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Exercise Induced Hypervolemia: Role of Exercise Mode

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EXERCISE INDUCED HYPERVOLEMIA: ROLE OF EXERCISE MODE

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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

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

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ABSTRACT

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The supine posture has been shown to limit exercise-induced plasma volume expansion. Differences in hydrostatic pressure gradients between the standing and seated position indicate that treadmill exercise might promote a greater plasma volume expansion than cycle ergometer exercise. To test this hypothesis ten subjects performed intermittent high intensity exercise (4 min at 85% VO_2max, 5 min at 40% VO_2max repeated 8 times) on separate days on the treadmill and cycle ergometer. Changes in plasma volume expansion were calculated from changes in hematocrit and hemoglobin. Stroke volume (SV), trans-thoracic impedance (Z_0), HR, and arterial blood pressure (non-invasive arm cuff, SBP & DBP) were assessed in the seated position before and postexercise. Z_o increased ($p<0.05$) as subjects started exercise (both treadmill and cycling), indicating a reduction in central blood volume (CBV), which returned to baseline towards the end of exercise. Postexercise Z_o returned to control levels within 30 min regardless of the previous exercise mode. A significant post-exercise hypotension was observed following cycle ergometer exercise ($p<0.05$) but not following treadmill

exercise. Plasma volume increased 6.1±1.0% and 7.0 ± 1.1% (p<0.05) following treadmill and cycle ergometer exercise, respectively. The increase in PV was similar for both exercise modes. Initial differences in central blood volume disappeared over the course of the exercise protocol and during recovery, possibly indicating that there is a postural threshold and moving beyond it yields no further effect. The lack of differences between modes of exercise on plasma albumin content and Z_0 indicate that the upright postures were not different from each other. As such, PV expansion following high intensity intermittent exercise appears to be independent of upright exercise mode.

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Exercise Induced Hypervolemia: Role of Exercise Mode

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Abstract

The supine posture has been shown to limit exercise-induced plasma volume expansion. Differences in hydrostatic pressure gradients between the standing and seated position indicate that treadmill exercise might promote a greater plasma volume expansion than cycle ergometer exercise. To test this hypothesis ten subjects performed intermittent high intensity exercise (4 min at 85% VO_2max, 5 min at 40% VO_2max repeated 8 times) on separate days on the treadmill and cycle ergometer. Changes in plasma volume expansion were calculated from changes in hematocrit and hemoglobin. Stroke volume (SV), trans-thoracic impedance (Z_0), HR, and arterial blood pressure (non invasive arm cuff, SBP & DBP) were assessed in the seated position before and postexercise. Z_0 increased ($p<0.05$) as subjects started exercise (both treadmill and cycling), indicating a reduction in central blood volume (CBV), which returned to baseline towards the end of exercise. Postexercise Z_0 returned to control levels within 30 min regardless of the previous exercise mode. A significant postexercise hypotension was observed following cycle ergometer exercise ($P<0.05$) but not following treadmill exercise. Plasma volume increased 6.1±1.0% and 7.0 ± 1.1% ($p<0.05$) following treadmill and cycle ergometer exercise, respectively. The increase in PV was similar for both exercise modes. Initial differences in central blood volume disappeared over the course of the exercise protocol and during recovery, possibly indicating that there is a postural threshold and moving beyond it yields no further effect. The lack of differences between modes of exercise on plasma albumin content and Z_0 indicate that the upright postures were not different from

each other. As such, PV expansion following high intensity intermittent exercise appears to be independent of upright exercise mode.

Introduction

Plasma volume (PV) expansion is a well documented adaptation to aerobic

training (1). It can also occur acutely (within 24 h) after intense intermittent exercise on

the upright cycle ergometer (13). This adaptation provides cardiovascular stability (4, 7)

and improved thermoregulatory function in subsequent exercise bouts (4). Exercise

posture plays an important role in the expansion of PV in response to endurance training

(18) or acutely following intense intermittent exercise (13). Specifically, cycle ergometry

training in the supine posture does not elicit an increase in PV (18). In addition, PV

expansion that normally occurs within 24 hr of a high intensity intermittent exercise

protocol performed in the upright cycling posture is abolished when the same protocol is

performed in the supine position (13). Clearly, exercise posture plays a role in inducing

PV expansion.

Albumin dynamics are closely related to posture at rest (23) and during exercise

(14). Plasma albumin content increases after upright cycle ergometry training (1) and

following high intensity intermittent exercise (5, 6). However, high intensity intermittent

exercise in the supine posture does not increase plasma albumin content or PV (13).

Nagashima et al. (14) suggested that in the upright posture decreased central venous

pressure (CVP) lowered lymphatic outflow resistance and thereby increased lymphatic

delivery of protein (albumin) to the vascular compartment. The increased plasma albumin

content elevates plasma colloid osmotic pressure, drawing water into the vascular space.

In support of Nagashima et al. (14), Wu and Mack (23) clearly illustrated the immediate,

yet reversible, impact of variations in central venous pressure on lymphatic albumin

return. These data (14, 23) support the idea that postures which reduce CVP enable PV expansion in response to exercise because of an increase in plasma albumin (13).

Since PV expansion is not produced by supine exercise and is able to be demonstrated in the upright posture, we attempted to cause a greater PV expansion than that produced by upright cycling. We chose treadmill running in anticipation that it would even further decrease CVP because of its completely upright posture. Treadmill running has also been previously shown to increase lymphatic outflow and albumin clearance (9). These data indicate that treadmill running may produce a PV expansion.

The purpose of this study was to determine if a purely upright mode of exercise (i.e., treadmill running) would expand PV more than high intensity intermittent upright cycle ergometry. We hypothesized that it would presumably due to the greater reduction in central blood volume and CVP.

Methods

Subjects

Ten healthy active college age students (six males and four females), who were not involved in any endurance training program, participated in the current study. Subjects filled out a medical history and gave written informed consent to the current protocol that was approved by the University Human Subjects Institutional Review Board. The subjects' physical characteristics are as follows: age: 24 ± 1 years, weight: 72 ± 4 kg, height: 172 ± 3 cm, cycle ergometer $\dot{V}_{O_{2max}}$: 52.3 ± 1.5ml•kg^{-1}•min^{-1} and treadmill $\dot{V}_{O_{2max}}$: 48.6 ± 1.9ml•kg^{-1}•min^{-1}. $\dot{V}_{O_{2max}}$ was determined by indirect calorimetry (Parvo Medics Truemax 2400, Salt Lake City, UT) using a graded exercise

protocol at least 10 days prior to any experiments. Female subjects were studied only during the first five days after the menstrual cycle (follicular phase) and trials were separated by at least 28 days. Experimental trials for male subjects were separated by at least 10 days.

Experimental protocol

On separate days subjects performed two identical trials of high intensity intermittent exercise, one on an upright (seated) cycle ergometer (Lode Excalibur, Groningen, Netherlands) and one on a treadmill (Trackmaster, Full Vision Inc, Newton, KS). Each trial consisted of two consecutive days. Diet and fluid intake were controlled for 16 hr prior to the first experimental day and throughout each trial. On day one subjects reported to the lab wearing shoes and shorts and a sports bra for women. They were allowed 30 min to consume a fixed breakfast and 10 ml•kg^{-1} water. Upon completion of breakfast, subjects rested in the upright seated posture for one hour during which time they were instrumented. A venous catheter was placed in a large antecubital vein while electrocardiogram electrodes and cardiac impedance tape were applied to the surface of the body. Placement of the cardiac impedance tapes was documented in detail to allow for replicate placement on day 2 and in the subsequent trial. After 60 min subjects voided their bladders and returned to the upright seated posture for another hour to allow equilibration of body fluid compartments. A small blood sample (one ml) was taken 45 min after being seated to compare with the 60 min blood sample to verify stable baseline hemoglobin and hematocrit. After 60 min of rest heart rate (HR), stroke volume (SV), cardiac output (Q) and transthoracic impedance (Z_0) were recorded (1500B EGK

Sanborn Series Hewlett Packard Medical Electronics Waltham, MA and Minnesota Impedance Cardiograph model 304 B, Surcom Inc, Minneapolis, MN). Blood pressure (BP) was measured noninvasively with an automated brachial artery arm cuff (Colin 685 STBP Monitor, South Plainfield, NJ) on the opposite arm. Finally, a second blood sample (20 ml) was drawn. Subjects then voided their bladder again and the entire urine sample was collected to measure volume, specific gravity, osmolality and electrolytes. Next the subject performed a high intensity intermittent exercise protocol (4 min of 85% $\dot{V}O_{2max}$ followed by 5 min of 40% $\dot{V}O_{2max}$ repeated 8 times) on either the treadmill or cycle ergometer. During exercise, HR (S810i, Polar Electro, Oy, Finland) and transthoracic impedance were recorded. Upon completion of exercise, subjects voided their bladders for a second urine sample and returned to the seated upright posture for a 30 min recovery period during which HR, SV, Q, Z_0 and BP were measured at 15 and 30 min of recovery. At 30 min postexercise a blood sample (20 ml) was drawn. A third urine sample was then collected. Upon completion of the first day of testing, subjects received a 590 ml electrolyte replacement drink, lunch, dinner, water, and were then dismissed. They were instructed not to participate in any athletic activity before returning to the lab the next day. On day 2, the same procedures were followed exactly as on day 1 except no exercise was performed.

Measurements

Blood Analysis. For each blood sample 0.5 ml whole blood was used to measure hematocrit (Hct) and hemoglobin concentration ([Hb]). Hematocrit was determined using a microhematocrit technique and hemoglobin concentration was measured using a

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cyanomethemoglobin method. Changes in plasma volume were calculated from changes

in Hct and [Hb] using the following equation (3):

$$\Delta PV = \left(\frac{[Hb]_{pre}}{[Hb]_t} \times \frac{\left(1 - \frac{Hct_t}{100}\right)}{\left(1 - \frac{Hct_{pre}}{100}\right)} \times 100 \right) - 100$$

where: ΔPV, change in plasma volume; pre is value at baseline; and t is the value at time

t (30 min or 24 h postexercise). The remaining blood was divided into two vacutainers:

lithium heparin and serum for centrifugation. Lithium heparin plasma was used to

determine plasma osmolality (freezing point depression, Advanced Osmometer

Advanced Instruments, Norwood, MA), total protein concentration (Pierce BCA,

Rockford, IL), albumin concentration (BCG Eagle Diagnostic, De Soto, TX) and plasma

cortisol (ELISA, IBL, Hamburg, Germany). Serum was used to determine plasma sodium

and potassium concentrations using ion selective electrodes (Nova Biomedical electrolyte

8+, Waltham, MA).

Urine analysis. Urine volume was measured with a graduated cylinder. Urine

osmolality (freezing point depression), urine specific gravity (refractometery), and

electrolytes (ion selective electrodes) were determined on all urine samples.

Diet Intervention

Subjects' diet and fluid intake were controlled for 16 h prior to and throughout the

two-day experimental testing. The diet consisted of five meals, dinner the night before,

breakfast, lunch and dinner on the day of exercise and breakfast on the day after.

Breakfast, lunch and dinner consisted of 8 kcal•kg body weight $^{-1}$, 10 kcal•kg body

weight $^{-1}$, and 12 kcal•kg body weight $^{-1}$, respectively. Subjects were instructed to consume at least 10 ml•kg body weight $^{-1}$ of water with each meal. To aid in rehydration, subjects were given 590 ml of an electrolyte replacement drink upon leaving the laboratory after the first day of testing.

Data Analysis

We enrolled 10 subjects in this study based upon a power analysis that indicated we could detect a true difference in plasma volume of 3% at a $p < 0.05$ statistical significance level. We utilized the Dill/Costill equation (3) to estimate the change in plasma volume because Evan's Blue dye was unavailable at the time of these studies, which prevented measurement of absolute plasma volume. We estimated baseline plasma volume equal to 50 ml•kg body weight $^{-1}$, plasma content estimations were based upon this initial assumption. Values for PV and plasma solute contents were normalized to body weight. Due to problems with an initial baseline blood sample only enough blood was collected for determination of Hct and Hb. As such, plasma albumin and plasma albumin content were only present for 9 subjects.

Repeated measures ANOVA (exercise mode and time) was used to examine differences between treadmill and cycle ergometer responses. Post-hoc analysis were performed using the Tukey minimum significant difference test. Statistical significance was established at a confidence level of $p < 0.05$.

Results

Subjects completed 97 ± 1% of the expected treadmill workout (4 min at 85% $\dot{V}_{O_{2max}}$, 5 min at 40% $\dot{V}_{O_{2max}}$) and 92 ± 2 % of the expected cycle ergometer power output.

The mean HR during the 8 bouts of treadmill exercise was 179 ± 2 beats•min^{-1}, while the mean HR during cycle ergometer exercise was significantly lower, 173 ± 2 beats•min^{-1}.

The effect of exercise on plasma variables is shown in Table 1. Hematocrit decreased 24 h after treadmill running and cycle ergometry ($p<0.05$). Hemoglobin also decreased 24 h after both modes of exercise ($p<0.05$). Both modes of exercise produced significant increases in plasma volume 24 h post exercise ($p<0.05$).   Plasma volume expansion induced by cycle ergometry exercise (7.0 ± 1.1 %) was similar to that of treadmill exercise (6.1 ± 1.0 %). Plasma cortisol concentration increased significantly 30 min post cycle ergometry exercise while plasma cortisol levels after treadmill running did not change.

Table 2 shows the estimated plasma contents. Both exercise modes produced a significant increase in plasma albumin content. Plasma albumin increases after exercise were similar between modes of exercise. The magnitude of increase in plasma volume following exercise was proportional to the increase in estimated plasma albumin content regardless of exercise mode ($p<0.05$, Figure 1).

Figure 2 shows Z_0 monitored during exercise. At the start of exercise, Z_0 increased above the baseline values determined while subjects rested prior to exercise in the upright seated position. Z_0 showed a slow rise over the remainder of the cycle ergometry exercise protocol ($p<0.05$). The treadmill exercise did not significantly increase Z_0 throughout the exercise. However, during exercise, Z_0 was not different between modes of exercise. Thirty minutes after cycle ergometry exercise Z_0 was elevated above baseline and was higher than the value measured 30 min and 24 h post

treadmill exercise (p<0.05, Table 3). Z_0 returned to levels seen at baseline after treadmill running. Baseline mean arterial pressure (MAP) was similar prior to cycle ergometry and treadmill exercise. However, cycle ergometry produced a significant hypotension 30 min post. Treadmill running did not produce a postexercise hypotension.

The effect of exercise on urine variables is shown in Table 4. The renal responses to acute exercise showed no postural effects; there were no differences between modes.

Discussion

The primary finding of the present study was that plasma volume expansion 24 h after high intensity intermittent exercise was similar for treadmill running and cycle ergometry exercise. During exercise, Z_0 significantly increased only during cycle ergometry, treadmill running did not produce a significant increase in Z_0 (Figure 2). Following exercise an increase in recovery Z_0 was only seen in response to cycle ergometry exercise. Transthoracic impedance represents the electrical impedance of the thoracic cavity and is known to reflect changes in thoracic blood volume (16, 17). An increase in Z_0 indicates a reduction of central blood volume. As such, our assumption that treadmill exercise would result in a greater reduction in central blood volume during exercise was not supported by the transthoracic impedance data during exercise. Only during cycle ergometry did central blood volume decrease and lymphatic return, although not directly measured, was probably increased (23).

Earlier research indicated that exercise in the supine posture did not result in plasma volume expansion 24 h after high intensity intermittent exercise (18). The lack of plasma volume expansion was attributed to an increase in central venous pressure,

possibly preventing lymphatic return. It is thought that an increase in lymphatic return

can contribute to an increase in plasma albumin, which can then exert a greater colloid

pressure, drawing in more water to the vascular space. We proposed that the more

upright posture of treadmill running versus that of upright cycle ergometry would result

in a greater increase in plasma protein content, presumably because of a larger reduction

in central venous pressure and a greater lymphatic delivery of protein to the vascular

compartment (9, 13, 19, 20). Both knee extension exercise (10) and treadmill running

increase lymphatic outflow and albumin clearance (9). These findings are supportive of

our hypothesis that treadmill running would be able to elicit a PV expansion. However,

the plasma volume expansion following the cycle ergometry and treadmill running were

similar. The similar PV expansions we are reporting are most likely because running

produced similar increases in plasma albumin content regardless of the Z_0 response to

exercise. The increase in plasma volume associated with the increase in plasma albumin

content was similar for each exercise mode. Figure 1 shows the treadmill and cycle

ergometry pooled data for albumin content.

We noted similar increases in Z_0 (estimating similar reductions in central blood

volume and CVP) and similar increases in plasma protein content. These data indicate

that postural influence on exercise-induced plasma volume expansion may have some

upper limit. Whereas, the change in posture from supine to the upright seated position

(cycle ergometry exercise) provides a significant effect on facilitating increased

lymphatic outflow, moving from the seated to the standing position has little additional

impact on reducing lymphatic outflow resistance or the redistribution of albumin to the

vascular compartment and have no additional effects on PV. A similar example of this optimal homeostatic response is seen with the reflex control of atrial natriuretic peptide (ANP). ANP is released from the atrial myocytes in response to increases in CVP. As such, plasma ANP levels are lowest in the standing position when CVP is low and higher in the supine posture when CVP is high (21). However, moving from the supine posture to the head-down tilt position does not increase plasma ANP levels further, despite the additional increase in CVP.

Alternatively, postexercise hypotension associated with cycle ergometry exercise is known to contribute to PV expansion (11). In this experiment, cycle ergometry exercise produced a significant postexercise hypotension while treadmill running did not. The postexercise hypotension may have contributed to the plasma volume expansion following cycle ergometry exercise and may have minimized the impact of posture on PV expansion. It is interesting to note that while cycling produced a hypotensive status and larger increase in recovery Z_0, treadmill running yielded a similar PV expansion. It is unclear why the PV expansions were equal. It is possible that the upright posture in treadmill running did impact the magnitude of PV expansion but that postexercise hypotension and greater pooling (greater increase in Z_0) following cycle ergometer exercise compensated for the postural differences. Regardless, further research is needed in order to determine the contribution of postexercise hypotension to PV expansion in differing modes of exercise.

Plasma cortisol concentrations were higher after cycle ergometry than treadmill exercise. The increased cortisol concentration may confound the postural effects of the

experiment. Cortisol is known to increase plasma albumin as well as PV (12). Yet there were no differences between modes of exercise on plasma albumin or PV increase. However, cortisol may have acted to increase plasma albumin beyond what would have occurred through a postural stimulus alone. The increased cortisol levels produced by cycle ergometry may have contributed to the PV expansion independently of posture.

PV expansion occurs from a combination of two major mechanisms, an increase in plasma albumin and an increase in water and sodium retention. Plasma albumin increases occur through several different mechanisms. In an acute setting (PV expansion in 24 h) there is known to be an increase in albumin redistribution from the interstitial space to the vascular compartment via the lymphatics (1). There is also known to be a reduction in transcapillary escape (8). Increases in albumin due to chronic training have also been attributed to increases in albumin synthesis (24). Water and sodium retention also contribute to the increase in plasma volume in an acute time frame. There is known to be a reduction in sodium excretion and urine output (2), possibly due to an increase in anti-diuretic hormone (ADH) as well as increase sodium retention (15). Sodium retention is shown to be triggered in two ways, reduced renal blood flow as a result of a drop in MAP (22) and aldosterone mediated sodium retention (15).

In this study, we speculate that the PV expansion seen, is due primarily to an increased plasma albumin content (Figure 1). The Z_0 data indicate that central blood volume did decrease, possibly increasing the lymphatic return of albumin to the vascular compartment. The decrease in MAP seen after exercise in the cycle ergometer may also have affected plasma albumin escape by reducing the transcapillary escape rate via a

decrease in capillary filtration pressure. We did see a significant decrease in urine output and urine sodium concentration, indicating that body water was retained. We did not measure ADH and therefore cannot provide any insight with regards to the mechanism behind the water and sodium retention.

In conclusion, acute exercise induced PV expansion occurs after treadmill running as well as upright cycle ergometry. However, treadmill running does not produce a greater expansion of PV than cycle ergometry. Presumably there was no difference in PV expansion between modes of exercise because there was no difference in Z_0 during exercise and there were no differences in plasma protein content 24 h after exercise. There may be confounding variables such as increased plasma cortisol and postexercise hypotension that contributed to the PV expansion independently from posture. There may also exist a threshold for postural changes on cardiovascular impact and moving beyond this threshold yields no further effects.

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Table 1. Plasma variables.

	Treadmill			Cycle Ergometer		
Variable	BL	P 30 min	P 24 hr	BL	P 30 min	P 24 hr
Body Weight, kg	71.8 ± 4.2	---------	71.7 ± 4.2	71.7 ± 4.3	----------	72.0 ± 4.7
Hct, %	44.3 ± 1.0	44.9 ± 0.9	43.4 ± 1.0*	44.7 ± 1.2	44.8 ± 1.0	43.2 ± 1.1*
Hb, g·dl^{-1}	14.7 ± 0.4	14.9 ± 0.4	14.1 ± 0.4*	14.8 ± 0.5	15.1 ± 0.5	14.3 ± 0.4*
Δ PV %	---------	-0.9 ± 1.5	6.1 ± 1.0*	---------	-1.6 ± 1.6	7.0 ± 1.1*
[Na]p, mM	139 ± 2	137 ± 2	139 ± 1	138 ± 2	140 ± 1	139 ± 1
[K]p, mM	3.9 ± 0.1	4.2 ± 0.2	4.0 ± 0.1	3.9 ± 0.1	4.2 ± 0.1*	4.0 ± 0.1
Posm, mOsm·kg^{-1}	287 ± 1	292 ± 2*	287 ± 1	287 ± 1	290 ± 1*	287 ± 1
TP, g·dl^{-1}	6.5 ± 0.3	7.3 ± 0.48	6.52 ± 0.16	6.67 ± 0.15	6.86 ± 0.27	6.66 ± 0.19
ALB, g·dl^{-1}	5.4 ± .01	5.63 ± 0.13	5.42 ± 0.11	5.44 ± 0.17	5.63 ± 0.18	5.55 ± 0.17
CORT, ng·ml^{-1}	169 ± 29	221 ± 56	186 ± 43	104 ± 20	219 ± 21*	120 ± 17

BL, baseline; P 30 min, 30 min postexercise; P 24 hr, 24 h postexercise; Hct, hematocrit; Hb, hemoglobin; ΔPV, change in plasma volume; [Na]p, plasma sodium; [K]p, plasma potassium; Posm, plasma osmolality; TP, plasma total protein; ALB, plasma albumin; CORT, plasma cortisol. Values are given as mean ± 1 SEM of 10 subjects. *$p < 0.05$ different from baseline.

Table 2. Estimated plasma content

	Treadmill			Cycle Ergometer		
Variable	BL	P 30 min	P 24 hr	BL	P 30 min	P 24 hr
ALB, g·kg^{-1}	2.71 ± 0.62	2.82 ± 0.07	2.87 ± 0.56*	2.77 ± 0.78	2.84 ± 0.09	3.02 ± 1.13*
TP g·kg^{-1}	3.23 ± 0.04	3.55 ± 0.24	3.46 ± 0.88*	3.33 ± 0.68	3.15 ± 0.37	3.59 ± 1.1*
Osm, mOsm	997 ± 56	1011 ± 74	1060 ± 64*	1033 ± 69	1019 ± 62	1099 ± 62*

BL, baseline; P 30 min, 30 min postexercise; P 24 hr, 24 h postexercise; ALB, estimated plasma albumin content; TP, estimated total protein content; Osm, estimated plasma osmolar content. Values are given as mean ± 1 SEM of 9 subjects. * $p < 0.05$ different from baseline.

Table 3. Resting cardiovascular variables

	Treadmill			Cycle Ergometer		
Variable	BL	P 30 min	P 24 hr	BL	P 30 min	P 24 hr
SBP, mmHg	114 ± 3	104 ± 2*	112 ± 3	117 ± 4	106 ± 3*	111 ± 3*
DBP, mmHg	64 ± 3	65 ± 3	66 ± 3	69 ± 4	65 ± 2	67 ± 2
MAP, mmHg	81 ± 3	79 ± 2	82 ± 3	85 ± 4	79 ± 2*	81 ± 2*
HR, bpm	62 ± 4	86 ± 3*	63 ± 3	59 ± 2	79 ± 2*	59 ± 2
SV, ml·beat^{-1}	113 ± 6	94 ± 5*	112 ± 4	117 ± 6	94 ± 6*	99 ±10
CO, L·min^{-1}	7.1 ± 0.5	8.1 ± 0.5*	7.0 ± 0.4	6.7 ± 0.4	7.6 ± 0.4	6.5 ± 0.2
TPR, RU	11.4 ± 0.7	9.8 ± 0.7	11.8 ± 0.7	12.5 ± 1.0	10.3 ± 0.5	12.2 ± 0.5
Z_0, ohms	27.2 ± 1.6	27.0 ±1.5	28.1 ± 1.5	27.5 ± 1.4	28.4 ± 1.6*†	27.0 ± 1.4†

BL, baseline; P 30 min, 30 min postexercise; P 24 hr, 24 h postexercise; SBP, systolic blood pressure in mmHg; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate; bpm, beats per min; SV, cardiac stroke volume; CO, cardiac output; TRP, total peripheral resistance; RU, resistance units, mmHg•min•L^{-1}; Z_0, transthoracic impedance. Values are given as mean ± 1 SEM of 10 subjects. *,$p<0.05$ different from baseline; † $p<0.05$ different from treadmill.

Table 4. Urine variables.

Variable	Treadmill			Cycle Ergometer		
	BL	P 30 min	P 24 hr	BL	P 30 min	P 24 hr
Uvol, ml	346 ± 48	23 ± 4*	297 ± 50	351 ± 39	37 ± 10*	352 ± 54
[Na]u, mM	46 ± 6	235 ± 12*	63 ± 8	51 ± 6	210 ± 23*	55 ± 9
Na Ex, mmols	14.0 ± 1.1	5.5 ± 1.0*	17.4 ± 3.9	18.3 ± 2.9	7.7 ± 1.8*	19.2 ± 3.9
Uosm, mOsm·kg^{-1}	176 ± 23	744 ± 27*	263 ± 49	162 ± 20	659 ± 40*	170 ± 19
[K]u, mM	10 ± 2	69 ± 6*	13 ± 4	9 ± 2	56 ± 9*	10 ± 2
Usg	1.005 ± 0.001	1.022 ± 0.001*	1.008 ± 0.001	1.005 ± 0.001	1.020 ±0.001*	1.005 ± 0.001

BL, baseline; P 30 min, 30 min postexercise; P 24 hr, 24 h postexercise; Uvol, urine volume; [Na]u, urine

sodium; Na Ex, urine sodium excretion; Uosm, urine osmolality; [K]u, urine potassium; Usg, urine specific

gravity. Values are given as mean ± 1 SEM of 10 subjects. *$p < 0.05$ different from baseline.

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Figure Legends:

Figure 1 . Relationship of the change in plasma volume 24 h following exercise and the change in estimated plasma albumin content. Individual data for each subject under both exercise modes. Regression line based upon only those data in which the change in plasma albumin content was greater than or equal to zero (n=16) for both exercise modes. Best fit line by least squares linear regression.

Figure 2. Changes in transthoracic impedance over the course of the exercise protocol. The first Z_0 value was collected prior to exercise. Values are given as mean $\pm$ 1 SEM of 10 subjects. *$p < 0.05$ different from baseline, time 0.
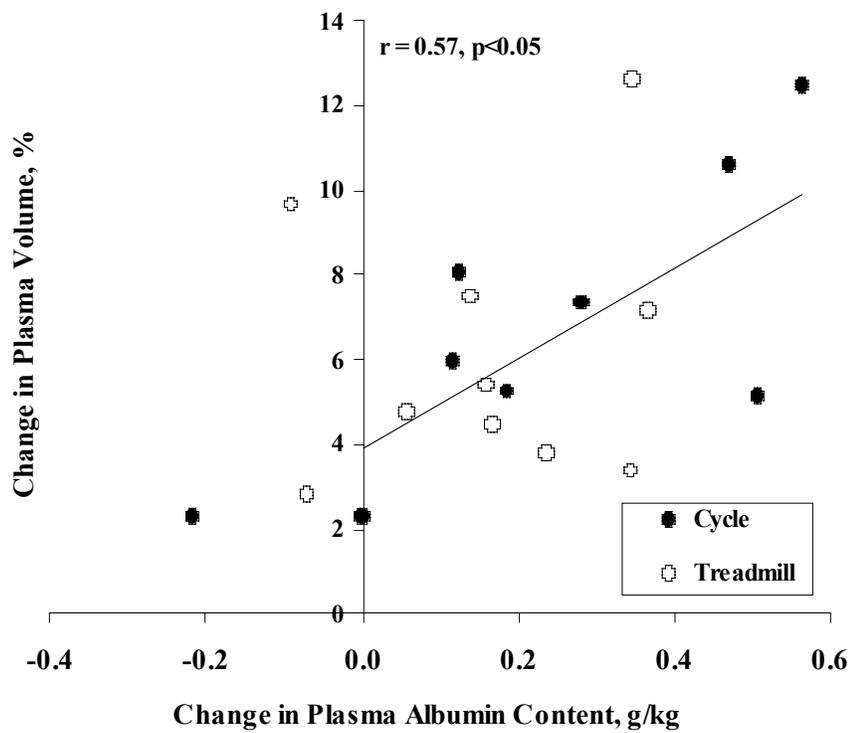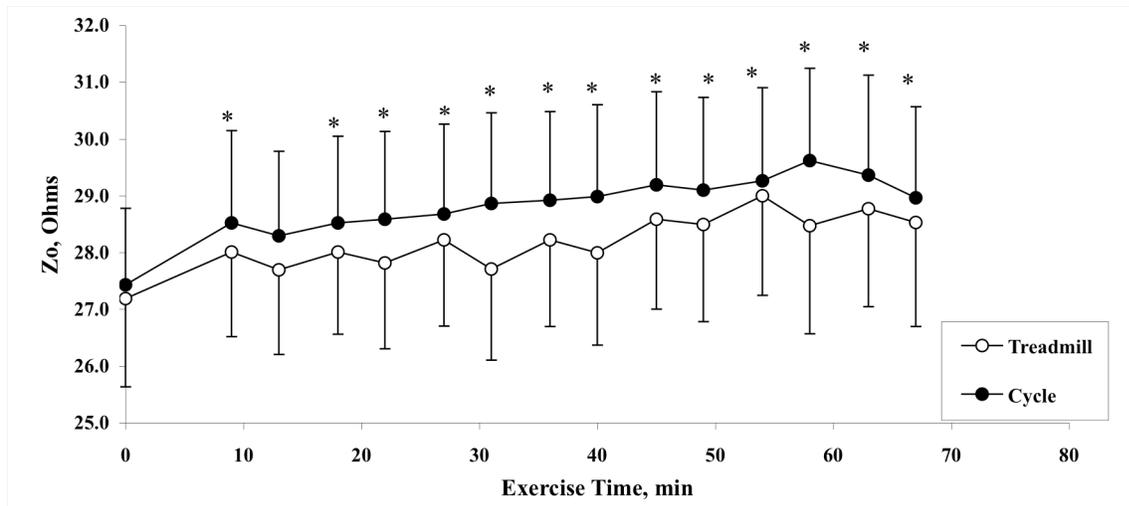
Figure 1.

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Figure 2.

Appendix A

Prospectus

Chapter 1

Introduction

Plasma volume (PV) expansion is a well documented adaptation to aerobic training (4). It is an adaptation to chronic endurance training and can also be seen acutely after intense intermittent exercise (20). Exercise posture plays a large role in the acute expansion of PV. Supine cycle ergometry will not elicit an increase in PV, however, upright cycle ergometry will increase PV 24 hours after exercise (20). Increased PV is an advantageous adaptation to exercise because it can increase maximal cardiac output ($\dot{Q}_{max}$) and allows the body to maintain blood flow to the working skeletal muscle and the skin for heat transfer (24).

It is well documented that albumin content of the plasma increases after upright cycle ergometry (8), (9). Supine cycle ergometry did not produce increases of plasma albumin and likewise no increase in PV (20). Albumin dynamics are closely related to posture during exercise. Nagashima et al.. (21) suggested in 1999 that upright posture during exercise decreases central venous pressure (CVP) and allows for increased lymph outflow. Decreases in CVP improve lymph outflow enabling a redistribution of albumin from interstitial space to vascular space. The increased plasma albumin increases colloid pressure, which draws water into the vascular space in order to maintain a concentration equilibrium. The results of Nagashima et al.. suggest that with further decreases in CVP there would be an even greater PV expansion.

Even though posture of exercise has been shown to play a significant role in PV expansion in cycle ergometry ((20), the effect of running on PV expansion is unknown.

If a running upright posture further decreases CVP, then it is possible to further augment lymph outflow and demonstrate a greater PV expansion.

Statement of Problem

The purpose of this study is to compare the effect of running versus upright cycle ergometer exercise on exercise-induced PV expansion.

Alternative and Null Hypothesis

I hypothesize that treadmill running at a similar exercise intensity and duration will elicit a greater hypervolemic response (i.e. greater PV expansion) than upright cycle ergometry. The null hypothesis is that there will be no difference in the hypervolemic response to treadmill running compared to that of cycle ergometer exercise.

Assumptions

Subjects will exercise sufficiently to induce hypervolemia. Treadmill running will elicit a hypervolemic effect. During data collection subjects will follow a controlled diet and will not exercise except as part of the study protocol. The exercise stimulus, as defined by a given percentage of their posture-specific maximal aerobic capacity, is equivalent for both exercise modes.

Delimitations

This study will be delimited to individuals who are currently active, not beginning novel exercise programs, not on medications, have no cardiovascular disease and are generally healthy. The study is also delimited to females during the first 5 d after the menstrual cycle.

Limitations

Limitations in this study could be subjects may not finish the $\dot{V}_{O_{2max}}$ protocol. Subjects may not finish the treadmill or cycle ergometer protocol. Subjects may eat/drink more than the controlled diet. Subjects may not eat enough of the controlled diet the night before. Females may be studied during a phase of the menstrual cycle other than the first 5 d after the menstrual period. Subjects may participate in heavy exercise outside of the study.

Significance

Hypervolemia is a well documented result of intense intermittent exercise (4). It is known that the acute expansion of PV is due to an increase in plasma albumin (20). Previous work done by Nagashima et al.. (21) showed that cycle ergometry in the upright position increased PV, but supine cycle ergometry did not. Because CVP was decreased in upright cycle ergometry and increased in supine cycle ergometry, elevated CVP may be a factor in the expansion of PV. Those results suggest that posture plays a significant role in albumin dynamics. Whether similar PV and CVP results would be obtained during running exercise has not been reported in the literature. Therefore, measuring CVP after running and upright cycling may provide further insight into CVP as a possible cause of PV expansion.

Chapter 2

Review of Literature

Water makes up about 45-74% (7) of the mass of the human body. Two-thirds of the total body water is found in intracellular fluid (ICF) while one-third is found in the extracellular fluid (ECF), a compartment that includes the fluid surrounding cells and the vascular space (7). Body water contains numerous materials found in solution. Due to the dissolved nature of these materials, they are available for transport throughout the body. Water moves throughout the body passively by osmosis. The make up of the ICF is dictated by the cell membrane's permeability and transport characteristics. Both the ICF and ECF are similar in total osmolality, about 286 mOsm/kg^{-1} water. The ECF is made up of the blood plasma and fluid outside the cells. The ECF is transported via circulating plasma and then transported through the capillary to the space outside the vascular space or interstitial fluid (ISF). Changes that occur in any of the fluid compartments of the body will result in a redistribution of the body water and a change in solute concentrations in all of the compartments.

The distribution of water in the compartments is dependent upon the quantity of the solutes in each compartment. Both water and the solutes move together as they are passively transported between compartments. Water moves by bulk flow across the capillary through fenestrations, clefts, membrane pores and by passive diffusion. Once inside the ISF, the hydrostatic and osmotic forces that regulate fluid movement are dependent upon gradients to move from the ISF to the ICF. The distribution of water in the body varies according with age, gender, weight, and lean body mass (7).

The lymphatic system, responsible for the transport of lymph, plays a role in the distribution of body water and plasma proteins. Fluids are transported from the ISF to the vascular system via the capillary. Capillary exchange is determined by the characteristics of the transcapillary pressures, protein concentrations, and the capillary membrane (2). These three factors can alter the amount of exchange that occurs between the lymphatic system and the ISF, and ultimately the vascular system. When capillary filtration is high, such as when there is a high venous pressure, with low plasma flow, plasma proteins do not enter the lymphatic system and remain plasma bound, increasing the colloid osmotic pressure (23). Increased colloid osmotic pressures will draw water into the ISF, increasing the plasma volume and contributing to a hypervolemia.

Hypervolemia is a result of chronic aerobic training (4, 8, 9, 25). Cross sectional studies have demonstrated that athletes have a greater blood volume (BV) and extracellular fluid compartment than sedentary populations (18). Both the plasma and red blood cell content of the blood are increased in response to exercise (3). Expansion of the blood volume following acute exercise has been observed and is primarily attributed to plasma volume expansion (13, 20). Plasma volume is also reported to increase as a result of a high intensity intermittent exercise protocol (1, 12, 31).

There are two physiological advantages of an increased PV. First, the increase in PV expands the total blood volume. During exercise, blood is used to serve two primary functions: transport oxygen to working skeletal muscle and transfer heat to the skin. Harrison et al. (14) demonstrated a loss of water from the intravascular space during exercise. This drop in BV is accompanied by an increase in core temperature (19) which

can lead to exercise related heat illness. It has been demonstrated that as BV decreased

due to exercise in a dehydrated state, stroke volume (SV), cardiac output ($\dot{Q}$) , skeletal

muscle blood flow and skin blood flow decreased while esophageal temperature rose (10,

11). Body temperature increases as skin blood flow is reduced. These data demonstrate

the negative effects of a decreased BV. With an increased BV, more body fluid can be

lost before the negative effects of lowered SV, $\dot{Q}$, skin blood flow and increased body

temperature inhibit performance.

Second, increasing BV will increase maximum oxygen uptake ($\dot{V}o_{2max}$) . $\dot{V}o_{2max}$ is

defined as $\dot{Q}_{max}$ times the maximal arterial-venous oxygen difference. $\dot{Q}_{max}$ will increase

with aerobic training as a result of increased SV (28). Increased SV is a byproduct of two

events, increased filling and stretch of the heart both of which are partly due to an

increased mean systemic filling pressure caused by larger circulating volume of blood.

Therefore, the result of increasing BV is an increased SV and subsequent rise in $\dot{Q}_{max}$ (28)

and $\dot{V}o_{2max}$.

There are several possible mechanisms that could lead to acute PV expansion

after exercise. An increase in albumin content of the plasma is well documented (4, 8,

21). It is understood currently that the increase in PV is associated with an increase in

plasma protein content, approximately 85% of total plasma protein is albumin (8). There

are several factors that contribute to an increase in plasma albumin content, i.e. a

redistribution of albumin from the interstitial to the intravascular space (25), a reduced

transcapillary escape rate (TER) of albumin and increased albumin rate of synthesis.

Plasma albumin content increases immediately after upright exercise (20). It is suggested by Nagashima et al. that this leads to an increase in plasma oncotic pressure (21) which directly increases PV as water is moved into the intravascular space. Since one gram of albumin binds approximately 18 ml of water (27, 30), the 10% increase in albumin content (≈15g) and subsequent PV expansion found by Convertino et al. (4) and Gillen et al. (8) is consistent with this hypothesis. Albumin content has been shown to increase within 1 h of recovery from exercise (20). This elevation of plasma albumin remains for 48 h post exercise (8).

The immediate increase in plasma albumin content occurs too rapidly to be completely attributed to an increase in albumin synthesis. It is hypothesized that it is due primarily to a redistribution of albumin from the interstitial space to the intravascular compartment (22). This redistribution of albumin is thought to result from increased lymph flow that is characteristic of upright exercise (20). Reed et al. (26) showed that 75-80% of intramuscular albumin was cleared via the lymphatics. Using these data, it can be understood that when lymph outflow is increased so will albumin clearance from exercising muscles. Nagashima et al. found similar data in 2001. Lymph flow was increased by skin hyperemia and muscle pumping (22). Havas et al. (17) found in 1997 that lymph flow was higher during knee extension exercises than at rest, demonstrating that muscle contractions augment lymph flow, aiding in albumin transport and distribution. Havas et al. have also demonstrated that there is higher albumin clearance in steady-state running than in rest (16). Higher albumin clearance rates during exercise are associated with increased lymph outflow. Results showed an initial increase in albumin

clearance during the first 15 min followed by a decline in clearance during the next 25 min and a further decrease after exercise. Despite the decreasing trend of albumin clearance during exercise, all measurements were still significantly higher than at rest throughout the exercise bout. During recovery following exercise, albumin clearance was equal to pre-exercise measures. Running therefore has been shown to increase lymph outflow and redistribute albumin to the vascular space. Nagashima et al. (20) demonstrated that supine exercise did not increase plasma albumin content. It is suggested that the elevated central venous pressure, seen in supine exercise, limits lymph flow, thus preventing a redistribution of albumin (30).

Haskell et al. (15) found a reduction in TER of albumin 24 h after upright exercise, which accompanied an increased plasma albumin content. A reduced TER of albumin acts to retain the redistributed albumin in the plasma. Yang et al. (33) reported that albumin synthetic rate increased 3-6 h after upright intense intermittent exercise. Nagashima et al. (20) demonstrated an increased albumin synthesis 24 hours post exercise. Fractional albumin synthesis rate seen after a single bout of exercise increased from 5.9 ± 0.5 to $6.4 \pm 0.5\%$ per day. This is insufficient to account for the elevated albumin content seen during the first four hours of recovery (33).

Hormones play a role in PV expansion and regulation. Nagashima et al. (21) reported in 1999 that aldosterone increased during exercise and remained higher than control for 2 hours into recovery. It was also reported that there was a decrease in sodium clearance and urine sodium/potassium clearance ratio, factors that contribute to water retention. Convertino et al. demonstrated water retention after 10 d of exercise training,

suggesting that extracellular fluid volume expansion contributes to PV expansion (5) .

These findings suggest an activated renin-angiotensin-aldosterone axis after intense

intermittent exercise. Even after one exercise bout, a significant reduction is seen in

sodium, chloride, and water excretions for 48 hr post exercise (6). In 2001 Nagashima et

al. (22) reported increased sodium reabsorption in the proximal tubules, possibly a result

of decreased renal blood flow. This same study also showed a baroreflex mediated

reduction in fluid regulating hormones after saline infusion, supporting the hypothesis

that changes in renal function and homeostatic control of volume regulating hormones

after intense intermittent exercise contribute to the expansion of PV.

Roy et al. (29) induced PV expansion by 15.8±2.2% in untrained athletes using a

6% Dextran or 10% Pentispan solution. The subjects then exercised for 90 min on a cycle

ergometer at 60% $\dot{V}_{O_{2max}}$. Exercise with no induced PV expansion resulted in significant

increases in plasma vasopressin (AVP), plasma rennin activity (PRA), aldosterone

(ALD), alpha atrial naturetic peptide (alpha-ANP), and the catecholamines

norepinephrine (NE) and epinephrine (EPI). Exercise with PV expansion blunted the

increases in AVP, PRA, ALD, NE and EPI, during the exercise itself. The concentration

of alpha-ANP was also lower during exercise following PV expansion, an effect that

could be attributed to the lower resting levels. No differences in osmolality were

observed between conditions.

Expansion of PV is also influenced heavily by the posture of the subject during

exercise. Nagashima et al. reported (21) that after intense intermittent exercise in the

upright position there was a 6.4% increase in PV at 22 h of recovery, whereas supine

exercise yielded no PV expansion. The authors hypothesized that posture affects lymph albumin dynamics.

The supine posture results in an increased CVP and is theorized to decrease lymph flow into the vascular space and thus decrease albumin redistribution into the plasma from the extracellular space. Mechanical reduction of CVP in the supine posture using lower body negative pressure enhances lymphatic delivery of albumin to the vascular compartment (32). In addition, a mechanical increase of CVP in the seated posture using lower positive pressure attenuates lymphatic delivery of albumin to the vascular compartment (32).

These observations provide indirect support for the hypothesis that lymphatic albumin distribution to the blood is altered by posture and may explain PV expansion following exercise. There exist no data on PV expansion while maintaining a completely weight bearing posture during exercise. It is expected that the upright posture during running would elicit a greater stimulus for PV expansion than that seen in the upright, seated position during cycle ergometer exercise due to a hypothesized greater decrease in CVP and potentially greater increase in lymph outflow.

Chapter 3

Methods

This study is designed to evaluate the effects of treadmill running and upright

cycle ergometry on blood volume. Subjects will perform a posture specific $\dot{V}_{O_{2max}}$ test

prior to data collection on the cycle ergometer/treadmill. $\dot{V}_{O_{2max}}$ will be achieved when the

subject meets two of the three qualifications: respiratory exchange ratio (RER) >1.1, max

heart rate is reached and/or a plateau in $\dot{V}_{O_{2max}}$ readings. These measures of $\dot{V}_{O_{2max}}$ will be

used to formulate a workload on the bike and treadmill for the acute exercise protocol.

For data collection subjects will complete a high intensity intermittent exercise protocol

on a treadmill and cycle ergometer in random order. The trials will be separated by a

minimum of 10 d for males and approximately 28 d for females. Testing on female

subjects will be conducted during the first 5 d after initiation of their menstrual cycle. The

primary dependent variables will be pre and post exercise PV, determined by changes in

hemoglobin, hematocrit and changes in albumin content.

Subjects

Ten college age students (five male and five female) will be recruited to

participate. Subjects will be excluded if they are on medications and currently highly

trained (defined as participating in a current competitive training program). Subjects will

be asked to maintain current fitness levels and not to begin new exercise programs or

terminate a current program. During the two-day data collection, subjects will limit their

exercise to that proscribed in the study. Female subjects will only be studied during the

first 5 d after the menstrual cycle (follicular phase). Subjects' health and risks will be

assessed through a screening questionnaire. Written informed consent will be obtained from all subjects. The BYU Institutional Review Board will approve all experimental procedures. Identities of subjects will be kept confidential.

Maximal Oxygen Capacity

All subjects will perform two $\dot{V}_{O_{2max}}$ exercise tests prior to data collection, a treadmill max test and upright cycle ergometer max test. The $\dot{V}_{O_{2max}}$ tests will be a ramped protocol to indicate submaximal workloads. The ramped cycle ergometer max protocol will begin at 100 W for males and 50 W for females. It will increase 1 W every 4 s until volitional exhaustion. The highest wattage achieved will be the max and percentages of max wattage will be derived to use in the submaximal protocol on the cycle ergometer. For the treadmill max test the subject will commence walking at 3.5 mph for a period of 5 min. Speed will increase to 5 mph and then raise 0.5 mph every 30 s until 7.5 mph is reached. If at 7.5 mph exhaustion is not reached, the incline will increase 0.5% grade every 30 s until exhaustion. $\dot{V}_{O_2}$measures from the treadmill will be recorded along with speed and grade in order to quantify intensity for data collection trials.

Cycle Ergometer Data Protocol

At least ten days after the protocol subjects will cycle for 5 min at 50 W as a warm-up. The wattage will be increased to that which yielded a 85% $\dot{V}_{O_{2max}}$ during the test for 4 min. This will be followed by 5 min at the wattage that elicited 40% $\dot{V}_{O_{2max}}$. Heart rates will be recorded. There will be 8 bouts of 85% and 40% $\dot{V}_{O_{2max}}$ followed by a 5 min cool down at 50 W.

Treadmill Data Protocol

Subjects will walk on a treadmill at self selected speed for 5 min to warm up. The intensity will increase to 85% of $\dot{V}_{O_{2max}}$ for 4 min followed by a 5 min period at 40% $\dot{V}_{O_{2max}}$. This will be repeated 8 times followed by walking at 3 mph as a cool down. Heart rate will be recorded throughout the protocol.

Dependent Variables

Blood sampling. All blood sampling is done via a 18-gauge IV catheter (Johnson and Johnson, Arlington, TX) placed in an superficial arm vein. Subjects must be seated for one hour prior to sampling to ensure a steady state in plasma volume and constituents. Sampling is done from free-flowing blood; fluid from the system dead space will be discarded prior to sampling and the dead space will be filled with normal saline after sampling. Samples are taken 1 hr before exercise, immediately before exercise, 30 min after, exercise and 24 hr after exercise. Sample 1 will be 2 ml, samples 2-4 will be 20 ml each. The catheter is flushed regularly with normal saline to prevent clotting of the catheter. Hemoglobin concentration, hematocrit, albumin, total protein, EPO, catecholamine, plasma osmolality, and electrolytes will be measured.

Plasma Volume. PV changes will be measured using two methods, Evans Blue Dye and an equation which calculates PV from Hb and Hct data determined from the blood analysis. Specifically, $\Delta PV = ((((Hb1/Hb_x)*(1-Hct_x)/100)/(1-Hct1)/100)*100)-100$

(1)

which uses hemoglobin concentration (Hb) from the first sample (Hb1) and 3 subsequent measures (Hb_x) and hematocrit (Hct) from the first sample (Hct1) and the 3 following measures (Hct_x).

Evans Blue Dye Method. This technique involves injection of an accurately determined volume of dye (specific gravity of dye is 1.0) into an arm vein and sampling blood for determination of dye dilution after complete mixing has occurred (at 10, 20, and 30 min). The amount of dye injected is 0.05mg of Evans Blue Dye per kg bodyweight. Plasma volume is determined from the product of the concentration and volume of dye injected, divided by the concentration in plasma after mixing. Blood volume is calculated from plasma volume and hematocrit concentration and corrected for peripheral sampling.

Urine Collection. Urine collections will be used to establish water retention. Subjects will be escorted to a private restroom where they will be asked to void into a container for the collection of urine at four times throughout the study. Time of urination, volume, osmolality, sodium and potassium concentrations will be recorded.

Cardiovascular Parameters. The following cardiovascular parameters are monitored to quantify the circulatory stress. Systolic (SBP) and diastolic (DBP) blood pressures (in units of mmHg) are measured with an automated arm cuff (Colin 685 STBP Monitor, South Plainfield, NJ). Mean arterial pressure (MAP) is calculated as (2xDBP + SBP)/3. An electrocardiogram (EKG) is used to determine heart rate (HR) and provides timing information for the ensemble averaging impedance cardiography and gating signals for Korotkoff sounds. Heart sounds recorded by a phonograph microphone are

used to verify cardiac cycle timing. SV is measured using impedance cardiography, which requires the placement of four EKG electrodes onto the subject's torso. CVP will be estimated from the impedance data.

The following time line provides an overview of the experimental protocols:

TIME	ACTIVITY	MEASUREMENT

DAY ONE

TIME	ACTIVITY	MEASUREMENT
7:00AM-7:30AM	Breakfast and water provided	Void at 7:00AM
7:30AM-8:45AM	Insert catheters and prep	Void at 8:45AM
8:45AM-9:45AM	Seated rest	HR, SV, BP, B, U, BW
9:45AM-11:00AM	Exercise	HR, BP, BW
11:00AM-11:30AM	30 minute period of seated rest	SV,BP, HR, BW, B, U

DAY TWO

TIME	ACTIVITY	MEASUREMENT
7:00AM-7:30AM	Breakfast and water provided	Void at 7:00AM
7:30AM-8:45AM	Insert catheters and prep	Void at 8:45AM
8:45AM – 9:45AM	Seated rest	HR, BP, B
9:45AM- 10:15AM	Plasma volume measurement	Inject dye at 9:45AM, B

BW= Bodyweight, U = Urine Collection, B = Blood Sample, HR = Heart Rate, BP = Blood Pressure, SV = Stroke Volume

Diet Intervention

Subjects will be provided with a controlled diet. The diet will consist of 5 meals, dinner the night before, breakfast, lunch and dinner on day of exercise and breakfast on day after. Breakfasts will consist of 8 kcal/kg body weight (BW) and 10 ml/kg BW of water. Lunch will be 12 kcal/kg BW and 10 ml/kg BW of water. Dinners will be 15 kcal/kg BW and 15 ml/kg BW of water.

Data Analysis

The number of replications (subjects) needed to detect a given true difference between means was determined from the following equation:

$n \geq 2 \cdot (s/d)^2 \cdot [t_{a[v]} + t_{2(1-P)[v]}]^2$ where n was the number of replicates; s true standard deviation; d, the smallest true difference that is desired to detect, t, significance level; v, degrees of freedom of the sample standard deviation; P, desired probability that a difference will be found to be significant or the desired power of the test; and $t_{a[v]} + t_{2(1-P)[v]}$, values from a two tailed t table with v degrees of freedom and corresponding to probabilities of s and 2(1-P). Determination of n is through an iterative process and requires some estimate of the variability of the measurement. For example, to detect a true difference in plasma volume of 3% (given p = 0.05) would require a minimal subject pool of 9. We expect to enroll 10 subjects that will allow us to detect a true difference in of plasma volume of 3% at a p<0.05 statistical significance level. To minimize variations because of body weight differences between individuals, values for PV and plasma solute contents are expressed as the value divided by the body weight (kg) measured on the morning before exercise.

A paired t test will be used to examine possible significant differences between treadmill PV samples and cycle ergometer PV samples. Statistical significance is established at a confidence level of p<0.05.

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