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The Ohio State University, 1988

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MECHANISM OF
EXERCISE-INDUCED PLASMA VOLUME EXPANSION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Maurie Joe Luetkemeier, B.S., M.A.

* * * * *

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1988

To My Parents,
who exemplify that
which I aspire
to become.

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- Luetkemeier, M.J. (1978). The effect of endurance swimming on the cardio-respiratory fitness level of middle-aged sedentary men and women. (Thesis Research Project)
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FIELDS OF STUDY

Major Field: Exercise Physiology

**Studies in : Plasma Volume, Dr. David R.Lamb
Cardiac Rehabilitation and Adult Fitness,
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CHAPTER I

INTRODUCTION

The regulation and maintenance of an appropriate blood volume is critical to survival. Fortunately, blood volume remains fairly constant over the long term despite a variety of conditions (e.g., dehydration, consumption of a salty meal) that elicit transient fluctuations. This stability in blood volume is achieved with a balance of neural, hormonal and osmotic influences that act on various organs (e.g., kidney, blood vessels, and skin) to restore fluid homeostasis once it is disturbed. Given the usual constancy of blood volume, it is quite surprising that individuals who engage in physical exercise training typically experience an 8 to 10% expansion in blood volume that seemingly persists as long as training continues (Convertino, Brock, Keil, Bernauer, and Greenleaf, 1980; Green, Jones, Hughson, Painter, and Farrance, 1987; Lijnen, Hespel, Eynde, and Amery, 1985).

Exercise-induced hypervolemia has been demonstrated using several different training protocols. Convertino et al. (1980) reported a 457 mL (8.1%) mean increase in blood volume following 8 d of stationary bicycling at 65% $\dot{V}O_{2\max}$ for 120 min. They found that the expansion in blood volume was caused primarily by an increase in plasma volume with little or no change in red cell volume. Similar

training programs and results were reported by Kirby and Convertino (1986), Green et al. (1987), and Greenleaf, Brock, Keil, and Morse (1983). In addition, Green et al. (1984) observed a 5% increase in blood volume with an associated 11.6% increase in plasma volume following 4 d of high intensity interval training. Furthermore, the subjects of Lijnen et al. (1985) spent 3-3.5 h/d doing on-road cycling, calisthenics, jogging and interval running and exhibited an 8.6% increase in blood volume with a 13% rise in plasma volume following only 2 d of training.

Hypervolemia induced by exercise training is associated with hemodynamic and thermoregulatory adaptations that are potentially advantageous. An expanded blood volume enables stroke volume and cardiac output to be significantly larger at rest and during submaximal and maximal exercise (Fortney, Nadel, Wenger, and Bove, 1981; Kanstrup & Ekblom, 1982). Although the hemoglobin concentration of the blood is lower, the improved hemodynamics compensate for the lower oxygen carrying capacity of the blood so that maximal aerobic capacity does not suffer (Kanstrup & Ekblom, 1982). With an expanded plasma volume, skin blood flow (Fortney et al., 1981) and sweat rate (Convertino, 1983) can be increased to improve heat dissipation.

Proposed Mechanisms for Training-Induced

Plasma Volume Expansion

Two contrasting explanations have been proposed to account for the hypervolemic adaptations that accompany exercise training. First, exercise training is said to promote the entry of protein into the blood, causing the colloidal osmotic pressure in the capillaries to

increase fluid uptake into the vasculature by osmosis (Convertino et al., 1980; Freund, Claybaugh, Dice and Hashiro, 1987; Senay & Kok, 1977). Second, exercise stimulates the release of arginine vasopressin and activates the renin-angiotensin-aldosterone system to facilitate expansion of plasma volume by reductions in renal blood flow, sodium clearance, and urine production (Costill, Branam, Fink, and Nelson, 1976; Geyssant et al., 1981; Kosunen & Pakavinen, 1976; Kraemer et al., 1988; Melin et al., 1980; Staessen et al., 1987).

Evidence for Increased Plasma Proteins

After 8 d of endurance training, 28 g of protein, primarily albumin, had entered the vascular space of the subjects of Convertino et al. (1980). He calculated that the addition of this much protein was sufficient to account for the concurrent increase of plasma volume that he observed. Convertino's findings are paralleled by McKeever, Schrug, and Convertino (1985), who also concluded that a significant portion of the increase in plasma volume that they observed in endurance-trained dogs could be attributed to additional plasma proteins.

Edwards and Harrison (1984) and Freund et al. (1987) have reported influxes of protein into the vasculature following a single exercise bout. However, this observation is not consistent with the finding of Costill et al. (1976) or Convertino et al. (1980), who reported no increase in total protein content following 1 and 4 d of training, respectively.

Evidence for Increased Vasopressin and Greater
Activity of the Renin-Angiotensin-Aldosterone System

Arginine vasopressin (AVP) is produced in the hypothalamus and secreted into the blood from the posterior pituitary. The release of AVP is triggered by a high plasma osmolality or a persistent fall in arterial blood pressure. Vasopressin promotes fluid conservation by increasing the permeability of the distal renal tubule cells to water, thus allowing the return of filtered water back into the peritubular circulation.

The concentration of AVP during exercise varies directly with relative changes in workload above a threshold of $\sim 40\% \dot{V}O_{2\max}$ (Wade, 1984). However, the probability that vasopressin contributes significantly to exercise-induced hypervolemia is remote because antidiuresis that is associated with exercise, as noted by Wade and Claybaugh (1980) and by Kachadorian (1970), occurs during a time when free water clearance is increasing; this is inconsistent with vasopressin activity. In addition, AVP concentration has been reported to return to preexercise levels within 1 h after exercise (Convertino et al., 1980), whereas the increase of plasma volume following exercise may continue for 48 h (Costill et al., 1976; Lijnen et al., 1985).

The renin-angiotensin-aldosterone system (R-A-A) is a hormonal pathway that is activated by the release of renin from the juxtaglomerular cells of the kidney. Renin acts enzymatically to convert angiotensinogen to angiotensin I, and angiotensin I is then converted to angiotensin II. Angiotensin II is a powerful

vasoconstrictor that inhibits fluid losses by decreasing glomerular filtration and sodium clearance. Angiotensin II also stimulates the release of aldosterone, a steroid hormone produced in the adrenal cortex. When aldosterone contacts the epithelial cells of the distal tubules of the kidney, aldosterone diffuses into the cytoplasm where it complexes with a highly specific receptor protein that actively facilitates the passage of aldosterone through the nuclear membrane into the cell nucleus (Williams, 1985). Within the nucleus, aldosterone induces DNA transcription of messenger RNA strands that diffuse into the cytoplasm and initiate the synthesis of new protein molecules. These new proteins augment sodium retention in the kidney by:

1. Increasing the permeability of the tubular epithelium to sodium.
2. Increasing the amount or activity of Na/K-ATPase within the peritubular capillary membrane.
3. Increasing mitochondrial oxidative metabolism in the tubular and peritubular epithelium, thereby supplying more energy to the sodium pump, which can then transport more sodium from the renal tubules to the peritubular capillaries (Guyton, 1986).

Vigorous exercise enhances all aspects of the R-A-A system, i.e., plasma renin concentration, plasma renin activity (the rate at which angiotensinogen is converted to angiotensin I), angiotensin II concentration, and aldosterone concentration (Staessen et al., 1987).

However, during recovery from exercise, plasma renin activity quickly returns to preexercise levels (Wade & Claybaugh, 1980; Convertino et al., 1980), whereas aldosterone remains elevated for up to 6 h (Costill et al., 1976; Wade, Ramee, Hunt, & White, 1987). Wade, Dressendorfer, O'Brien, & Claybaugh (1981) found a reduced sodium excretion rate and an elevated aldosterone secretion rate up to 20 h after vigorous exercise. With the persistence of aldosterone activity, it seems reasonable to hypothesize that each training session evokes the release of aldosterone that facilitates sodium and water conservation throughout recovery until training is performed the next day.

Development of the Experimental Hypotheses

Convertino et al. (1980) demonstrated that the hypervolemic adaptations associated with exercise training progress rapidly during the first 4 d of training and then remain constant during the next 4 d. Likewise, McKeever, Schurg, Jarrett, and Convertino (1987), who studied horses, reported a similar plateau in plasma volume after 8 d of endurance training. These data are suggestive of a biphasic mechanism. The first phase facilitates the actual enlargement of the vascular volume, whereas the second enables the plasma volume to remain elevated at some new plateau.

Ultimately, exercise-induced plasma volume expansion appears to be associated with the addition of solute, presumably sodium or protein, to the blood. This increased solute increases the total osmolar content of the plasma and thus expands the volume. Sodium appears to be the predominant solute effector early in training. The

rate of urinary sodium output drops dramatically during and immediately following a single bout of endurance exercise (Costill et al., 1976; Lijnen et al., 1985). A decrease in urinary sodium closely parallels a concomitant decrease in the percent of filtered sodium excreted but is not related to changes in glomerular filtration rate (Castenfors, 1977). This observation indicates that the retention of sodium occurs in the renal tubule; an action that is consistent with aldosterone activity. Furthermore, Costill et al. (1976) indicated that during the initial 48 h following an endurance exercise bout, the 9% increase they observed in plasma volume was related nearly exclusively to the retention of sodium by aldosterone with little or no influence of changes in plasma protein. Similarly, Convertino et al. (1980) noted that increases in total plasma sodium during training were nearly maximized by the fourth day, whereas significant accumulation of protein did not develop until the eighth day.

Therefore, it is hypothesized that increased sodium retention related to elevated aldosterone activity is the primary contributor to early training-induced hypervolemia. It is further hypothesized that a later movement of protein into the vasculature permits blood volume to remain expanded.

Purpose

The purpose of this study was to determine if the early development of training-induced expansion of plasma volume was closely associated with the activity of aldosterone, and if the maintenance of expanded plasma volume later in training was better associated with increased concentrations of plasma proteins. As one approach to this

problem, an attempt was made to competitively inhibit the action of aldosterone by the administration of spironolactone. It was hypothesized that spironolactone-treated subjects would exhibit a lesser expansion of plasma volume early in training when compared to nonmedicated exercising controls. Furthermore, it was hypothesized that exercised spironolactone-treated subjects would eventually expand their plasma volume by means of a delayed increase in plasma proteins.

CHAPTER II

REVIEW OF LITERATURE

The primary purpose of this study was to examine the hypothesis that aldosterone exerts a significant influence on the early expansion of plasma volume during endurance exercise training. It was further hypothesized that an influx of protein into the vasculature during later training contributes to maintenance of the expanded plasma volume. Research from the areas of physiology, biochemistry, endocrinology, and exercise physiology have been integrated in this chapter to provide an overview of the major topics.

Included in the first section is a discussion of the Renin-Angiotensin-Aldosterone (RAA) System, a major hormonal pathway involved in the stability of proper fluid balance. The review will focus on mechanisms for activating the RAA System, generation and degradation of the hormones, and the relationship between renin, angiotensin, and aldosterone. This section will conclude with a discussion of aldosterone, its affect on sodium conservation, and its inhibition by spironolactone.

Plasma proteins are also osmotically active and can influence plasma volume by increasing the colloidal osmotic pressure within the capillaries. The influx of protein during an acute exercise bout as

well as during prolonged training will be discussed in the second section of this chapter.

Some researchers suggest that the hypervolemia that accompanies exercise training is related to an increase in body temperature. Others maintain that the mechanisms for heat-induced and exercise-induced hypervolemia are separate. Research that supports both of these viewpoints will be discussed.

Finally, the review of literature will conclude with a discussion of artificially induced alterations in blood volume, postural changes that influence plasma volume, and exercise-induced hypervolemia in animals.

The Renin-Angiotensin-Aldosterone (RAA) System

Some investigators have speculated that the hypervolemic adaptations that accompany exercise training are largely caused by the fluid-conserving influence of the renin-angiotensin-aldosterone system (Costill, Branam, Fink, & Nelson, 1976; Wade, Hill, Hunt, and Dressendorfer, 1985). The RAA system exerts influence on the kidney to conserve fluid by promoting vasoconstriction of the kidney arterioles and sodium retention within the nephron. It is theorized that exercise-induced plasma volume expansion is related to the persistent actions of aldosterone that is released in high concentrations during exercise.

Mechanisms for Activation of RAA

Activation of the RAA system begins with renin release, which is directly related to the relative intensity of dynamic work (Convertino, Keil, and Greenleaf, 1983; Pedersen, Kornerup and Larsen,

1981; Wade & Claybaugh, 1980). Galbo (1983) determined that the elimination of renin by the liver is unrelated to hepatic blood flow. Therefore, he concluded that the increase in renin activity associated with exercise is most likely due to an increased rate of renin production, rather than to a decreased rate of removal.

The stimulus for renin release has not been clearly defined during exercise but is presumably related to beta adrenergic receptor activation by circulating catecholamines (Galbo, 1983). In a study by Kotchen et al. (1971), the responses of norepinephrine and renin to graded exercise were highly correlated, and the threshold exercise intensities required to elicit both responses were similar. Persuasive evidence for this theory came from Williams and Foster (1985), who ascertained that the beta-adrenergic blocking agent, propranolol, successfully blocked the increase in renin activity, whereas exogenous norepinephrine enhanced it. However, the direct relationship between catecholamines and renin was recently questioned by Guezennec, Defer, Cazorla, Sabathier, and Ihoste (1986), who found that the postural and environmental differences associated with swimming and running strongly influenced the catecholamine-renin correlation.

Thus, the bulk of the literature supports the view that with cycling exercise, renin is released in proportion to the intensity of the exercise, and this release is prompted by elevated concentrations of circulating catecholamines. Because renin initiates the RAA system, it was determined that for the present study, multiple measurements of renin activity during and after exercise would be

helpful in assessing the magnitude and timing of hormonal influences on blood volume.

The Generation and Degradation of Angiotensin II

The second stage of the RAA System involves the generation of Angiotensin II. The hydrolysis of angiotensinogen (also called renin substrate) to angiotensin I by the enzymatic activity of renin has been generally considered to be the rate-limiting step in the pathway from renin substrate to angiotensin II. Moreover, the generation of angiotensin I has been assumed to be dependent on the plasma renin concentration. However, during exercise, this relationship may be less direct. Kosunen and Pakarinen (1976) reported that 30 min following maximal running, plasma renin concentrations were approximately five times higher than plasma renin activity. They suggested that angiotensin I generation was limited by substrate and not renin. In their discussion, Kosunen and Pakarinen (1976) cautioned that their results may have been exaggerated because the in vitro generation of angiotensin I during the analysis was performed at an optimal pH of 6.0 rather than normal body pH of 7.4.

The interactions of renin, angiotensin, and aldosterone during exercise were recently investigated by Staessen et al. (1987). They observed that the plasma concentration of angiotensin II rose in proportion to the increase in activity of plasma renin at 30% and 60% of relative $\dot{V}O_{2\max}$ but increased much more than renin activity or aldosterone at peak effort. This finding opposed the prevailing belief that changes in angiotensin II concentrations nearly paralleled the activity of renin and raised speculation that either

the production or elimination of angiotensin II was in some way altered by maximal exercise. Staessen et al. (1987) explained that the activity of angiotensinase A₁, the primary enzyme involved with the degradation of angiotensin II, was pH dependent and may be reduced by 50% with a fall in pH from 7.4 to 7.0. They suggested that the metabolic acidosis produced by severe anaerobic exercise might inhibit the usually rapid degradation of angiotensin II, thereby leading to its accumulation.

Most researchers feel that the generation of angiotensin II from renin substrate is dependent on the concentration of circulating renin. However, conditions within the cell during high intensity exercise may modify this relationship and ultimately affect the way fluids are conserved. The moderate workload that was used for the present dissertation project was presumably not great enough to elicit a disproportional rise in angiotensin II relative to renin.

The Relationship Between Renin and Aldosterone

Numerous studies have been conducted to determine the relationship between renin activity and plasma aldosterone during exercise. Generally, it is agreed that renin activity, by its effect on angiotensin II, is correlated with aldosterone release and that a direct relationship is maintained throughout a wide range of exercise intensities (Costill et al., 1976; Staessen et al., 1987; Pederson et al., 1981). In addition, high aldosterone concentrations feed back negatively on renin release so that sodium homeostasis is better maintained (Williams & Foster, 1985).

Increases in aldosterone are not totally dependent on increases in renin activity or angiotensin II. Aldosterone secretion from isolated, perfused, dog adrenal glands was increased five fold by adding potassium or reducing sodium in the perfusate; furthermore, aldosterone secretion stimulated by either angiotensin II or potassium was negatively affected by perfusate osmolality, even when sodium concentration was reduced (Schneider, Radke, Ulderich, & Taylor, 1985).

The exercise-induced increase in circulating aldosterone is unaffected by the administration of captopril, a drug which blocks the conversion of angiotensin I to angiotensin II (Wade, Ramee, Hunt, & White, 1987). Accordingly, although angiotensin II is usually assumed to be the primary mediator of aldosterone secretion, an alternative stimulus, namely ACTH, may have acted in the absence of angiotensin II. ACTH can provoke the release of aldosterone independently of angiotensin II, and the rise in circulating ACTH during exercise is similar to that of aldosterone. This reasoning was supported by evidence from Raff, Shinsako, Wade, Keil, and Dallman (1985), who demonstrated that ACTH was as potent as angiotensin II in stimulating aldosterone release.

Renin and aldosterone under most circumstances vary together, but aldosterone release cannot always be predicted by renin concentration. Therefore, during the present study, measurements of renin and aldosterone were made simultaneously. Comparison of the two measurements was made to determine how their relationship was affected by treatments involving both exercise training and aldosterone

inhibition by spironolactone. It was hoped that this analysis would help identify compensatory mechanisms that the body initiates to overcome the inhibition of aldosterone.

Aldosterone and Sodium Conservation

Aldosterone has been linked to exercise-induced hypervolemia because of its effect on sodium conservation by the kidney and sweat glands. Costill et al. (1976) followed urinary sodium excretion and total plasma sodium subsequent to 1 h of stationary cycling and showed a decline in urinary sodium and an accumulation of total plasma sodium lasting up to 48 h. They also observed an elevation in plasma aldosterone concentration and plasma renin activity, but a return of these variables to pre-exercise levels within 6 h following exercise. They could not provide an explanation for the discrepancy in time between the recovery of urinary sodium (48 h) and aldosterone (6 h). They speculated that either the activity of aldosterone persisted long after the aldosterone concentration declined, or sodium reabsorption was mediated by some unidentified factor.

Wade et al. (1985) and Wade et al. (1981) reported that the urinary excretion and plasma concentrations of aldosterone were persistently elevated during repeated long distance running. Increases in plasma aldosterone were correlated with decreases in urinary sodium. Moreover, the reduction of plasma aldosterone and the return of sodium excretion to basal conditions required 70 h. Wade et al. (1985) concluded that aldosterone mediated the decrease in urinary

sodium and that an accumulation of sodium and an expansion of plasma volume was likely under these circumstances.

Thus, relative to the hypotheses under investigation in the present study, it was anticipated that sodium conservation would be compromised if aldosterone action were inhibited by the administration of spironolactone (see following section). Therefore, it was predicted that plasma volume expansion under spironolactone treatment would be impaired unless alternative mechanisms to achieve fluid conservation were initiated.

Spironolactone

It was assumed that the effect of aldosterone on sodium retention in the distal tubule could be assessed during exercise by comparing blood and urinary responses of experimental groups who either received or did not receive spironolactone. This assumption was based on the literature describing the effectiveness of spironolactone in blocking the actions of aldosterone. The actions of spironolactone and mechanisms mediating those actions were reviewed by Corvel et al. (1981) and are summarized in the following section.

Spironolactone was introduced in 1960 as a therapeutic antialdosterone agent in treating hyperaldosteronism and essential hypertension. Spironolactone has long been considered a direct competitor with aldosterone because the antagonism provided has been dose dependent, specific, and operational at the receptor site. When spironolactone was added to the perfusate going into a kidney preparation that was stimulated by aldosterone, urinary sodium/potassium ratios varied directly with the spironolactone dose.

Spironolactone specifically blocks the effects of the mineralocorticoids. Spironolactone labelled with ^3H has a high affinity for the mineralocorticoid binding sites within rat kidney preparations. During the adherence of spironolactone to these binding sites, no translocation of steroid-receptor complexes into the nucleus is observed. Spironolactone has also been shown to inhibit aldosterone biosynthesis in in-vitro adrenal glandular tissue.

The blockade of aldosterone by spironolactone has not been well studied during endurance exercise. However, Bevegard, Castenfors, and Danielson (1977) did investigate the effects of long-term treatment with spironolactone on circulatory parameters during moderate exercise in hypertensive patients. After four months of treatment with 100 mg/d spironolactone, blood pressures at rest and during exercise were significantly lower, while cardiac output, stroke volume, and heart rate both at rest and during exercise were unaffected. The calculated resting total peripheral resistance (TPR) dropped, but the exercising TPR remained unchanged. In addition, resting plasma renin activity was increased following spironolactone treatment.

Spironolactone was administered to subjects in the present study to elucidate the contribution of aldosterone to the hypervolemic adaptations that accompany endurance training. The reported effectiveness in inhibiting the actions of aldosterone suggested that the use of spironolactone could help distinguish that portion of exercise-induced plasma volume expansion that is attributed to aldosterone.

Postural Effects on RAA During Exercise

Postural changes have been shown to have a substantial effect on measurement of plasma volume both at rest and during exercise. Senay and Pivarnik (1985) suggests that these changes reflect a difference in hydrostatic pressure that would drive fluid out of the vascular space when changing from supine to upright position. Hagan, Diaz, and Horvath (1978) calculated a 16.2% decrease in plasma volume after 35 min of standing from a previously supine position.

Hagan et al. (1978) subsequently studied the effects of shifting from one of three postures (supine, low sitting, and upright standing) to an upright sitting posture on a stationary bicycle followed by 45 min of cycling. The changes in plasma volume that corresponded to the positional changes were 17.3%, 9.0%, and 2.2%, respectively, for the supine, low sitting, and upright sitting posture. The additional loss of plasma volume that occurred during the cycling was 11%, 7.1%, and 2.7% for the prior supine, low sitting, or upright sitting positions. Similar findings were reported by Edwards and Harrison (1984) in subjects who changed from a sitting to a standing position prior to running. Therefore, it is crucial that the posture of subjects before and during measurements of plasma volume be standardized to control for the influence of postural changes.

Plasma Protein

Plasma proteins exert an osmotic pressure that is necessary for the retention of fluid within the vascular space. Each gram of protein is capable of holding approximately 15 mL of fluid (Convertino

et al., 1980). Therefore, any additional protein present in the bloodstream will increase the colloidal oncotic pressure and pull fluid from the interstitium.

Convertino et al. (1980) reported a 28 g increase in total plasma protein following 8 d of endurance cycling. They calculated that 390-420 mL of additional plasma volume could be retained within the vascular space as a result of this protein influx. With sequential monitoring of plasma protein content they showed that the influx of protein was not apparent until the eighth day of training and could not have accounted for early changes in plasma volume. Similarly, McKeever et al. (1985) reported a 21.7 g increase in plasma proteins and a concomitant 472 mL expansion in plasma volume in greyhound dogs following 14 d of treadmill walking. Both Convertino et al. (1980) and McKeever et al. (1985) identified albumin as the specific protein that constituted the majority of the additional protein.

Conflicting results have been reported with regard to whether protein molecules enter or escape the vasculature during a single bout of exercise. Senay (1979) observed an influx and an efflux, respectively, of protein in the same subjects performing the same work in first a cool environment (20° C) and then a hot environment (40° C). He theorized that the cutaneous vascular bed at hot temperatures was more dilated than at cool temperatures, allowing proteins to exit through the pores in the endothelium. Furthermore, he attributed the buildup of proteins during cool temperatures to a corresponding increase in lymph flow, which transports proteins from the

interstitial space back into circulation. The latter hypothesis was supported by Olszewski, Engeset, Jaeger, Sokolowski, and Theodorsen (1977), who studied leg lymph flow and protein dynamics during moderate exercise and hot water exposure. They determined that lymph flow and total protein content within the lymph increased during both conditions. However, it could not be determined from their study whether the increase in lymph protein was over and above what was lost from the plasma through filtration.

Differences in protein flux during exercise have also been noted between subjects who were heat acclimatized prior to exercising and those who were not. In general, protein influx has been observed when heat acclimatization was included with exercise training as part of the treatment (Harrison, Edwards, Graveney, Cochrane, and Davies, 1981; Sawka, Francesconi, Pimental, and Pandolf, 1984; Senay & Kok, 1977), and protein efflux was experienced when heat acclimation was not part of the treatment (Novosadova, 1977; Costill et al., 1976; McMurray, 1983; Fortney & Senay, 1979). A noteworthy exception to this generalization was reported by Costill and Fink (1974). They imposed dehydration in non-acclimatized subjects by exposing them to extreme heat (65-75° C) or to vigorous exercise at room temperature. In both cases, protein was added to the bloodstream both during and following the treatment. However, the heat-induced dehydration treatment resulted in a significantly greater influx of protein than did the exercise-induced dehydration.

Alterations in the movement of the albumin and globulin components of plasma proteins were studied by Pivarnik, Montain,

Graves, and Pollack (1988). They observed that no net movement of protein into or out of the vasculature resulted from maximal cycling. However, there was a shift favoring albumin influx and globulin efflux so that the final composition of the plasma protein mixture favored additional albumin.

In summary, protein has been noted to both exit and enter the vasculature in response to exercise. Shifts in body fluid volumes are said to follow fluctuation in plasma proteins. Therefore, the influence of protein on body fluid dynamics can be evaluated by monitoring these fluctuations in plasma proteins in relation to concurrent shifts in plasma volume.

Comparison of Hypervolemia Induced by Exercise or Heat

Plasma volume expansion is an adaptive response to both repeated heat exposure (acclimatization) and endurance exercise training (Fortney & Senay, 1979; Harrison et al., 1981; Kirby & Convertino, 1986). Many of the adaptations in cardiovascular and thermoregulatory responses to exercise are similar for both heat acclimatization and exercise training. Furthermore, because of the increase in body core temperature that is inherent to endurance training, it has been difficult to isolate which adaptations, if any, are unique to either mode. Convertino, Greenleaf, and Bernauer (1980) attempted to separate the effects of exercise and thermal factors by training one group for eight consecutive days, 2 h/d, on a stationary bicycle ergometer at a relative load of 60-65% $\dot{V}O_2$ max. He exposed the second group to a hot, humid environment (42° C, 93% relative humidity) for a similar time and duration as the exercise condition.

During the two treatments, the heat stress and the exercise intensity were altered so that rectal temperatures were maintained at 38.5° C. Comparisons between the two conditions on a variety of variables were made each day, and it was observed that the heat exposure group consistently had a lower heart rate, plasma osmolality, and plasma vasopressin response, but had a significantly greater drop in plasma volume after each session. There was a significant interaction effect in total body sweat rate such that the heat exposure group sweated less in the first several days but finished the acclimatization program sweating more than the exercise training group. Both groups experienced a hypervolemic adaptation after training, but that of the exercise group was greater than that for the heat exposure group. Finally, the exercise group experienced a greater influx of total protein into the bloodstream than did the heat exposure group. It was concluded that 40% of the hypervolemia induced by exercise training was attributed to a thermal stimulus, whereas the remaining 60% appeared to be contributed by additional factors related to the exercise.

Senay and Kok (1977) addressed this same problem by first training subjects for 48 d with daily, 4 h bicycle rides in a cool environment and then heat acclimatizing those same subjects for the next 12 d by 4 h daily exposures to 65-70° C heat. Periodically during the study, the subjects performed a standardized 4 h stair stepping test in both "cool" and "hot" environments. Heart rate, rectal temperature, and $\dot{V}O_2$ were lower during the initial 2 h of the test that was conducted in the hot environment following the endurance

training segment compared to the test in the hot environment that was performed prior to training. In addition, the total amount of protein and the potassium concentration in the vasculature rose slightly during the 4 h test, whereas hematocrit was lower. During the "hot" test following 12 additional days of heat acclimatization, subjects demonstrated a further lowering of heart rate, rectal temperature, and $\dot{V}O_2$. However, sweat rate was lower during the first 2 h of the tests and greatly increased during the final 2 h. In addition, subjects experienced a progressive influx of protein into the plasma that Senay (1979) suggested was largely responsible for allowing hematocrit values to remain stable throughout the trial.

Senay (1979) hypothesized that two strategies were evoked by the subjects as they progressed from endurance training to acclimatization. He characterized the first strategy, which was operating primarily following endurance training, as the development of an increased fluid reservoir that allowed for greater sweat output to meet the demands of temperature regulation. He pointed out that this strategy was wasteful in humid conditions because of the large amount of water that is lost but not evaporated. The second strategy, developed as a function of acclimatization, was characterized by a reduction of resting plasma volume but an ability to rapidly increase plasma volume at the beginning of exercise in the heat through the transportation of protein from the interstitium to the vascular space.

In the present study, the training was performed in a cool environment; therefore, the plasma protein changes were expected to be more consistent with the adaptations that Senay (1979) described in

the "first strategy". These changes are characterized by an expanded plasma volume and a greater sweat rate. Since no heat stress was included as part of the treatment, the adaptations that Senay (1979) describes as the "second strategy" were not expected to develop.

Models of Simulated Hypo- and Hypervolemia

In studies that have demonstrated a hypervolemic response, whether induced by exercise training or heat exposure, it was difficult, if not impossible, to disassociate the effects of the hypervolemia from the additional adaptations that were associated with the treatment. In the following studies, a change in an individual's volemic status was acutely induced by either infusing fluid into or withdrawing fluid from the bloodstream.

Kanstrup and Ekblom (1982) evaluated the relative importance of blood volume on $\dot{V}O_{2\max}$ by sequentially imposing hypervolemia with reduced hemoglobin concentration [Hb] and hypovolemia with normal [Hb]. Thus, oxygen carrying capacity of the blood was similar under both conditions. Hypervolemia was evoked by infusing 700 mL of 6% dextran in saline, thus expanding blood volume from 5.5 to 6.2 L while lowering [Hb] from 14.4 to 12.8 g/dL. Submaximal and maximal exercise were performed immediately after infusion and compared to a control condition previously performed. Fluid infusion was associated with a lower hematocrit and heart rate and a higher $\dot{V}O_2$ during both submaximal and peak exercise. The second condition, hypovolemia with reduced [Hb], was imposed after 2 weeks of recovery by whole blood letting of 800 mL. After equilibration, blood volume and [Hb] dropped from 5.43 L to 5.14 L and 16.1 to 14.3 g/dL, respectively. The

exercise test following the blood withdrawal condition was associated with a $\dot{V}O_{2\max}$ that was lower than either the control or postinfusion conditions. This suggests that blood volume per se can influence exercise performance beyond its predicted role in oxygen delivery, perhaps by influencing perfusion pressure in exercising limbs.

A similar study was conducted by Fortney et al. (1981), but the order of the conditions was reversed. The first condition was accomplished by withdrawing and saving 10% of the subject's original blood volume. Following approximately 90 min of equilibration, it was noted that the original volume was completely restored. It was observed that the fluid that moved into the vasculature was isotonic and relatively protein poor. The second condition, hypervolemia with reduce [Hb], was imposed 2 weeks after the first. To produce hypervolemia, the blood that was withdrawn two weeks prior was reinfused into the subjects, resulting in significant increases in plasma volume, red cell number, and protein content. A comparison of these two conditions against a control condition was made during a 30 min cycling bout at 60% $\dot{V}O_{2\max}$ in 35° C heat. It was determined that blood withdrawal resulted in near maintenance of blood volume and stroke volume with a relative reduction in skin blood flow at a given core temperature. The latter circumstance was linked with a decreased ability to dissipate heat that resulted in heat storage. Under hypervolemia, cardiac output, stroke volume, and forearm skin blood flow were enhanced, but core temperature during the exercise was not altered. Therefore, these alterations in hemodynamics and oxygen transport that occurred when blood volume was expanded by infusion,

may also be expected to develop when plasma volume is expanded with exercise training.

Sawka, Hubbard, Francesconi, and Horstman (1983) infused subjects on two occasions either with 200 mL of saline or saline with 50 g of human albumin to observe the effects of hypervolemia on heat tolerance and exercise performance. No differences were found for performance time to exhaustion, final heart rate, or final rectal temperature between the two conditions. The albumin-infused group was found to have an expanded plasma volume and lower heart rates at various time intervals during the condition. Thus, the addition of protein to the blood has been shown to positively effect plasma volume and heart rate during exercise.

Acute volume expansion was also studied by Raff et al. (1985), who added a quantity of isotonic saline sufficient to enlarge the blood volume 10% in dogs during mechanical circulation. Volume expansion was associated with a significant reduction in the amount of aldosterone that was released in response to angiotensin II and ACH stimulation. This demonstrates that aldosterone release can be affected by changes in plasma volume.

It is noted from the studies included in this section that alterations in blood volume result in concurrent changes in circulatory hemodynamics. The addition of isosmotic fluid composed of either dextran or human protein in saline is capable of expanding and maintaining an elevated blood volume. This provides some evidence that osmotically rich fluid will remain within the vascular space for an extended period of time.

Variation Due to Species

As with most areas of mammalian physiological research, much of what is learned from experimentation on one species carries over somewhat to other species. The following studies are examples of research that examined research hypotheses similar to those of the current study.

McKeever et al. (1985) and McKeever, Schrug, Jarrett, and Convertino (1987) demonstrated an exercise-induced hypervolemic adaptation in greyhound dogs and horses similar to that which has been described in humans, yet the mechanisms that mediated these changes were quite species specific.

In the greyhound dogs, unlike humans, blood and plasma volume increases were accompanied by a concomitant increase in red cell volume such that hematocrit, hemoglobin concentration, and mean corpuscular hemoglobin concentrations were unchanged following training. McKeever et al. (1985) explained that these changes were indicative of repeated splenic contractions that contribute a large quantity of erythrocytically rich blood to the circulation. In addition, the posttraining analysis revealed an increase in osmotic content of the blood reflective of significant increases in total plasma sodium and albumin. The increase in fluid was most probably related to the 328 mL/d increase in water intake that overshadowed the 87 mL/d increase in urine output.

In contrast, the expansion of blood volume in the horse was not associated with additional water intake but rather a decrease in urine output mediated by a conservation of urea (McKeever et al., 1987).

The authors speculated that the conservation of urea could be facilitated by the microflora within the cecum of the horse, where recycling of amino acids has been demonstrated.

The species variation that was observed in the horse and dog supports the importance of conducting separate research on humans. Although the animal adaptations that were reported did not resemble the human response, this information may be helpful in identifying the potential mechanisms that regulate plasma volume.

CHAPTER III

METHODS AND PROCEDURES

Twenty-four healthy males between the ages of 18 and 45 were recruited to participate in the study. Each candidate was required to have a base level of three months prior aerobic training. Candidates attended an orientation meeting where a detailed description of procedures was presented prior to participation in the research project. The candidates demonstrated their willingness to participate as subjects by providing informed consent (Appendix A). A medical evaluation was performed prior to the study to screen out those individuals who were at excessive risk of sustaining an injury or developing a complication as a result of participating in the study.

The subjects were randomly assigned to one of four experimental conditions. Two of the groups (SED) refrained from any vigorous exercise and kept their ordinary daily physical exertion to a minimum. The two remaining groups (EX) performed stationary bicycling for 2 h each day for 7 d at an intensity requiring 65% $\dot{V}O_{2\max}$. Training sessions were conducted in a laboratory where the temperature was 21-24° C and the relative humidity ranged between 30-50%. The cycling was performed using the subject's own bicycle mounted on a Velodyne Road Simulator (Frontline Technologies, Irvine, California). In an attempt to inhibit the activity of aldosterone on sodium reabsorption,

one exercise training group (EX/DRUG) and one sedentary group (SED/DRUG) took 100 mg of spironolactone daily in four 25 mg oral doses, one with each meal and one before bed, whereas the two remaining groups (EX/NO DRUG, SED/NO DRUG) received no medication.

The number of subjects within each group was as follows:

1. Sed/No Drug ($n=5$); did not exercise and received no medication.
2. Sed/Drug ($n=7$); did not exercise and received 100 mg spironolactone daily.
3. Ex/No Drug ($n=6$); participated in the exercise training program and received no medication.
4. Ex/Drug ($n=6$); participated in the exercise training program and received 100 mg of spironolactone daily.

Aldosterone is competitively inhibited by spironolactone (AldactoneTM), a potassium-sparing diuretic that is used in the treatment of moderate hypertension. Spironolactone competes with aldosterone for receptor binding sites on the transporter protein molecules located within the kidney tubule. These proteins facilitate sodium transport across the nuclear membrane, but when bound by spironolactone, these transporter proteins are rendered inactive. Results from a study by Bevegard et al. (1977) indicate that aldosterone activity both at rest and during moderate exercise can be blocked by 100 mg of spironolactone per day.

Experimental Paradigm

The time course of the experiment, consisting of a baseline pretest, a control period and a training period, is shown in Figure 1.

Pre = Baseline Pretest Day
 M = Measurement Day
 Post = Posttest Day

Figure 1. The timing of measurements

Control Period

Preliminary testing was performed to assess the subjects' initial height, weight, and maximal oxygen uptake. These tests were performed on all subjects during the first day of a 3 d control period. On that day, the subjects collected urine from all voids following their initial morning void until testing began (approximately 8 h). Three hours prior to testing, the subjects came to the laboratory and were given a standardized meal consisting of two 240 mL cans of Ensure™ Nutritional Beverage (Ross Laboratories, Columbus, Ohio) and 360 mL water. Two cans of Ensure provided 500 kcal, of which 55%, 31%, and 14% were carbohydrate, fat, and protein respectively.

The subjects' heights and body weights were measured along with resistance and reactance scores from tetrapolar bioelectrical impedance (Lukaski, Bolonchuk, Hall & Siders, 1986). Measurements of bioelectrical impedance were taken for use in another research study. An 18 mL blood sample was drawn with Vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey) after the subjects sat for

a minimum of 15 min. Maximal oxygen uptake was measured according to the procedures described in the research techniques section.

Following testing, the subjects who were assigned into EX/DRUG or SED/DRUG were given their prescriptions and instructed to begin taking their medication.

During the three day control period all subjects abstained from vigorous physical exercise, except for the maximal oxygen uptake test on the first day of the period. They were asked to choose a daily diet that they could repeat throughout the study and were provided with dietary exchange lists for sodium and calories to help them maintain a consistent calorie and sodium intake. They were also asked to discontinue taking any medications other than the spironolactone and to refrain from drinking caffeinated or alcoholic beverages. Subjects were allowed to drink fluids ad libitum.

The Training Period

The training period consisted of seven consecutive days during which subjects in the exercise training groups came to the laboratory and rode their bicycles for daily 2-h training sessions. Oxygen uptake was monitored, and workload adjustments were made each 30 min to maintain a 65% $\dot{V}O_{2\max}$ training intensity. If a subject could no longer continue a 65% intensity beyond a minimum threshold of 60 min, further exercise on that day was terminated. Members of the two sedentary groups came to the laboratory only when needed for testing procedures.

Measurement Days During The Training Period

Days 1, 3, and 7 of the training period were designated as measurement days. Fig. 2 provides a summary of measurements taken on each of these three days.

Figure 2. Summary of measurements for days 1, 3, and 7

Beginning after the first void in the morning, urine was collected throughout the measurement day and into the subsequent day until 24 h after the onset of the measurement-day exercise. In addition, a standardized meal was consumed 3 h prior to the scheduled testing time. This meal was identical to that consumed on the baseline pretest day.

Upon arrival at the laboratory, an 18 gauge catheter was inserted into an antecubital vein. The catheter was capped with a rubber tipped injection cap and locked with 0.5 mL of 10 units/mL heparinized saline. The subject then sat in a chair for a minimum of 15 min prior to testing. A resting blood sample was taken without stasis while the subjects remained seated. Weight was measured, as

was resistance and reactance from tetrapolar bioelectrical impedance (Lukaski, et al., 1986).

After the completion of the resting measurements, members of the EX/DRUG and EX/NO DRUG began their rides. Members of the SED groups remained inactive. During the ride, blood was drawn at 30 min and 120 min. These same procedures were performed in the SED groups at corresponding times. Immediately following the ride, both sedentary and exercise training groups collected a voided urine sample, were weighed, and underwent bioelectrical impedance measurements. Collections of blood and urine and measurements of body weight and bioelectrical impedance were made again at 30, 60, and 120 min into recovery.

The subjects were not allowed to eat or drink until the measurements at 120 min into recovery were completed. Following the 2 h recovery period, the intravenous catheter was removed, and the subject was allowed to leave the lab, after being reminded to eat a diet containing the same calorie content and approximate sodium content as they had eaten during the control period. Fluid consumption was allowed ad libitum, and urine was collected until the subjects returned to the laboratory the following day for the 24 h measurements. These measurements were identical to the resting baseline preexercise measurements except blood was drawn into Vacutainer tubes (Becton-Dickenson, Rutherford, New Jersey) with a single venipuncture, and no standardized meal was consumed.

Posttraining Testing

A final test day included, with two additions, measurements identical to those taken on the pretest day. The additions were the inclusion of a blood volume measurement and a test to determine total body water.

Total body water was assessed with the aid of a 15 mL tracer dose of deuterium-oxide added to 345 ML of water. The subjects were given the water-deuterium mixture in two approximately equal volumes. The first contained all of the deuterium oxide, which they drank without hesitation. The second contained only distilled water, which they poured in the first cup and then drank, swirling the water in their mouths before each swallow. After finishing the water, the subjects were given paraffin to chew to stimulate the production of a 5 mL saliva sample that was used as a background sample in the assessment of total body water. The subjects were then allowed to leave the lab but were instructed not to eat or drink anything until after the initial testing session was over. They returned 3 h later and were asked to produce a second saliva sample that was used to determine the dilution of the deuterium in the total volume of body water, as described below under Measurement Techniques.

Measurement Techniques

Height

Height (cm) was measured while the subject stood barefoot with his back against a wall. The subject's head was positioned so that the line between the lower orbit of the eye and the external acoustic meatus of the ear was parallel with the floor. Instructions to the

subjects were "look straight ahead, take a deep breath, stand up as tall as possible without raising your heels off the floor." The measurement was taken during maximal inhalation.

Body Weight

Body weight was measured to the nearest 110 g on a physician's scale (Detecto Scales, Inc., Brooklyn, New York) while the subjects wore only cycling shorts or underwear.

Total Body Water by D₂O Dilution

Total body water analysis was performed by deuterium (D₂O) dilution technique described by Davis, Lamb, Burgess, and Bartoli (1987). Saliva samples used in the analysis were collected 3 h after consuming 15 g of deuterium in 345 mL of water. The subjects were given paraffin to chew to stimulate the production of a 5 mL saliva sample. Once collected, the samples were centrifuged at 4000 RPM for 15 min in a refrigerated centrifuge (Accuspin, Beckman Instruments, Fullerton, California). The supernatant was drawn off and frozen at -80 °C until the analysis was performed.

The analysis of deuterium oxide for the determination of total body water was performed using the following methods. Two and one-half mL of saliva were placed into the center moat of a Conway diffusion dish while 2.5 mL of distilled water was placed in the outer ring. The dish was sealed with parafilm and tightly capped. The dish was incubated in a water bath for 48 h at 37° C, after which it was removed and allowed to cool for 1 h. One mL of the distilled water from the outer moat (containing one-half of the the D₂O sample) was placed into the temperature-controlled sampling cell of the infra-red

spectrophotometer, and absorbances were read against a blank of distilled water. The diffusion procedure was performed in duplicate, and the analysis of each equilibration dish was performed in duplicate. Average absorbance values for the two dishes were computed and used to determine D_2O concentration by comparison with a standard curve produced from pooled saliva spiked with known concentrations of D_2O from 50 to 500 ppm.

Blood Collection and Handling

At each sampling period, 18 mL of blood were withdrawn. On days when multiple samples were required, blood was drawn from an intravenous catheter with a 20 mL syringe and transferred immediately into two chilled tubes. Five mL of blood were placed into a tube containing no additives to be used for assays requiring serum. The remaining 13 mL of blood were transferred to a tube containing 0.2 mL of 7.5% disodium dihydrogen ethylenediamine tetra-acetate dihydrate (EDTA) (GFS Chemicals, Columbus, Ohio) to be used for assays requiring plasma. Single blood draws were made with Vacutainer tubes with or without EDTA as described above for multiple draws.

A 100 μ L aliquot of EDTA-treated blood was transferred into a screw-topped polypropylene tube for hemoglobin analysis. EDTA-treated blood was also used to fill three 75 mm capillary tubes for hematocrit determination. Remaining EDTA-treated tubes were centrifuged immediately in a refrigerated centrifuge (Accuspin, Beckman Instruments, Fullerton, California) at 3000 RPM, 4° C, for 20 min. Aliquots of the cold plasma were placed in polypropylene screw-topped tubes for the analysis of renin.

The tubes containing no anticoagulant were allowed to clot for 30 min prior to centrifugation at 3000 RPM, 4° C for 20 min. Aliquots of serum were placed in polypropylene screw-topped tubes for the analyses of sodium, potassium, osmolality, aldosterone, creatinine, albumin, and total protein. All specimen tubes, including the hemoglobin tube, were stored at -80° C until the analyses were performed.

Hematocrit

The three capillary tubes were spun at 1,500 RPM for 10 min in an International Micro-capillary Centrifuge (Model MB, International Equipment, Needham Heights, Massachusetts). The lengths of the total column and the red cell column were measured with a Brown and Sharpe (Model 599-573, North Kingstown, Rhode Island) digital caliper with measurement accuracy of 0.01 mm. Hematocrit (HCT) was calculated according to the following formula:

$$\text{HCT} = 100 \times (\text{red cell column length} / \text{total column length}) \times 0.8736$$

The constant, 0.8736, represents a combined correction factor for venous sampling and trapped plasma. The triplicate readings of hematocrit were averaged to yield the final value. (Refer to Table 1 for precision of the measurement.)

Hemoglobin

Hemoglobin was determined by a kit from Sigma Diagnostics (Sigma Chemical Co., St. Louis, Missouri). The determination was

Table 1

Precision of the measurements

	Coefficient of Variation (%)	
	Intraassay ^A	Interassay ^B
Aldosterone	2.07*	11.97
Albumin	0.77	2.62
Creatinine - Serum	2.11	11.33
Creatinine - Urine	0.58	6.15
Hematocrit	1.10*	
Hemoglobin	1.10	1.50
Osmolality - Serum	1.19	1.76
Osmolality - Urine	1.20	1.61
Potassium - Serum	0.27	1.35
Potassium - Urine	0.25	1.25
Protein - Serum	1.38	1.72
Renin Activity - Plasma	**	13.34
Sodium - Serum	0.41	1.42
Sodium - Urine	0.24	1.48

A = The intraassay coefficient of variation from 10 or more determinations of the same control sample performed on the same day.

* = The average coefficient of variation on duplicate readings from all samples.

B = The interassay coefficient of variation from day-to-day analyses of the same control sample.

** = Quality control was determined by comparing daily assays of the manufacturer's control sample to values determined by the manufacturer.

based on the conversion of hemoglobin to cyanmethemoglobin. The assay was performed by adding 10 μ L of whole blood to 2.5 mL of Drabkin's Reagent. The tubes were vortexed and allowed to stand at room temperature for 15 min. The absorbance of the solutions at 540 nm was recorded with a spectrophotometer (Model DU-6, Beckman Instruments, Fullerton, California). Simple linear regression was used to derive a regression equation from the hemoglobin standards, and this equation was used to determine the hemoglobin concentration of control and unknown samples. (See Table 1 for precision of the measurement.)

Albumin

Serum albumin was analyzed with a kit from Sigma Diagnostics (Sigma Chemical Co., St. Louis, Missouri). The method was based on the principle that albumin binds to bromcresol green to produce a blue-green color with an absorbance peak at 628. The assay was performed by adding 20 μ L of standard, control, or sample serum to 2.0 mL of bromcresol-green reagent. The tubes were mixed gently and read at an absorbance of 628 nm at exactly 1 min. Albumin concentrations were calculated by comparing the absorbance of control and unknown samples to the absorbance of protein standard solution (Catalog #540-10, Sigma Chemical, St. Louis, Missouri) with an albumin concentration of 5 g/dL. (See Table 1 for precision of the measurement.)

Total protein

Total protein concentration in serum was performed using a modified formulation of biuret reagent (See Appendix C) and protein standard solution. A standard curve was prepared from an 8 g/dL stock (Catalog #540-10, Sigma Chemical, St. Louis, Missouri) to yield

standards with protein concentrations of 4,5,6,7 and 8 g/dL. The procedure used was identical to the one described by Sigma Diagnostics, where 20 uL of standard, control, or unknown sample were added to 1 mL of Biuret's reagent. The tubes were vortexed and allowed to stand for 15 min. The absorbance of the sample was read at 540 nm on a Beckman DU-6 spectrophotometer (Beckman Instruments, Fullerton, California). The concentrations of the control and unknown samples were calculated from the standard curve. (Refer to Table 1 for the precision of the measurement.)

Sodium and Potassium

Serum sodium and potassium concentrations were determined by flame photometry (Model 943, Instrumentation Laboratories, Lexington, Massachusetts). The intraassay and interassay coefficients of variation are recorded in Table 1.

Osmolality

Serum osmolality was determined by freezing point depression (Model 504 u Osmette Osmometer, Precision Systems, Kansas City, Missouri). (Refer to Table 1 for the precision of the measurement.)

Serum Creatinine

Serum creatinine was assayed by a kit from Sigma Diagnostic (Catalog #555-1, Sigma Chemical Co., St. Louis, Missouri). The analysis was based on the original Jaffe reaction, where creatinine causes alkaline picrate to change color from yellow to orange, which can be detected by a spectrophotometer at 500 nm. Because a number of substances, including proteins and other chromagens, also cause this color change to occur, the assay was improved by including a second

reading of the tubes following acidification with acetic acid. It was observed that under acid conditions the creatinine-picrate color faded faster than color from interfering substances and could be differentiated if measured again within 5 min. Therefore, the difference in color intensity before and after acidification was proportional to creatinine concentration.

The procedure was performed by pipetting 75 uL of standard, control, or unknown sample in duplicate into individual 1.0 mL polystyrene cuvettes (Sarstedt Corp, Princeton, New Jersey). Each cuvette was then filled with 750 uL of alkaline-picrate. The tubes were read on a spectrophotometer (Model DU-6, Beckman Instruments, Fullerton, California) at 500 nm after 10 min at room temperature. Next, 250 uL of 0.1 M acetic acid were added to each cuvette, and the absorbance was read a second time after 5 min at room temperature. The final absorbance was subtracted from the initial, and the remainder was used to calculate the creatine concentration from the regression equation derived from the standard curve. (Refer to Table 1 for the precision of the measurement.)

Aldosterone

Aldosterone concentration was determined by radioimmunoassay using a kit from Diagnostic Products Corporation (Catalog #CON6, Los Angeles, California). The assay was performed by pipetting 200 uL of calibrator, control, or unknown serum in duplicate into aldosterone-antibody coated test tubes. Then 1 mL of buffered ^{125}I -aldosterone was added to each tube, and the tubes were set in a water bath at 37°C for 180 min. Following the 180 min incubation, all tubes except the

tubes used to assess total radioactivity were removed from the bath, inverted over a hot sink, and allowed to drain on paper toweling. The tubes were counted for 1 min on a gamma counter (Model 5500, Beckman Instruments, Fullerton, California). Aldosterone concentrations were calculated from a logit-log representation of the calibration curve. (Refer to Table 1 for the precision of the measurement.)

Renin activity

Renin activity was measured by the radioimmunoassay kit from the Clinical Assays Division of Baxter Travenol Diagnostics, Inc., Cambridge, Massachusetts (Catalog #4 Ca553-553). The plasma renin activity measurement involved an initial incubation of plasma to generate angiotensin I followed by quantitation of angiotensin I by radioimmunoassay.

The generation step was performed by adding maleate generation buffer and converting enzyme inhibitor to control or unknown samples. Half of the sample was transferred into a separate tube and placed in an ice bath at 4° C. The original tube with the remaining sample was placed in a warm water bath at 37° C. Both tubes were allowed to incubate for 90 min.

The radioimmunoassay procedure included the preparation of a standard curve from which the control and unknown angiotensin I content in both the 37° and 4° series samples were interpolated. The background (4° series) was then subtracted from the corresponding 37° C generation. This value was corrected mathematically for dilutions of the sample and the time of generation to yield the final plasma renin activity in ng/mL x h.

The control sample that was included with the kits was analyzed each day that the assay was performed. The angiotensin I level determined by the manufacturer for the control sample was 4.4 ± 1.0 ng/mL x h. The values for the control sample in the present study ranged from 3.64 to 5.21 ng/mL x h.

Urine Studies

Urine collections were measured for volume with a standard graduated cylinder and for rate by dividing the volume by the number of minutes that elapsed during the collection. A sample of each urine collection was used for the determination of urine concentrations of sodium, potassium, osmolality, and creatinine.

Sodium and Potassium

Urinary sodium and potassium concentrations were determined by flame photometry using an Instrumentation Laboratories flame photometer (Model 943, Instrumentation Laboratory, Lexington, Massachusetts). Duplicate determinations were accepted if the coefficient of variation of the pair did not exceed 1.0%. A 1:2 or 1:4 dilution of the sample with distilled water was made if the sodium or potassium concentration exceeded 200 mEq/L, which approached the limit of linearity of the instrument as set by the manufacturer. (Refer to Table 1 for the precision of the measurement.)

Osmolality

Urine osmolality was measured by freezing point depression (Model 504, U Osmette Osmometer, Precision Systems, Kansas City, Missouri). Duplicate determinations were accepted if the coefficient of variation did not exceed 1.0%. A 1:2 dilution of the sample with

distilled water was made if the osmolality of the sample exceeded 1000 mOsmoles. (Refer to Table 1 for the precision of the measurement.)

Creatinine

The analysis of urinary creatinine concentration was similar to that for serum creatinine, except a 1:15 dilution of the samples was made prior to the analysis. In addition, the standard curve was prepared with a 15 mg/dL stock solution, yielding standards with concentrations of 2.5, 5, 7.5, 10, 12.5, and 15 mg/dL. Duplicate determinations of the sample were accepted if the coefficient of variation between the duplicates did not exceed 3%. A 30:1 dilution of the sample was made if the urine creatinine concentration exceeded the high standard. (Refer to Table 1 for the precision of the assay.)

Maximal Oxygen Uptake

The test to determine maximal cycling oxygen uptake was performed while the subject rode his personal bicycle mounted to a Velodyne Road Simulator. The test began at an initial workload of 150 W and progressed 50 W every 2 min. The test was terminated when a subject could no longer maintain the workload and his power output fell by 25 W for longer than 15 s. Inspired volumes were measured with a Rayfield gas meter, while expired O₂ and CO₂ concentrations were measured with an Applied Electrochemistry S3-A Oxygen Analyzer (Pittsburg, Pennsylvania) and a Beckman LB-2 CO₂ Analyzer (Fullerton, California), respectively. The instruments were calibrated immediately prior to each test using calibration gas taken from a tank that had been analyzed by the Micro-Scholander method. Heart rates during the test were monitored with a CIC heartwatch pulse meter

(Computer Instruments Corporation, Hempstead, New York), the accuracy of which was tested prior to the study by comparison with an electrocardiograph.

Blood Volume

Blood volume was assessed by radioactively labelling red blood cells with ^{51}Cr . Thirty mL of the subject's blood was withdrawn from an antecubital vein. Of this total, 25 mL was added to a mixture of 5 mL of acid citrate dextrose, 100 uCi of ^{51}Cr -sodium chromate (NaCrO_4), and 100 mg of ascorbic acid, and incubated for 10 min. The remaining 5 mL were transferred to a heparinized tube and used to assess background radiation and hematocrit. The 25 mL sample was centrifuged and decanted to separate labelled red cells from free Cr in plasma. The labelled red cells were then brought up to 40 mL with saline and divided into two 20 mL aliquots. One of the samples was diluted 1:50 with water and used as a standard, whereas the other sample was reinjected into the subject and allowed to circulate for 30 min. Following equilibration, a 6 mL blood sample was drawn from the contralateral arm into a tube containing EDTA, from which duplicate 2 mL samples were taken. Scintillation counting was conducted for 5 min on duplicate tubes containing 2 mL of sample, standard, or blank.

Statistical Analyses

A three way analysis of variance was performed on measured variables to test the main and interaction effects of the independent variables, drug status, exercise status, and days. Least square means were used as the measure of central tendency because of unequal numbers of subjects within the experimental groups. A posthoc least

significance difference test was performed on interaction effects to determine differences between levels of the independent variables. Rejection of the null hypotheses was made at the $P < 0.05$ level.

CHAPTER IV

RESULTS

Each of 24 subjects completed one of four experimental protocols to determine the relationships between training-induced changes in plasma volume and corresponding changes in the activity of aldosterone and changes in plasma protein content. The four experimental protocols represented the crossing of two independent variables, DRUG and EXERCISE, each composed of two dichotomous levels, i.e., NO DRUG/SED, NO DRUG/EX, DRUG/SED, and DRUG/EX. Repeated measurements of the dependent variables were performed on three predetermined days during the 11 d experiment and at seven different times within each day.

The results of blood analyses that are presented in this chapter, with the exception of the aldosterone and renin data, are measurements taken 24 h after treatment sessions performed on days one, three and seven. These blood samples were taken at rest following ad libitum rehydration. It was felt that these measurements best describe the net effect of the bodily responses that were initiated to restore and elevate plasma volume after each bout of exercise. In the text, these measurements will be called day two, four and eight, referring to blood samples taken 24 h after treatment sessions performed on days one, three and seven. The results of all

renin and aldosterone analyses will be presented to demonstrate when peak responses occurred and how blood levels changed during the initial 2 h of recovery following exercise.

The urinary variables that are presented in this chapter, with the exception of sodium clearance and the fractional excretion of sodium, are cumulative measurements compiled from voided samples collected throughout each 24 h measurement period beginning before the exercise or corresponding control session on days one, three and seven. In the text, these cumulative samples will be referred to as day one, three, and seven. The sodium clearance and the fractional excretion of sodium values that are presented were calculated from each of 7 blood and urine collections made on days one, three, and seven.

Age and Physical Characteristics

The average age and initial physical characteristics, including blood and plasma volumes, of the subjects are listed in Table 2. There were no statistically significant differences in these characteristics between the groups at the onset of the study.

Exercise Sessions

The duration and intensity of each exercise session were intended to be 120 min and 65% of $\dot{V}O_{2\max}$, respectively. Premature subject exhaustion during some exercise sessions resulted in actual durations that were slightly less than the intended time. Likewise, slight differences between predicted and actual metabolic requirements of the workloads resulted in actual work intensities that deviated somewhat from 65% of $\dot{V}O_{2\max}$. A comparison of the actual exercise time

and percentage of $\dot{V}O_{2\max}$ during each training session is presented in Table 3. There were no significant differences between the groups on any day for either variable.

Table 2

Means ($\pm$ SE) for pretest measurements

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Age (Years)	32.2 (1.80)	27.8 (1.33)	26.1 (0.59)	25.1 (0.62)
Height (cm)	183.4 (1.24)	176.6 (0.98)	177.5 (0.97)	177.1 (0.80)
$\dot{V}O_{2\max}$ (mL $\times$ kg ⁻¹ $\times$ min ⁻¹)	56.8 (2.02)	57.7 (0.92)	60.5 (1.20)	59.8 (2.00)
Blood Volume (mL)	4818 (234)	4844 (125)	4564 (223)	4520 (356)
Plasma Volume (mL)	2969 (137)	2995 (107)	2790 (125)	2790 (259)
Red Cell Volume (mL)	1849 (98)	1849 (26)	1774 (106)	1730 (104)

Table 3

Means ($\pm$ SE) for duration and intensity of exercise sessions during training

Protocol Day	NO DRUG (n=6)		DRUG (n=6)	
	Duration in min	$\dot{V}O_{2\max}$	Duration in min	$\dot{V}O_{2\max}$
One	115 (6)	65 (6)	110 (6)	67 (6)
Two	112 (7)	65 (7)	111 (7)	68 (6)
Three	113 (6)	63 (6)	115 (6)	65 (6)
Four	120 (6)	61 (7)	120 (6)	66 (8)
Five	120 (6)	63 (7)	110 (6)	64 (7)
Six	110 (6)	66 (7)	116 (6)	65 (7)
Seven	120 (6)	66 (6)	120 (6)	63 (7)

Plasma Volume

Training was associated with a mean increase in plasma volume of 16.6% (470 mL) and 11.6% (290 mL) for subjects who participated in the NO DRUG/EX and DRUG/EX conditions, respectively (see Table 4).

Table 4

Means ($\pm$ SE) for percent change in plasma volume

Protocol Day	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Two	3.9 (2.1)	6.4 (3.4)	-1.8 (3.4)	1.5 (2.6)
Four	7.0 ^{ax} (2.0)	17.1 ^{bx} (2.0)	-3.8 ^{ay} (2.0)	7.1 ^{by} (5.4)
Eight	5.7 ^a (3.0)	16.6 ^b (2.3)	4.3 ^a (2.3)	11.6 ^b (3.4)

Note. When collapsed across DRUG and NO DRUG conditions, composite means for SED groups^a were significantly different from EX groups^b ($p < 0.05$). When collapsed across EX and SED conditions, composite means for NO DRUG groups^x were significantly different from DRUG groups^y ($p < 0.05$).

From the results of the three-way analysis of variance using DRUG, EXERCISE, and DAY as independent variables, two significant two-way interactions (DRUG by DAY, EXERCISE by DAY) were identified (see Figs. 3 & 4). The DRUG by DAY interaction indicates that plasma volume increased faster in subjects who did not take spironolactone compared to those who did. Pairwise comparisons of means for each of the three days determined that the percent increase in plasma volume was greater for subjects in the NO DRUG condition on day 4 than it was for subjects in the DRUG condition. Composite means on day four for NO DRUG and DRUG groups when collapsed across EX and SED conditions were $12.0\% \pm 3.7$ and $1.8\% \pm 3.5$, respectively. Differences between DRUG and NO DRUG groups on days two and eight were insignificant (Fig. 3).

Figure 3. Change in plasma volume (%); interaction of DRUG and DAY.

Figure 4. Changes in plasma volume (%); interaction of EXERCISE and DAY.

The interaction of EXERCISE status with DAY indicates that the expansion of plasma volume by those who participated in the exercise training program progressed rapidly over the initial three days and remained elevated throughout the remainder of the study (Fig. 4). Significantly higher values were attained by exercisers compared to non-exercisers on days four and seven, but not on day two. When collapsed across DRUG and NO DRUG conditions, the composite means for EX vs. SED groups were $12.1\% \pm 3.4$ vs. $1.7\% \pm 3.7$ on day four and $14.1\% \pm 3.2$ vs. $5.0\% \pm 3.2$ on day eight.

Blood Volume and Red Cell Volume

Mean percent changes in blood volume are presented in Tables 5 and 6. The statistical analysis of blood volume changes mimics the results for plasma volume, i.e., significant two-way interactions were present between DRUG status and DAY and between EXERCISE status and DAY. Unlike plasma volume, no significant differences were evident between DRUG vs NO DRUG means collapsed over exercise conditions on any of the three days. In addition, only the difference on day four between means of the two exercise groups collapsed over drug conditions was significantly different.

Table 5

Means ($\pm$ SE) for percent change in blood volume

Protocol Day	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Two	3.2 (3.6)	3.9 (3.3)	-0.9 (3.0)	-0.5 (3.7)
Four	4.3 ^a (4.2)	9.9 ^b (3.3)	-4.0 ^a (3.4)	2.8 ^b (3.7)
Eight	4.0 ^c (3.6)	9.6 ^d (3.3)	2.5 ^c (3.0)	7.5 ^d (3.3)

Note. When collapsed across DRUG and NO DRUG conditions, the composite means for SED groups^a vs. EX groups^b ($p < .01$), and SED groups^c vs. EX groups^d ($p < .01$) were significantly different.

Results of the analysis of red cell volume are presented in Table 6. No statistically significant effects were noted.

Table 6

Means ($\pm$ SE) for percent change in red cell volume

Protocol Day	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Two	2.0 (3.6)	0.0 (3.3)	0.6 (3.0)	-3.1 (3.8)
Four	0.0 (4.2)	-1.4 (3.3)	-4.5 (3.4)	-3.4 (3.8)
Eight	1.0 (3.6)	-1.3 (3.3)	0.1 (3.0)	1.2 (3.3)

Total Body Water and Body Weight

Means for total body water on day eight were: NO DRUG/SED 49.8 ± 4.7 L, NO DRUG/EX 44.8 ± 4.3 L, DRUG/SED 46.6 ± 4.0 L, and DRUG/EX 45.1 ± 4.3 L. A baseline test was performed during the pretest, but the results were not presented because of procedural errors during the collection of the sample.

There was a main effect of DAY on body weight, i.e., the total subject population was slightly heavier on day 4 than on days 2 and 8. The mean weights ($\pm$ SE) of all subjects for days 2, 4, and 8, were 71.1 ± 2.8 kg, 71.7 ± 2.8 kg, and 71.1 ± 3.0 kg, respectively (Table 7). There were no significant effects of EXERCISE or DRUG treatments on body weight.

Table 7

Means ($\pm$ SE) for body weight (kg)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	74.7 (3.6)	69.2 (2.5)	71.7 (3.8)	70.5 (2.5)
Day Two	74.6 ^a (3.4)	69.0 ^a (2.8)	71.2 ^a (3.9)	69.5 ^a (2.4)
Day Four	75.1 ^b (2.2)	69.7 ^b (2.3)	71.6 ^b (2.6)	70.2 (2.6)
Day Eight	74.8 ^c (2.7)	68.7 ^c (1.9)	71.4 ^c (4.0)	69.9 ^c (2.9)

Note. When collapsed over NO DRUG and DRUG conditions and EX and SED conditions, The composite means for groups^{a, c} were significantly different than groups^b ($p < 0.05$).

Plasma Constituents

Plasma osmolar, sodium, protein, and albumin concentrations on days two, four, and eight, are listed in Tables 8, 10, 12, and 14. These concentrations were also multiplied by the plasma volume to provide an estimate of the total plasma osmolar, sodium, protein, and albumin contents (Tables 9, 11, 13, 15).

Serum Osmolality

A significant main effect of DAY was detected for serum osmolality (Table 8). This effect was consistent across both levels of DRUG and EXERCISE. The means ($\pm$ SE) for all subjects on days two, four and eight were 288.0 ± 1.2 , 288.6 ± 1.2 , and 292.5 ± 1.2 mOsmol/L (Fig. 5). Posthoc comparisons showed that the overall mean at day eight was significantly greater than for days two and four.

Table 8

Means ($\pm$ SE) for serum osmolality (mOsmol/L)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	285 (2.4)	288 (3.9)	286 (3.9)	285 (3.0)
Day Two	293 ^a (3.0)	288 ^a (2.3)	286 ^a (2.2)	284 ^a (2.3)
Day Four	292 ^b (2.6)	288 ^b (2.3)	287 ^b (2.2)	287 ^b (2.3)
Day Eight	293 ^c (2.6)	296 ^c (2.7)	290 ^c (2.2)	290 ^c (2.3)

Note. When collapsed across DRUG and NO DRUG conditions as well as EX and SED conditions, composite means for DAY groups^a were significantly differently different than DAY groups^b and ^c ($p < .03$ and $p < .01$). There was no significant difference between DAY groups^b and DAY groups^c.

Figure 5. Serum osmolality (mOsmol/L): main effect of DAY.

From the analysis of total osmolality (Table 9, Fig. 6) a significant EXERCISE by DAY interaction was identified that was similar to that detected in the analysis of percent changes in plasma volume. The total osmolality was significantly higher in the combined EX groups compared to the combined SED groups on day four ($928 \text{ mOsmol} \pm 83$ vs. $840 \text{ mOsmol} \pm 93$) and day eight ($966 \text{ mOsmol} \pm 83$ vs. $882 \text{ mOsmol} \pm 79$) but not on day two (Fig. 6). It should be noted that changes in total osmolality are heavily influenced by changes in plasma volume, which are used in the calculation of total osmolality.

Table 9

Means ($\pm$ SE) for total serum osmolality (mOsmol)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	846 (90)	887 (76)	798 (88)	799 (80)
Day Two	877 (22)	917 (16)	787 (15)	795 (19)
Day Four	913 ^a (22)	1006 ^b (16)	768 ^a (17)	849 ^b (19)
Day Eight	917 ^c (18)	1034 ^d (19)	847 ^c (15)	896 ^d (16)

Note. When collapsed across DRUG and NO DRUG conditions, the composite means for SED groups^a and EX groups^b were significantly different ($p < .0001$), and the composite means for SED groups^c and EX groups^d were significantly different ($p < .0001$).

Serum Sodium

A significant main effect of EXERCISE on concentrations of serum sodium was detected (Table 10). A comparison of the composite means collapsed across the DRUG and NO DRUG treatments and across DAYS

Figure 6. Total serum osmolality (mOsmol); interaction of EXERCISE and DAY.

two, four and eight determined that exercising subjects had significantly lower serum sodium concentrations than non-exercising controls; 138.1 ± 1.1 vs 140.5 ± 1.2 . Because there was no interaction between DAY and EXERCISE, the effect presumably occurred within the first measurement day and persisted throughout the remainder of the study. This presumption is supported by the fact that the baseline pretest means for the combined EX groups was higher than that for the combined SED groups (140.3 ± 1.2 mEq/L), and 139.0 ± 1.1 mEq/L, respectively.

Table 10

Means ($\pm$ SE) for serum sodium concentration (mEq/L)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	139.6 (0.7)	140.1 (1.6)	138.5 (0.9)	140.6 (1.0)
Day Two	141.2 ^a (1.1)	137.9 ^b (0.9)	139.5 ^a (0.8)	138.0 ^b (0.9)
Day Four	141.8 ^a (1.0)	139.2 ^b (0.9)	140.0 ^a (0.8)	137.7 ^b (0.9)
Day Eight	140.6 ^a (1.0)	138.0 ^b (0.9)	139.7 ^a (0.8)	138.4 ^b (0.9)

Note. When collapsed across DRUG and NO DRUG conditions as well as across DAY two, four, and eight, SED groups^a were significantly different than EX groups^b ($p < .0001$).

The effects that were identified in the analysis of total serum sodium content (Table 11) were consistent with those that were determined for plasma volume partly because plasma volume is used in the calculations of total sodium. Significant interaction effects

Figure 7. Total sodium content (mEq); interaction of EXERCISE and DAY.

Figure 8. Total sodium content (mEq); interaction of DRUG and DAY.

were detected between DAY and both DRUG and EXERCISE (Figs. 7 & 8). The combined means collapsed across DRUG and NO DRUG conditions for EX vs. SED groups, respectively, were 447.8 ± 6.1 mEq vs. 411.2 ± 6.8 mEq on day four and 452.6 ± 5.7 mEq vs. 423.8 ± 5.8 mEq on day eight.

Table 11

Means ($\pm$ SE) for total serum sodium content (mEq)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	415 (9)	421 (9)	386 (8)	392 (8)
Day Two	427 ^u (11)	437 ^u (8)	382 ^v (7)	382 ^v (9)
Day Four	447 ^{aw} (11)	486 ^{bw} (8)	375 ^{ax} (8)	409 ^{bx} (9)
Day Eight	439 ^{cy} (9)	478 ^{dy} (8)	408 ^{cz} (7)	428 ^{dz} (8)

Note. When collapsed across DRUG and NO DRUG conditions, SED groups^a and EX groups^b ($p < .01$), and SED groups^c and EX groups^d ($p < .05$), are significantly different from each other. When collapsed across EX conditions, NO DRUG groups^u and DRUG groups^v ($p < .0001$), NO DRUG groups^w and DRUG groups^x ($p < .0001$), and NO DRUG groups^y and DRUG groups^z ($p < .0001$) are all significantly different from each other.

Serum Albumin

From the analysis of serum albumin concentration, a significant DRUG by DAY interaction was noted (Table 12, Fig. 9). When DRUG and NO DRUG groups were compared across EX and SED conditions, the DRUG group had a significantly higher serum albumin concentration on day four than the NO DRUG group; 4.36 ± 0.04 g/dL vs. 4.10 ± 0.04 g/dL. No differences were noted between EX conditions.

Figure 9. Serum albumin (g/dL); interaction of DRUG and DAY.

Table 12

Means ($\pm$ SE) for serum albumin concentration (g/dL)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	4.45 (.04)	4.21 (.13)	4.29 (.08)	4.21 (.10)
Day Two	4.38 (.07)	4.13 (.06)	4.38 (.05)	4.24 (.05)
Day Four	4.21 ^X (.06)	3.99 ^X (.06)	4.35 ^Y (.05)	4.39 ^Y (.05)
Day Eight	4.23 (.06)	4.03 (.06)	4.27 (.05)	4.15 (.06)

Note. When collapsed across DRUG and NO DRUG conditions, the composite means of NO DRUG group^X and DRUG group^Y are significantly different ($p < .0001$).

A significant DAY by EXERCISE interaction was present in the analysis of total albumin content (Table 13, Fig. 10). When collapsed across DRUG and NO DRUG conditions, composite means for EX and SED groups were significantly different on day four and eight but not on day two. The composite means for the EX vs. SED groups on days four and eight were 133.9 ± 1.6 g vs. 125.0 ± 1.6 g and 133.2 ± 1.5 g vs. 128.2 ± 1.5 g. The analysis for total albumin content is consistent with the findings that were reported for plasma volume changes, indicating a positive association between the two variables.

Figure 10. Total albumin content (g); interaction of EXERCISE and DAY.

Table 13

Means ($\pm$ SE) for total serum albumin (g)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	131.9 (13.7)	129.1 (9.7)	119.3 (4.8)	117.0 (10.3)
Day Two	132.4 (2.8)	130.2 (2.1)	119.8 (1.9)	118.9 (2.4)
Day Four	131.6 ^a (2.3)	138.8 ^b (2.1)	118.3 ^a (1.9)	128.9 ^b (2.1)
Day Eight	132.1 ^c (2.3)	139.0 ^d (2.1)	124.2 ^c (1.9)	127.4 ^d (2.1)

Note. When collapsed across DRUG and NO DRUG conditions, SED groups^a and EX groups^b ($p < .001$), and SED groups^c and EX groups^d ($p < .05$) are significantly different.

Serum Protein

Both of the independent factors DRUG and EXERCISE had significant main effects on serum protein concentrations (Table 14). The composite scores for DRUG and NO DRUG groups when collapsed across DAY two, four and eight and across EX and SED conditions were 6.07 ± 0.08 g/dL and 6.39 ± 0.07 g/dL. The composite values for EX vs. SED groups when collapsed across DRUG and NO DRUG conditions and across DAY two, four and eight were 6.12 ± 0.07 g/dL vs. 6.35 ± 0.08 g/dL, respectively. Given the fact that both EXERCISE groups experienced an expansion of plasma volume, this finding indicates that training was associated with a dilution of plasma proteins.

Table 14

Means ($\pm$ SE) for serum protein concentration (g/dL)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	6.35 (.07)	6.26 (.17)	6.41 (.12)	6.12 (.17)
Day Two	6.32 ^a (.23)	6.15 ^b (.18)	6.54 ^a (.16)	6.24 ^b (.18)
Day Four	6.06 ^a (.20)	5.90 ^b (.18)	6.60 ^a (.16)	6.40 ^b (.18)
Day Eight	6.05 ^a (.19)	5.96 ^b (.18)	6.51 ^a (.16)	6.09 ^b (.17)

Note. When collapsed across DRUG and NO DRUG conditions and across DAY two, four and eight, there was a significant difference between SED groups^a and EX groups^b.

Unlike the total serum osmolarity, sodium and albumin content, there were no interaction effects in the analysis of total serum protein content. There was a significant main effect of DAY (see Fig. 11), indicating that the increase in total protein was experienced by all experimental groups consistently. When collapsed across DRUG and NO DRUG as well as the EX and SED conditions, the composite means for days two, four and eight were 184.6 ± 1.9 g, 190.2 ± 1.9 g, and 192.9 ± 1.7 g, respectively.

Figure 11. Total serum protein content (g); main effect of DAY.

Table 15

Means ($\pm$ SE) for total serum protein (g)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Baseline	188.6 (20.0)	192.8 (15.5)	178.4 (7.5)	170.2 (15.0)
Day Two	191.5 ^a (4.6)	194.4 ^a (3.4)	179.0 ^a (3.2)	173.3 ^a (3.9)
Day Four	191.0 ^b (4.6)	206.1 ^b (3.4)	180.1 ^b (3.5)	183.4 ^b (3.4)
Day Eight	189.7 ^b (3.7)	206.4 ^b (3.4)	189.7 ^b (3.2)	185.7 ^b (3.4)

Note. There is a main effect of DAYS from 2 to 4 ($p < .04$); from 2 to 8, ($p < .003$)

Correlations Between Plasma Volume and Total Serum Osmolar,
Sodium, Albumin, and Protein Contents

Changes in the total plasma contents of sodium, protein, and albumin reflect the predominant influence of changes in the plasma volume, which are used in the calculation of these variables. An attempt was made to test the influence of total sodium, osmolar, albumin, and protein content on plasma volume by multiple linear regression, but multicollinearity of the variables precluded valid interpretations (Tables 16-18).

Table 16

Correlations between plasma volume, total serum osmolar content, total serum sodium content, total serum albumin content, and total serum protein content on day 2

	PV	OSM	NA	ALB	PRO
Plasma Volume (PV)	1.00	0.94	0.96	0.68	0.53
Total Serum Osmolar Content (OSM)		1.00	0.99	0.96	0.97
Total Serum Sodium Content (NA)			1.00	0.97	0.97
Total Serum Albumin Content (ALB)				1.0	0.98
Total Serum Protein Content (PRO)					1.00

Table 17

Correlations between plasma volume, total serum osmolar content, total serum sodium content, total serum albumin content, and total serum protein content on day 4

	PV	OSM	NA	ALB	PRO
Plasma Volume (PV)	1.00	0.96	0.96	0.49	0.33
Total Serum Osmolar Content (OSM)		1.00	0.99	0.94	0.94
Total Serum Sodium Content (NA)			1.00	0.94	0.95
Total Serum Albumin Content (ALB)				1.0	0.95
Total Serum Protein Content (PRO)					1.00

Table 18

Correlations between plasma volume, total serum osmolar content, total serum sodium content, total serum albumin content, and total serum protein content on day 8

	PV	OSM	NA	ALB	PRO
Plasma Volume (PV)	1.00	0.94	0.91	0.74	0.33
Total Serum Osmolar Content (OSM)		1.00	0.98	0.94	0.89
Total Serum Sodium Content (NA)			1.00	0.94	0.89
Total Serum Albumin Content (ALB)				1.0	0.92
Total Serum Protein Content (PRO)					1.00

Aldosterone

Figs. 12, 13, & 14 represent the fluctuations in aldosterone concentration that occurred as subjects participated in the experiment. Values from every blood sampling period were used in the analysis to determine when significant effects between groups were present. The differences in aldosterone concentration (Fig. 12) among the groups on the first day were predominantly affected by EXERCISE status. Immediately after exercise, there was a 4.4 fold increase in aldosterone concentrations for exercisers compared to nonexercisers. The main effect of EXERCISE remained significant throughout 30 min of recovery.

Figure 12. Serum aldosterone (ng/dL) on day one.
D, E, or Dx E signify an effect of DRUG,
EXERCISE, or DRUG x EXERCISE.

Figure 13. Serum aldosterone (ng/dL) on day three. D and E signify main effects of drug or exercise.

Figure 14. Serum aldosterone (ng/dL) on day seven. D and E signify main effects of DRUG and EXERCISE.

Beginning on day one, a main effect of DRUG became evident (Fig. 12). This effect persisted throughout the remainder of the study and was not significantly different across levels of EXERCISE. The exercise training that was performed on days three and seven caused a rise in aldosterone concentration among exercisers that remained in effect throughout at least 2 h of recovery (Figs. 14 & 15).

Renin

The pattern of renin activity responses to exercise and to spironolactone paralleled that of aldosterone (Figs. 15, 16, & 17). Each exercise session caused a rise in renin activity that remained elevated for 1-2 h into recovery. Beyond day two, subjects who took spironolactone had significantly higher renin activity than those who did not. Significant interactions between DRUG and EXERCISE were noted on the resting samples taken on days one and seven. This suggests that the impairment of aldosterone activity imposed by spironolactone administration weakened the negative feedback that aldosterone usually exerts on renin.

Kidney Function

The results for variables that reflect changes in kidney function are presented in Tables 19-23. Fig. 18 depicts the significant three-way interaction (DRUG by EXERCISE by DAY) that exists in the analysis of daily urine volumes (Table 19). A three-way interaction occurs when the interactions of two variables changes at the different levels of the third. At day one, exercise had an apparent diuretic effect on subjects taking spironolactone, whereas it

RENIN ACTIVITY

Figure 15. Renin activity (ng/mL x h⁻¹) on day one. D and E signify main effects of DRUG and EXERCISE

Figure 16. Renin activity ($\text{ng/mL} \times \text{h}^{-1}$) on day three.

Figure 17. Renin activity (ng/mL x h⁻¹) on day seven.

had the opposite effect on subjects without spironolactone. However, by the end of the third day, exercise status had no significant effect on urine output.

Table 19

Means ($\pm$ SE) for 24 h urine output (mL)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Day One	2129 ^a (347)	1093 ^b (258)	1012 (349)	1862 (351)
Day Three	1421 (347)	1010 (294)	1725 (239)	1903 (306)
Day Seven	1646 (283)	1950 (258)	1718 (239)	1680 (258)

Note. There was a significant difference between Group^a and Group^b ($p < .03$).

Creatinine clearance (Table 20) is assumed to be a reasonable estimate for glomerular filtration rate. Fig. 19 represents a plot of creatinine clearance accumulated throughout the 24 h measurement day. Each individual sample is appropriately weighted for the time between measurements. No main or interaction effects were evident in the analysis of cumulative creatinine clearance (Table 20).

24 HOUR URINE OUTPUT

Figure 18. Changes in 24 hour urine output (mL).

CREATININE CLEARANCE

Figure 19. Changes in creatinine clearance (L/24h).

Table 20

Means ($\pm$ SE) for creatinine clearance (L/24 h)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Day One	156 (22)	132 (15)	157 (22)	175 (21)
Day Three	132 (22)	139 (17)	188 (12)	144 (16)
Day Seven	137 (15)	134 (15)	132 (19)	133 (13)

Free water is the excess water that is excreted when the kidneys produce urine that is osmotically dilute when compared to plasma, and free water clearance is the rate that free water is excreted. Free water clearance is positive when water is being taken out of the plasma and negative when extra solutes are being removed. In the analysis of cumulative 24 h free water clearance, no main or interaction effects on free water clearance were detected (Table 21).

Table 21

Means ($\pm$ SE) for free water clearance (mL/24 h)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Day One	-1988 (523)	-1923 (334)	-1459 (496)	-1395 (382)
Day Three	-1724 (523)	-1975 (321)	-2747 (331)	-1408 (270)
Day Seven	-1911 (302)	-1979 (402)	-1802 (270)	-1827 (270)

The analysis of 24 h urinary sodium output (Table 22) produced a significant three-way interaction (Fig. 20). Means for 24 h sodium output were consistently lower for exercisers, but these effects were most dramatic after day one for the NO DRUG condition and after day three for the DRUG condition ($p < 0.05$). The 24 h sodium outputs for EX and SED subgroups by the end of day seven were very similar, suggesting that training-induced enhancement of sodium conservation occurred only in the first few days of training.

Table 22

Means ($\pm$ SE) for 24 h serum sodium output (mEq)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Day One	253 ^a (33)	85 ^b (24)	134 (33)	120 (33)
Day Three	174 (33)	109 (28)	247 ^c (33)	104 ^d (29)
Day Seven	177 (27)	155 (24)	190 (23)	153 (24)

Note. Group^a is significantly higher than Group^b ($p < .002$), and Group^c is significantly higher than Group^d ($p < .001$).

The analysis of 24 h, urinary potassium output is presented in (Table 23, Fig. 21). No main or interaction effects were detected among any of the groups. This finding suggests that the sodium retention was achieved by exercisers without a concurrent exchange for potassium.

24 HOUR URINARY SODIUM OUTPUT

Figure 20. Changes in 24 h urinary sodium output (mEq/24h).

Figure 21. Changes in potassium output (mEq/24 h).

Table 23

Means ($\pm$ SE) for 24 h urinary potassium output (mEq)

Variable	NO DRUG/SED (n=5)	NO DRUG/EX (n=6)	DRUG/SED (n=7)	DRUG/EX (n=6)
Day One	99.3 (10.8)	83.1 (8.0)	53.4 (10.8)	81.2 (10.9)
Day Three	73.1 (10.8)	86.4 (9.2)	99.5 (7.4)	101.0 (9.5)
Day Seven	83.7 (8.8)	97.1 (8.0)	79.3 (7.4)	76.1 (8.0)

The results of sodium clearance and the fractional excretion of sodium are presented in Figs. 22-27. Both variables are reflective of the sodium conservation that is enhanced by each exercise session. The similarity of the response in sodium clearance and the fractional excretion of sodium between the NO DRUG/EX and DRUG/EX groups indicates that the sodium conservation that was associated with exercise was not altered by the administration of spironolactone.

Figure 22. Change in sodium clearance on day one (mL/min).

Figure 23. Change in sodium clearance on day three (mL/min).

Figure 24. Changes in sodium clearance on day seven (mL/min).

Figure 25. Change in the fractional excretion of sodium on day one (%).

Figure 26. Change in the fractional excretion of sodium on day three (%).

Figure 27. Change in the fractional excretion of sodium on day seven (%).

CHAPTER V

DISCUSSION AND CONCLUSIONS

A review of the literature provided the basis for developing the research hypothesis that the action of aldosterone on the kidney to conserve sodium and water was the primary mechanism accounting for the early hypervolemic adaptation that accompanies aerobic training. However, sufficient controversy remained regarding the role of additional fluid conserving influences to warrant a more thorough investigation. Therefore, the present study was undertaken to broaden our comprehension of the importance of aldosterone and plasma proteins to the expansion and maintenance of an elevated plasma volume in response to exercise training. To address this problem, the present research project was designed to capitalize on the fact that spironolactone, a commonly prescribed hypertensive medication, is effective in causing sodium and water loss by competitively inhibiting the action of aldosterone.

It was hypothesized that increases in plasma volume would correspond early in the training with increases in aldosterone and plasma sodium content, that spironolactone treatment would minimize this association, and that later increases in plasma volume would correspond more closely to late increases in plasma protein content. A 2 x 2 factorial design was implemented using dichotomous levels of

the two independent variables, DRUG and EXERCISE. The experiment was conducted over eleven days, with the exercise training being performed on the fourth through the tenth days. Repeated measurements of blood and urinary variables were conducted at seven points on each of three measurement days, i.e., days 1, 3, and 7, with final measurements taken 24 h after the start of exercise, i.e. on days 2, 4, and 8.

Plasma, Blood and Red Cell Volumes

The posttraining changes in plasma volumes for subjects who were randomly assigned to participate in the EXERCISE condition increased by $14.1 \pm 3.2\%$ compared to a $5.0 \pm 3.2\%$ increase for subjects who were assigned to the SEDENTARY condition. No distinction was noted in the overall response to exercise training between subjects who did or did not take spironolactone during the course of the experiment except that the group who took the medication expanded their plasma volume later in the training protocol (Fig. 4). These results are consistent with the findings of Convertino et al. (1980), Kirby and Convertino (1986), and Greenleaf et al. (1983). This finding could not be explained by differences in the way that group conserved sodium or by differences in any of the osmotically active solutes that were measured.

The administrated dose of spironolactone did not consistently increase 24 h urine production (Table 19), did not increase 24 h urine sodium output (Fig. 20), did not decrease urinary potassium output (Table 23), did not systematically increase sodium clearance (Fig 22), and did not increase the fractional excretion of sodium (Fig. 23). These results suggest that the spironolactone treatment was

ineffective in blocking aldosterone action on sodium retention. On the other hand, the facts that both plasma aldosterone concentrations (Figs. 12-14) and plasma renin activities (Figs. 15-17) were consistently higher in spironolactone-treated subjects indicate that the drug caused an oversecretion of renin and aldosterone. The increased aldosterone may have been sufficient to overwhelm any aldosterone-blocking action of spironolactone. Therefore, although spironolactone treatment was effective in altering aldosterone and renin levels, it was not effective in totally blocking the action of aldosterone. Thus, the possibility that increased aldosterone activity may have caused much of the expansion of plasma volume observed in both NO DRUG/EX and DRUG/EX conditions cannot be eliminated.

The expansion of plasma volume was associated with a concurrent change in total blood volume. The difference in blood volume between the EX and SED groups in this study, $8.5 \pm 1.1 \%$ vs. $3.0 \pm 3.2 \%$, was comparable to the 8.0% increase from baseline conditions that was reported by Convertino et al. (1980).

No main or interaction effects were noted in the analysis of red cell volume. The stability of red cell volumes that was experienced by the subjects in the present study supports the results of studies that conducted similar training programs (Convertino et al., 1980; Greenleaf et al., 1983).

Body Weight and Total Body Water

Greenleaf et al. (1983) have proposed that an increase in fluid intake was the primary mechanism that accounted for exercise-induced

expansion of plasma volume. In the current study, subjects did not experience any noticeable gain in body water or body weight, nor did they demonstrate an increase in urine output. However, the variability inherent in the measurement of total body water may have obscured an exercise-induced increase in body water. Furthermore, because the EX subjects expended approximately 10,500 kcal of exercise-associated energy during the 7 d of training, it is also conceivable that up to 1 kg of body weight loss not associated with fluid loss was replaced by excess fluid consumption over the 7 d of the experiment. Alternatively, if the absence of increases in body water and body weight accurately reflects the absence of an increase in fluid retention during exercise training, it appears that the expansion of plasma volume resulted from a shift of water from either the intracellular or interstitial space to the plasma.

Plasma Constituents

The concentrations in plasma of both sodium and protein were lower on days two, four and eight in exercising subjects than in those who remained sedentary. In the case of sodium, this training effect was apparent during the first measurement day and persisted for the duration of the study (Table 11). Because only a small increase in plasma volume in the exercisers occurred on that first day, presumably some of the dilution of plasma sodium can be accounted for by ingestion of hypotonic fluids during recovery from exercise. If plasma volume levels were totally dependent on sodium, this sodium dilution in plasma would not have persisted. These data do not support the findings of Costill et al. (1976), who noted no

differences in sodium concentration the day following a 60 min moderate exercise bout.

Despite the apparent dilution of plasma sodium, the serum osmolality was not affected by exercise on day two (Table 8). This cannot be explained by an influx of protein because protein concentrations and contents were also slightly lower in the exercisers. One can speculate that some other osmotically active particle entered the blood stream and provided the additional osmotic pressure to retain fluids within the vasculature. McKeever et al. (1987) attributed the exercise-induced expansion of plasma volume in horses to an accumulation of urea. Urea has not been shown to evoke a significant osmotic influence, but further research on this topic is warranted. Osmolar concentration also became greater in the exercise group compared to the sedentary control group at day eight. This increase in osmolality took place during a concomitant expansion of plasma volume. This increase in osmolality was not related to any of the variables measured.

Beyond the first measurement day, sodium concentrations stabilized between the two exercise groups, and the values for the entire subject population demonstrated very little fluctuation. Given the fact that plasma volume expanded significantly on day four and remained elevated through day eight, it is very plausible that sodium exerted a major influence on fluid retention during this time. On the other hand, plasma protein concentration progressively dropped from day to day, reflective of the dilution of a solute within a greater volume of solvent.

Aldosterone and Renin

The responses of aldosterone and plasma renin activity to exercise and spironolactone were closely associated throughout the investigation (Figs. 12-17). In those subjects who exercised, peak levels for both hormones were attained immediately after exercise, and return of those values to baseline required 1-2 h. Subjects who took spironolactone had higher concentrations of both aldosterone and renin beginning on the second day and lasting throughout the remainder of the study. Bevegard and Danielson (1977) also noted an increase in the renin response to exercise in subjects who were taking spironolactone.

When interpreting these results it is important to reconsider the action of spironolactone on aldosterone and the relationship of aldosterone to renin. Spironolactone does not block the release of aldosterone, but rather it competes directly for binding sites on the kidney nephron, thus inhibiting the sodium and fluid retaining effects. Renin stimulates the release of aldosterone, and the activity of aldosterone acts negatively on further renin stimulation. Given the lower activity of aldosterone associated with the inhibition of spironolactone, negative feedback on renin release would be diminished, and higher renin values would be expected (Bevegard & Danielson, 1977). Higher renin levels would in turn stimulate greater aldosterone release. With the higher levels of aldosterone and renin that were noted both at rest and exercise, it is quite plausible to conclude that oversecretion of aldosterone may have compensated for any inhibitory actions that were offered by spironolactone. The

effective spironolactone dosage that has been determined for use with clinical patients who have primary hypertension ranges from 100 to 400 mg/d. No effective dosage has apparently been determined that will completely block the actions of aldosterone during exercise.

Urinary Variables

The major difference between exercisers and non-exercisers in their urinary responses to exercise training involved the capacity to conserve sodium. Sodium clearance was reduced during 4 h of recovery from exercise regardless of drug treatment, especially during the first three days of training (Figs. 22-24). The statistical analysis of these data, however, made no allowance for the time interval that was represented by each value. With this in mind, the comparison of 24 h sodium outputs (Fig. 21, Table 23) may represent a more valid assessment of sodium conservation. Twenty-four hour sodium outputs for exercisers were lower than they were for sedentary subjects on each of the three measurement days. When interpreting these results however, one must consider the three-way interaction of DRUG by EXERCISE by DAY. Thus, it appeared that sodium conservation was less effective on day one in the spironolactone subgroup of the exercise condition than in the non-spironolactone subgroup. This may reflect a successful inhibition of aldosterone in the spironolactone subgroup at this time. This conclusion is supported by the fact that there was no difference in the aldosterone response during the first measurement day between the two subgroups of the exercise condition.

Sodium conservation was equally effective between the two subgroups of the exercise condition on days three and seven. This

finding is consistent with the hypothesis that spironolactone takers who participated in exercise training overcame the inhibition of spironolactone by over secreting aldosterone.

Creatinine clearance values for the subjects did not differ significantly between the four experimental groups (Table 20, Fig. 19). Therefore, the training-induced reductions in sodium output are presumed to result from postglomerular sodium retention rather than from differences in glomerular filtration rate. This finding is consistent with the idea that training caused increased sodium conservation by increasing the activity of aldosterone.

It is worthy to note that potassium output did not increase in conjunction with the retention of sodium. Potassium is usually exchanged for sodium under the influence of aldosterone activity. It is accepted that sodium can be exchanged for H^+ , resulting in the same degree of sodium conservation as when exchanged for potassium. During exercise of this intensity, some degree of anaerobic metabolism would be occurring, and H^+ would be in high concentration. Therefore, it is plausible that sodium was conserved via an exchange with hydrogen rather than potassium.

Conclusions

The hypothesis that prompted this investigation was that the early phase of the exercise-induced expansion of plasma volume would be associated with increased activity of aldosterone, whereas the later phase of sustained maintenance of an expanded plasma volume would be associated with an increase in circulating plasma proteins. It was hoped that the administration of spironolactone would make it

possible to reduce the activity of aldosterone and thereby help distinguish the proportion of the plasma volume expansion that can be attributed to aldosterone effects. Unfortunately, contrary to the results of a small pilot study, spironolactone administration in the current investigation seems to have been inadequate to influence the effects of aldosterone on postglomerular sodium retention.

Therefore, the principal evidence obtained to support the notion of an early involvement of aldosterone and its effects on plasma volume expansion was the maintenance of a constant plasma sodium concentration with a concomitant increase in plasma volume on days four and eight. However, the data for day two speak against the importance of aldosterone and plasma sodium as being critical to plasma volume expansion. On day two, plasma volume was unchanged in the face of a decreased plasma sodium concentration in exercised subjects. Therefore, the precise role of aldosterone in the early phase of exercise-induced plasma volume expansion remains obscure.

With respect to the role of plasma proteins, the fact that little change in plasma protein content accompanied early increases in plasma volume supports the concept of only minor involvement of plasma proteins in the early phase of plasma volume expansion. During the late phase of plasma volume expansion, plasma protein content did increase in the exercised groups. Although no increase in protein content occurred in the NO DRUG/SED group, an increase was observed from day four to day eight in the DRUG/SED group. Therefore, the statistical analysis could not distinguish between combined EXERCISE and SEDENTARY groups in this late phase plasma protein adaptation.

Accordingly, based on the present results, the role of plasma proteins on exercise-induced plasma volume expansion remains promising, but indeterminate.

REFERENCES

- Bevegard, S., Castenfors, J., & Danielson, M. (1977). The effects of four months' treatment with spironolactone on systemic blood pressure, cardiac output and plasma renin activity in hypertensive patients. Acta Medica Scandinavica, 202, 373-377.
- Castenfors, J. (1977). Renal function during prolonged exercise. Annals New York Academy of Sciences, 301, 151-159.
- Convertino, V.A., Greenleaf, J.E., & Bernauer, E.M. (1980). Role of thermal and exercise factors in the mechanism of hypervolemia. Journal of Applied Physiology, 48(4), 657-664.
- Convertino, V.A. (1983). Heart rate and sweat responses associated with exercise-induced hypervolemia. Medicine and Science in Sports and Exercise, 15(1), 77-82.
- Convertino, V.A., Brock, P.J., Keil, L.C., Bernauer, E.M., & Greenleaf, J.E. (1980). Exercise training induced hypervolemia: Role of plasma albumin, renin, and vasopressin. Journal of Applied Physiology, 48(4), 665-689.
- Convertino, V.A., Keil, L.C., & Bernauer, E.M. (1981). Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. Journal of Applied Physiology, 50, 123-128.
- Convertino, V.A., Keil, L.C., & Greenleaf, J.E. (1983). Plasma volume, renin and vasopressin responses to graded exercise after training. Journal of Applied Physiology, 54, 508-514.
- Corvel, P., Claire, M., Oblin, M.E., Georing, K., & Rossier, B. (1981). Mechanism of the antimineralocorticoid effects of spironolactone. Kidney International, 20, 1-6.
- Costill, D.L., Branam, G., Fink, W., and Nelson, R. (1976). Exercise induced sodium conservation: Changes in plasma renin and aldosterone. Medicine and Science in Sport, 8(4), 209-213.
- Costill, D.L., & Fink, W.J. (1974). Plasma volume changes following exercise and thermal dehydration. Journal of Applied Physiology, 37(4), 521-525.

- Davis, J.M., Lamb, D.R., Burgess, W.A., & Bartoli, W.P. (1987). Accumulation of deuterium oxide in body fluids after ingestion of D₂O-labeled beverages. Journal of Applied Physiology, 63(5), 2060-2066.
- Edwards, R.J., & Harrison, M.H. (1984). Intravascular volume and protein responses to running exercise. Medicine and Science in Sports and Exercise, 16(3), 247-255.
- Fagard, R., Grauwels, R., Groeseneken, D., Lijnen, P., Staessen, J., Vanhees, L., & Amery, A. (1985). Plasma levels of renin, angiotensin II, and 6-ketoprostaglandin F_{1α} in endurance athletes. Journal of Applied Physiology, 59(3), 947-952.
- Fortney, S.M., Nadel, E.R., Wenger, C.B., & Bove, J.R. (1981). Effect of acute alterations of blood volume on the circulatory performance in humans. Journal of Applied Physiology, 50(2), 292-298.
- Fortney, S.M., & Senay, L.C. (1979). Effect of training and heat acclimation on exercise responses of sedentary females. Journal of Applied Physiology, 47(5), 978-984.
- Freund, B.J., Claybaugh, J.R., Dice, M.S., & Hashiro, G.M. (1987). Hormonal and vascular fluid responses to maximal exercise in trained and untrained males. Journal of Applied Physiology, 63(2), 669-675.
- Galbo, H. (1983). Hormones and metabolic adaptations to exercise. New York: Thieme-Stratton Inc.
- Geyssant, A.G., Geelen, G., Denis, C.H., Allevard, A.M., Vincent, M., Jarsaillon, E., Bizollon, C.A., Iacour, J.R., and Gharib, C. (1981). Plasma vasopressin, renin activity, and aldosterone effects of exercise training. European Journal of Applied Physiology, 46, 21-30.
- Green, H.J., Jones, L.L., Hughson, R.L., Painter, D.C., & Farrance, B.W. (1987). Training-induced hypervolemia: Lack of an effect on oxygen utilization during exercise. Medicine and Science in Sports and Exercise, 19(3), 202-206.
- Green, H.J., Thomson, J.A., Ball, M.E., Hughson, R.L., Houston, M.E., & Sharratt, M.T. (1984). Alterations in blood volume following short-term supramaximal exercise. Journal of Applied Physiology, 56(1), 145-149.
- Greenleaf, J.E., Brock, P.J., Keil, L.C., & Morse, J.T. (1983). Drinking and water balance during exercise and heat acclimation. Journal of Applied Physiology, 54(2), 414-419.

- Greenleaf, J.E., Convertino, V.A., & Mangseth, G.R. (1979). Plasma volume during stress in man: Osmolality and red cell volume. Journal of Applied Physiology, 47(5), 1031-1038.
- Guezennec, C.Y., Defer, G., Cazorla, G., Sabathier, C., & Ihoste, F. (1986). Plasma renin activity, aldosterone and catecholamine levels when swimming and running. European Journal of Applied Physiology, 54, 632-637.
- Guyton, A.C. (1986). Textbook of Medical Physiology. Philadelphia: W.B. Saunders.
- Hagen, R.D., Diaz, F.J., & Horvath, S.M. (1978). Plasma volume changes with movement to supine and standing positions. Journal of Applied Physiology, 45, 414-418.
- Harrison, M.H., Edwards, R.J., Graveney, M.J., Cochrane, L.A., & Davies, J.A. (1981). Blood volume and plasma protein responses to heat acclimatization in humans. Journal of Applied Physiology, 50(3), 597-604.
- Hespel, P., Lijnen, P., Fiocchi, R., Lissens, W., Moerman, E., & Amery, A. (1987). Effects of calcium antagonism on the resting and exercise-stimulated renin-aldosterone axis. Methods and Findings in Experimental Clinical Pharmacology, 9(7), 461-467.
- Jones, N.L., & Rebuck, A.S. (1973). Rebreathing equilibration of CO₂ during exercise. Journal of Applied Physiology, 35, 538-541.
- Kachadorian, W.A., & Johnson, R.E. (1970). Renal responses to various rates of exercise. Journal of Applied Physiology, 28(6), 748-752.
- Katzman, P.L., Hulthen, U.L., and Hokfelt, B. (1987). Catecholamines, renin-angiotensin-aldosterone and cardiovascular response during exercise following acute and long term calcium antagonism with felodipine in essential hypertension. Journal of Cardiovascular Pharmacology, 10, 439-444.
- Kanstrup, I., & Ekblom, B. (1982). Acute hypervolemia, cardiac performance, and aerobic power during exercise. Journal of Applied Physiology, 52(5), 1186-1191.
- Kraemer, W.J., Armstrong, L.E., Hubbard, R.W., Marchitelli, L.J., Leva, N., Rock, R.B., & Dziados, J.E. (1988). Response of plasma human atrial natriuretic factor to high intensity submaximal exercise in the heat. European Journal of Applied Physiology, 57, 399-403.
- Kirby, C., & Convertino, V.A. (1986). Plasma aldosterone and sweat sodium concentration after exercise and heat acclimation. Journal of Applied Physiology, 61(3), 967-970.

- Kotchen, T.A., Hartley, L.H., Rice, T.W., Mougey, E.H., Jones, L.G., & Mason, J.W. (1971). Renin, norepinephrine, and epinephrine responses to graded exercise. Journal of Applied Physiology, 31(2), 178-184.
- Kosunen, K.J., & Pakarinen, A.J. (1976). Plasma renin, angiotensin II, and plasma and urinary aldosterone in running exercise. Journal of Applied Physiology, 41(1), 26-29.
- Kosunen, K., Pakarinen, A., Kuoppasalmi, K., Naveri, H., Rehuman, S., Staderskjold-Nordenstam, C.G., Harkonen, M., & Adlercreutz, H. (1980). Cardiovascular function and the renin-angiotensin-aldosterone system in long-distance runners during various training periods. Scandinavian Journal of Clinical Laboratory Investigation, 40, 429-435.
- Lijnen, P., Hespel, P., Eynde, E.V., & Amery, A. (1985). Biochemical variables in plasma and urine before and after prolonged physical exercise. Enzyme, 33, 134-142.
- Lukaski, H.C., Bolonchuk, W.W., Hall, C.B., & Siders, W.A. (1986). Validation of tetrapolar bioelectrical impedance method to assess human body composition. Journal of Applied Physiology, 60(4), 1327-1332.
- Maher, J.T., Jones, L.G., Hartley, L.H., Williams, G.H., & Rose, L.I. (1975). Aldosterone dynamics during graded exercise at sea level and high altitude. Journal of Applied Physiology, 39, 18-22.
- Maresh, C.M., Wang, B.C., & Goetz, K.L. (1985). Plasma vasopressin, renin activity and aldosterone response to maximal exercise in active college females. European Journal of Applied Physiology, 54, 398-403.
- McKeever, K.H., Schurg, W.A., & Convertino, V.A. (1985). Exercise training-induced hypervolemia in greyhounds: Role of water intake and renal mechanisms. American Journal of Physiology, 248, R422-R425.
- McKeever, K.H., Schurg, W.A., Jarrett, S.H., & Convertino, V.A. (1987). Exercise training-induced hypervolemia in the horse. Medicine and Science in Sports, 19(1), 21-27.
- McMurray, R.G. (1983). Plasma volume changes during submaximal swimming. European Journal of Applied Physiology, 51, 347-356.

- Melin, B., Eclache, J.P., Geelen, G., Annat, G., Allevard, A.M., Jarsaillon, E., Zebidi, A., Legros, J.J., & Gharib, C. (1980). Plasma AVP, neurophysin, renin activity, and aldosterone during submaximal exercise performed until exhaustion in trained and untrained men. European Journal of Applied Physiology, 44, 141-151.
- Novosadova, J. (1977). The changes in hematocrit, hemoglobin, plasma volume and proteins during and after different types of exercise. European Journal of Applied Physiology, 36, 223-230.
- Olszewski, W., Engeset, A., Jaeger, P.M., Sokolowski, J., & Theodorsen, L. (1977). Flow and composition of leg lymph in normal men during venous stasis, muscular activity, and local hyperthermia. Acta Physiologica Scandinavica, 99, 149-144.
- Pederson, E.B., Kornerup, H.J., & Iarsen, J.S. (1981). Responsiveness of the renin-aldosterone system during exercise in young patients with essential hypertension. European Journal of Clinical Investigation, 11, 403-408.
- Pivarnik, J.M., Montain, S.J., Graves, J.E., & Pollack, M.L. (1988). Alterations in plasma volume, electrolytes and protein during incremental exercise at different pedal speeds. European Journal of Applied Physiology, 57, 103-109.
- Raff, H., Shinsako, J., Wade, C.E., Keil, L.C., & Dallman, M.F. (1985). Acute volume expansion and decreased adrenocortical sensitivity to ACTH and angiotensin. American Journal of Physiology, 249, R611-R616.
- Sawka, M.N., Francesconi, R.P., Pimental, N.A., & Pandolf, K.B. (1984). Hydration and vascular fluid shifts during exercise in the heat. Journal of Applied Physiology, 56(1), 91-96.
- Sawka, M.N., Hubbard, R.W., Francesconi, R.P., & Horstman, D.H. (1983). Effects of acute plasma volume expansion on altering exercise-heat performance. European Journal of Applied Physiology, 51, 303-312.
- Schneider, E.G., Radke, K.J., Ulderich, D.A., & Taylor, R.E. (1985). Effect of osmolality on aldosterone secretion. Endocrinology, 116(4), 1621-1626.
- Senay, L.C. (1979). Effects of exercise in the heat on body fluid distribution. Medicine and Science in Sports, 11(1), 42-48.
- Senay, L.C., & Kok, R. (1977). Effects of training and heat acclimatization on blood plasma contents of exercising men. Journal of Applied Physiology, 43(4), 591-599.

- Senay, L.C., & Pivarnik, J.M. (1985). Fluid shifts during exercise. In: R.L. Terjung (ed), Exercise and Sport Sciences Reviews, 335-387, New York: MacMillan.
- Shigeoka, J.W., Colice, G.L., & Ramirez, G. (1985). Effect of normoxemic and hypoxemic exercise on renin and aldosterone. Journal of Applied Physiology, 59(1), 142-148.
- Skipka, W., Boning, D., Deck, K.A., Kulpmann, W.R., & Meurer, K.A. (1979). Reduced aldosterone and sodium excretion in endurance-trained athletes before and during immersion. European Journal of Applied Physiology, 42, 255-261.
- Staessen, J., Fagard, R., Hespel, P., Lijnen, P., Vanhees, L., & Amery, A. (1987). Plasma renin system during exercise in normal men. Journal of Applied Physiology, 63(1), 188-194.
- Wade, C.E. (1986). Response, regulation, and actions of vasopressin during exercise: A review. Medicine and Science in Sports and Exercise, 16(5), 506-511.
- Wade, C.E., & Claybaugh, J.R. (1980). Plasma renin activity, vasopressin concentration, and urinary excretory responses to exercise in men. Journal of Applied Physiology, 49(6), 930-936.
- Wade, C.E., Dressendorfer, R.H., O'Brien, J.C., & Claybaugh, J.R. (1981). Renal function, aldosterone, and vasopressin excretion following repeated long distance running. Journal of Applied Physiology, 50(4), 709-712.
- Wade, C.E., Hill, L.C., Hunt, M.M., & Dressendorfer, R.H. (1985). Plasma aldosterone and renal function in runners during a 20 day road race. European Journal of Applied Physiology, 54, 456-460.
- Wade, C.E., Ramee, S.R., Hunt, M.M., & White, C.J. (1987). Hormonal and renal responses to converting enzyme inhibition during maximal exercise. Journal of Applied Physiology, 63(5), 1796-1800.
- Williams, J.D., & Foster, D.W. (1985). Textbook of Endocrinology. Philadelphia: W.B. Saunders.
- Wolf, J.P., Nguyen, N.U., Dumoulin, G., & Berthelay, S. (1986). Plasma renin and aldosterone changes during twenty minutes' moderate exercise. European Journal of Applied Physiology, 54, 602-607.

APPENDIX A
INFORMED CONSENT FORM

THE OHIO STATE UNIVERSITY

Protocol No. _____

CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

I, _____, hereby authorize or direct D.R. Lamb or associates or assistants of his choosing, to perform the following treatment or procedure (describe in general terms).

A determination of plasma volume involving the withdrawal of a 25 mL blood sample (< 1 oz) and the reinfusion of the red blood cells from that sample tagged with a trace amount of radioactive Cr₅₁, three blood sampling periods where a total of 8 ozs of blood will be withdrawn through a sterile non-toxic teflon tube inserted into a forearm vein, three 24 hr urine collections, eighteen cardiac output determinations involving approximately 15 seconds of rebreathing a gas mixture containing 14% carbon dioxide and 86% oxygen while riding a stationary bicycle ergometer at a workload representing 65% maximal effort, two maximal oxygen uptake measurements requiring volitional maximum effort on a stationary bicycle ergometer while respiratory gases are collected and continuous electrocardiographic monitoring is observed, two determinations of total body water involving the ingestion of a total of 1 1/2 ozs of deuterium oxide (D₂O); a stable isotope of water, and eighteen measurements of total body water where bioelectrical impedance is determined from passing a painless electrical signal through the body.

The experimental (research) portion of the treatment or procedure is:

FACTOR 1:

Performing seven 120 min. exercise bouts on a stationary bicycle ergometer at a workload representing 65% maximal effort.

FACTOR 2:

Taking either a 25 mg dose of spironolactone 4 times daily for 12 days while the investigation is in progress, or taking an equal sized placebo. Spironolactone is a commonly used high blood pressure medication that is considered safe when taken in doses of 25-100 mg four times daily.

This is done as part of an investigation entitled: The relationship between aldosterone, vasopressin, renin, plasma proteins, atrial natriuretic factor, and exercise induced plasma volume expansion.

1. Purpose of the procedure or treatment: To determine the mechanism whereby aerobically trained individuals maintain a chronically elevated plasma volume.
2. Possible appropriate alternative methods of treatment: Not to participate in the study.

3. Discomforts and risks reasonably to be expected: Spiro-lactone administration along with excessive intake of potassium may cause high blood levels of potassium. However, frequent determinations of potassium levels will be assessed throughout the test and appropriate measures will be taken if hyperkalemia is observed. Some enlargements and tenderness of the external genitalia and breasts may occur from spironolactone administration, but these changes are normally reversible. The risks of volitional maximum graded exercise tests, including irregular heartbeats, heart attack, or death are 1/20,000. Other risks may include minor bruising or infection from blood sampling, muscular soreness from exercise training, and the remote possibility of temporary confusion and nausea associated with dueterium consumption.
4. Possible benefits for subjects/society: Subjects will be made aware of their maximal oxygen uptake and other measures of physical fitness status. The investigation will also yield greater understanding of the factors that contribute to an expansion of blood volume in aerobically trained individuals.
5. Anticipated duration of subject's participation: 12-days.

I hereby acknowledge that _____ has provided information about the procedure described above, my rights as a subject, and he/she answered all questions to my satisfaction. I understand that I may contact him/her should I have additional questions. He/She has explained the risks described above and I understand them; he/she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study may be made available to the sponsor of this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given to me concerning this treatment or procedure.

In the unlikely event of injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of The Ohio State University. I also understand that the costs of such treatment will be at my expense and that financial compensation is not available. Questions about this should be directed to the Human Subject Review Office at 292-9046. I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed: _____
(Signature of Project Director or his/her
Authorized Representative)

APPENDIX B
BIOMEDICAL SCIENCE REVIEW COMMITTEE FORM

BIOMEDICAL SCIENCES REVIEW COMMITTEE
RESEARCH INVOLVING HUMAN SUBJECTS
THE OHIO STATE UNIVERSITY

☐ Original Review
☒ Continuing Review
☐ Five-Year Review

ACTION OF THE REVIEW COMMITTEE

OCT 20 REC'D

With regard to the employment of human subjects in the proposed research:

86H0292 THE RELATIONSHIP BETWEEN ALDOSTERONE VASOPRESSIN, RENIN, PLASMA
PROTEINS, ATRIAL NATRIURETIC FACTOR AND EXERCISE INDUCED PLASMA
VOLUME EXPANSION, David R. Lamb, Health, Physical Education and
Recreation

THE BIOMEDICAL SCIENCES REVIEW COMMITTEE HAS TAKEN THE FOLLOWING ACTION:

☒ APPROVED	☐ DISAPPROVED
☐ APPROVED WITH STIPULATIONS*	☐ WAIVER OF WRITTEN CONSENT GRANTED

*Stipulations stated by the Committee- have been met by the investigator and,
therefore, the protocol is APPROVED.

It is the responsibility of the principal investigator to retain a copy of each signed consent form for at least four (4) years beyond the termination of the subject's participation in the proposed activity. Should the principal investigator leave the University, signed consent forms are to be transferred to the Human Subjects Review Committee for the required retention period. This application has been approved for the period of one year. You are reminded that you must promptly report any problems to the Review Committee, and that no procedural changes may be made without prior review and approval. You are also reminded that the identity of the research participants must be kept confidential.

Date: October 19, 1987

Signed: Thomas M. O'Donoghue
Chairperson

APPENDIX C
MODIFIED FORMULATION OF BIURET REAGENT

Biuret Reagent

Prepare According to Formula

K-Na Tartrate	96.0 g
Distilled Water	6 L
CaSO ₄ 5H ₂ O	24.0 g
NaOH, 2.5 N (100g NaOH/L)	3,608 mL
KI	10.0 g

Add ingredients together and bring up to 10 L volume with distilled water.

APPENDIX D
ANOVA TABLES FOR SIGNIFICANT EFFECTS

Table 24

ANOVA for percent change in plasma volume

Sources	df	SS	F	PR > F
DRUG	1	631.8	5.15	0.03
EXERCISE	1	904.2	7.36	0.01
DRUG x EXERCISE	1	2.2	0.02	0.89
ID (DRUG x EXERCISE)	20	2455.3		
DAY	2	571.3	13.11	0.0001
DRUG x DAY	2	170.3	3.91	0.03
EXERCISE x DAY	2	136.2	3.12	0.05
DRUG x EXERCISE x DAY	2	16.3	0.38	0.69
DAY x ID (DRUG x EXERCISE)	36	784.6		

Table 25

ANOVA for percent change in blood volume

Sources	df	SS	F	PR > F
DRUG	1	336.0	5.29	0.032
EXERCISE	1	258.9	4.08	0.057
DRUG x EXERCISE	1	0.0	0.00	0.979
ID (DRUG x EXERCISE)	20	1270.1		
DAY	2	231.0	8.24	0.001
DRUG x DAY	2	91.3	3.62	0.055
EXERCISE x DAY	2	97.4	3.37	0.046
DRUG x EXERCISE x DAY	2	2.7	0.09	0.990
DAY x ID (DRUG x EXERCISE)	36	520.7		

Table 26

ANOVA for body weight

Sources	df	SS	F	PR > F
DRUG	1	173.2	0.19	0.671
EXERCISE	1	1116.7	1.20	0.286
DRUG x EXERCISE	1	349.2	0.38	0.547
ID (DRUG x EXERCISE)	20	18596.8		
DAY	2	22.7	7.44	0.002
DRUG x DAY	2	0.5	0.17	0.845
EXERCISE x DAY	2	3.6	1.18	0.318
DRUG x EXERCISE x DAY	2	0.9	0.29	0.747
DAY x ID (DRUG x EXERCISE)	39	59.6		

Table 27

ANOVA for serum sodium

Sources	df	SS	F	PR > F
DRUG	1	14.1	1.14	0.298
EXERCISE	1	86.8	7.05	0.015
DRUG x EXERCISE	1	5.9	0.48	0.498
ID (DRUG x EXERCISE)	20	246.0		
DAY	2	4.6	0.51	0.605
DRUG x DAY	2	5.7	0.63	0.536
EXERCISE x DAY	2	1.2	0.13	0.880
DRUG x EXERCISE x DAY	2	1.5	0.16	0.851
DAY x ID (DRUG x EXERCISE)	39	176.1		

Table 28

ANOVA for total serum sodium

Sources	df	SS	F	PR > F
DRUG	1	44478.7	2.53	0.127
EXERCISE	1	8123.3	0.46	0.504
DRUG x EXERCISE	1	499.0	0.03	0.867
ID (DRUG x EXERCISE)	20	351391.1		
DAY	2	10509.3	13.66	0.0001
DRUG x DAY	2	3132.1	4.07	0.026
EXERCISE x DAY	2	2691.4	3.50	0.041
DRUG x EXERCISE x DAY	2	124.4	0.16	0.851
DAY x ID (DRUG x EXERCISE)	35	13462.3		

Table 29

ANOVA for serum osmolality

Sources	df	SS	F	PR > F
DRUG	1	297.9	3.12	0.092
EXERCISE	1	7.8	0.08	0.777
DRUG x EXERCISE	1	5.5	0.06	0.811
ID (DRUG x EXERCISE)	20	1907.1		
DAY	2	249.2	3.82	0.031
DRUG x DAY	2	12.7	0.19	0.824
EXERCISE x DAY	2	93.4	1.43	0.251
DRUG x EXERCISE x DAY	2	30.5	0.47	0.630
DAY x ID (DRUG x EXERCISE)	38	1238.6		

Table 30

ANOVA for total serum osmolar content

Sources	df	SS	F	PR > F
DRUG	1	207610.0	2.88	0.105
EXERCISE	1	60978.7	0.85	0.368
DRUG x EXERCISE	1	4941.3	0.07	0.796
ID (DRUG x EXERCISE)	20	1439793.8		
DAY	2	65569.6	20.43	0.0001
DRUG x DAY	2	7071.5	2.20	0.126
EXERCISE x DAY	2	12413.5	3.87	0.031
DRUG x EXERCISE x DAY	2	2031.2	0.63	0.537
DAY x ID (DRUG x EXERCISE)	35	54570.7		

Table 31

ANOVA for serum albumin

Sources	df	SS	F	PR > F
DRUG	1	0.31	2.44	0.134
EXERCISE	1	0.39	3.05	0.096
DRUG x EXERCISE	1	0.10	0.77	0.391
ID (DRUG x EXERCISE)	20	2.56		
DAY	2	0.14	3.54	0.039
DRUG x DAY	2	0.15	3.73	0.033
EXERCISE x DAY	2	0.03	0.74	0.482
DRUG x EXERCISE x DAY	2	0.03	0.66	0.523
DAY x ID (DRUG x EXERCISE)	39	0.79		

Table 32

ANOVA for total serum albumin

Sources	df	SS	F	PR > F
DRUG	1	1808.6	1.42	0.247
EXERCISE	1	255.1	0.20	0.659
DRUG x EXERCISE	1	0.5	0.00	0.984
ID (DRUG x EXERCISE)	20	25458.5		
DAY	2	330.9	6.47	0.004
DRUG x DAY	2	14.3	0.28	0.757
EXERCISE x DAY	2	280.0	5.47	0.009
DRUG x EXERCISE x DAY	2	36.5	0.71	0.497
DAY x ID (DRUG x EXERCISE)	35	895.2		

Table 33

ANOVA for serum protein

Sources	df	SS	F	PR > F
DRUG	1	1.82	9.79	0.005
EXERCISE	1	0.87	4.69	0.043
DRUG x EXERCISE	1	0.12	0.62	0.439
ID (DRUG x EXERCISE)	20	3.70		
DAY	2	0.28	1.59	0.218
DRUG x DAY	2	0.38	2.11	0.135
EXERCISE x DAY	2	0.02	0.13	0.880
DRUG x EXERCISE x DAY	2	0.06	0.35	0.706
DAY x ID (DRUG x EXERCISE)	39	3.47		

Table 34

ANOVA for total serum protein

Sources	df	SS	F	PR > F
DRUG	1	3147.2	1.07	0.314
EXERCISE	1	315.4	0.11	0.747
DRUG x EXERCISE	1	686.9	0.23	0.634
ID (DRUG x EXERCISE)	20	58924.8		
DAY	2	755.4	5.42	0.009
DRUG x DAY	2	148.7	1.07	0.355
EXERCISE x DAY	2	302.8	2.17	0.129
DRUG x EXERCISE x DAY	2	105.3	0.76	0.477
DAY x ID (DRUG x EXERCISE)	35	2439.1		

Table 35

ANOVA for 24 h urine output

Sources	df	SS	F	PR > F
DRUG	1	151934.9	0.17	0.684
EXERCISE	1	6340.5	0.01	0.934
DRUG x EXERCISE	1	1615685.4	1.81	0.194
ID (DRUG x EXERCISE)	20	17853901.9		
DAY	2	700244.3	0.87	0.428
DRUG x DAY	2	1732766.3	2.16	0.133
EXERCISE x DAY	2	190031.4	0.24	0.791
DRUG x EXERCISE x DAY	2	2829738.6	3.52	0.042
DAY x ID (DRUG x EXERCISE)	31	12446687.8		

Table 36

ANOVA for 24 h sodium output

Sources	df	SS	F	PR > F
DRUG	1	6.1	0.00	0.981
EXERCISE	1	71618.6	7.04	0.015
DRUG x EXERCISE	1	1268.7	0.12	0.728
ID (DRUG x EXERCISE)	20	203472.8		
DAY	2	3890.6	0.55	0.585
DRUG x DAY	2	13141.7	1.84	0.175
EXERCISE x DAY	2	15696.6	2.20	0.128
DRUG x EXERCISE x DAY	2	31158.2	4.37	0.021
DAY x ID (DRUG x EXERCISE)	31	110482.1		