effects by a paired t-test as outlined by Sokal (47). The null hypothesis was rejected when P<0.05. Nonsignificant differences were denoted by NS.

Results

Hypervolemic Responses

Mean values (±S.E.) and percent change for plasma volume, blood volume, red cell volume, hematocrit, hemoglobin concentration, hemoglobin content, and mean corpuscular hemoglobin concentration (MCHbCn), for before and after training are listed in Table 1. Plasma volume increased by 472 ml (27.5%, P<0.05) after 14 days of training with a concomitant blood volume increase of 767 ml (20.6%, P<0.05) and a hemoglobin content increase of 89.4 g (26.4%, P<0.05). Resting hematocrit, red cell volume, hemoglobin concentration, and MCHbCn did not change.

Fluid Intake and Renal Function Responses

Mean values (±S.E.) and percent changes for these parameters are listed in Tables 2 and 3. Following 14 days of training, the rate of water intake increased by 328 ml/day (33.6%, P<0.05) while the rate of urine output
TABLE 1. Hypervolemic Responses to Exercise Training

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Training Mean ± S.E.</th>
<th>Post-Training Mean ±S.E.</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Volume (ml)</td>
<td>3727 ± 886</td>
<td>4495 ± 1038</td>
<td>+20.6*</td>
</tr>
<tr>
<td>Plasma Volume (ml)</td>
<td>1716 ± 401</td>
<td>2188 ± 419</td>
<td>+27.5*</td>
</tr>
<tr>
<td>Red Cell Volume (ml)</td>
<td>2012 ± 487</td>
<td>2266 ± 560</td>
<td>+12.7</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>53.9 ± 0.9</td>
<td>50.3 ± 2.1</td>
<td>-6.7</td>
</tr>
<tr>
<td>Hemoglobin (g/100ml)</td>
<td>19.8 ± 0.3</td>
<td>19.2 ± 0.6</td>
<td>-1.6</td>
</tr>
<tr>
<td>Hemoglobin (g)</td>
<td>339.2 ± 80.8</td>
<td>428.6 ± 99.5</td>
<td>+26.4*</td>
</tr>
<tr>
<td>MCHbCN (%)</td>
<td>36.6 ± 0.1</td>
<td>38.2 ± 0.4</td>
<td>+4.2</td>
</tr>
<tr>
<td>Body Weight (Kg)</td>
<td>29.2 ± 5.2</td>
<td>28.5 ± 4.5</td>
<td>-2.4</td>
</tr>
</tbody>
</table>

* P<0.05 compared to pre-training value.
### Table 2. Fluid Intake and Renal Function Responses to Training

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Training Mean ± S.E.</th>
<th>Post-Training Mean ± S.E.</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Intake (ml/day)</td>
<td>1005 ± 487</td>
<td>1333 ± 175</td>
<td>+33.6*</td>
</tr>
<tr>
<td>Urine Output (ml/day)</td>
<td>450 ± 92</td>
<td>537 ± 85</td>
<td>+20.8*</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>62.9 ± 16.6</td>
<td>61.5 ± 16.4</td>
<td>-2.2</td>
</tr>
<tr>
<td>Osmotic Clearance (ml/min)</td>
<td>1.89 ± 0.44</td>
<td>2.09 ± 0.55</td>
<td>+10.6</td>
</tr>
<tr>
<td>Free Water Clearance (ml/min)</td>
<td>-1.58 ± 0.39</td>
<td>-1.72 ± 0.50</td>
<td>-8.6</td>
</tr>
<tr>
<td>Sodium Clearance (ml/min)</td>
<td>0.31 ± 0.07</td>
<td>0.59 ± 0.10</td>
<td>+90.3*</td>
</tr>
<tr>
<td>Potassium Clearance (ml/min)</td>
<td>9.38 ± 1.43</td>
<td>7.87 ± 2.85</td>
<td>-16.1</td>
</tr>
</tbody>
</table>

* P<0.05
Table 3. Urine Osmotic and Electrolyte Concentrations and Contents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Training Mean ± S.E.</th>
<th>Post-Training Mean ± S.E</th>
<th>%Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>1837 ± 261</td>
<td>1682 ± 304</td>
<td>-8.4</td>
</tr>
<tr>
<td>Osmotic Content (mOsm/24hrs)</td>
<td>823 ± 191</td>
<td>921 ± 253</td>
<td>+11.9</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>157.8 ± 31.1</td>
<td>243.4 ± 63.9</td>
<td>+54.2*</td>
</tr>
<tr>
<td>Sodium (mEq/24hrs)</td>
<td>69.8 ± 15.8</td>
<td>129.6 ± 24.0</td>
<td>+85.7*</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>143.3 ± 25.9</td>
<td>114.0 ± 31.5</td>
<td>-20.2</td>
</tr>
<tr>
<td>Potassium (mEq/24hrs)</td>
<td>63.8 ± 12.8</td>
<td>62.3 ± 23.1</td>
<td>-2.2</td>
</tr>
</tbody>
</table>

*P<0.05
increased by 87 ml (20.8%, P<0.05). The changes in glomerular filtration rate (-2.2%), solute free water clearance (-8.6%), and osmotic clearance (+10.6%) were not distinguishable from pre-training values. The urine osmolality decreased (11.9%, NS) while, due to an increase in the volume of urine produced in each 24-hour period, the total osmotic content of the urine increased (11.9%, NS). Sodium clearance increased by 0.29 ml/min (90.3%, P<0.05). The urine [Na+] increased (90.3%, P<0.05) as did the total urine sodium excretion (85.7%, P<0.05). Training had the opposite effect on potassium clearance, which decreased by (16.1%, NS). There was a decrease in the urine [K+] (20.23%, NS) and no change in the potassium content of the urine.

Plasma Solutes

Mean (±S.E.) values for plasma solute concentrations and contents are summarized in Table 4. There were no significant changes in the plasma osmotic, sodium, total protein, albumin, globulin, and creatinine concentrations. Plasma potassium concentration increased 0.80 mEq/l (17.0%, P<0.05). The total plasma osmotic content increased by 162 mOsm (31.2%, P<0.05). Plasma sodium content increased by 65.2 mEq (24.1%, P<0.05) and plasma potassium content increased by 3.9 mEq (47.7%, P<0.05). There was a 21.7 g
Table 4. Plasma Solute Concentrations and Contents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-Training Mean ± S.E.</th>
<th>Post-Training Mean ± S.E.</th>
<th>Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality (mOsm/Kg)</td>
<td>302 ± 2.5</td>
<td>305 ± 4.6</td>
<td>+1.0</td>
</tr>
<tr>
<td>Osmotic Content (mOsm)</td>
<td>519 ± 125</td>
<td>681 ± 156</td>
<td>+31.2*</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>157.2 ± 3.4</td>
<td>153.5 ± 1.4</td>
<td>-2.4</td>
</tr>
<tr>
<td>Sodium (mEq)</td>
<td>270.6 ± 68.5</td>
<td>335.8 ± 63.0</td>
<td>+24.1*</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>4.7 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>+17.0*</td>
</tr>
<tr>
<td>Potassium (mEq)</td>
<td>8.2 ± 2.4</td>
<td>12.1 ± 2.7</td>
<td>+47.7*</td>
</tr>
<tr>
<td>Total Protein (g/100ml)</td>
<td>6.49 ± 0.46</td>
<td>6.04 ± 0.29</td>
<td>-6.9</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>112.2 ± 31.9</td>
<td>133.9 ± 26.1</td>
<td>+19.3</td>
</tr>
<tr>
<td>Albumin (g/100ml)</td>
<td>2.88 ± 0.21</td>
<td>2.76 ± 0.13</td>
<td>-4.2</td>
</tr>
<tr>
<td>Albumin (g)</td>
<td>49.5 ± 11.7</td>
<td>61.6 ± 14.5</td>
<td>+24.4*</td>
</tr>
<tr>
<td>Globulin (g/100ml)</td>
<td>3.61 ± 0.59</td>
<td>3.28 ± 0.25</td>
<td>-9.1</td>
</tr>
<tr>
<td>Globulin (g)</td>
<td>62.7 ± 22.0</td>
<td>72.4 ± 11.7</td>
<td>+15.5</td>
</tr>
</tbody>
</table>

*P<0.05
increase in the plasma total protein content (19.3%, NS) primarily due to a 12.1 g increase in the albumin content (24.4%, P<0.05) with a smaller contribution from an increase (15.5%, NS) in the plasma globulin content.

Body weight decreased in three of the dogs and increased in one dog during the training period but the mean change (-2.4%) was not statistically significant.

**Discussion**

In the present study, a plasma volume increase of 27.5% was observed after two weeks of training. This is larger than values reported in humans (14, 15, 16) where plasma volume increased 12% after 8 days of training. In humans it appears that the increased blood volume associated with exercise training is primarily due to an expansion of the plasma volume since there was no change in red cell volume or hemoglobin content, despite a decrease in hematocrit and hemoglobin concentration (14, 15, 35). The blood volume increase observed in the present study (+20.6%) was due to an expansion of the plasma volume with an additional contribution from an increase in the red cell volume and the hemoglobin content. While the 12.7% increase in the red cell volume seen in the dogs is not statistically
significant, it may be physiologically significant and may reflect a contribution from repeated splenic contraction.

As reported in human subjects (15, 40) resting total plasma protein, albumin, and globulin concentrations did not change with training. However, as a result of the plasma volume expansion, there was an increase in the resting total protein content, primarily through an increase in the albumin content. Because 1 g of protein binds 14-15 ml of water (15, 42) we can account for 326 ml of the 472 ml plasma volume expansion observed in the present study. Proposed mechanisms which may account for this increase in resting total protein content include either an increase in net protein synthesis (15) or a net shift of protein from the interstitial compartment to the vascular space (43). Proportional increases in body weight, plasma volume and plasma protein content may indicate a net increase in the synthesis of protein. While values for total body water and body composition were not obtained in the dogs, the lack of change in body weight following training would appear to be supportive of a net shift of protein from other compartments as suggested by Senay et al. (43) rather than an increase in total body water. A chronic net shift, proportional to the increase in plasma volume, would maintain a constant body
weight and plasma protein, albumin, and globulin concentrations, but would increase their respective plasma contents. The data obtained in this experiment demonstrates that there was an increase in both the rate of water intake (328 ml/day) and the rate of urine output (87 ml/day). Although only a gross estimate of water balance, the difference between water intake and urine output increased 241 ml/day from a pre-training value of 555 ml/day to a post-training value of 796 ml/day. Using previously published data obtained from exercising dogs (22), a water loss of approximately 200-220 ml/day can be attributed to increased respiratory, salivary, and nasal discharge losses during exercise. The remaining 20 to 40 ml/day when multiplied by 14 days would suggest a net gain of 280-560 ml of body water which was consistent with the measured plasma volume gain of 472 ml. These data suggest that the plasma volume increase may be indicative of a total body water expansion rather than the hypothesized shift of protein from the extracellular to the intravascular space (15). Although in the present study there was no change in body weight in the dogs following training, the results indicate a net gain in body water retention which may have cancelled any loss in body weight associated with conditioning. Such a weight loss may have occurred in these dogs as the total amount of food given
to each dog was not increased over the course of the training period despite an increase in the caloric output.

In the present study, water intake increased 33%. This appears to be the sole route for increasing fluid volume as urine output increased 20.8%. This is consistent with the mechanism proposed by Greenleaf et al. (24) that drinking is the primary route for the fluid volume increases associated with exercise training in humans and that changes in plasma osmolality, angiotensin II, and plasma volume can independently affect the rate of water consumption through the sodium ion-osmotic-vasopressin pathway (SOV) and the renin-angiotensin II-aldosterone (RAA) pathway.

Both the RAA system and the SOV system may have influences on renal function during muscular activity since the activity of both systems increases during acute exercise and repeated elevations in the activity of both systems might cause chronic changes in renal function in humans (15). More specifically, with increases in aldosterone and vasopressin activity, one would expect a decrease in the loss of sodium and water via the kidneys. The opposite is reflected in the dogs of the present study where urine output and sodium clearance both increased. These results may be partly explained by data of Dorn et al. (20) who demonstrated that experimentally elevated levels of cerebral spinal fluid
[Na+] in dogs increased renal excretion of sodium with no changes in GFR. Furthermore, the intracerebroventricular administration of angiotensin has produced an increase in renal sodium excretion (2, 44). This natriuresis was most pronounced in hydrated, volume expanded animals and was highly correlated with the [Na+] in the cerebral spinal fluid (3). In another study where dogs were pretreated with aldosterone, Andersson et al. (1) found that the natriuresis was not secondary to reduced aldosterone secretion and suggested that elevations in [Na+] and angiotensin activity are the primary mediators for the increase in renal excretion of sodium. Andersson (4, 5) also suggests that there may be a common juxtaventricular sensory target for both angiotensin and elevated CSF [Na+] and that connections may exist between these receptors and the hypothalamus. However, the manner in which they act upon renal sodium excretion is not clear.

The negative values for free water clearance observed in our study are indicative of an excess of solute being removed from the plasma by the kidneys. In the present study, the increases in urine volume and sodium clearance, with no change in GFR, may be associated with renal mechanisms that are uniquely important to the maintenance of plasma sodium and osmotic concentrations in the dog since,
unlike the renal conservation mechanisms associated with training-induced hypervolemia in human subjects, the dogs exhibited both a natriuresis and a diuresis.

The results of the present study suggest that the primary mechanism for the hypervolemia associated with endurance exercise training in the dogs is an increase in the rate of water intake. The contribution of renal control to the hypervolemic response appears to be minimal.
CHAPTER 4

EXERCISE TRAINING-INDUCED HYPERVOLEMIA IN THE HORSE

Summary

The purpose of this study was to determine if a chronic hypervolemia would accompany endurance exercise training in the horse. Six mature previously sedentary horses were utilized for this study. During the 5-week experiment, 5 of the horses were trained for 14 days on a treadmill ergometer at a constant treadmill speed of 5.6 km/hr and a constant grade of 12.5%. One horse was trained by lunging at a trotting pace in a round pen. Following training, plasma volume increased by 4.7 liters (29.1%, P<0.05). Although daily water intake did not change during the training period, 24-hr urine output decreased by an average of 3.5 l/day (-24.5%, P<0.05). Resting glomerular filtration rate and the rate of sodium clearance were not altered by training. However, potassium and osmotic clearance were decreased by training (P<0.05) while free water clearance was increased (P<0.05). Plasma potassium concentration was significantly decreased following the two weeks of training suggesting that the decrease in potassium clearance was primarily a response to a net potassium deficit. These data suggest that renal control mechanisms
affecting water reabsorption via the reabsorption of osmotically active substances other than sodium provide the primary route for the training-induced hypervolemia seen in horses.

**Introduction**

Brenon (8, 9) observed that the hematocrit and hemoglobin concentrations were lower in horses that were in peak track condition and the values increased when the horses were removed from the track. Fluctuations similar to this occur in humans where exercise training and heat acclimation induce an expansion of plasma volume (14, 33, 35). Bed rest studies in humans have shown that detraining produces a reduction in plasma volume (25).

Repeated exercise training produces a plasma volume expansion in man (14, 35) and in Greyhound dogs (32). This adaptation to training may provide an increased vascular volume to meet the increased cardiovascular and thermoregulatory needs of an individual during acute exercise (14, 15, 33, 35). In man, suggested physiological mechanisms responsible for this hypervolemic response include repeated elevations in renin and vasopressin activity with chronic enhancement of renal sodium and water retention (15, 17). However, it has been demonstrated in man (24) and Greyhound dogs (32) that the rate of water intake increases significantly with exercise training and heat acclimation, suggesting that drinking appears to be a significant
contributing mechanism associated with the defense of total body water and extracellular volume.

Increased blood volume in response to exercise training has been documented in the horse (36) and the physiological implications have been studied extensively (37). As in humans, this expansion of blood volume appears to be due primarily to an expansion of the plasma volume. This increase would provide a greater vascular fluid volume which may insure the provision of an adequate venous return and cardiac output during acute athletic activity (15). Such an increase in cardiac output is required during exercise to meet both the increased circulatory and thermoregulatory needs of the exercising animal (15, 37). While the observation has been made that trained horses have an expanded blood volume (36, 37) and a great deal of information has been published on the importance of blood volume to the performance capacity of the equine athlete (36, 37) no data are available which describe the physiological mechanisms which are responsible for the exercise training-induced hypervolemia.

The purpose of the present study was to determine if the increase in plasma volume that accompanies exercise training is due to mechanisms associated with net water retention by measuring changes in 24-hr fluid intake and urine output.
Materials and Methods

Six mature horses, 4 geldings and 2 stallions ranging in age from 16 months to 4 years were used for this study. Previous conditioning was minimal in all animals and all had been sedentary for approximately two months prior to the experimental period. All the horses were examined to insure that they were in good health and were given routine worming treatments and vaccinations prior to the experiment. The horses were housed in individual metabolism pens for the entire experiment. They were fed an alfalfa cube diet, and were given steamed flaked milo as an energy supplement. The amount of feed given to each animal was kept constant with each animal receiving approximately 7.26 kg of hay and 0.9 kg of grain per day in two equal portions fed at 7:00 AM and 4:00 PM. Water was given to the horses four times daily at 4:00 AM, 9:00 AM, 3:00 PM and 9:00 PM. To avoid losses via evaporation or from the animals playing in their buckets, water was given to the horses and then removed after each horse had been allowed to drink to satiety.

An outline of the experimental period can be found in Figure 1. The 5-week experiment contained 5 phases: a 7-day housing adaptation period, a 7-day pre-training control period, a 15-day exercise training period, and a 7-day post-training recovery period. The volume of water intake was measured each day and the volumes were used to compute one value representing average consumption rates for the 7-day
EXPERIMENTAL PROTOCOL

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercise Training Period</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-7 -6 -5 -4 -3 -2 -1</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>PV</td>
<td>PV</td>
<td>PV</td>
<td>PV</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>U</td>
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<td>U</td>
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</tr>
</tbody>
</table>

PV = Plasma Volume. U = Urine Volume and Sample.
B = Blood Sample. W = 24-HR Water Volume

Figure 2. Experimental Protocol - Horses
control period, the first 7-days of exercise, the second 7-days of exercise, and the 7-days of recovery. Urine was collected for 24-hours on two days during the control period, on day 8 and 15 of the exercise period, and on day 1 and 7 of the recovery period. The two control period volumes were averaged to eliminate day-to-day variations as were the volumes from day 15 of the exercise period and day 1 of the recovery period. Aliquots of urine were taken from each of the pooled 24-hour samples for later analyses. Plasma volume determinations and blood samples were collected 7 days prior to training, the day prior to the start of training, on day 8 of the training period, and on days 1 and 7 of the recovery period. The horses were exercised daily during the 15-day training period with the exception of day 8 which was used for collecting samples.

The exercise training for 5 of the horses was performed on a treadmill ergometer (Talbot Carlson Inc.). One horse would not exercise on the treadmill and was lunged at a trotting pace in a round pen for a daily duration equal to the duration used for the horses trained on the treadmill. Each horse was trained at a constant treadmill speed of 5.6 km/hr and grade of 12.5%. To avoid injury, daily training duration was increased with moderation. The following protocol was used to increase the exercise time: an average of 5 minutes on day 1, 9 minutes on day 2, 10
minutes on day 3, 12 minutes on day 4, 15 minutes on days 5, 6, and 7, and 20 minutes for days 8 through 14.

Resting plasma volume was determined by a modified Evans blue dye dilution method (23). Total blood volume and red cell volume were calculated from the plasma volume and hematocrit. The venous hematocrit was corrected for whole body hematocrit by multiplication with the factor 0.91 (13). Hemoglobin concentration was determined by using standard cyanmethemoglobin procedures (Sigma Kit #525, Sigma Chemical Company) and the results were used to calculate hemoglobin content and mean corpuscular hemoglobin concentration (MCHbCn).

Plasma and urine creatinine concentrations were determined by standard analytical procedures (44). Endogenous creatinine clearances were calculated and the results were used as an estimate of glomerular filtration rate. Plasma osmolality and urine osmolality were determined via freezing point depression osmometry (Osmette Model A, Precision Systems). The data obtained were used to calculate osmotic and free water clearance. Plasma and urine sodium and potassium concentrations were measured by a sodium/potassium ion-sensitive electrode analyzer (Nova 1, Nova Biomedical) and the results were used to calculate clearances for both substances.

Total plasma protein concentration was determined using a modified Lowry procedure (34). Plasma albumin
concentration was determined using standard procedures (21). Total plasma globulin concentration was calculated by subtracting the albumin concentration from the concentration of total protein.

Resting plasma osmotic, protein and electrolyte contents were calculated by multiplying their concentration by the plasma volume. In addition, total urine osmotic and electrolyte contents were calculated by multiplying their concentrations by the urine volume.

The individual values for the data collected on the two pre-training control days were averaged to eliminate day-to-day variations within the control period. A one-way analysis of variance was performed on the data using methods of analysis outlined by Sokal (47). In addition, mid-training, end of training, and recovery values were statistically compared to the control mean by a paired t-test (48). The null hypothesis was rejected when \( P < 0.05 \). Nonsignificant differences were denoted by NS.

Results

Vascular Volume Responses to Training

Mean (\( \pm \)S.E.) values for blood volume, plasma volume, red cell volume, hematocrit, hemoglobin concentration, hemoglobin content, and mean corpuscular hemoglobin concentration for the before, mid-training, after training, and recovery are listed in Table 1. Blood volume increased by
<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>Ex-8</th>
<th>R + 1</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Volume (ml)</td>
<td>25251 ± 3710</td>
<td>34480 ± 4296</td>
<td>31033 ± 1088</td>
<td>29347 ± 3061</td>
</tr>
<tr>
<td>Plasma Volume (ml)</td>
<td>16135 ± 3710</td>
<td>20375 ± 1711</td>
<td>20827 ± 646</td>
<td>19156 ± 1647</td>
</tr>
<tr>
<td>Red Cell Volume (ml)</td>
<td>9129 ± 1997</td>
<td>14105 ± 2723</td>
<td>10206 ± 1069</td>
<td>10158 ± 1833</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>39.4 ± 4.4</td>
<td>44.0 ± 3.3</td>
<td>37.4 ± 2.9</td>
<td>38.5 ± 4.7</td>
</tr>
<tr>
<td>Hemoglobin (g/100ml)</td>
<td>15.4 ± 1.6</td>
<td>15.2 ± 1.2</td>
<td>13.5 ± 1.0</td>
<td>14.6 ± 1.9</td>
</tr>
<tr>
<td>Hemoglobin (g)</td>
<td>2477 ± 413</td>
<td>3104 ± 459</td>
<td>2807 ± 208</td>
<td>2802 ± 309</td>
</tr>
<tr>
<td>MCHbCn (%)</td>
<td>39.1 ± 1.2</td>
<td>34.0 ± 0.9</td>
<td>37.4 ± 1.2</td>
<td>38.5 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for before training (CONTROL), mid-training (Ex-8), after training (R + 1), and after recovery (R + 7). MCHbCn, mean corpuscular hemoglobin concentration. *P < 0.05 compared to control value.
9229 ml (36.5%, P<0.05) following 7 days of training via a 
4240 ml (26.2%, P<0.05) increase in the plasma volume and a 
4976 ml (54.5%, P<0.05) increase in red cell volume. Hemato­
crit increased (4.6%, P<0.05) following 7 days of training 
as did the hemoglobin content, which increased by 627 g 
(25.3%, P<0.05). Mean copuscular hemoglobin concentration 
decreased (-5.1%, P<0.05) following the first 7 days of 
training.

Following the second week of training there was a 
5782 ml (22.9%, P<0.05) change in the blood volume, 
primarily from a 4692 ml (29.1, P<0.05) increase in the 
plasma volume. Approximately 90.4% of the final plasma 
volume increase was reached by the 7th day of training. 
After two weeks of training there were no differences 
between control and post-training values for red cell 
volume, hematocrit, hemoglobin content, or mean copuscular 
hemoglobin concentration. However, two weeks of training 
did produce a 1.9 g/100 ml (-12.3%, P<0.05) decrease in the 
hemoglobin concentration.

After 7 days of recovery plasma volume remained 3021 
ml (18.7%, P<0.05) greater than before training. However, 
following the 7 days of recovery, there were no differences 
between before training and after recovery values for blood 
volume, red cell volume, hematocrit, hemoglobin
concentration and content, and mean corpuscular hemoglobin concentration.

Fluid Intake and Renal Function Responses to Training

Mean (±S.E.) values for water intake; urine output; gross water retention; glomerular filtration rate; osmotic, free water, sodium and potassium clearance; and the urine osmotic and electrolyte concentrations and contents are listed in Tables 2 and 3. There were no changes in 24-hr water intake during either of the two weeks of the training period. However, water intake decreased by 4.6 l/day (-12.7%, P<0.05) during the recovery period. There were significantly lower levels of urine production during both the first and second weeks of the training period with urine output decreasing by 2.9 l/day (-20.3%, P<0.05) following the first 7 days of conditioning. Urine output decreased further during the second week of training, dropping by 3.5 l/day (-24.5%, P<0.05) from the control period level. Gross water retention, the difference between water intake and urine output, increased by 3.8 l/day (17.4%, P<0.05) after 7 days of training and remained elevated by approximately the same amount (3.5 l/day, P<0.05) during the second week of training. Following 7 days of recovery, gross water retention was 2.3 l/day (-10.5%, NS) lower than the level observed during the control period.
Table 6. Fluid Intake and Renal Function Responses

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>Ex-8</th>
<th>R + 1</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Intake (l/day)</strong></td>
<td>36.1 ± 3.5</td>
<td>37.4 ± 4.5</td>
<td>36.3 ± 5.4</td>
<td>31.5* ± 3.9</td>
</tr>
<tr>
<td><strong>Urine Output (l/day)</strong></td>
<td>14.3 ± 3.5</td>
<td>11.4* ± 3.0</td>
<td>10.8* ± 2.7</td>
<td>11.9* ± 4.2</td>
</tr>
<tr>
<td><strong>Gross Water Retention (l/day)</strong></td>
<td>21.9 ± 2.1</td>
<td>25.7* ± 2.6</td>
<td>25.4* ± 3.4</td>
<td>19.6 ± 0.9</td>
</tr>
<tr>
<td><strong>Glomerular Filtration Rate (ml/min)</strong></td>
<td>399.3 ± 50.7</td>
<td>480.6 ± 175.4</td>
<td>405.1 ± 90.5</td>
<td>470.2 ± 141.5</td>
</tr>
<tr>
<td><strong>Osmotic Clearance (ml/min)</strong></td>
<td>32.2 ± 3.0</td>
<td>29.4* ± 4.1</td>
<td>27.3* ± 3.0</td>
<td>28.3* ± 5.2</td>
</tr>
<tr>
<td><strong>Free Water Clearance (ml/min)</strong></td>
<td>-25.1 ± 1.9</td>
<td>-21.5 ± 2.5</td>
<td>-19.7* ± 2.6</td>
<td>-20.1* ± 3.1</td>
</tr>
<tr>
<td><strong>Sodium Clearance (ml/min)</strong></td>
<td>0.62 ± 0.34</td>
<td>0.74 ± 0.34</td>
<td>0.74 ± 0.47</td>
<td>0.94 ± 0.95</td>
</tr>
<tr>
<td><strong>Potassium Clearance (ml/min)</strong></td>
<td>221.0 ± 43.9</td>
<td>155.3* ± 26.4</td>
<td>185.6* ± 45.4</td>
<td>222.7 ± 93.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for before training (CONTROL), mid-training (Ex-8), after training (R + 1), and after recovery (R + 7). * P < 0.05 compared to control value.
Table 7. Urine Concentrations and Contents

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>Ex-8</th>
<th>R + 1</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>1027 ± 174</td>
<td>1079 ± 103</td>
<td>1027 ± 181</td>
<td>991 ± 157</td>
</tr>
<tr>
<td>Osmotic Content (mOsm/24hrs)</td>
<td>14201 ± 620</td>
<td>12127* ± 1768</td>
<td>10729* ± 984</td>
<td>11279* ± 2137</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>9.0 ± 5.2</td>
<td>12.9 ± 7.1</td>
<td>14.8 ± 10.2</td>
<td>17.1 ± 19.2</td>
</tr>
<tr>
<td>Sodium (mEq/24hrs)</td>
<td>124.7 ± 66.9</td>
<td>142.2 ± 65.2</td>
<td>144.9 ± 91.6</td>
<td>188.0 ± 193.9</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>95.7 ± 3.4</td>
<td>82.2* ± 3.7</td>
<td>94.9 ± 4.6</td>
<td>98.8 ± 10.5</td>
</tr>
<tr>
<td>Potassium (mEq/24hrs)</td>
<td>1371.5 ± 315.6</td>
<td>934.9* ± 200.7</td>
<td>1030.2* ± 270.1</td>
<td>1166.2* ± 411.7</td>
</tr>
</tbody>
</table>

Values are means ± SE for before training (CONTROL), mid-training (Ex-8), after training (R + 1), and after recovery (R + 7). * P < 0.05 compared to control value.
Renal osmotic clearance rate was decreased by 2.8 ml/min (-8.7%, P<0.05) after 7 days of training and by 4.8 ml/min (-15.2, P<0.05) following the second week of exercise. Following 7 days of recovery, osmotic clearance continued to take place at a rate that was lower (-12.1%, P<0.05) than that observed during the control period. There was no observable change in the urine osmolality during the entire experimental period. The total amount of osmotic substances excreted in the urine during 24 hours decreased by 3472 mOsm/24hrs (24.4%, P<0.05) with training and did not return to control levels following the 7 day recovery period. Free water clearance was significantly more positive by 5.4 ml/min (21.5%, P<0.05) after two weeks of conditioning and remained elevated during the recovery period (19.9%, P<0.05). There were no changes in either the rate of sodium clearance, the sodium concentration of the urine, or in the total sodium excreted per 24/hours following either of the two weeks of training or the week of recovery. The potassium clearance rate was decreased by 65.7 ml/min (-29.7%, P<0.05) following the first week of training and while the rate increased during the second week, it was still 35.4 ml/min (16.0%, P<0.05) below the rate calculated for the control period. Potassium clearance had returned to pre-training levels by the end of the recovery period. The urine potassium concentration was significantly lower (-14.1%, P<0.05) following the first
week of exercise. By the end of the second week of training and after the week of recovery, there were no distinguishable variations in the potassium concentration of the urine. The amount of potassium excreted by the kidneys in 24 hours decreased sharply after the first week of training (-31.8%, P<0.05) and was significantly depressed for the remainder of the experiment. There were no observable changes in glomerular filtration rate following either of the training or the recovery periods.

Plasma Solutes

Mean (±S.E.) values for plasma solute concentrations and contents are summarized in Table 4. Plasma osmolality was decreased by 8 mOsm/kg (-2.8%, P<0.05) following the second week of training and remained below (2.1%, P<0.05) the control level following recovery. However, plasma osmotic content increased by 1157 mOsm (20.3%, P<0.05) after training, remaining elevated (16.5%, P<0.05) above control levels after the recovery period. Plasma sodium concentration was increased (1.5%, P<0.05) after the first week of training but, was decreased (-1.6%, P<0.05) after the second week. Sodium concentration was still significantly lower (P<0.05) following the recovery period. Resting plasma sodium content was increased by 607 mEq (27.2%, P<0.05) after training and remained elevated (P<0.05) above control levels following the week of recovery. After the two weeks
Table 8. Plasma Solute Concentrations and Contents

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>EX-8</th>
<th>R + 1</th>
<th>R + 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality (mOsm/Kg)</td>
<td>282 ± 2</td>
<td>286 ± 3</td>
<td>274* ± 5</td>
<td>276* ± 2</td>
</tr>
<tr>
<td>Osmotic Content (mOsm)</td>
<td>4541 ± 560</td>
<td>5829* ± 523</td>
<td>5698* ± 127</td>
<td>5291* ± 461</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>138.7 ± 0.8</td>
<td>140.8* ± 0.8</td>
<td>136.5* ± 1.0</td>
<td>137.7* ± 0.9</td>
</tr>
<tr>
<td>Sodium (mEq)</td>
<td>2235 ± 278</td>
<td>2870* ± 256</td>
<td>2842* ± 73</td>
<td>2638* ± 223</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>4.29 ± 0.12</td>
<td>4.16 ± 0.23</td>
<td>3.86* ± 0.36</td>
<td>3.71* ± 0.27</td>
</tr>
<tr>
<td>Potassium (mEq)</td>
<td>69.3 ± 9.4</td>
<td>84.4 ± 7.3</td>
<td>78.6 ± 10.3</td>
<td>71.0 ± 4.9</td>
</tr>
<tr>
<td>Total Protein (g/100ml)</td>
<td>7.92 ± 0.97</td>
<td>7.73 ± 0.56</td>
<td>7.18 ± 1.51</td>
<td>6.04* ± 1.25</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>1164 ± 159</td>
<td>1576* ± 182</td>
<td>1492* ± 318</td>
<td>1140 ± 163</td>
</tr>
<tr>
<td>Albumin (g/100ml)</td>
<td>4.04 ± 0.22</td>
<td>4.27* ± 0.30</td>
<td>4.12* ± 0.25</td>
<td>4.17* ± 0.21</td>
</tr>
<tr>
<td>Albumin (g)</td>
<td>652 ± 89</td>
<td>870* ± 102</td>
<td>858* ± 66</td>
<td>797* ± 68</td>
</tr>
<tr>
<td>Globulin (g/100ml)</td>
<td>3.25 ± 0.96</td>
<td>3.55 ± 0.71</td>
<td>3.05 ± 1.70</td>
<td>2.35* ± 0.62</td>
</tr>
<tr>
<td>Globulin (g)</td>
<td>512 ± 132</td>
<td>726* ± 170</td>
<td>634 ± 354</td>
<td>433 ± 433</td>
</tr>
</tbody>
</table>

Values are means ± SE for before training (CONTROL), mid-training (Ex-8), after training (R + 1), and after recovery (R + 7). * P < 0.05 compared to control value.
of training the plasma potassium concentration fell by 0.43 mEq/l (-10.0%, P<0.05). Potassium continued to decrease in the recovery period and was 0.58 mEq/l (13.5%, P<0.05) lower than control levels at the end of the experiment. There was no change in the plasma potassium content over the course of the experiment.

Resting total protein and globulin concentrations did not change during the exercise phase of the experiment. However, at the end of the recovery period, the total protein concentration exhibited a decrease of 1.88 g/100ml (-23.7%, P<0.05), primarily through a concurrent 27.7% (P<0.05) decrease in the globulin concentration. The albumin concentration was increased by 0.23 g/100ml (P<0.05) by the end of the first week of training and remained significantly elevated through the rest of the experiment. Following the second week of training there was a 328 g increase in the plasma resting total protein content (28.2%, P<0.05) primarily due to a 206 g increase in the albumin content (31.6%, P<0.05), with a smaller contribution from an increase (122 g, NS) in the globulin content. While there was no distinguishable change in the plasma globulin content associated with the second week of the training period there was a significant (42%, P<0.05) increase associated with the first week of training. The total protein content returned to its control level during the recovery period, primarily
through a decrease in the globulin content, which fell (-15.4%, NS) below its control value. Albumin content did not return to its pre-training level, remaining 22.2% (P<0.05) above its control value.

Discussion

In the present study a plasma volume increase of 29.1% was observed after two weeks of training. This is similar to the 27% increase observed in Greyhound dogs after 14 days of training (32), but larger than the 12% expansion reported in humans (14, 15, 16) after 8 days of training. In humans the increased blood volume associated with exercise training was primarily due to an expansion of the plasma volume since there was no change in red cell volume or hemoglobin content despite a decrease in hematocrit and hemoglobin concentration (15, 16, 35). After the first week of training in the present study, blood volume increased 9229 ml through both a 4242 ml increase in the plasma volume and a 4976 ml increase the red cell volume. Hematocrit and hemoglobin content were elevated significantly after 7 days of exercise and may reflect a contribution from splenic contraction. It is possible that this red cell volume contribution was temporary and due to excitement at the time that blood was drawn because, after two weeks of training, the blood volume increase was only 5782 ml greater than control levels and was primarily the result of an 4692 ml
increase in the plasma volume with no change in red cell volume. Thus, it is unlikely that there was a change in the total volume of red blood cells.

The fact that 90% of the expansion of plasma volume occurs within 7 days of training suggests a fairly rapid physiological mechanism. This conclusion is supported by work with human subjects (15) where 71% of the 12% plasma volume expansion seen after 8 days of training was observed within the first 3 days of exercise. However, unlike the results seen in humans, plasma volume did not return to control levels after 7 days of recovery. Even so, the decrease in plasma volume seen during the recovery period was sufficient to lower the mean blood volume to a level that was not distinguishable from the level observed in the control period. While data was not collected after subsequent weeks of recovery, the trend was such that the plasma volume may have returned to pre-training levels in a relatively short time. This is supported in part by the fact that gross water retention decreased during the recovery period to below the level observed during the control period.

Resting plasma albumin concentration increased slightly in the present study which is different from the results seen in humans and canines (15, 32, 41) where the concentration did not change with training. Similar to the results observed in humans and greyhounds is the observation
that after 14 days of training there were no changes in the resting total protein and globulin concentrations. However, there was an increase in the total protein content, primarily through an increase in the albumin content. Because 1 g of protein binds 14-15 ml of water (15, 42) we can account for 4592 ml of the 4692 ml plasma volume expansion observed in the present study. Since the increase in plasma sodium and osmotic content was not proportional to the total plasma volume increase, the increase in protein content appears to be the primary mechanism. Proposed mechanisms which may account for this increase in total protein content include either a shift of protein from the interstitial compartment to the vascular space (43) or an increase in net protein synthesis (15). Although a protein shift from the extravascular space to the intravascular space is a possible mechanism, it would suggest that there must be a protein deficit in the extravascular compartment. However, this proposed extravascular protein deficit appears unlikely since it would be nonconducive to a net water retention. Data from recent studies using humans (15, 17) and Greyhounds (32) strongly supports the hypothesis that this increase in total protein content appears to be due to a net increase in total protein synthesis since the data reflected a net increase in total body water rather than a vascular
expansion at the cost of an interstitial or intracellular fluid deficit.

The water intake observed in the control period of the present study was consistent with previously reported values (10, 50). In the present study, exercise training did not cause a change in the rate of water consumption. However, urine output was decreased with exercise training, dropping by an average of 3.5 l/day from the average level observed during the pre-training control period. Although only a gross estimate of water balance, the difference between water intake and urine output increased an average of 3.5 l/day from a pre-training control value of 21.9 l/day to a post-training value of 25.4 l/day. Using previously published data obtained from exercising horses (10, 46), approximately 3.0 to 3.3 l/day can be attributed to increased sweating and respiratory losses during exercise. The remaining 0.5 to 0.2 l/day when multiplied by 14 days would suggest a net gain of 2.8 to 7.0 liters of body water, compartmentalized in the vascular space, which was consistent with the measured plasma volume gain of 4.7 liters. These data suggest that the plasma volume increase seen in horses may be indicative of a total body water expansion, similar to the expansion reported in the results from studies using humans (15) and dogs (32).

Urine output decreased 24.5% in the present study. This appears to be the sole route for increasing fluid
volume, since water intake did not change. This is consistent with the mechanism proposed by Convertino et al. (15) that renal control mechanisms are the primary route for the fluid volume increases associated with exercise training in humans. However, this is in contrast to the results seen in dogs (32) where drinking appears to be the primary route for exercise-training induced increases in fluid volume and those seen in humans (23) where water intake increases primarily to replenish exercise induced losses. Both the renin-angiotensin II-aldosterone (RAA) system and the sodium ion-osmotic-vasopressin system (SOV) may have influences on renal function during muscular activity since the activity of both systems increases during acute exercise and repeated elevations in the activity of both systems might cause chronic changes in renal function in humans (15). More specifically, with increases in aldosterone and vasopressin activity, one would expect a decrease in the loss of sodium and water via the kidneys. As postulated, training produced a decrease in urine output by the horses of the present study but, as the results indicated sodium clearance did not change, suggesting that aldosterone may not be active in the mechanism and since free water clearance increased, then vasopressin likewise may not be implicated in the mechanism. Therefore, the horse may not use either mechanism to produce the training-induced hypervolemia and uses some other mechanism
which produces a decrease in the clearance of total osmotic substances.

Although there was no change in the total amount of sodium excreted during either of the two weeks of training, there was a significant decrease in the excretion of potassium. The data from the present study indicates that plasma potassium levels had decreased after 14 days of training, suggesting that the horses had entered a net potassium deficit. It has been reported that a significant amount of potassium is lost in the sweat of exercising horses (10, 46) and in the present study, sweating was visually observed to increase with training. This increased loss was not countered through an increase in intake since there was no potassium supplementation provided and the amount of feed given to the horses during the training period was not changed. Therefore, the decrease in the clearance of potassium by the kidneys would appear to be in response to a net deficit in the balance of potassium as indicated by a chronic lowering of the plasma potassium concentration.

Unlike the renal conservation mechanisms associated with training-induced hypervolemia in humans, where water and sodium are the primary substances that are reabsorbed by the kidneys, the mechanisms associated with the horses of the present study appear to be concerned primarily with the conservation of water and potassium. Since the results of
the present study indicate that there were no chronic training-induced changes in glomerular filtration rate one would postulate that the major renal conservation mechanisms which contributed to the training-induced hypervolemia in the horse are those which control post-glomerular loss of water by the kidney. This appears to be due to some mechanism other than aldosterone or vasopressin with some other solute being conserved.
CHAPTER 5

GENERAL CONCLUSIONS

Four Greyhound dogs and six horses were utilized to study the physiological mechanisms associated with the development of an exercise training-induced hypervolemia. The animals were used in two separate experiments and were exercised for 14 days on a treadmill ergometer and the results were used to formulate conclusions regarding the physiological and practical implications related to the phenomenon.

Based upon the human literature (14, 15, 16) the following hypotheses regarding the general physiological mechanisms associated with the hypervolemic response were formulated (see Figure 3):

1). That exercise training would produce an increase in plasma volume through an increase in net water retention and total body water.

2). That the increase in net water retention would come about through an increase in water intake and/or a decrease in urine output.

3). That water intake increases might come about through the stimulation of hypothalamic thirst receptors by repeated exercise-induced elevations in plasma osmolality and arginine vasopressin activity.
Figure 3. Predicted Physiological Mechanism for the Hypervolemic Response to Exercise Training
4). Since acute exercise at an intensity above 65% \( \dot{V}O_2 \) max produces significant increases in plasma renin and arginine vasopressin activity in man (15), we theorized that we would see a chronic decrease in both sodium and free water clearance and consequentially a decrease in urine output.

The results, as reported in this dissertation, indicate that exercise training will cause an expansion of the blood volume of the Greyhound dog and the horse. Physiologically the result is similar in man, the dog, and the horse, however, the mechanisms by which this adaptation is reached appears to differ in each of the species.

Based upon recent findings by Convertino and Kirby (17), it appears that in man (Figure 4), renal mechanisms predominate the hypervolemic response with primary contributions from the reabsorption of sodium and water. Water intake increased proportionally with the increase in sweat production and did not add to the increase in total body water (17). The contribution of vasopressin to the renal mechanism was ruled out since free water clearance was observed to increase (17). Although resting plasma aldosterone levels did not change with exercise and heat acclimation, sodium clearance was reduced, therefore Convertino and Kirby (17) suggested that in man, the mechanism may involve an increased sensitivity to circulating aldosterone.
Figure 4. Suggested Mechanism in Humans for the Hypervolemic Response to Exercise Training
In the dog, as outlined in Figure 5, water intake appears to be the primary mechanism for the increase in fluid volume. The suggested mechanism (32) for the increase may involve exercise-induced increases in plasma osmolality and angiotensin II which stimulate thirst receptors in the hypothalamus. Even though repeated exercise produced a diuresis and a natriuresis in the dog, the chronic increase in water consumption urinary losses and was large enough to produce a net increase in total body water.

In the horse (Figure 6), renal control mechanisms appear to predominate the hypervolemic response with those that control the retention of solutes other than sodium predominating over those that control sodium and free water clearance. Drinking did not contribute to the increase in total body water, however, during the recovery period the average volume consumed in 24-hrs appeared to decrease, suggesting that a reduction in consumption may be associated with the reduction in water retention and blood volume observed during recovery.

In conclusion, the material presented in this dissertation provides an examination of the basic physiological mechanisms associated with the exercise-induced expansion of the vascular compartment. Further research is needed in the dog and horse to understand which endocrine controls might be implicated in the hypervolemic response.
Figure 5. Suggested Mechanism in the Greyhound Dog for the Hypervolemic Response to Exercise Training
Figure 6. Suggested Mechanism in the Horse for the Hypervolemic Response to Exercise Training
APPENDIX A

DETERMINATION OF PLASMA VOLUME

The procedures listed in this appendix were provided by Dr. V. A. Convertino as a part of the Laboratory Techniques in Exercise Physiology Class (PHED 550), they are presented here with his permission.

Reagents

1. Disodium Hydrogen Phosphate, \( \text{Na}_2\text{HPO}_4 \) (anhydrous), 2\% (2 gm/100ml).

2. Teepol-phosphate: Add 2 gm \( \text{Na}_2\text{HPO}_4 \) to 3 ml Teepol. Make up to 100 ml with distilled water (Teepol 610 concentrate, Particle Data Laboratories, LTD).

3. Potassium Phosphate, Monobasic, \( \text{KH}_2\text{PO}_4 \) (anhydrous), 8\% (8 gm/100ml).

4. 1:1 acetone-water solution.

5. Solka-Floc SW-40A (James River Corporation, Berlin, New Hampshire). Suspend approximately 1 gm Solka-Floc in 100 ml of 2\% \( \text{Na}_2\text{HPO}_4 \) (reagent 1).

6. Standard Evans Blue Dye T-1824, same lot number as that used for injection. Dilute 1 ml to 50 with distilled water. Refrigerate.


Procedure

1. Preparation of Column

   A. Prepare one column for the standard and one for each plasma sample. Columns should be about 1 cm diameter and may be conveniently made from broken 50 ml burettes.
B. Insert approximately 100mg of glass wool in each column, wash the tube down with distilled water, and pack the glass wool firmly above the small constriction with the aid of a large stirring rod. The height of the glass wool should be about 0.5 cm.

C. Using a 10-ml pipette (upside down), pipette about 12 ml of the Solka-Floc suspension into the column so that the height of the Solka-Floc is 3-5 cm.

D. Wash each column with approximately 10 ml of Na₂HPO₄ to pack the column. Use a circular motion to wash down the sides of the column.

2. Test

A. Fill the syringe with Evans Blue Dye T-1824 and weigh along with needle and cap. Following the injection of the dye, the syringe along with the needle and cap should be weighed once again. The difference between the weights represents the amount of dye injected.

B. Inject I.V., 0.3 mg T-1824/kg body weight as a 0.1% solution in isotonic saline. (Each 5 ml vial contains 22.6 mg of Evans Blue Dye).

C. Draw a heparinized blood sample to yield 1 ml plasma 15 min after injection. If circulation time is prolonged, the sample should be taken 20-25 min after injection.

D. Collect a sample for hematocrit determination at this time.

E. Note: When a sample has been done within the last 10 days a blood sample is also drawn prior to injection.

3. Preparation of Plasma Samples

A. TEST Pipette 1 ml TEST plasma into a 50-ml Erlenmeyer flask.

B. STANDARD Pipette 1 ml normal plasma (i.e., containing no dye) into a 50-ml Erlenmeyer flask. Add exactly 0.2 ml of the STANDARD to the plasma. Mix by swirling gently and allow to stand for 2 minutes.
C. Blank If T-1824 blood volume has been done within the last 10 days, pipette 1 ml of subject's plasma obtained prior to dye injection into a 50-ml Erlenmeyer flask (Otherwise, the plasma blank is zero).

D. Add 15 ml Teepol-phosphate to each flask, washing down any plasma from the sides of the flask. Then swirl gently for about 15 seconds to mix thoroughly.

4. Extraction

A. Transfer the contents of each flask gently onto the pulp column with a Pasteur pipette so that the surface is not disturbed.

B. Rinse the flask with 5 ml Teepol-phosphate and add this to the column.

C. When the level of the solution has reached that of the Solka-Floc, add at least 10 ml 2% Na$_2$HPO$_4$ to the column. This "wash" must be sufficient to remove all interfering substances, or else cloudiness or an appreciable artifactual color in the final eluate will result.

D. Allow the "wash" to pass through until the fluid level is just above the Solka-Floc.

5. Elution

A. Must be carried out at once.

B. Pipette 0.5 ml 8% KH$_2$PO$_4$ into a 10-ml volumetric flask and place the flask under the delivery needle to collect the eluate. This with the Na$_2$HPO$_4$ from the column, buffers the pH of the eluate to 7.0.

C. Gently transfer approximately 5 ml of the acetone-water solution (pH adjusted that day) to the column and allow it to pass down until the blue front passes through the column to the flask.

D. Allow the columns to stand for 15 minutes before completion of the elution.

E. Add additional acetone-water (4-5 ml) to the column and continue the elution until the eluate fills the flask almost to the 10 ml mark, by which time the column should have lost all blue coloration.
F. Bring the volume of to the mark with acetone-water and mix the contents of the flask by inversion.

G. Read the solutions in a spectrophotometer in 1-cuvets (with tops to prevent evaporation) at 615 mu.

Calculations

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

Where:

- \( V \) = Volume T-1824 injected
- \( D \) = Dilution of STANDARD (0.2ml 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

\[ \frac{\text{plasma volume} \times 100}{100 - (0.91 \times \text{hematocrit})} = \text{blood volume} \]

Where:

0.91 is a correction factor to convert venous hematocrit to "whole body hematocrit"

Red Cell Volume = Blood Volume - Plasma Volume


