POSTEXERCISE HEMODYNAMICS: INTERACTIONS OF SEX, TRAINING STATUS, AND FLUID REGULATION

by

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In general, postexercise hypotension is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output. These hemodynamic changes are present immediately after a single bout of moderate-intensity dynamic exercise in healthy (sedentary and endurance exercise-trained) and hypertensive humans. The mechanisms underlying this postexercise hypotension are currently under investigation; however, the overall hemodynamic response may be altered in response to different factors related to sex, training status, and fluid regulation. The purpose of this dissertation was to investigate the contribution of endogenous hormones associated with the normal menstrual cycle and training status and sex on postexercise hemodynamics and to better understand how fluid replacement and

heat-stress affect postexercise hemodynamics in a group of highly trained men. In Chapter IV, the contribution of the menstrual cycle and sex to postexercise hemodynamics was investigated. The results showed that postexercise hemodynamics are largely unaffected by sex and factors associated with the menstrual cycle. In Chapter V, the role of heat-stress and fluid replacement on the postexercise cardiac hemodynamics in a group of endurance exercise-trained men was investigated. These data suggest that fluid replacement and heat-stress mitigate the previously observed fall in cardiac output during exercise recovery in trained men. In Chapter VI, the study investigated the hemodynamic profile in well-hydrated sedentary and trained men and women during recovery from exercise. In contrast to previous research, the results showed a lack of variation in the postexercise hemodynamic response across categories of subjects as there was no evidence of a sex and training interaction. Thus, it appears that factors such as heat-stress and fluid replacement can alter postexercise hemodynamics in trained men; however, factors such as menstrual cycle, sex, and training status do not seem to influence the hemodynamic recovery profile. Yet substantial variation in the postexercise response across individuals remains unexplained. This dissertation contains my previously published and my co-authored material.

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CHAPTER I

INTRODUCTION

Statement of the Problem

In general, postexercise hypotension is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output. These hemodynamic changes are present immediately after a single bout of moderate-intensity dynamic exercise in normotensive and hypertensive humans. The mechanisms underlying postexercise hypotension are currently under investigation; however, the overall hemodynamic response may be altered with factors associated with the menstrual cycle and following exercise in a warm environment and dehydration. As well, endurance exercise-trained men may show different postexercise hemodynamics compared to the general population. The contribution of these factors to the hemodynamics following exercise is unknown.

The purposes of the studies outlined in this dissertation are twofold: 1) to investigate the contribution of factors such as endogenous hormones, sex, and training status on postexercise hemodynamics and 2) to further understand how fluid replacement and exercise in a warm environment affects postexercise hemodynamics in a trained group of men. Specifically, these studies investigate the role of thermoregulation and fluid balance on the reduction in postexercise arterial pressure. The studies in Chapters IV and VI outline the menstrual cycle and training status and their role in blood pressure regulation following exercise. Chapter V investigates the contribution of heat-stress (warm environment) and oral fluid replacement on central and peripheral postexercise

hemodynamics in a group of endurance exercise-trained men. Chapter IV could not have been completed without the contribution of Jenni L. McCord, Ph.D., and John R. Halliwill, Ph.D. Chapter V could not have been completed without the contribution of John R. Halliwill, Ph.D. Chapter VI could not have been completed without contributions by Zachary Barrett-O'Keefe, Santiago Lorenzo, M.S., and John R. Halliwill, Ph.D.

Significance

The research objectives outlined in this dissertation advance the basic scientific and mechanistic literature of postexercise hemodynamics. These studies can further the clinical knowledge of hypertension and the use of exercise as a non-pharmacological anti-hypertensive therapy. The American College of Sports Medicine has recognized the importance of daily exercise in prevention of hypertension and the maintenance of blood pressure at a healthy level (Pescatello *et al.*, 2004). Several studies have suggested that the effect of exercise on blood pressure reduction is additive (Beaulieu *et al.*, 1993). Postexercise reductions in blood pressure show the greatest duration and magnitude in hypertensives (MacDonald, 2002a). Regular endurance training with supplemental resistance exercise is recommended for control of blood pressure throughout the lifespan (Pescatello *et al.*, 2004). Understanding the basic mechanisms associated with the postexercise reduction in blood pressure is important for insights into hypertension. The overall goal of this research is to further understand cardiovascular regulation following exercise in humans under different individual and environmental conditions.

CHAPTER II

REVIEW OF THE LITERATURE

This review of the literature will first address the basic hemodynamic response seen after dynamic exercise, called "postexercise hypotension." The mechanisms of postexercise hypotension (Part I) and the factors that influence these hemodynamic changes during exercise recovery will be discussed. The following reviews tackle various factors that alter fluid regulation and their role in cardiovascular regulation postexercise. The menstrual cycle (Part II), exercise-induced dehydration (Part III) (fluid replacement and exercise in a warm environment), and cardiac hypertrophy (Part IV) (endurance-trained athlete) will be the focus of the remaining part of the review of the literature. The review of postexercise hypotension will shed light on the lacking knowledge in the literature to aid in development of the specific hypotheses for each of the studies presented in this dissertation. The final section of the literature review will discuss clinical significance of orthostatic intolerance in endurance-trained athletes and a brief overview of hypertension and the use of exercise as an anti-hypertensive non-pharmacological therapy.

Part I: Postexercise Hypotension and the Associated Postexercise Hemodynamics

Postexercise Hypotension

Historical Perspective

Postexercise hypotension was first documented over 100 years ago. In 1897 Hill reported elevations in heart rate and sustained reduction in mean arterial pressure after 400 m sprints (Hill, 1897). A second published observation in 1907 demonstrated decreases in mean arterial pressure in athletes (rugby football players) directly after exercise (Gordon, 1907). Many years later Kral and colleagues (1966) confirmed these results and observed an immediate reduction in blood pressure after swimming. These researchers made simplistic measurements (mean arterial pressure and heart rate) and clever, insightful observations that brought attention to the importance of exercise in the regulation of blood pressure in healthy and normotensive individuals. Fitzgerald (1981) anecdotally noticed that after running his usually elevated blood pressure was reduced for a sustained period (minutes to hours after exercise). It was Fitzgerald that first referred to the drop in arterial pressure following exercise as "postexercise hypotension".

Introduction

Postexercise hypotension is defined as the reduction in systolic or diastolic arterial pressure below preexercise values (Kenney & Seals, 1993). Further, postexercise hypotension is a sustained decrease in mean arterial pressure from resting values for several minutes to hours following an acute exercise bout (Halliwill, 2001). For the last 20 years numerous studies have documented the occurrence of postexercise hypotension in both normotensive (Somers *et al.*, 1985; Coats *et al.*, 1989; Piepoli *et al.*, 1993a; Piepoli *et al.*, 1993b; Forjaz *et al.*, 2000) and hypertensive individuals (Bennett *et al.*, 1984; Hagberg *et al.*, 1987; Cleroux *et al.*, 1992a; Floras & Wesche, 1992) and in different age groups, races (Pescatello *et al.*, 2004; Enweze *et al.*, 2007), and sexes (Carter *et al.*, 2001; Kenny & Jay, 2007). The reduction in mean arterial pressure postexercise can persist in healthy normotensive subjects for up to 2 h (Halliwill, 2001) and for approximately 12 h in hypertensives (Hagberg *et al.*, 1987; Kenney & Seals,

1993) regardless of the exercise intensity. Mean arterial pressure is typically reduced 5 to 10 mmHg following exercise in sedentary and endurance-exercise trained humans (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002a). However, reductions in arterial pressure in hypertensives and borderline hypertensives are significantly larger (in comparison to normotensives) reaching upper limits of 20 mmHg after exercise (Wilcox *et al.*, 1982; Bennett *et al.*, 1984; Halliwill, 2001; Brandao Rondon *et al.*, 2002; MacDonald *et al.*, 2002). The postexercise pressure reductions are present with supine, seated, and standing postures; however, the magnitude of the decrease is more pronounced and prolonged with the orthostatic influence of upright posture (Kenney & Seals, 1993; Raine *et al.*, 2001).

Overall Duration and Onset of the Response

The onset of postexercise hypotension has been shown to occur immediately (Piepoli *et al.*, 1993a) after the cessation of the exercise, whereas other studies have shown the nadir at 20 min (Kaufman *et al.*, 1987; Brown *et al.*, 1993), and 30 to 60 min following exercise (Floras & Wesche, 1992; MacDonald *et al.*, 1999b). When studies have compared postexercise mean arterial pressure (ambulatory blood pressure) to blood pressure in activities of daily living, the reduction has been shown to last for more than 12 h in hypertensive subjects (Pescatello *et al.*, 1991; Kenney & Seals, 1993).

Overall Hemodynamic Changes during Postexercise Hypotension

Once dynamic exercise has ended, the reduction in arterial pressure is sustained for a significant portion of time and is due to a persistent elevation in systemic vascular conductance. Typically, postexercise hypotension is achieved through an increase in systemic vascular conductance and not a fall in cardiac output (Kenney & Seals, 1993; Halliwill, 2001; MacDonald *et al.*, 2002). Following exercise, the muscle pump is inactive (passive recovery in the supine position) and plasma volume is decreased due to 1) an increased sweat loss during exercise and 2) a loss of fluid into the interstitial space due to higher arterial pressures during dynamic aerobic exercise. Venous return is

decreased from these factors and is also affected by vasodilation in the compliant vascular beds. The decrease in venous return directly lowers central venous volume and reduces cardiac filling pressure (cardiac preload). Typically, cardiac output (product of stroke volume and heart rate) is elevated during recovery from exercise due to the maintenance of stroke volume and elevation in heart rate. Despite the reduction in preload, stroke volume is maintained due to a pressure reduction in the circulatory system (afterload). A rise in the inotropic cardiac response helps prevent the fall in stroke volume and together with the increase in heart rate there is a rise in cardiac output postexercise. Thus, an increase in systemic vascular conductance causes an increase in blood flow postexercise and this is the primary mechanism responsible for the reduction in arterial pressure postexercise. Thus, postexercise hypotension is commonly characterized by a rise in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output (adapted from a review article (Halliwill, 2001)). Typically, postexercise hypotension is mediated through neurohumoral factors that affect vascular responsiveness. This has been the model to explain postexercise hemodynamics in the general population (healthy sedentary and normally active). The later discussions will address the postexercise hemodynamics in the trained and hypertensive populations (Hagberg *et al.*, 1987; Senitko *et al.*, 2002).

Measured Cardiovascular Parameters

Cardiac Output

Cardiac output has been measured following exercise in hypertensive and normotensive humans (Hagberg *et al.*, 1987; Piepoli *et al.*, 1993a; Piepoli *et al.*, 1994; Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b). In a healthy normotensive population, cardiac output has generally been shown to increase postexercise due to increases in heart rate and contractility (Halliwill, 2001), whereas stroke volume does not seem to change postexercise (Floras & Wesche, 1992; Hara & Floras, 1992; Halliwill, 2001). Hagberg et al. (1987) showed reduced cardiac output following exercise in a seated recovery position in older hypertensives due to possible reduced myocardial contractility. The same fall in

cardiac output was present in endurance-trained men during recovery from exercise (Senitko *et al.*, 2002).

Blood and Plasma Volume

The reduction in pressure postexercise has been suggested to aid plasma volume recovery following exercise (Hayes *et al.*, 2000). The decrease in central venous pressure is thought to be due, in part, to reductions in plasma volume following exercise. Plasma volume is reduced primarily due to sweat losses and loss of plasma into the interstitial space during exercise. However, plasma volume is usually unchanged or recovers faster than mean arterial pressure during the hypotensive period (MacDonald *et al.*, 1999b; MacDonald *et al.*, 2000a). Arterial pressure was reduced in hypertensive humans whereas, plasma volume remained unchanged postexercise (Cleroux *et al.*, 1992b). Postexercise hypotension has been found to persist for greater than one hour following moderate dynamic exercise and during this time period, plasma volume tends to recover quickly in the normotensive individual. Taken together, these data suggest that a reduction of plasma volume does not appear to play a key role in postexercise hypotension. However, the fall in mean arterial pressure may be related to exaggerated loss of plasma volume after exercise (i.e., higher sweat loss in a trained population).

Peripheral Vascular Conductance

Several mechanisms appear to underlie the sustained peripheral vasodilation following exercise. Forearm (Coats *et al.*, 1989; Cleroux *et al.*, 1992a; Piepoli *et al.*, 1994; Halliwill *et al.*, 2000) and calf (Hara & Floras, 1992; Halliwill *et al.*, 1996a; Halliwill *et al.*, 2000) vascular resistance has been shown to decrease with systemic vascular resistance. Thus, the increased vascular conductance is not only present in the exercising muscle (i.e., legs) but also in the inactive vascular beds (i.e., arms), suggesting that increases in vascular conductance is a whole body phenomenon (Coats *et al.*, 1989; Cleroux *et al.*, 1992a; McCord *et al.*, 2006a; McCord & Halliwill, 2006).

Cutaneous, Splanchnic, and Renal Blood Flow

There seems to be no contribution of the renal and splanchnic vascular beds to the overall increase in systemic vascular conductance, as vascular conductance in these compliant circulations was unchanged after exercise in normotensive individuals (Pricher *et al.*, 2004). Core body temperature remains elevated after dynamic exercise (Brown *et al.*, 1993) and this elevation in thermal load may alter cutaneous blood flow and contribute to the sustained vasodilation that is associated with postexercise hypotension. Franklin *et al.* (1993) showed that a warm environment produced increased and sustained reduction in mean arterial pressure compared to the thermoneutral and cold conditions. This implicates cutaneous blood flow as a possible contributing factor in postexercise hypotension. However, the timing of heat dissipation and postexercise hypotension are not well correlated. Wilkins *et al.* (2004) showed different timing between postexercise skin blood flow and the reduction in arterial pressure. Thus, it appears that the contribution of the cutaneous circulation cannot account for the reductions in mean arterial pressure after exercise. In addition, the timing of cutaneous vascular conductance also differs from leg vascular conductance, so it appears that skeletal muscle vasodilation and not cutaneous blood flow is responsible for postexercise hypotension. The direct effects of exercise in a warm environment and fluid replacement on postexercise hemodynamics will be discussed in Part III of the literature review.

Potential Mechanisms

The hallmark of postexercise hypotension is a sustained vasodilation that suggests that sympathetic vascular regulation is altered following exercise (Halliwill *et al.*, 1996a; Halliwill, 2001). There is a growing body of research to support that postexercise hypotension in humans is mediated by multiple and redundant mechanisms (Halliwill, 2001). The specific mechanisms that underlie postexercise hypotension, 1) neural and 2) local (Halliwill *et al.*, 1996a; Halliwill *et al.*, 2000) will be discussed in detail in the following paragraphs.

Baroreflex Mediated Reduction in Sympathetic Activity

The baroreflex exerts finite control over the cardiovascular system by tightly regulating arterial pressure under resting and most exercising conditions. The baroreflex is reset to higher pressure during exercise (Raven *et al.*, 1997; Raven *et al.*, 2001). Halliwill *et al.* (1996a) showed that the arterial baroreflex was reset to operate at a lower pressure following exercise in humans. Results from this study suggest that for a given diastolic pressure there was less muscle sympathetic nerve activity following exercise compared to the sham protocol.

Lower body negative pressure (Bennett *et al.*, 1984) was shown to increase baroreceptor sensitivity following exercise. In agreement with these results, baroreflex sensitivity with the Oxford method after maximal exercise was increased 60 min postexercise (Somers *et al.*, 1985; Piepoli *et al.*, 1993a), but R-R interval gain in the first portion of recovery was shown to decrease. Carotid heart rate gain was augmented in the hypotensive period in humans following moderate exercise (Halliwill *et al.*, 1996b). Carotid-cardiac baroreflex responses were assessed by applying external neck pressure/ neck suction and analyzing R-R interval. Discrepancies in the results may exist due to the different methodologies such as pharmacological intervention (Oxford method) (Somers *et al.*, 1985; Piepoli *et al.*, 1993a) versus directly targeting the carotid baroreceptors with neck suction (Halliwill *et al.*, 1996b) which was used in order to generate baroreflex curves. Authors suggested that there could be a possible change at the level of the central nervous system or possible inhibitory input from the low pressure cardiopulmonary baroreceptors due to hypovolemia in the postexercise period (Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b).

Halliwill *et al.* (2000) blocked sympathetically medicated vasoconstriction with an α-adrenergic antagonist (phentolamine) in humans. Following exercise and the sham period there was no alteration in postexercise hypotension; however, the decreases in sympathetic activity following exercise contributed to a 30% reduction in systemic vascular resistance. Results showed that there were no regional effects on vascular resistance and or mean arterial pressure despite the sympathoinhibition (Halliwill *et al.*,

2000). Recent work in the animal model (spontaneous hypertensive rats) suggested a contribution of central neural mechanism to postexercise hypotension (via sympathetic nerve activity). Specifically, substance P receptors in the nucleus tractus solitarius (Chen *et al.*, 2002) and GABA receptors in the rostral ventrolateral medulla (Kajekar *et al.*, 2002) were shown to play a role in mediating postexercise hypotension. However, the central nervous system mechanisms involved with baroreflex resetting during and after exercise in humans is unknown (Halliwill, 2001).

Ineffective Transduction of Sympathetic Activity

In addition to the reduction in sympathetic nerve activity, there is reduced vascular responsiveness to a given level of α-adrenergic receptor stimulation as vascular resistance is reduced for a given level of sympathetic nerve activity postexercise (Halliwill *et al.*, 1996a; Halliwill *et al.*, 2003). A locally acting vasodilator could be contributing to postexercise hypotension. Inhibition could occur at the presynpatic or postsynaptic level. Ineffective transduction of sympathetic nerve activity into vasoconstriction could be due to a decreased neurotransmitter release or downregulation of the sensitivity of the receptors (hyporesponsiveness) at the level of the arterial smooth muscle (Halliwill *et al.*, 1996a). To investigate this idea, calf vascular resistance and muscle sympathetic nerve activity was measured before and after a sham and exercise protocol (Halliwill *et al.*, 1996a). For a given level of muscle sympathetic activity, calf vascular resistance was less responsive after exercise compared to the sham condition. These data suggest that with equal muscle sympathetic nerve activity, after exercise, the vasoconstrictor mechanism is ineffective at producing a certain degree of vascular resistance. Halliwill *et al.* (2003) suggest that a local influence (possible vasodilator) may be acting to disrupt the sympathetic signal down this pathway and tested the hypothesis that hyporesponsiveness of the α_1- or α_2-adrenergic receptors were responsible for the postexercise vasodilation in humans. Phenylephrine (α_1-agonist) and clonidine (α_2-agonist) were administered intraarterially and results showed no difference in vasoconstriction before and after exercise. These data suggest vascular responsiveness to

α-adrenergic agonists is maintained during the hypotensive period in humans. The following sections will review the role of local vasodilators in mediating postexercise hypotension.

Norepinephrine/Epinephrine Release

Prior results suggest that norepinephrine spill over is known to be increased or unchanged in normotensives following exercise (MacDonald *et al.*, 2002). Halliwill *et al.* (1996b) showed norepinephrine was increased and epinephrine was unchanged in normotensive humans following moderate dynamic exercise. Hypertensive men and women showed no increase in urinary catecholamines postexercise (Brownley *et al.*, 1996).

Nitric Oxide and Vascular Sensitivity

It has been proposed that certain vasodilators, such as nitric oxide, could potentially modify α-adrenergic responsiveness. The release and production of nitric oxide is associated with wall stress and has been shown to be upregulated after exercise (Jungersten *et al.*, 1997). Nitric oxide has been show to contribute to postexercise hypotension in the animal model (Patil *et al.*, 1993); however, current work has shown no role for nitric oxide in humans. Halliwill *et al.* (2000) examined the effects of nitric oxide on postexercise hypotension after exercise and a sham period, in which L-NMMA was used to block the production of nitric oxide. Following blockade there was no alteration in postexercise hypotension. This suggests that postexercise hypotension is independent of the release and/or production of nitric oxide in normotensive humans.

Potential Vasodilator/Vasoconstrictor Substances

A number of other vasodilators have been implicated in the postexercise increase in vascular conductance (Halliwill, 2001). Adenosine, adrenaline, atrial natriuretic factor, and potassium were thought to have the capacity to act on vascular smooth muscle and mediate postexercise hypotension. However, most data has suggested that these

substances have little role in the contribution of postexercise hypotension, as the half-life of these vasodilators does not seem to be in line with the timing of postexercise hypotension (MacDonald, 2002a). Vasoconstrictors such as renin, angiotensin II, and anti-diuretic hormone do not seem to show any direct mechanistic link to postexercise hypotension (Wilcox *et al.*, 1982).

Histamine

Recently, a sustained histamine-receptor dependent vasodilation has been shown to be responsible for the increase in vascular conductance postexercise. Blockade of H_1- and H_2-receptors with fexofenadine and ranitidine have been shown to decrease postexercise vasodilation and subsequently mitigate the reduction in arterial pressure postexercise (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a; McCord & Halliwill, 2006). H_1-receptors seem to be responsible for the early part (first 30 min) (Lockwood *et al.*, 2005b) and the H_2-receptors seem to mediate the late phase response (~60 to 90 min) of the hypotension (McCord *et al.*, 2006a).

Prostaglandins

Blockade of cyclooxygenase enzymes prevents production of prostaglandins. Following exercise, the inhibition of prostaglandins immediately reduces calf blood flow (Cowley *et al.*, 1984; Cowley *et al.*, 1985) and this suggests a role for prostaglandins in postexercise hyperemia. In flow restricted models, prostaglandins were thought to decrease vascular resistance for 40 min postexercise (Morganroth *et al.*, 1977). Recently, prostaglandins were blocked with ibuprofen after a bout of dynamic exercise and no difference was observed in arterial pressure or femoral vascular conductance between control and blockade through 90 min postexercise (Lockwood *et al.*, 2005a). This research suggests that the sustained postexercise vasodilation does not appear to be dependent on prostaglandins.

The Animal Model

Postexercise hypotension is observed in both human and animal models (hypertensive rats (Patil *et al.*, 1993; Kulics *et al.*, 1999) and conscious rabbits (Howard & DiCarlo, 1992)). Animal studies have shown that postexercise hypotension occurs in rats after running at 70% of VO_{2max} (Kulics *et al.*, 1999) and spontaneous running bouts (Shyu & Thoren, 1986). Treadmill running for 40 min at 60-70% VO_{2peak} produced greater reductions in mean arterial pressure in rats compared to the 20 min bout (Overton *et al.*, 1988). Spontaneous (Shyu & Thoren, 1986) and forced (Overton *et al.*, 1988) running has produced postexercise hypotension in rats.

The mechanisms for decreases in arterial pressure postexercise have been studied extensively in the animal model; there are differences and similarities in the mechanisms underlying the postexercise responses between animals and humans. Hypertensive rats show reductions in lumbar sympathetic nerve activity (Kulics *et al.*, 1999), whereas other studies have shown increases (Kenney & Morgan, 1993) during nerve stimulation. Collins & DiCarlo (1993) suggested the possible influence of cardiac afferent nerves on postexercise hypotension.

Vascular responsiveness to α-adrenergic stimulation is reduced following exercise in rats (Patil *et al.*, 1993; Rao *et al.*, 2002). In addition, there appears to be a role for nitric oxide (Rao *et al.*, 2002) in reducing vascular sensitivity (Patil *et al.*, 1993), as increased iliac blood flow was mediated by decreased adrenergic sensitivity, demonstrating that local vasodilation mediated by nitric oxide may mediate postexercise hypotension in rats. The vasoconstriction postexercise was found to be reduced following α-adrenergic receptor agonist in rats (Howard & DiCarlo, 1992; Patil *et al.*, 1993; Rao *et al.*, 2002). This research suggested that the α-adrenergic receptors are hyporesponsive to neurotransmitter after exercise and may mediate the postexercise vasodilation, but this is not the case in humans. Data from humans and animal studies have advanced the understanding of the basic hemodynamic mechanisms and have given clinical perspective to postexercise hypotension.

Exercise Factors that Affect the Postexercise Hypotensive Response

Studies have shown that magnitude and duration of postexercise hypotension can be modified by different types, intensities, and durations of exercise.

Exercise Intensity

Typically, exercise protocols at 40 -100% of maximal exercise capacity have been shown to consistently produce postexercise hypotension (Somers *et al.*, 1985; Coats *et al.*, 1989; Floras *et al.*, 1989; Franklin *et al.*, 1993; Piepoli *et al.*, 1993a; Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b). Postexercise hypotension is thought to be independent of the exercise intensity, but conflicting results do exist. MacDonald *et al.* (1999a) compared three different submaximal exercise intensities (50 to 70% VO_{2peak}) when the duration was held constant and no differences in postexercise hypotension were seen in normotensive subjects. Piepoli *et al.* (1994) was the first to report a greater reduction in mean arterial pressure following a maximal incremental test (25 W, 5 min stages) compared to moderate (12.5 W, 5 min stages) and mild (constant at 50 W) exercise in sedentary normotensive individuals. Forjaz *et al.* (1998) confirmed these results over a wider range of exercise intensities (30, 50, and 80% of VO_{2peak}). Recently, the same group of researchers found that the magnitude of the reduction was dependent on the exercise intensity (30, 50, and 75% of VO_{2peak}), as the lower intensity (30%) produced the smallest reduction in arterial pressure compared to the other intensities (50 and 75%) postexercise (Forjaz *et al.*, 2004). Higher intensity treadmill running (70 and 93 % VO_{2max}) was proven to show larger reductions in arterial pressure postexercise compared to lesser intensities (Kenny & Niedre, 2002). Subsequent results showed greater decreases in mean arterial pressure with increased exercise intensity (70 and 85% VO_{2max}), whereas the lower intensity (55% VO_{2max}) produced the smallest decreases in mean arterial pressure after exercise (Kenny *et al.*, 2003). Thus, recent studies suggest the postexercise reduction in arterial pressure is dependent on the exercise intensity.

Duration of the Exercise

Postexercise hypotension has been documented over short (10 min) (Bennett *et al.*, 1984) and long (170 min) exercise sessions (Seals *et al.*, 1988). However, 20 to 60 min of dynamic exercise is typically used, as this duration has been shown to produce consistent reductions in postexercise arterial pressure (Wilcox *et al.*, 1982; Somers *et al.*, 1985; Kaufman *et al.*, 1987; Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b; MacDonald *et al.*, 1999b; Halliwill *et al.*, 2000; Halliwill, 2001). Some research has pointed to enhanced benefits with longer exercise protocols in the hypertensive (Bennett *et al.*, 1984) and normotensive individual (Forjaz *et al.*, 2000). MacDonald *et al.* (2000a) observed no difference in postexercise hypotension following exercise duration ranging from 10 to 45 min at a constant intensity of 70% of VO_{2peak} in normotensive and borderline hypertensive subjects. Mach *et al.* (2005) found a relationship between exercise duration and the reduction of postexercise systolic blood pressure. Exercise at 50% of VO_{2peak} at the longer durations (40 and 80 min) showed pronounced decreases compared to 10 and 20 minutes of exercise at the same intensity. The difference in overall intensity of the exercise bout in these two studies (MacDonald *et al.*, 2000a; Mach *et al.*, 2005) may explain the conflicting results. In addition, this study is limited by the fact that systolic blood pressure was the only measurement of pressure (Mach *et al.*, 2005).

Type of Exercise

Dynamic aerobic exercise consistently reduces arterial pressure postexercise and is typically the mode of exercise that is used to study postexercise hypotension. Cycling (Somers *et al.*, 1985; Coats *et al.*, 1989; Floras *et al.*, 1989; Cleroux *et al.*, 1992a), swimming (Kral *et al.*, 1966), and treadmill running (Kaufman *et al.*, 1987; Hara & Floras, 1995) at submaximal to maximal intensity have been shown to elicit postexercise hypotension. Postexercise hypotension has been noted after whole body dynamic exercise (Fitzgerald, 1981; Floras & Wesche, 1992; Hara & Floras, 1992, 1995) and after leg and arm ergometry (Somers *et al.*, 1985; Floras *et al.*, 1989). MacDonald *et al.*

(2000b) examined arm and leg ergometry at the same intensity and found no differences in the magnitude of blood pressure reduction postexercise. Thus, postexercise hypotension is not dependent on the amount of activated muscle mass during exercise. It seems that in most cases, shorter and less intense exercise bouts produce inconsistent reductions in postexercise hypotension in normotensive individuals (Halliwill, 2001).

Resistance Exercise

Resistance training has been shown to produce a hypotensive response during exercise recovery. A smaller number of studies have investigated the effects of resistance exercise on acute reductions in blood pressure. Data suggests that resistance exercise reduces postexercise mean arterial pressure, but the hypotensive response lasts for shorter durations (~1 min and up to 60 minutes postexercise) (Hill & Butler, 1991; MacDonald *et al.*, 1999b; Simao *et al.*, 2005; Polito *et al.*, 2007). Thus, it seems that resistance training does elicit a hypotensive response that is shorter in duration compared to dynamic aerobic exercise.

Subject Characteristics and Study Population

The mechanisms, magnitude, and duration of postexercise hypotension can be dependent on subject characteristic and subject populations. The following sections will address the role of population differences on the overall postexercise hemodynamic response.

Sex

The occurrence of postexercise hypotension has been found in men and women. Historically, male subjects were used to establish the occurrence of postexercise hypotension. Recently, the effect of sex on postexercise hypotension has been evaluated with conflicting results (Carter *et al.*, 2001; Senitko *et al.*, 2002; Kenny & Jay, 2007). Studies specifically addressing the role of sex on postexercise hypotension seem to show no relationship as reductions in blood pressure were of similar magnitude between men

and women (Senitko *et al.*, 2002; Journeay *et al.*, 2007). Carter *et al.* (2001) showed that following inactive recovery women had greater decreases in mean arterial pressure following 3 minutes of cycling at 60%VO_{2max} compared to men. Kenny and Jay (2007) found that women showed a greater reduction in mean arterial pressure (5 to 7 mmHg) during recovery relative to men and this was later confirmed (Fisher *et al.*, 1999; Deschenes *et al.*, 2006). The pattern and magnitude of postexercise hypotension between sexes will be further examined in Chapter IV of this dissertation.

Age

Limited data exists on the role of age on postexercise hypotension. Postexercise hypotension does not appear to be dependent on age as its occurrence has been observed in young, middle aged (Kaufman *et al.*, 1987), and older men and women (Hagberg *et al.*, 1987). Hagberg *et al.* (1987) showed older (60-69 yr) hypertensives (16 men versus 8 women) demonstrate blood pressure reduction following exercise. Kaufman *et al.* (1987) found no differences in a young and older hypertensive population of men. Currently, it seems that the reduction in arterial pressure following exercise is not dependent of the age on the subjects; however, a study examining an equal number of young and old men and women is warranted.

Ethnicity

Pescatello *et al.* (2003) compared postexercise hypotension in a limited number of white and black women and found the reduction in arterial pressure to be less among the black women. Recently, Enweze *et al.* (2007) showed that postexercise blood pressure was not reduced in African-American women compared to preexercise baseline values. This area warrants further investigation with consideration of different races and inclusive of both men and women.

Training Status

The role of training status has been addressed and it appears that postexercise hypotension is not a phenomenon of a sedentary lifestyle. Endurance-trained populations demonstrate reductions in blood pressure following dynamic exercise (Roltsch *et al.*, 2001; Senitko *et al.*, 2002; Dujic *et al.*, 2006; McCord & Halliwill, 2006) compared to sedentary individuals. However, eight endurance-trained men showed altered responses as cardiac output was decreased and systemic vascular conductance was unchanged postexercise when compared to sedentary men and women and trained women (Senitko *et al.*, 2002). Recently, in a study by Dujic *et al.* (2006) elite Croatian Soccer players were maximally exercised for 9 to 13 min during a running field exercise and measurements of central hemodynamics were taken before and after exercise with similar results to Senitko *et al.* (2002). Currently, limited studies exist on the mechanisms associated with postexercise hemodynamics in a large sample of highly trained and sedentary individuals. The role of training status on postexercise hypotension will be addressed in the final chapter of this dissertation (Chapter VI).

Part II: The Menstrual Cycle

The Role of Female Sex Hormones in the Cardiovascular System

Endogenous Hormones

Female sex steroids, estrogen and progesterone, are known to exert complex effects on physiological functions and specifically influence cardiovascular regulation. To date, the role of estrogen and progesterone has been examined with respect to the endogenous hormones (the menstrual cycle) and exogenous hormones (oral contraceptives). Cardiovascular risk increases dramatically after the loss of estrogen associated with menopause, which suggests a protective effect of estrogen on cardiovascular function (Du *et al.*, 1995). It is now established that estrogen and progesterone have dramatic effects on the cardiovascular system, specifically on vascular regulation by altering the sympathetic neural control to the peripheral circulation

(Charkoudian, 2001). The following sections will outline the actions of estrogen and progesterone on cardiovascular regulation and postexercise hypotension.

Normal Menstrual Cycle

An average menstrual cycle lasts between 20 and 45 days and averages 28 days. The hormonal changes that modulate the menstrual cycle are divided into three main phases: the follicular, ovulatory, and luteal. The follicular phase starts with menstruation and ends with ovulation and is characterized by relatively low levels of estrogen and progesterone and is further subdivided into the early and late follicular phases. Prior to ovulation, there is a marked increase in estrogen. Ovulation is the result of the sudden surge of luteinizing hormone. The ovulatory phase is characterized by elevated levels of estrogen with relatively low levels of progesterone. The luteal phase occurs from ovulation to the start of menstruation. In this phase, both estrogen and progesterone levels are typically elevated and this phase is further subdivided into the early and late luteal phases. If conception does not occur hormonal concentrations fall dramatically and this causes menstruation (shedding of the lining of the uterus) and the entire cycle begins once again (Frankovich & Lebrun, 2000).

Estrogen

In women, estrogens are primarily secreted by the ovaries and to a lesser extent by the adrenal glands. Estrogens are a group of 18-carbon steroids which include estradiol and in humans two less potent estrogens called estrone and estriol (Frankovich & Lebrun, 2000). Estrogen has been shown to act as a calcium channel blocker (Collins, 1996) and through this mechanism can cause an increase in blood flow through endothelium-dependent vasodilation (English *et al.*, 1998; Sanada *et al.*, 2001). Further, estrogen has been found to control the production and release of nitric oxide (Cicinelli *et al.*, 1996). Estrogen receptors are located in the endothelium, (Deroo & Korach, 2006), smooth muscle of the peripheral vasculature (Karas *et al.*, 1994), and coronary arteries (Sarrel, 1990; Herrington, 2000; Sanada *et al.*, 2001). Estrogen improves the function of

the vascular endothelium by promoting an increase in blood flow and vasodilation; thus, decreased estrogen levels may increase peripheral resistance and cause a decrease in blood flow to the exercising muscle (Collins, 1994; Collins *et al.*, 1995; Collins, 1996). Higher estradiol levels have been shown to have less β-receptor sensitivity to catecholamines and this lowers the cardiovascular response to stress (Sita & Miller, 1996). Ettinger *et al.* (1998) examined changes in muscle sympathetic nerve activity in response to static handgrip exercise during the early follicular and pre-ovulatory phases of the menstrual cycle. During the pre-ovulatory phase, (elevated estrogen) there was a significant decrease in muscle sympathetic nerve activity. Minson *et al.* (2000) assessed cardiovagal and sympathetic baroreflex in the early follicular and mid-luteal phases of the normal menstrual cycle. Sympathetic neural outflow, noradrenaline levels, and sympathetic baroreflex sensitivity were elevated in the mid-luteal phase, whereas cardiovagal baroreflex sensitivity was unaffected by menstrual phase. Thus, estrogen not only alters baroreflex sensitivity and muscle sympathetic nerve activity, but may alter arterial pressure during rest and recovery from exercise.

Progesterone

The other major group of female hormones is the progestin group, the main form of which is endogenous progesterone. Physiological alterations have been present during the luteal phase of a normal ovulatory menstrual cycle when progesterone and estrogen are elevated. Progesterone is known to have antiestrogenic properties; in other words, it tends to antagonize the actions of estrogen (Frankovich & Lebrun, 2000). Body temperature is typically increased 0.3 to 0.5 °C during the luteal phase compared to the follicular phase when plasma concentrations of estrogen and progesterone are the lowest (Carpenter & Nunneley, 1988). Exogenous progesterone has been shown to cause an increase in body temperature and these changes have not been seen with the administration of estrogen (Stachenfeld *et al.*, 2000). However, metabolic heat production is not different during the follicular and luteal phases (Frascarolo *et al.*, 1990). Progesterone appears to promote a vasoconstriction in skin microvasculature

(Charkoudian, 2001) and is also known to oppose the systemic vasodilatory actions of estrogen (Sarrel, 1990). These effects are seen indirectly through an elevated internal temperature threshold shift through higher brain centers. In addition to a greater temperature threshold, larger sweat rates have been reported in the luteal phase (Grucza *et al.*, 1993). Progesterone may increase cardiac excitability during the luteal phase (Rosano *et al.*, 1996). Higher heart rate and minute ventilation (Schoene *et al.*, 1981) and increased ratings of perceived exertion for the same exercise suggests a greater cardiovascular strain during the luteal phase (Hessemer & Brück, 1985b; Pivarnik *et al.*, 1992; Birch & Reilly, 1997). Progesterone is thought to contribute to postovulatory fluid retention involving aldosterone and the renin-angiotensin system (Oian *et al.*, 1987). Progesterone exerts complex physiological effects and along with estrogen may alter postexercise hemodynamics. The influence of estrogen and progesterone in postexercise hemodynamics is currently unknown and needs to be examined.

The Role of Sex Steroids on Vascular Volumes and Fluid Regulation

Plasma volume is known to fluctuate between different phases of the normal menstrual cycle (Turner & Fortney, 1984; Fortney *et al.*, 1988; Fortney *et al.*, 1994). During the normal menstrual cycle plasma volume peaks twice, once within two days of estimated ovulation and additionally, at the end of the menstrual cycle in the mid-luteal phase (Fortney *et al.*, 1994). High levels of estrogen have been shown to cause an expansion and restoration in plasma volume (Turner & Fortney, 1984; Fortney *et al.*, 1988; Tankersley *et al.*, 1992; Fortney *et al.*, 1994; Stachenfeld *et al.*, 1999b; Stachenfeld *et al.*, 2001; Stachenfeld & Taylor, 2004). The plasma volume contracts during the mid-luteal phase (Turner & Fortney, 1984; Oian *et al.*, 1987; Tollan *et al.*, 1990; Fortney *et al.*, 1994; Stachenfeld *et al.*, 1999b; Stachenfeld *et al.*, 2000), approximately one week after ovulation (Stachenfeld *et al.*, 1999a). However, hormonally mediated changes in plasma volume and hemoglobin concentration do not seem to impact exercise performance (De Souza *et al.*, 1989; Frankovich & Lebrun, 2000).

The Influence of the Menstrual Cycle on Postexercise Hypotension

The role of hormones in postexercise arterial pressure regulation is not well understood. It has been proposed that changing levels of endogenous and exogenous estrogen and progesterone may alter the pattern, timing, and hemodynamics during recovery from exercise. Specifically, the possibility exists that increased estrogen (associated with the ovulatory phase) may enhance peripheral vasodilation postexercise and exacerbate postexercise hypotension during this menstrual phase.

Birch *et al.* (2002) examined the effects of exogenous hormones (monophasic oral contraceptives) on postexercise hypotension in 15 women. Authors found no differences in postexercise hemodynamics following moderate cycling exercise for 60 min. Decreases in mean arterial pressure were seen through 60 min postexercise, but there were no differences in the postexercise hemodynamic pattern between the high and low exogenous hormone doses. Postexercise heart rates were elevated during the high compared to the low dose; however, there was no difference in blood flow after exercise, with the exception of increased calf blood flow during the low dose. This study was the first to examine exogenous hormone administration on postexercise hypotension, but the impact of exogenous hormones are known to differ from that of endogenous hormones and further examination is warranted.

Esformes *et al.* (2006) examined the postexercise hypotension following 30 min of moderate cycling exercise in a small sample of eight women during three separate phases of the normal menstrual cycle (early follicular, late follicular, and mid-luteal). The authors reported that the pattern of postexercise hypotension was the same during the late follicular and mid-luteal phases of the menstrual cycle, but postexercise hypotension was enhanced (greater decrease in arterial pressure) during the early follicular phase. Central hemodynamics did not differ at rest or between menstrual phases during recovery from exercise. This study failed to measure estradiol and progesterone concentration in order to verify menstrual phase. In addition to this limitation, Esformes *et al.* (2006) did not investigate the contribution of skeletal muscle and cutaneous blood flow. Finally, postexercise hemodynamics has not been studied during the ovulatory phase when

plasma volume is known to fluctuate. A more comprehensive study is necessary to explore many of these issues. The influence that estrogen and progesterone have on the peripheral vasculature during recovery from exercise has yet to be determined. In Chapter IV, the influence of endogenous hormones (associated with the normal menstrual cycle) on postexercise hypotension will be examined.

Part III: Exercise-Induced Dehydration (Fluid Replacement and Exercising in a Warm Environment)

Dehydration during Exercise

Introduction

Water losses during exercise in the heat occur primarily from sweating. Warm and hot conditions increase exercise related sweat loss and cause dehydration (> 2 % loss in body weight). Dehydration describes negative water balance and hypohydration describes water balance with an overall decrease in volume (Greenleaf & Castle, 1971). Water and electrolyte imbalances that are not properly replaced during exercise can lead to decreases in performance and can adversely affect health (Casa *et al.*, 2005). Dehydration causes elevations in core temperature, heart rate, and perceived exertion ratings during heat-stress (Sawka & Coyle, 1999) and these measures increase as the level of dehydration worsens. Aerobic exercise is known to be negatively affected by dehydration (in the absence of heat strain) and performance is worsened when exercise is conducted in a hot environment. Elevations in body temperature initiate the heat loss mechanisms of increased skin blood flow and increased sweat loss (Sawka *et al.*, 1996b). This displacement of blood flow to the skin during dehydration makes it difficult to maintain central venous pressure and cardiac output in order to support metabolic demands and thermoregulation (Sawka *et al.*, 2001). These next sections will focus on the effects of a warm environment and dehydration in the context of fluid regulation and cardiovascular parameters during and following exercise.

Temperature Responses during Exercise-Induced Dehydration

Thermal receptors are distributed throughout the body's core and skin, with a high concentration in the hypothalamus where much of the temperature integration (Sawka *et al.*, 1996b) and heat loss responses are initiated. Most of the thermal inputs are sensed by receptors in the deep body core and to a lesser extent by receptors located on the skin (Fortney & Vroman, 1985). Water and electrolyte balance are key to thermoregulation and exercise performance in the heat. Plasma hyperosmolality and hypovolemia may reduce heat loss responses during exercise heat-stress (Sawka *et al.*, 1992). The increased core temperature during dehydration has been linked to plasma hyperosmolality and hypovolemia and this contributes to a decreased sweat rate and lower skin blood flow (Fortney *et al.*, 1981b). Body water loss of ~1 % elevates core temperature during exercise (Sawka *et al.*, 1996b). Graded increases in core temperature are seen with more severe water deficits; for every percent loss of body water there is an elevation of 0.1 to 0.25 °C (Montain & Coyle, 1992a). The excessive rise in temperature associated with dehydration is due to inadequate sweating (Greenleaf & Castle, 1971). Thus, dehydration lowers sweat response when the heat loss mechanism is needed the most. The rise in core temperature during dehydration may be due in part to increased metabolic heat production or decreased heat loss mechanisms (Sawka *et al.*, 2007). During dehydration, local sweating and skin blood flow are reduced for a given core temperature (Kenney *et al.*, 1990).

Body Fluid Balance during Exercise-Induced Heat-stress and Dehydration

Total body water can have an influence on thermoregulatory processes and exercise performance in hot environments. Total body water stays generally constant in thermoneutral conditions, but will dramatically decrease under these conditions of elevated temperature (Sawka *et al.*, 2001). Body fluids are stored within and outside of cells; these are called intracellular (60% of fluid volume) and extracellular (the other 40 % of body fluids). The extracellular compartment is made up of the intravascular and

interstitial fluid compartments. Total body water is approximately 60 % of body mass (ranging from 45 to 75 %) (Mack & Nadel, 1996).

Heat-stress poses a threat to the maintenance of body fluid volume and the redistribution of body water to conserve intravascular volume is essential during exercise in the heat. The distribution of fluid between the intra- and extravascular spaces depend on Starling forces (osmotic gradient) (Mack & Nadel, 1996). Dehydration results in reduction of intravascular volume and compartment size and an increase in plasma osmolality (Costill *et al.*, 1975). In addition to the overall loss of fluid associated with exercise-induced sweating, there is a substantial loss of electrolytes from various fluid compartments. Incomplete fluid replacement decreases total body water through free fluid exchange that affects each fluid compartment (Durkot *et al.*, 1986) and different body organs. Nose *et al.* (1983) dehydrated rats (reducing body weight by 10 %) and found that dehydration resulted in water redistribution largely from the intra- and extracellular spaces of muscle and skin in order to conserve total blood volume. Plasma volume losses due to dehydration are known to vary from 8 to 27 % (Costill & Sparks, 1973). Plasma volume reductions with no change in osmolality can increase core temperature and ultimately impair heat loss mechanisms during exercise (Fortney *et al.*, 1981a; Fortney *et al.*, 1981b). Maintenance of body fluid balance during heat acclimation (blood volume expansion) and a decrease in sweat Na^+ concentration, improves thermoregulatory and cardiovascular function during exercise and heat-stress (Mack & Nadel, 1996).

Mechanisms of Sweating and Sweat Rate during Exercise

When ambient temperature increases there is a greater reliance upon evaporative heat loss mechanisms (sweating) to defend core temperature during exercise. Thermoregulatory sweating can begin almost immediately after the initiation of exercise (Van Beaumont & Bullard, 1963). Increased sweating is achieved first by the recruitment of sweat glands and next by increased rates of sweat gland secretion (Sato & Dobson, 1970). The glands of the back and chest have the greatest sweat rates in

comparison to the glands of the limbs. Sweat glands are innervated by postganglionic sympathetic fibers which are nonmyelinated cholinergic C fibers (Sawka *et al.*, 1996b). Sweat glands primarily respond to cholinergic stimulation (through acetylcholine) but are also responsive to circulating epinephrine as sweat glands have α- and β- adrenergic receptors (Sato & Sato, 1981).

Sweat evaporation is the main way in which heat is lost. Sweat rates can be increased by exercise intensity, duration, environmental temperature, body weight, and size (Barr & Costill, 1989), genetic predisposition, metabolic rate, heat acclimation state, and the amount of clothing worn (Sawka *et al.*, 1996b). Typical sweat rates range between 0.5 to 2.0 L/hr. However, there is a great deal of variability for sweat loss between individuals, activities, and workrates (Sawka *et al.*, 2007). Daily fluid requirements for sedentary to very active persons range from 2 to 4 L/d and 4 to 10 L/d (Sawka & Montain, 2000). Athletes performing vigorous exercise in the heat often have sweat rates of 1 to 2 L/h (Costill, 1977). The highest sweat rates were measured at 2.8 L/h in a laboratory setting and 3.7 L/h during field activities for elite runners in hot conditions (Armstrong et al. 1986). Women generally have lower sweat rates and electrolyte losses than men (Frye & Kamon, 1981; Sawka *et al.*, 1983).

The reduction in sweat rate with dehydration is thought to be due to inadequate interstitial fluid volume, changes in osmotic content of the body fluids, and possible impairment of the central or local stimuli to the sweat glands (Greenleaf & Castle, 1971). Fluid losses associated with dehydration come from all body fluid compartments, including the intravascular compartment (Costill *et al.*, 1975). In cooler environments the capacity for heat loss via radiation and convection reduces the need for evaporative cooling and sweat losses are dramatically reduced (Sawka *et al.*, 2007). As the temperature increases there is a greater reliance on evaporative cooling and the need for sweat loss is higher (Sawka *et al.*, 2001). Inadequately replaced sweat loss will cause deficits in total body water.

Heat Loss Mechanisms and Skin Blood Flow

It has been suggested that the ability to dissipate heat and heat acclimatization is directly related to training status (MacDougall *et al.*, 1974). Heat is transferred via blood flow from the muscle to the skin. Once sweat responses are initiated, skin blood flow effectively delivers heat to the periphery to be removed by evaporative cooling. Skin blood flow is affected by local temperature of the skin and overall temperature of the core (Sawka *et al.*, 1996b).

Blood flow to acral regions (such as the lips, hands, and feet) is controlled by noradrenergic sympathetic vasoconstriction and the vasodilation is a result of withdrawing vasoconstrictor activity. In non-acral skin (i.e., limbs, trunk, and back) changes in skin blood flow are controlled by two branches of the sympathetic nervous system: 1) noradrenergic vasoconstrictor nerves and 2) cholinergic active vasodilator nerves (Blair *et al.*, 1960). Cutaneous active vasodilation is mediated by cholinergic nerve activation through release of an unknown cotransmitter and not through acetylecholine (Kellogg *et al.*, 1995). The neurotransmitter acetylcholine mediates sweating and was thought to be involved in active vasodilation due to the fact that sweating and active vasodilation occurs at similar thresholds during heat-stress. At present, vasoactive intestinal peptide is thought to be the cotransmitter. When core and skin temperature rise with heat-stress, vasoconstrictor tone is absent (Kellogg, 2006). As core temperature continues to rise, sweat response and cutaneous active vasodilation becomes fully engaged. This active vasodilator system is responsible for 80 to 95% of the increase in skin blood flow (Rowell, 1977). Nitric oxide has been shown to play a role in active vasodilation during heat-stress (Kellogg *et al.*, 1999). When nitric oxide synthase was blocked, skin blood flow was drastically reduced; thus, nitric oxide is needed for active vasodilation to reach full capacity (Kellogg *et al.*, 1998). Wilkins *et al.* (2003) suggested that nitric oxide may have a synergistic vasodilatory relationship with the neurotransmitters that affects cutaneous active vasodilation. Wong *et al.* (2004) demonstrated that H_1-receptors play a role in the generation of nitric oxide during active cutaneous vasodilation in humans (with passive heating) under hyperthermic conditions.

Dehydration and Cardiovascular Responses during Exercise

The primary cardiovascular challenge during exercise in the heat is to provide enough blood flow to both the exercising muscle (to support metabolic processes) and cutaneous vasculature (to dissipate heat). The effects of dehydration on cardiovascular responses during exercise have been well studied. During exercise the rate of heat production increases above baseline due to thermal heat generated by skeletal muscle contraction. As core temperature rises, the heat loss mechanisms are initiated and the rate of heat dissipation increases. With the continuation of exercise, heat loss mechanisms increase (core temperature rises more slowly) which effectively balances heat production and a steady-state is reached (Sawka *et al.*, 1996b). If heat loss mechanisms are not effective at removing heat (i.e., due to the environment or changes in fluid balance) core temperature will continue to rise.

Dehydration results in a decrease in blood volume (from sweating), reduced central venous pressure, and cardiac filling, which reduces stroke volume and increases heart rate. Dehydration during submaximal exercise (in the absence of heat strain) elicits increases in heart rate and decreases in stroke volume with little change in cardiac output (Saltin, 1964). These cardiovascular responses are worsened with dehydration and heat strain (Gonzalez-Alonso *et al.*, 1997). Moderate dehydration (3 to 4 % loss of body weight) ultimately causes decreases in cardiac output because heart rate is not enough to offset the decline in stroke volume (even at prolonged submaximal exercise). This upward rise in heart rate associated with exercise in the heat is commonly known as "cardiovascular drift". Cardiovascular drift is exacerbated and occurs earlier during heat-stress at higher exercise intensities (Montain *et al.*, 1998). The magnitude of dehydration is directly related to hyperthermia and cardiovascular drift (Montain & Coyle, 1992b). The combination of upright exercise in the heat results in a competition for limited cardiac output between the central and peripheral circulation (Sawka *et al.*, 1996a) and this is intensified during dehydration when blood volume is more drastically reduced (Gonzalez-Alonso *et al.*, 2000). In severe dehydration and heat-stress (when cardiac output is decreased) there are reductions in muscle blood flow. However, this is

accompanied by increased oxygen extraction by the working muscle (Gonzalez-Alonso *et al.*, 1999a; Gonzalez-Alonso *et al.*, 1999b).

As body temperature increases, vasodilation occurs in the compliant cutaneous vascular beds, thus decreasing venous resistance and overall pressure (Sawka *et al.*, 1996b). Splanchnic and renal blood flow is reduced during exercise in the heat to compensate for the peripheral pooling of blood and this mechanism effectively maintains cardiac output and arterial pressure (Rowell *et al.*, 1965; Rowell *et al.*, 1971; Rowell *et al.*, 1972). If heat-stress is superimposed on dehydration, renal blood flow is decreased during orthostatic stress sooner compared to the thermoneutral condition (Minson *et al.*, 1999). An increase in cardiac contractility may act to defend stroke volume; however, if these mechanisms cannot supply enough blood flow to skin and muscle, exercise performance will be reduced (Sawka *et al.*, 1996b).

Exact Fluid Replacement during Exercise

The goal of fluid replacement during exercise is to prevent a 2 % loss or greater in body weight. During exercise, when subjects are euhydrated, the human thirst mechanisms may be insufficient to promote fluid intake and maintain water balance (Maresh *et al.*, 2004). Thirst is not a good indicator of body water requirements until deficits reach 2 % or more (Adolph, 1947). Failure to replenish electrolyte balances after exercise will prevent euhydration. However, high fluid intake (> 1 L/h) can be difficult to achieve during exercise and can result in accumulation of fluid in the bowel and colon, and paired with movement, abdominal discomfort is often described. Thus, oral fluid replacement should be started immediately and consumed over time and not administered in bolus amounts. Frequent and consistent hydration during exercise training can alleviate symptoms of abdominal discomfort through increased intestinal absorption (Noakes, 1993) and improved gastric emptying (Maughan & Leiper, 1999). Oral fluid replacement that replaces ~81 % of loss after exercise-induced dehydration restores plasma volume and normalizes heart rate to baseline values (Costill & Sparks, 1973). Fluid replacement during exercise attenuates hyperthermia due to increases in skin blood

flow (Montain & Coyle, 1992a) and prevents the decline in stroke volume and cardiac output during prolonged exercise (~2 h) (Hamilton *et al.*, 1991). Water has been shown to be effective at maintaining body weight and preventing plasma volume losses during exercise (Sanders *et al.*, 1999). No differences in gastric emptying or total fluid absorption were evident in the duodenum or the jejunum between water, isotonic, hypotonic, or hypertonic solution (Gisolfi *et al.*, 1998). It is recommended that 1.5 L of fluid should be consumed for every kilogram loss in body weight (Shirreffs & Maughan, 1998). Intravenous fluid replacement is advised for severe dehydration when losses in body weight are greater than 7 % (Casa *et al.*, 2000).

The Effects of Dehydration on Athletic Performance

Dehydration does not alter anaerobic performance (Cheuvront *et al.*, 2006) but decreases muscular endurance, maximal aerobic power, and overall exercise performance (Burge *et al.*, 1993; Sawka *et al.*, 2001). Aerobic metabolic rate for a given power output decreases with increases in ambient temperature. Exercise-induced losses in body weight of < 3 % does not alter VO_{2max} in moderate climates, but larger losses in the same climate has been shown to alter VO_{2max}. In hot environments relatively small losses of body weight (2 to 4 %) produce large reductions in VO_{2max} (Craig & Cummings, 1966). For a given level of dehydration, exercise capacity (measured by time to exhaustion) is decreased to a greater extent than VO_{2max}. Mechanisms for reduced aerobic performance include: increased cardiovascular (Montain *et al.*, 1998) and thermal strain, increased ultilization of glycogen (due to less free fatty acid utilization from increased catecholamines) and higher accumulation of metabolites. Running performance is impaired with a 2 % loss in body weight and to a greater extent at longer distances (Armstrong *et al.*, 1985).

A Hot Environment and Postexercise Hemodynamics

In addition to the known mechanisms of postexercise hypotension (see review on postexercise hypotension (Part I)), the need to dissipate heat accumulated during the

exercise period is another possible cause for the decreased arterial pressure following exercise. Heat and dehydration are thought to exacerbate postexercise hypotension and potentially reduce orthostatic tolerance (Halliwill, 2001) through increased sweat loss (greater loss of plasma volume) and increased blood distribution to the cutaneous circulation for heat loss mechanisms. It is currently unknown what role exercise in a warm environment plays on the recovery hemodynamics associated with postexercise hypotension.

Franklin *et al.* (1993) assessed postexercise hypotension under three different environmental conditions during recovery from cycling exercise. Eleven males underwent experimental sessions involving 30 min of baseline measurements in a thermoneutral condition, followed by 30 min of thermoneutral exercise at 70 % of VO_{2peak}. Recovery in the warm condition caused elevations in core and skin temperature at 60 min postexercise, which worsened postexercise hypotension. In the thermoneutral and cold conditions, core temperature and mean arterial pressure returned to baseline at 60 min postexercise. Thus, persistence of vasodilation during exercise recovery and the magnitude of postexercise hypotension were dependent on elevations in skin and core temperature. It seems that thermoregulatory mechanisms do play a role in postexercise hypotension. Franklin *et al.* (1993) did not address the impact of exercise-induced thermal stress on postexercise hemodynamics. Cardiac output and skin and muscle blood flow were not measured in this study, and a control group was not used.

Early studies observed elevations in heart rate, core and skin temperature (Brouha, 1960; Mathews *et al.*, 1969), and pressor responses (Brouha, 1960) during recovery from heat-stress but did not observe the associated postexercise hemodynamics. Kilgour *et al.* (1993) examined the effect of exercise-induced thermal stress on postexercise hemodynamics. Eight male normally active subjects performed cycling exercise (60 % of VO_{2max}) for 30 min under control and a heat-stress condition (wearing a thermal suit during exercise and recovery). Metabolic and temperature measurements, along with stroke and cardiac index, heart rate, and arterial pressure were taken before, during, and after exercise. These researchers found a decline in stroke index to below

preexercise values in both control and heat conditions. Heart rate recovery was slower in the heated condition which accounted for the increase in cardiac index above preexercise levels. Systemic vascular resistance was lower postexercise in both conditions, but returned to baseline within 5 min of recovery during control condition and remained lower throughout recovery following heat-stress. These authors concluded that the baroreflex may have compensated for the fall in cardiac index by increasing contractility during the recovery period. This study failed to characterize the peripheral hemodynamics during recovery from heat-stress. This study is limited to normally active men and does not evaluate the effects in women or an endurance exercise-trained population. The complete hemodynamic profile during supine exercise recovery in the heat and the impact of exercise-induced dehydration on an extended recovery time period is unknown.

Fluid Replacement and Postexercise Hemodynamics

Research exists on the effect of fluid replacement and rehydration on core temperature during exercise recovery (Mitchell *et al.*, 2000), but little is known about the influence of fluid replacement on postexercise hemodynamics. The persistence of a sustained peripheral vasodilation, and not postexercise hypovolemia, has been shown to be the underlying mechanism of postexercise hypotension (Piepoli *et al.*, 1993a; Halliwill *et al.*, 1996a). Despite this fact, the role of fluid replacement, plasma volume, and sweat loss on postexercise hypotension has not been addressed. Fluid ingestion and intravenous rehydration have been shown to partially correct the fall in postexercise arterial pressure (Davis & Fortney, 1997; Charkoudian *et al.*, 2003). However, other studies have shown mean arterial pressure postexercise in a hot environment to be tightly regulated and not different between conditions (Brouha, 1960). Previous observations have shown that postexercise hemodynamics in endurance-trained men is secondary to altered cardiac hemodynamics (Senitko *et al.*, 2002; Dujic *et al.*, 2006). The reasons for these hemodynamic differences remain unclear and may be due to inadequate fluid replacement during exercise and greater sweat rates in trained men (upwards of 2.0 L/h in

runners) (Costill, 1977; Frye & Kamon, 1981; Myhre *et al.*, 1985; Sawka *et al.*, 2007) that may ultimately reduce preload during recovery from exercise. However, these studies failed to document hydration status (i.e., pre to postexercise changes in overall body weight) and amount of oral fluids during exercise was ad libitum (Senitko *et al.*, 2002) or completely restricted (Dujic *et al.*, 2006). It is known that ab libitum drinking results in incomplete fluid replacement (Bean & Eichna, 1943; Adolph, 1947; Sawka *et al.*, 2001). Currently, limited studies exist on the role that hydration plays in determining postexercise hemodynamics in a group of endurance exercise-trained men. The influence of fluid replacement following exercise in thermoneutral and a warm environment in trained men will be examined in Chapter V (Study 2).

Part IV: Cardiac Hypertrophy and the Endurance Exercise-Trained Athlete

The Athlete's Heart

Cardiac Adaptation to Endurance Training

When subjected to repeated, sustained exercise the human heart will grow and adapt (Fagard, 2003; Thompson, 2004). Changes common among the hearts of athletes are increases in atrial and ventricular volume, atrial and ventricular mass, diastolic ventricular filling, and overall stroke volume (Oakley, 2001; Sharma, 2003). These changes arise from sustained and increased cardiac output which alters the loading conditions of the heart (Fagard, 2003) during chronic physical training (Sharma, 2003). A linear relationship has been seen between oxygen uptake and cardiac output (Blomqvist & Saltin, 1983) and athletes that train at high oxygen uptake impose a chronic volume load on the heart, causing elevated filling pressure during training and resulting in an overall increase in end-diastolic volume (Levine *et al.*, 1991). The increase in chamber compliance offers a huge benefit to an athlete in facilitating a large volume of blood to the working muscles (Levine *et al.*, 1991); an example of this, removing the pericardium in racing dogs increased cardiac output and improved aerobic power (Stray-Gundersen *et al.*, 1986). The main hemodynamic feature during exercise in endurance-trained athletes is an elevation in blood pressure due to increased cardiac output (from

increases in stroke volume) with relatively little change or slightly decreased systemic vascular conductance. This effectively increases the pressure load on the heart (Fagard, 2003). The changes in cardiac function seem to be secondary to changes in structure. Cardiac adaptations are related to an athlete's sex, size, type, (Fagard *et al.*, 1984) and the intensity of the athlete's primary sport (Pelliccia *et al.*, 1991). Echocardiography and then magnetic resonance imaging has confirmed these findings (Fagard, 2003; Sharma, 2003). These changes manifest as large ventricular depolarization (QRS complexes) and sinus bradycardia on an electrocardiogram (Blomqvist & Saltin, 1983). Three months of competitive athletics produced changes in cardiac structure and function in endurance athletes. Endurance athletes showed biatrial enlargement and biventricular dilation which enhanced biventricular function (Baggish *et al.*, 2007). It seems likely that the structure of an athlete's heart may differ based on training state. The definition of "an athlete", for the purpose of this review is defined as an individual that competes at the county or national level in regular organized endurance activity (Oakley, 2001; Sharma, 2003). This section reviews the structural and functional changes in the heart of an athlete and the physiological changes that are associated with these structural alterations.

Cardiac Hypertrophy in Athletes

The large majority of studies have been done on young (18 to 35 yr) male Caucasian athletes (Sharma, 2003) where evaluations of the wall thickness of the left ventricular have been assessed. The right ventricle has been measured to a lesser extent due to the difficulty with reproducibility with echocardiography. Cardiac dimensions are increased in athletes, and although the differences are small and there is overlap between age-matched controls, the changes are significant. The left ventricular wall thickness and cavity size are increased 15 to 20 % and 10 % compared to age and sex-matched non-athletes (Maron, 1986; Sharma, 2003). Pelliccia *et al.* (1991) studied 1000 Italian Olympic athletes across 25 sports and showed that left ventricular wall thickness range from 5 to 15 mm and 2 % of athletes had values that exceeded the upper normal limits. Interestingly, these 2 % of athletes were all men and have large body surface areas and

were all participating in endurance sports (rowing, cycling and canoeing). Twin studies have revealed a genetic influence on left ventricular wall thickness (Fagard, 1997).

Athlete Bradycardia

Bradycardia (reduced resting heart rate) is a hallmark characteristic of the trained athlete. This athletically-induced bradycardia is due to increased parasympathetic (Seals & Chase, 1989) and reduced sympathetic tone (Hammond & Froelicher, 1985). An ambulatory electrocardiography evaluation of 35 male athletes found that sleeping heart rate ranged from 24 to 48 beats/min compared to 33 to 63 beats/min in the control subjects (Viitasalo *et al.*, 1982). There is little evidence that bradycardia is harmful (Oakley, 2001). However, some athletes develop symptoms of syncope (fainting) (Convertino, 1987) in response to training and require de-training after periods of high performance.

Left Ventricle

One meta-analysis examined 135 male runners (training more than 100 km/wk for a number of years) against 173 controls and found that the left ventricles of athletes had larger internal diameter and wall thickness (48 % greater) (Fagard, 1996). This result was confirmed by a large scale study involving 1451 athletes (Pluim *et al.*, 2000). The same trend was seen in male cyclists when compared against a control group as wall thickness was 19 % greater (Fagard, 1996).

At rest, left ventricular filling rate and posterior wall movement was not different in runners, cyclists, swimmers, and triathletes compared to controls. Thus, left ventricular diastolic function (at rest) is unchanged in athletes (Fagard, 1997). However, some evidence suggests that left ventricular end-diastolic function is enhanced during exercise in athletes (Fagard *et al.*, 1987; Fagard, 1997) and this alteration promotes filling of the ventricle when heart rate increases and the filling period shortens (Fagard, 2003).

Right Ventricle

The right ventricle has not been studied in the same detail as the left ventricle due to the limitations of echocardiography to produce accurate reproducible measurements (Fagard, 2003). Scharhag *et al.* (2002) used magnetic resonance imaging to evaluate cardiac structure in 21 male endurance athletes and confirmed increased left ventricular mass and found increases in right ventricular end-diastolic volume (25 %) and right ventricular mass (37 %) compared to age-matched controls. In addition, both groups showed the same right and left ventricular ratio. It seems that endurance training results in balanced cardiac muscular development on the left and right sides.

Cardiac Function

A number of large-scale studies have revealed that left systolic function (measured through echocardiography) is not different between athletes and age- and sex-matched control subjects (Blomqvist & Saltin, 1983; Fagard *et al.*, 1987; Fagard, 1996; Pluim *et al.*, 2000). Differences have been observed in resting ejection fraction and peak ejection fraction. These values remain within normal limits (Fagard, 2003) and do not seem to change with continued athletic training (Fagard *et al.*, 1983).

Effect of Age and Sex on Cardiac Adaptation

The first large study of cardiac adaptations in women was completed by Pelliccia *et al.* (1996). In this study 600 national level female athletes were compared with age-matched controls. The mean left ventricular wall thickness was 8.2 mm and cavity size was 46 mm, which was 6 and 14 % larger than sex- and age-matched controls. Similar findings have been confirmed by Sharma *et al.* (2002) in adolescent female athletes. No female athlete of any age group has been shown to have wall thickness greater than 12 mm, unlike findings in men (Pelliccia *et al.*, 1991). Absolute left ventricular mass is reported to be higher in men than in women runners (Mumford & Prakash, 1981). These sex-specific differences may be partially explained by circulating endogenous males hormones which promote increased muscle mass and allow for training at greater

intensities (Sharma, 2003). However, no differences have been seen in competitive men and women runners with equal training volume and oxygen uptake per lean body mass (Morales *et al.*, 1992).

Effect of Type of Sport on Cardiac Adaptation

Chronic endurance training (running, swimming, and cycling) increases cardiac preload; these athletes have the greatest left ventricular cavity size (Sharma, 2003). Rowers (both adult and adolescent athletes) have the largest left ventricular wall thicknesses, this indicates that left ventricular hypertrophy is stimulated by both isotonic and isometric exercise (Sharma, 2003). Athletes with the greatest left ventricular wall thickness also tend to have the greatest left ventricular cavity size (Pelliccia *et al.*, 1991; Sharma *et al.*, 2002).

Left Ventricular Performance during Exercise and Recovery

Left ventricular performance has been assessed during and after exercise under a range of intensities and durations. Seals *et al.* (1988) found that after prolonged exercise healthy subjects displayed increased left ventricular shortening and reduced left ventricular end-diastolic diameter, these factors likely contributed to the reduced left ventricular performance. Cardiac performance was evaluated (two-dimensional echocardiography) in 21 athletes before and after an Ironman triathlon. Decreases in left ventricular diastolic dimension and fractional shortening were observed (Douglas *et al.*, 1987). Goodman *et al.* (2001) examined left ventricular function with radionuclide angiography after 150 min of cycling at 60 % of VO_{2max} in 15 healthy men. Peak filling rate was increased through 30 min postexercise; however, ejection fraction was similar to prexercise values. Stein *et al.* (1978) showed an increase in end-diastolic dimension and fractional shortening during the initial minutes of exercise recovery. Left ventricular function following moderate dynamic exercise in high-level endurance-trained athletes has yet to be evaluated over an extended postexercise period.

Clinical Significance

Orthostatic Intolerance in Athletes

Greenleaf *et al.* (1981) said: "Trained men can run, but they can't stand". Orthostatic tolerance is defined as the ability to maintain consciousness during standing or following quick postural adjustments (Raven & Pawelczyk, 1993). The current thought is that athletes that have high VO_{2max} values (> 65 ml·kg $^{-1}$·min $^{-1}$) have lower orthostatic tolerance compared to sedentary controls (Raven & Pawelczyk, 1993). To address this issue, Stevens *et al.* (1992) had 8 men undergo a formal 8 mo exercise endurance-training program. Following exercise training, VO_{2max} and blood volume were increased and there was less orthostatic tolerance to lower body negative pressure. Thus, chronic exercise-training is a main predictor of orthostatic intolerance in endurance-trained individuals (Convertino, 1987). Four factors associated with endurance exercise-training are thought to contribute to the development of orthostatic intolerance with athletes: 1) increased leg vascular compliance, 2) ventricular hypertrophy, 3) increased total blood volume, and 4) reduced arterial baroreflex responsiveness (Raven & Pawelczyk, 1993). It is thought that syncope following exercise is an exaggerated form of postexercise hypotension (Krediet *et al.*, 2004). It is logical to assume that the endurance athlete prone to dizziness following exercise may be at risk of syncopal episodes.

Increased Left Ventricular Compliance with Endurance Training

Chronic endurance-training preserves myocardial compliance (Arbab-Zadeh *et al.*, 2004) and mitigates central arterial stiffening in humans (Seals, 2003). Levine *et al.* (1991) proposed orthostatic intolerance was due to structural and mechanical adaptations (i.e., increased ventricular compliance) due to chronic volume load on the heart during endurance training. Measurement of pulmonary capillary wedge pressure (Swan-Ganz catheter) and left-ventricular end-diastolic volume (echocardiography) was used to assess chamber compliance in response to lower body negative pressure in 13 men (7 athletes and 6 control subjects). They hypothesized that orthostatic intolerance in athletes was

due to greater compliance of the left ventricle so that there were large decreases in stroke volume for any given decrease in filling pressure. Results confirmed that athletes had increased chamber compliance which reduced end-diastolic volume over the same range of filling pressures (steeper slope of the Frank-Starling curve). During exercise, operating on a steeper portion of the Starling curve is a greater advantage as this allows for increased diastolic reserve and a greater change in stroke volume for a given change in filling pressure. However, this cardiac morphological change increases the prevalence of syncopal episodes in trained athletes when subjects are not exercising. Endurance-trained athletes show unexplained decreases in cardiac output and stroke volume postexercise (Senitko *et al.*, 2002). The possibility exists that the same fall in preload pressure produces a greater fall in stroke volume due to the effect of training-induced cardiac hypertrophy and operation on a steeper portion of the Frank-Starling pressure-volume cardiac curve. It is important to assess the postexercise hemodynamics postexercise when the exercise bout was superimposed on heat-stress as alterations in preload and afterload could further increase the prevalence of orthostatic intolerance in a trained population. The role of left ventricular compliance in postexercise hemodynamics will be examined in Chapter VI of this dissertation.

The Use of Exercise for Anti-Hypertensive Nonpharmacological Therapy

The overall goal of these studies is to further understand the mechanisms underlying postexercise hypotension and cardiovascular regulation following exercise. Postexercise hypotension is thought to be an effective therapy against hypertension, and direct mechanistic research furthers the understanding of blood pressure regulation in the healthy individual and those with disease (MacDonald, 2002a). Hypertension is one of the most common medical conditions in North America and is associated with increased incidence of cardiovascular events, disease and mortality. Hypertension is a public health concern with 58.4 million (28.7 %) American adults have been diagnosed with clinical hypertension (Hajjar & Kotchen, 2003). Hypertension is defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg or being on current

anti-hypertensive pharmaceutical treatment. Hypertension increases with age and is higher among men compared to women at younger age but this trend reverses in older women. Daily moderate intensity endurance exercise is recommended as a primary prevention in the control of hypertension (Pescatello *et al.*, 2004). Regular exercise in the post-collegiate years has been proven to protect against hypertension later in life (Paffenbarger *et al.*, 1991). Reductions in blood pressure following moderate exercise are clinically significant for hypertensives during daytime hours (Pescatello *et al.*, 2004). It is encouraging that the sustained and greatest magnitude reduction in mean arterial pressure has been seen in hypertensive patients (MacDonald, 2002a). A further detailed knowledge of the mechanisms of postexercise hypotension is needed for a clearer insight into hypertension and cardiovascular regulation in humans.

Specific Aims

The studies discussed in Chapters IV through VI were designed to address the following specific aims:

1. Study 1 entitled "Effects of the menstrual cycle and sex on postexercise hemodynamics" (Chapter IV) was designed to investigate the role of postexercise hemodynamics in eumenorrheic women with a specific examination related to thermoregulation and fluid balance in three different phases of the menstrual cycle compared to men.

2. Study 2 entitled "Postexercise cardiac hemodynamics in endurance exercise-trained men" (Chapter V) was designed to assess the influence of fluid replacement and exercise in a warm environment on postexercise hypotension. Postexercise hemodynamics was examined in a group of endurance exercise-trained men to determine the effects of oral fluid replacement and exercise in a warm environment during exercise on cardiac output during recovery from exercise.

3. Study 3 entitled "Variations in postexercise hemodynamics across sedentary and endurance-trained men and women" (Chapter VI) was designed to investigate central (i.e., heart structure and filling capacity) and peripheral (skin and leg blood flow) hemodynamics in well hydrated sedentary and trained men and women during recovery from exercise.

Hypotheses

The following hypotheses were tested:

1. In Chapter IV four main hypotheses were tested. First, the postexercise response in the ovulatory phase would be associated with enhanced vasodilation in skeletal muscle vascular beds and would show unchanged vasodilation in cutaneous vascular beds relative to the early follicular phase. Second, the postexercise response in the mid-luteal phase would be associated with similar vasodilation in muscle vascular beds and elevated skin blood flow relative to the early follicular phase. Third, the postexercise response in the mid-luteal phase would be associated with lower stroke volume and cardiac output relative to the other phases. Fourth, there would be similar postexercise hemodynamic responses between men and the early follicular phase but that there would be differences between men and the ovulatory and mid-luteal phases.

2. The study in Chapter V tested three main hypotheses. First, adequate fluid replacement during exercise in a group of trained men would prevent the postexercise fall in cardiac output. Second, under conditions of no fluid replacement and exercise in a warm condition, further decreases in cardiac output would be observed compared to fluid replacement conditions. Lastly, it was hypothesized that the magnitude of postexercise hypotension would not be different between conditions.

3. In Chapter VI two main hypotheses were tested. First, there would be variation in postexercise hemodynamics across groups, but the magnitude of postexercise hypotension would remain the same between trained and sedentary individuals. Second, differences in postexercise cardiac output response across subjects would be correlated to plasma volume, core temperature, central venous pressure, and left ventricular end-diastolic diameter.

CHAPTER III

EXPLANATION OF THE METHODOLOGY

Participating Subjects

Subjects

The experimental protocols described in this dissertation were approved by the Institutional Review Board of the University of Oregon (Protocol #C1-27-04F-C, #C2-117-08F and #C2-117-08F) and were administered in accordance with the guidelines as set forth by the Office of Human Subjects Compliance of the University of Oregon and the Declaration of Helsinki.

A total of ninety-two subjects participated in three studies (Study 1 – Chapter IV, Study 2 – Chapter V, and Study 3 – Chapter VI) outlined in this dissertation. All subjects were healthy with no allergies, non smoking, normotensive, and were free of cardiovascular disease. In Study 1, fourteen women between the ages of 19 and 33 yr participated in this study. These subjects were normally menstruating with menstrual cycles lasting 30 ± 2 days and had not used oral contraceptives in the previous 6 mo before the study. In Study 2, fourteen men between the ages of 20 and 40 yr participated in this study. All men were "endurance-exercise trained" (strenuous endurance exercise > 5 days/wk or > 5 h/wk). In Study 3, a total of sixty-four subjects [(34 men and 30 women (between 18 and 40 yr)] participated in this study. On the basis of their exercise habits over the previous 12 mo, subjects were classified as "sedentary" (no regular physical exercise) or "endurance-exercise trained" (strenuous endurance exercise > 5 days/wk or > 5 h/wk). Subjects were not taking any medications with the exception of Study 3 where the female subjects were permitted to take oral contraceptives. In Study 3,

female subjects were studied during the early follicular phase of the menstrual cycle or during the placebo phase of oral contraceptive use; this accounted for the influence of endogenous and exogenous hormones, as these factors are known to have an impact on cardiovascular parameters (see Part II of the literature review). All women subjects in Study 1 and 3 had negative pregnancy tests on each day of participation in the study. In Study 2, all data was collected in Eugene, Oregon, during the winter months (January and February) when subjects were naturally unacclimated to heat. The laboratory temperature was maintained between 20 and 22 °C for each study unless otherwise stated. Each subject gave written informed consent before participating in the study.

Screening Visit/Peak Aerobic Power

Maximal oxygen power was measured in the initial screening visit for each subject and was used for the determination of workload for the study days in the protocol described below. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands). The test consisted of 1-min of workload increments to determine peak oxygen uptake (VO_{2peak}). Specifically, after a 2-min warm-up period of easy cycling (40-120 W), workload increased at 20, 25, or 30 W every minute until volitional fatigue. Selection of the workload increment was subjective based on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen uptake (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). The combination of these two types of testing equipment has been shown to measure maximal oxygen uptake accurately (Bassett *et al.*, 2001). The peak test was stopped and a cool-down period (~50-100 W) was initiated after the subject's pedaling dropped below a cadence of 60 rpm. Respiratory exchange ratio of 1.10 or greater confirmed peak aerobic power was reached. All subjects reached subjective exhaustion (rating of perceived exertion) on the Borg (Borg, 1970) scale of 19-20 within the 9 to 15 min period.

Determining Moderate Exercise Intensity on the Cycle Ergometer

After the subjects rested for 15-20 min, they returned to the cycle ergometer for assessment of the workload corresponding to a steady-state VO_2 of 60 % of VO_{2peak}. Workload that would produce 60 % of VO_{2peak} was estimated from the following equation:

[Target VO_2 (ml/min)/12] – 25 = estimated workload (watts)

Steady state oxygen consumption was obtained within ~3-5 min. The workload was adjusted if needed to achieve one that produced steady state VO_2 of 60 % of VO_{2peak}. This workload was used on each of the study days for the 60 min exercise bout. This workload has been shown to produce a sustained (~2 hr) postexercise hypotension (Halliwill, 2001; Pricher *et al.*, 2004).

Questionnaire

For each of the studies outlined in this dissertation, self-report questionnaires were used to determine average activity level for each subject over the 6 to 12 mo before participation in the study. Subjects self-reported activity levels on two questionnaires (Baecke *et al.*, 1982; Kohl *et al.*, 1988) during the 15-20 min rest period before the start of the 60 % of VO_{2peak} verification test. Quantifying physical activity and energy expenditure can be difficult. Generally, pedometers, portable heart rate monitors, accelerometers, and activity diaries can be used. Pedometers can be inaccurate based on positioning and location of the pedometer and can be poor at quantifying certain types of movement. Portable heart rate monitors and daily activity logs reflect collection of short-term physical activity information (Saris & Binkhorst, 1977; Saris *et al.*, 1977a; Saris *et al.*, 1977b). Questionnaires are the most widely used measurement of physical activity and this method reflects physical activity over a longer period of time (Baecke *et al.*, 1982). Physical activity questionnaires are known to be reliable and valid at reporting information about daily energy expenditure and weekly physical activity levels (Suzuki *et al.*, 1998). Thus, for the purpose of this research, self-reported activity level

questionnaires were found to be a good way of describing the habitual physical activity levels of the subjects over 6 to 12 mo before the study.

Determination of Body Composition

DEXA

In order to classify subjects as trained or sedentary in Chapter VI (Study 3), subjects underwent measurement of body composition with a dual-energy x-ray absorptiometry (DEXA) scan. A DEXA scan (Total Body Analysis, ver. 3.6y, Lunar, Madison, WI) is considered to be the gold standard for measuring body composition and was used to estimate regional (lower body) muscle mass. This technique (which has been long recognized for its effectiveness in measuring bone density) is known to demonstrate excellent precision in the measurement of bone-free lean tissue (i.e., muscle) and fat. Bony landmark sites described by Heymsfield *et al.* (1990) were used to obtain the summed lower extremity (appendicular) muscle mass (i.e., bone-free lean tissue for both legs, in kilograms). DEXA has the advantage over hydrodensitometry of providing a measure of bone mineral mass in addition to fat mass and fat free mass, thereby providing a more comprehensive determination of body composition by examining a three compartmental model of determining body composition (Kohrt, 1998).

Other Acceptable Methods

Hydrostatic weighing or densitometry is a method used for assessing density of the human body. Once density is known, equations are used to convert readings into percent fat. Hydrostatic weighing is considered to be more accurate at estimating the percent body fat than anthropometric measures such as skinfolds or bio-electrical impedance. Limitations of this technique depend on the experience of the investigator, accuracy of the equipment, and experience of the subject to expel all air and achieve a true residual volume. The reliability of body densitometry is considered to be high ($r \geq 0.95$) (Ward *et al.*, 2007).

Air displacement plethysmography can be used to measure body volume in order to measure human body composition. This commercial device called the BODPOD™ uses the relationship between pressure and volume to derive the body volume of a subject seated inside a fiberglass chamber. Derivation of body volume, together with measurement of body mass, permits calculation of body density and subsequent estimate of percent fat and fat-free mass (Fields *et al.*, 2002; Ball & Altena, 2004). This method has been validated against hydrostatic weighing and DEXA, and although the methods seem to be well correlated, the amount of difference seems to increase with body fatness (Kohrt, 1998). It is advised that even with the use of these sophisticated methods, differences between methods exist and the determination of body composition is at best an indirect estimation.

Skinfolds are measured using large calipers for certain anatomical landmarks, mainly triceps, subscapula, pectoralis, umbilicus, suprailiac, biceps, and thigh. Skinfold thickness is determined at each site and usually repeated for a duplicate and triplicate measurement (especially if the measurements differ by 1 mm or more). Values are then averaged with the outlying values removed. Based on the sum of the skinfolds, equations are used based on sex and ethnicity to calculate a percentage body fat. Limitation with this technique exists in the reliability of its measurements, as sources of error exist with experience of the technician (Kohrt *et al.*, 1992). Minimal to moderate dehydration and increased distribution to the cutaneous circulation (that is often associated with exercise) can directly under and overestimate the skinfold measurement and thus, has the potential to add large amount of error into the results. The issue of measurement error is less of a consideration with hydrostatic weighing and DEXA. DEXA can accurately measure body composition even with dehydration of 7-10 % loss in body weight and thus, for the purpose of the research, the DEXA was deemed the best measurement technique to determine body composition (Kohrt, 1998).

Subject Monitoring

Heart rate and arterial pressure were monitored throughout all experimental procedures before, during, and after exercise. Heart rate was monitored using a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA). Arterial pressure was measured in the arm by using an automated oscilometric device (Dinamap Pro100 vital signs monitor, Critikon, Inc., Tampa, FL). This method of measuring arterial pressure is comparable to the "gold standard" of arterial pressure determination, an intra-arterial catheter (Hunyor & Nyberg, 1978; van Egmond *et al.*, 1985; Parati *et al.*, 1989). Exercising arterial pressure (in all studies) was obtained through manual auscultometric device due to the error in diastolic blood pressure with an automated oscilometric device (Griffin *et al.*, 1997). Mean arterial pressure was calculated as diastolic blood pressure + [(systolic blood pressure – diastolic blood pressure) *1/3].

Monitoring Hydration Levels

Urine Specific Gravity

It was important to ensure equal hydration status when assessing the role of fluid replacement under different study conditions. In Study 2 (Chapter V), hydration status was estimated in duplicate from urine specific gravity (Hand refractometer, NSG Precision Cells, Inc., Farmingdale, NY, USA). The refractory index is a ratio of the velocity of light in air to the velocity in urine; the change in velocity deviates (refracts) the path of light and the extent of which is read from a scale on the refractometer reference glass (Chadha *et al.*, 2001). The degree of refraction is proportional to the number and type of particles dissolved in the urine, providing valid indirect estimate of urine specific gravity. In addition, hydration can be assessed via hormonal blood samples and osmolality of the urine. However, urine specific gravity is known to accurately assess steady-state hydration and for our purposes it was an inexpensive and reliable technique (Sawka *et al.*, 2007) for assessing hydration level at the beginning of each study.

Measurement of Body Weight

Body weights were measured to ensure fluids levels were maintained postexercise in all studies presented in this dissertation. Body weight is a simple and effective tool to assess fluid balance when an accurate scale that can determine body weight to the nearest 5 g is used (Sartorius EB6CE-I, Precision Weighing Balances, Bradford, MA, USA). Body weight is a good measure of changes in total body water. In Study 2, it was critical to assess hydration status in order to assess the effects of heat and dehydration on postexercise hemodynamics (see timing of measurement above). Acute changes in body weights during and after exercise can be used to calculate accurate sweat rates and alterations in hydration status due to changes in environmental conditions and exercise. Pre- to postexercise changes in body weight corrected for urine output and volume of oral fluids consumed were used in Study 2 to assess sweat loss during the protocol. With a sensitive scale and when proper techniques are used (i.e., nude weights) body weight can provide an accurate assessment of hydration status and total body water changes during exercise. Women generally need consistent body weight measurements over several days to establish a true baseline (due to the fluctuations in body water status during the menstrual cycle) (Bunt *et al.*, 1989). This method accurately predicts fluid replacement needs for specific exercise modes and intensity and under altered environmental conditions (Sawka *et al.*, 2007).

Measurements and Techniques

Cardiac Output

Cardiac output is routinely measured by several direct and indirect techniques and these methods compare well with one another. In all of the studies outlined in this dissertation, cardiac output was estimated using an open-circuit acetylene (C_2H_2) washin method developed by Stout *et al.* (1975), modified by Gan *et al.* (1993) and validated in humans against the direct Fick approach (Johnson *et al.*, 2000). Thus, the acetylene rebreathing technique has gained acceptance in the scientific community (Johnson *et al.*,

2000). Cardiac output can be determined from the following equation of the Fick principle:

$$Q = VC_2H_2 / [C_vC_2H_2 - C_aC_2H_2]$$

where: VC_2H_2 = rate of C_2H_2 production (ml min^{-1})

$C_vC_2H_2$ = C_2H_2 content of mixed venous blood (ml 100 min^{-1})

$C_aC_2H_2$ = C_2H_2 content of mixed arterial blood (ml 100 min^{-1})

This method allows for the noninvasive, reliable, and valid estimation of cardiac output and was the best choice for the measurement of cardiac output in each of the studies outlined in this dissertation. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services) and tidal volume was measured via a pneumotach (model 3700, Hans Rudolf, Kansas City, MO) linearized by the technique of Yeh *et al.* (1982) and calibrated by using test gas before each study. Gas concentrations of inspired and expired helium and acetylene are measured for each breath. The difference of these gas concentrations equal the volume of the gas that is taken up by the pulmonary circulation. Cardiac output calculations have been described previously (Johnson *et al.*, 2000). Stroke volume was determined from cardiac output/heart rate. Systemic vascular conductance was calculated as cardiac output/mean arterial pressure and expressed as ml · min^{-1} · $mmHg^{-1}$.

Other Acceptable Methods

Alternative techniques include: direct Fick method (gold standard), indicator dilution techniques (including thermodilution), transthoracic cardiac electrical impedance, Doppler ultrasound to determine geometry of the heart, and foreign-gas (CO_2 rebreathing and nitrous oxide rebreathing) (Ehlers *et al.*, 1986). The addition of an inert reference gas such as helium or argon to the rebreathing mixture along with the use of the mass spectrometer has improved the resolution of the foreign-gas (usually acetylene)

rebreathing technique (Cander & Hanowell, 1963). The alternative techniques will be described in the following paragraphs.

Measurement of cardiac output using the direct Fick method is based on the principle that total uptake and release of any substance by an organ is the product of blood flow to the organ and the arteriovenous concentration difference of the substance (Fick, 1870) and is based on the following original equation $VO_2 = Q \times [C_aO_2 - C_vO_2]$. This principle can be used with a number of markers such as soluble gases (Ehlers *et al.*, 1986). The direct Fick method requires insertion of pulmonary and (usually) radial arterial catheters in order to determine arterial to venous oxygen difference. Pulmonary artery catheters are inserted via an introducer catheter under local anesthesia through an antecubital vein during continuous electrocardiogram and pressure monitoring. The correct position of the pulmonary artery catheter is verified with the wedge position and pressure tracing. The radial artery catheter is inserted under local anesthesia and is taped into place, and this allows multiple arterial blood-gas samples to be taken. Catheters are continuously flushed with heparinized saline to prevent clotting and obtain accurate pressure tracings (Johnson *et al.*, 2000).

The indicator dilution technique involves the principle of the introduction of a predetermined amount of substance at a single point in the blood stream and then analyzing the flowing blood at a point downstream to obtain a time dilution curve. The substance is introduced at a point in the circulation where uniform mixing is known to occur with the total blood flow prior to the measurement of the dilution curve. The average volume flow is inversely proportional to the area under the curve. In the method of indicator dilution-dye, indocyanine green (ICG) is typically used and can be injected into the right atrium. The advantages in this technique is that, after set-up, measurements can be made in less than 1 min, but repeated measurements are limited as 50 % of the dye is cleared by the kidneys 10 min post-injection (Ehlers *et al.*, 1986).

Impedence cardiography is another common non-invasive way to measure cardiac output; however, the theory behind determination of cardiac output and methods associated with this technique have been questioned (Critchley, 1998). In this technique,

cardiac output can be calculated by measuring transthoracic impedance and measuring changes in electrical conductivity due to the blood flow in the chest. Electrical impedance variations are measured as voltage or high-frequency current in the chest. Two sets of electrodes are placed around the neck and at the lower chest. This produces information about the dynamic variations in cardiac output. This method allows for real time determination of cardiac performance. However, patient movement can cause motion artifact and pulmonary blood flow may interfere with the measurements (Ehlers *et al.*, 1986).

Echocardiography can be used to estimate cardiac output. For this method subjects are positioned in the left lateral position in order to obtain the parasternal-long axis view for measurement of aortic ring diameter and left ventricular and left atrial dimensions with a 3-MHz transducer. Cardiac output can then be calculated from the product of stroke volume and heart rate (where stroke volume is calculated from the product of the mean time velocity integral and the cross-sectional area of the aortic orifice) (Hara & Floras, 1992).

CO_2 rebreathing to equilibrium can estimate cardiac output by the indirect Fick method (Collier, 1956). In this technique the subject breathes air though a large 3-way stopcock. Next a 5 L anesthesia bag is connected to one arm of the stopcock and a measured amount of the desired concentration of CO_2 in air is place in the bag. The instaneous concentration of CO_2 in the airway is recorded continuously through a catheter sampling system and a rapid infrared CO_2 analyzer. The subject continues to breathe normally throughout the procedure. The stopcock is then turned at the end of a normal expiration in order to connect the subject to the 5 L bag. The stopcock is turned again 20 seconds later to reconnect the subject to breathing room air. The CO_2 tracing is inspected for a plateau which is defined as an expiratory CO_2 level which differs from the preceding inspiratory level by less than 0.1 %. Calculations are then made with the Fick equation to calculate cardiac output (Collier, 1956).

Nitrous oxide (N_2O) rebreathing (Ayotte *et al.*, 1970) can be used to measure cardiac output due to high solubility of this gas. The volume of N_2O is calculated as the

difference between the amount of N_2O available at the start of rebreathing and the amount left unabsorbed at the end of the rebreathing procedure. A 13 L rubber bag is filled with a measurement volume (~5 L) of a gas mixture containing 10 % N_2O, 5 % Freon 14 (CF_4), 20 % O_2, and 65 % N_2. The volume of N_2O available for absorption is calculated from the volume of gas in the bag and the concentration of N_2O. The rebreathing procedure lasts until equilibrium is reached between gas in the bag and gas in the lungs. At this time, the volume of gas in the bag plus lung volume is calculated. Analyses of N_2O and CF_4 concentrations are made with a gas chromatograph with a thermal conductivity detector and a chart recorder gives values for N_2O, CF_4 and CO_2 from the other constituents of the gas mixture. The final record will show initial and final gas concentrations (Ayotte *et al.*, 1970).

Limitations

Acetylene rebreathing is limited by the assumption of the solubility of C_2H_2 in blood. Changes in hematocrit and blood temperatures can change solubility and can cause significant errors in the determination of cardiac output (Rowell, 1983). Acetylene rebreathing increases ventilation and the subject can get uncomfortable, both of which could potentially change hemodynamics (Petrini *et al.*, 1978; Liu *et al.*, 1997). Another drawback to the acetylene technique is the buildup of carbon dioxide as a result of rebreathing and the subsequent dyspnea. This may be problematic at higher intensities of exercise or with longer rebreathing time in patients with ventilation mismatch problems such as aging and obstructive airway disease (Johnson *et al.*, 2000). The nitrous oxide method is only suitable for subjects with normal lungs and is applied best during exercise. Obstructive airway diseases that can alter the ventilation-perfusion ratios may also interfere with this method. These foreign-gas methods all measure pulmonary capillary blood flow and not blood shunted past the lungs. All respiratory methods of measuring pulmonary blood flow assume adequate mixing of inspired air with alveolar air and equilibrium between alveolar and arterial gas concentrations (Ayotte *et al.*, 1970). For the purpose of these studies, and resting measurements before and after exercise,

acetylene is an acceptable and a proven reliable measure of cardiac output (Johnson *et al.*, 2000) and was used in each of the studies presented in this dissertation.

Core Temperature

Core body temperature can be measured through a number of different techniques. In each of the studies presented in this dissertation, internal body temperature was assessed by an ingestible pill telemetry system (HQInc, Palmetto, FL) (Coyne *et al.*, 2000; Wilson *et al.*, 2004). This system is a relatively new technique for obtaining core body temperature. In this system the subject swallows a small, disposable, silicon-coated pill that contains a crystal quartz oscillator which transmits a low-frequency radio wave to an external receiver. The ingestible-pill telemetry system approximates esophageal temperature very closely and has been reported to be a better measurement tool when compared to rectal temperature as an index of core temperature during passive heating and cooling and exercise (O'Brien & Montain, 2003; Low *et al.*, 2007). The intestinal sensor is easily ingestible and is not affected by subject movement. This technology reduces the sanitary and safety concerns of esophageal and rectal thermistors during situations where subjects are ambulatory. The ingestible pill was found to be a good alternative to esophageal temperature during specific time points due to comfort consideration (e.g., exercise) although it has been reported to have a slower response time (Lee *et al.*, 2000). All subjects ingested the pill (CorTemp™) between 6 and 10 h before the start of each study visit. This time lapse allows for the pill to move through the stomach and into the intestinal tract for more accurate measurements that are unaffected by oral fluid consumption. Each pill comes with a serial and calibration number that is programmed into the CorTemp™ data recorder. The temperature sensor wirelessly transmits the intestinal tract temperature to the data recorder. The data recorder converts the signal into a digital format. Subjects will harmlessly pass the pill through the intestinal tract within 24 to 48 h after ingestion.

Other Acceptable Methods

There is no one true core temperature due to the temperature differences among different sites in the core. It is known that temperatures at all core sites are within 1 °C of central blood temperature at thermal steady state. A site that is ideal for measuring core temperature should be unbiased to environmental conditions and should have the capabilities to show small changes in central arterial blood temperature (Sawka *et al.*, 2007). There are a number of current techniques used for research purposes in order to measure core body temperature, these include: sublingual, tympanic, oral meatus, and right atrial blood temperatures. However, esophageal and rectal temperatures are two measurement sites of core body temperature that are most commonly used for thermoregulation studies (Lee *et al.*, 2000).

Esophageal temperature is considered to be the gold standard and is preferred because of the deep body location that is close to the left ventricle, aorta, and direct blood flow to the central thermoreceptors in the hypothalamus. However, limitations with this method arise due to difficulty of insertion of the thermistor (pain and vomiting with probe insertion), irritation to nasal passages and throat, overall discomfort, and difficulty ingesting oral fluids. In this technique, local anesthetic is given to the subject and the probe is inserted into the nasal passage and subjects are instructed to swallow in order to place the probe deep into the esophagus close to heart level at the level of the left atrium, where the heart and esophagus are in contact and this area is virtually isothermal for several centimeters (Lee *et al.*, 2000). Esophageal temperature responds rapidly to changes in central blood temperature.

Rectal temperature has gained acceptance in the research community due to its ease of use and its stable measurements during non-dynamic conditions. However, this technique can be influenced by changes in leg blood flow (Saltin & Hermansen, 1966) and may have a longer response time compared with other techniques during rapid changes in core temperature. In this technique, the rectal thermistor probe is inserted 15 cm up into the rectum (5 to 20 cm past the anal sphincter). This technique can be limited by subject movement and in situations such as exercise and spaceflight the crew has

reported discomfort, and repositioning of the thermistor has been reported to interfere with movement which causes missing data (Lee *et al.*, 2000). Rectal temperatures tend to be higher and respond slower to changes in core temperature (i.e., during exercise) when compared to temperature measurements at different sites (Sawka *et al.*, 2007). These differences are most likely due to relatively lower blood flow rates.

Sublingual temperature is widely used clinically but less commonly in physiological research. The tongue is an effective heat exchanger with central blood due to high blood flow per gram of tissue. Sublingual temperature may be lowered by evaporation when the subject breathes through the mouth (Sawka *et al.*, 2007) and thus is not ideal for core temperature measurement during exercise.

Thus, for the purpose of this research intestinal temperature was the best method for the measurement of core body temperature as it produces similar valid and reliable results compared to rectal and esophageal temperatures, and measurements are easily obtained and are unaffected by subject movement.

Femoral Blood Flow

Femoral blood flow can be measured by a number of different techniques that will be discussed in the following sections. Femoral blood flow was determined through measurements of femoral artery diameter and velocity using an ultrasound probe (10-Mhz linear-array vascular probe, GE Vingmed System 5, Horton, Norway). Doppler ultrasonography measures the diameter of the artery and the mean velocity of the blood to determine the overall blood flow of the artery. The ultrasound probe generates a frequency-modulated constant voltage sine wave output and measures the returning sonic wave echoes. The difference is determined and a picture of the artery is generated to measure the diameter of the vessel and determine the mean blood velocity. The entire width of the femoral artery was insonated with an angle of 60 °. For each of the outline studies, velocity measurements were recorded immediately before diameter measurements were obtained. Leg blood flow was then calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs,

and reported in ml/min. Femoral vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as ml · min $^{-1}$· mmHg $^{-1}$.

Other Acceptable Methods

Venous occlusion plethysmography and thermodilution are common techniques used to quantify arterial blood flow, and give accurate results (LeJemtel *et al.*, 1992). Venous occlusion plethysmography is a common, inexpensive, and noninvasive techniques that works well to quantify skeletal muscle blood flow. This technique is used to assess blood flow in extremities by measuring calf girth in response to changes in venous volume (Greenfield *et al.*, 1963; Rowell & O'Leary, 1990). With this method, venous occlusion is produced by inflating a cuff to low pressure (helium for external pressure) on a given extremity. Changes in calf volume are measured using mercury in-Silastic strain gauges. The point of maximal calf circumference is measured and marked to determine appropriate size and placement of the strain gauge. Venous collecting cuff pressure is measured using a pressure transducer attached to the collecting cuff and positioned in line with the venous collecting cuff and air source. The pressure transducer is calibrated using a mercury mamometer (Hiatt *et al.*, 1989). During the occlusion, the volume of the limb increases at a rate equal to arterial inflow.

Thermal dilution technique can be used to determine blood flow by assessing temperature changes between two femoral vein catheters. In this technique, the injectate is kept close to 0 °C. The thermistor catheter is inserted through the venous catheter, which has four infusion holes and is advanced into the vein until the thermistor is located at a distance of 12 cm proximal to the infusion holes. The thermistor is connected and a temperature signal is recorded continuously for the entire infusion period. Flow is calculated with an equation that requires mass of blood and infusion of solution, blood temperature before infusion, blood temperature during infusion, temperature of infusion solution and specific heat of blood (Andersen & Saltin, 1985). This technique is more invasive compared to venous occlusion plethysmography and Doppler ultrasound and is best for measuring blood flow during exercise (LeJemtel *et al.*, 1992).

Limitations

Both venous occlusion plethysmography and Doppler ultrasound provide a means for determining relative changes in flow in the upper and lower extremities. Plethysmography measures flow that is derived from the primary arterial supply, whereas a Doppler measures flow in a single artery. When the Doppler technique is used to quantify leg blood flow the probe must be positioned at the level of the common femoral artery (above the branching and bifurcation) so that blood flow values are reflective of the entire leg. This technique requires an experienced researcher in order to produce reproducible diameters, velocities and overall blood flow. Thus, Doppler ultrasound is an acceptable technique for quantifying leg blood flow at rest and was used throughout these studies.

Left Ventricular Diameter

Measurement of the geometry of the left ventricle is commonly measured in humans with medical imaging. Two-dimensional guided M-mode transthoracic images were obtained from the left parasternal position by using a cardiac ultrasound machine (GE Vingmed) equipped with a 2.5 Mhz vector array cardiac probe, as performed previously (Lawler *et al.*, 1998). Transducer positioning was optimized to obtain the least oblique image at the mid-ventricular short-axis papillary muscle level. Measurements over five to ten consecutive cardiac cycles were used to estimate left ventricular end-diastolic diameter for a given experimental time point.

Left Ventricular Filling Pressure

Ideally, in order to get an index of left ventricular compliance and filling pressure, along with measures of left ventricular end-diastolic volume and end-systolic volume, invasive cardiac catheterization is used. Cardiac catheterization gives a measure of pulmonary capillary wedge pressure (Swan-Ganz catheter) and right atrial pressure. In

this technique a balloon-tippled fluid-filled catheter is placed under fluororscopic guidance through an antecubital vein and guided into the pulmonary artery. All intracardiac pressures are referenced to atmospheric pressure, with the pressure transducer zero reading set at 5 cm below the sternal angle. The mean pulmonary capillary wedge pressure is visually determined at end expiration and is used as an index of left ventricular end-diastolic pressure (Perhonen *et al.*, 2001). This is the gold standard for measurement of ventricular compliance and this technique allows the generation of Frank-Starling cardiac curves with pulmonary capillary wedge pressure (mmHg) plotted against a volume (Arbab-Zadeh *et al.*, 2004). This is an invasive technique and is usually performed on very small number of subjects. Thus, for the purpose of these studies, non-invasive echocardiography was used to evaluate left ventricular end-diastolic volume as a measure of filling capacity, before and after exercise and this was deemed an adequate index of filling capacity and ventricular compliance.

Other Acceptable Methods

Magnetic resonance imaging (MRI) can be used to measure left ventricular parameters (left ventricular volumes and left ventricular ejection fraction). MRI seems to be a slightly superior technique in clinical studies on left ventricular dimensional and functional changes as the measurements are more reproducible compared to two-dimensional echocardiography (2D-echo) (Strohm *et al.*, 2001). However, MRI is not widely available and thus, can be extremely costly and for the purpose of this research 2D-echo was an adequate technique to determine left ventricular end-diastolic volumes.

Central Venous Pressure

In order to determine filling pressure at the right side of the heart, primarily two techniques that are measures of direct and indirect filling pressure can be used. At the beginning of the study visit (Study 3), the subjects underwent the placement of an

intravenous catheter for blood sampling and measurement of peripheral venous pressure. A 2.5-cm, 22-gauge flexible catheter was placed in the antecubital area in the right arm using sterile techniques. The catheter was attached to a single-use sterile pressure transducer and continuously flushed with pressurized saline with heparin to prevent clotting (3 ml h^{-1}, 2 units ml^{-1}). A pressure transducer was attached and used to measure peripheral venous pressure; central venous pressure was estimated by the method of Gauer & Sieker (1956). This method required the subject to lie in the right lateral decubitus position with the right arm dependent. This positioning established a continuous hydrostatic gradient between the vena cava and the peripheral venous catheter such that changes in peripheral venous pressure accurately reflect changes in central venous pressure (Convertino *et al.*, 1991). Resting peripheral venous pressure was monitored continuously and measured for 2 min with a pressure transducer (Edwards Lifesciences LLC, Irvine, CA) (once a steady-state value was reached) and was recorded onto a chart recorder. With the subject in the lateral decubitus position, the transducer was positioned and marked at the level of the midsternum (xiphoid process) and taped into place. Care was taken to ensure repeatable location of the transducer for pre and postexercise central venous pressure measurements.

Other Acceptable Methods

A peripheral inserted central catheter (PICC) is considered to be the gold standard for measuring central venous pressure. In this technique, the basilic or antecubital vein is located by ultrasound. A catheter is then inserted peripherally and advanced to the level of the superior vena cava. The location is verified by measuring the distance from the antecubital fossa to the midclavicular line, and inserting the catheter to that length (verification can also be done through an X-ray). After the insertion, subjects rest in a supine position and are attached to a pressure transducers in order to record continuous pressure readings (Charkoudian *et al.*, 2003). Limitations to this method include the invasiveness and the time consuming nature of the procedure. PICC lines are usually placed in a hospital by a physician or IV nurse; however, this technique requires no

change in body position (compared to the Gauer method) by the subject in order to measure central venous pressure. The PICC line is a direct measure of central venous pressure and reproducibility and results of this technique are superior. The Gauer technique tends to be a better reflection of overall changes in central venous pressure. However, Gauer & Sieker (1956) found that when the methods describe above was compared to catheterization of the central veins, results were identical. Thus, for the purpose of the research, the Gauer method was deemed to be an acceptable technique for the measurement of central venous pressure in a large subject population.

Skin Blood Flow

Currently, no adequate means exists to quantify total skin blood flow. For the purpose of this research, an index of skin blood flow was derived from measuring red blood cell flux values via laser-Doppler flowmetry (DRT4, Moor Instruments LTD, Devon, England). The technique is based on the Doppler shifting and scattering of monochromatic light by moving red blood cells in the microvasculature, where the output signal is linearly related to blood flow. Laser-Doppler measures one direction of red cell velocity and thus no direct measurement of flow can be obtained. Flow velocity is a function of tissue volume, which in terms of the skin changes with flow. Laser-Doppler flowmetry monitors red blood cells flux (blood flow) in a single 1.0 mm^3 volume of tissue. Each integrated probe has one optic fiber emitting a laser light surrounded by eight receiving fibers in a 2 mm ring. The integrated probe provides a great surface area measured by the laser-Doppler system compared to single point laser-Doppler probes. For each of the protocols laser-Doppler probes were placed on the skin of the right ventral forearm and the anterior right thigh. Skin blood flows were expressed as cutaneous vascular conductance, calculated as laser-Doppler flux/mean arterial pressure, and normalized to the maximal values achieved during local heating to 43 °C at the end of the protocol (Minson *et al.*, 2001).

Other Acceptable Methods

Historically, the early measures relied on estimated thermal conductivity in conjunction with the Fick Principle to calculate transcutaneous heat transfer. Problems with the method exist because skin temperature over the body surface is highly variable and simple averaging by area without accounting for local conductance can lead to errors. The primary technique for measuring regional skin blood flow is venous occlusion plethysmography, but measurement is confined to the limbs (Rowell, 1983). Limb blood flow, which includes skin, muscle, and bone are expressed in absolute units of flow rate per 100 ml of limb The mercury strain gauge has fully replaced the traditional water-filled plethysmograph and the photoelectric plethysmograph due to its mobility. However, it is highly sensitive to movement and thus makes its use difficult during moderate to heavy exercise.

Xenon (Xe)-clearance rate technique can be used to estimate skin blood flow. Because Xe is known to be lost in sweat this method is used in nonsweating skin. Additional criticisms of this technique include: hyperemic effects of injection trauma, accumulation of Xe in the subcutaneous adipose so washout is a two-compartment model, as well the coefficient of distribution for Xe between cutaneous and other tissues is not known. Lastly, there is a countercurrent exchange of Xe between arteries and veins. However the Xe -clearance technique was found to be comparable in detecting skin blood flow when compared to venous occlusion plethysmography. In terms of measurement of skin blood flow, laser-Doppler flowmetry is the newest technique and is considered to be the gold standard (Rowell, 1983).

Techniques for Determining Blood and Plasma Volume

Evans Blue Dye

A number of techniques based tracers and dyes can be used to measure total vascular volumes. Evans blue dye (T-1824) dilution was used to measure plasma volume prior to exercise on each study visit in the women subjects in Study 1. Evans blue dye determines plasma volume by staining the plasma proteins (albumin, globulins,

fibrinogen, and connective tissues). After 40 min of quiet supine rest, a control blood sample was drawn and 15 mg of dye was injected via the indwelling catheter followed by a complete saline flush. The technique can be started and dye injected once a stable body position has been established, as vascular volumes take approximately 30 to 40 min to reach equilibrium (Davy & Seals, 1994). Plasma dye concentration was measured in blood samples obtained at 10, 20, and 30 min post-injection by spectrophometry (Beckman Spectrophotometer DU7400, Beckman Instruments; Fullerton, CA). Although no samples were overtly turbid, we used an extrapolation of the 780-720 nm baseline absorbance to 619 nm to correct for artifact as suggested by Farjanel et al. (Farjanel *et al.*, 1997). Preexercise blood volume was calculated from plasma volume and hematocrit. Samples were immediately measured in triplicate for hematocrit (i-Stat Portable Clinical Analyzer, Abbott Laboratories, East Windsor, NJ) and hemoglobin concentration (Hemocue AB, Angelholm, Sweden).

Limitations

The Evans blue dye technique can be limited in its accuracy as a portion of dye is thought to be unbound or becomes bound to extravascular albumin. The method (due to the variable loss of dye from the vascular space) can cause an overestimation of plasma volume. The measurement error seems to persist even with improvement techniques of dye-extraction and when multiple data points are used (back-extrapolation procedure) (Greenleaf *et al.*, 1979). This technique is most suitable for clinical measurement of plasma volume.

Carbon Monoxide (CO) Uptake Method

In Study 3, absolute blood and plasma volume was estimated by measuring total body hemoglobin via a CO uptake method as previously described (Burge & Skinner, 1995). This technique is based on dilution of a tracer, inhaled CO which binds to and changes the color of hemoglobin (Gore *et al.*, 2005) and measures circulating mass of hemoglobin. This was done at the end of the protocol with the subjects in a stable body

(supine) position. The dosage of CO administered in this study was 1.25 ml per kg body weight (up to a maximum dose of 100 ml). This dosage is predicted to raise HbCO by ~ 6.5 % to levels around 8 %. Subjects breathed 100 % O_2 from a Douglas bag for 4 min on an open circuit to flush N_2 from their lungs and then were switched to a rebreathing circuit with a 3 L anesthesia bag that was pre-filled with 100 % O_2. The rebreathing circuit was equipped with a soda-lime CO_2 scrubber (to prevent the buildup of CO_2 in the circuit) and had a fresh O_2 supply so that subjects stayed hyper-oxygenated throughout the procedure. After becoming accustomed to the rebreathing circuit (2 to 4 min), a calibrated volume of ultra high purity (> 99.9 % pure) CO was injected into the rebreathing circuit with a glass syringe over a 15 to 30 second period. Subjects continued to breathe on the circuit for an additional 10 min. Blood samples were obtained before and 10 min after the CO was added to the rebreathing circuit in order to measure the percent of hemoglobin bound to CO (% HbCO). Hemoglobin, hematocrit, and CO-hemoglobin were measured for the purpose of estimating blood volume and plasma volume. % HbCO and hemoglobin were measured spectrophotometrically with the diode-array spectrophotometer OSM3 hexoximeter (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary centrifuge method (Autocrit Ultra 3, Becton Dickson, USA). Samples were collected in 3 ml heparizined arterial blood gas syringes (Smiths Medical ASD, Inc., Keene, NH).

Other Acceptable Methods

The Chromium (^{51}Cr) for Measurement of Red Blood Cell Volume method is considered to be the gold standard and is reliable, reproducible, and easy for routine clinical use (Gore *et al.*, 2005). The biological radioactive chromium tracer is added to whole blood in vitro and then is injected and is rapidly taken up by the red cells. The red cells are known to retain their radioactivity without significant loss to the plasma for periods of 24 h or more post-injection. The exchange between red cells and plasma is negligible for 24 h, thus, this isotope appears ideal for the tagging of red blood cells and the measurement of circulating red cell volume. Samples are removed post-injection,

centrifuged, and then counted (Gray & Sterling, 1950). Sources of error for this technique include the following: error in pipetting and reinjection, blood resuspension, scintillation count error, and hematocrit biological variability (Fairbanks *et al.*, 1996).

Albumin Labeled with Radioactive Iodine (^{131}I or ^{125}I) for Measurement of Plasma Volume

The albumin labeled with radioactive iodine (^{131}I or ^{125}I) method is recommended for clinical use. However, if health risks are a concern, Evans blue or CO rebreathing can be used. In the albumin labeled with radioactive iodine technique a sample of blood is drawn and diluted with 0.9 % NaI and then human albumin labeled with radioactive iodine is injected back into the circulation. Over an hour long period five post-injection blood samples are drawn and sampled for hematocrit and the plasma level of protein and ^{131}I is determined by measurement with a scintillation counter (Sear *et al.*, 1953). Sources of error for this technique and the radioactive chromium method are known to depend on the variability of the tracer (Gore *et al.*, 2005).

Limitations to Blood Volume Measurements with Dilution and Tracers

The methods used to measure blood volume are all considered to be indirect and based on dilution of tracers either injected or inhaled into the circulation. All standard techniques are considered to be somewhat invasive and costly.

The accuracy and reliability of the CO oximeter are dependent on CO remaining in the rebreathing apparatus and in the circulation. Gas leaks in the rebreathing apparatus, noseclip or at the mouthpiece, inadequate CO dose, and using a rebreathing bag with excessive volume are known sources of error for this technique. Adequate CO dose becomes essential when estimating % HbCO levels with commercial CO-oximeters that display readings to only a single decimal place. Using a recommended dose of 1.25 to 1.5 ml per kg body weight (Burge & Skinner, 1995) and avoiding low doses in women help reduce measurement error. Despite the known sources of error with this technique it has been shown to be one of the better techniques for estimating hemodynamic volumes.

In a recent meta-analysis this technique has the lowest measurement error of 2.2 % (coefficient of variation) compared to 2.8 % for volume of red blood cells chromium, 6.7 % for volume of red blood cells CO, and 6.7 % for volume of red blood cells using the Evans blue dye technique. The clearly smaller magnitude of measurement errors for CO rebreathing and chromium technique indicated that these techniques are better compared to Evans blue dye and the CO red blood cell technique. Despite the small measurement error there is continued criticism that includes the claim that this technique overestimates hemoglobin mass, as inspired CO is thought to leave the vascular space and potentially bind to myoglobin (Sawka *et al.*, 1992). Arguing against this point is the fact that at relatively low HbCO saturation (which are observed at the end of the technique), HbCO remains stable and oxygen rather than CO is taken up by myoglobin (Clark & Coburn, 1975). As well, the low rate of extravascular loss of CO appears to remain constant between individuals and therefore has little effect on the random error. This method has been used frequently throughout the literature and is a reliable method for measuring blood and plasma volume (Gore *et al.*, 2005). Thus, for the purposes of the studies presented in this dissertation Evans blue dye and the carbon monoxide uptake method were deemed appropriate measurement of vascular volume across study visits and subject populations.

Venous Blood Sampling

In each study, a catheter was placed in each subject in order to draw blood samples for analyses and infuse dye (Study 1) and saline. After disinfecting the antecubital region of the arm, an intravenous catheter was placed by inserting a 20, 22, or 24 gauge needle into the vein. Once the catheter was in place, the needle was removed and the catheter was held in place with medical tape and co-flex medical wrap. After every blood draw, the sampling line and catheter were cleared with non-lactated, non-dextrose saline solution (0.9 %) in the exact volume that matched the blood draw. Blood samples were obtained from the sampling line into a sterile syringe. The blood was then

sampled, stored on ice or transferred into a vacuum sealed test tube (with an anti-coagulant) with the help of a transfer device.

Percent Changes of Plasma and Blood Volume

The overall changes in plasma and blood volume were determined in each study in order to assess recovery of vascular volumes postexercise. Changes in blood and plasma volume were estimated from changes in hematocrit and hemoglobin using the method of Dill & Costill (1974). The relative change in plasma and blood volume was calculated from the following formula:

$$\%\Delta \text{ Plasma Volume} = (\{[Hb_1]/[Hb_2]\} \times [100 - Hct_2)/100 - Hct_1)] -1) \times 100$$

$$\%\Delta \text{ Blood Volume} = 100 \times [(Hb_1/Hb_2)-1]$$

Where Hb is the hemoglobin concentration and Hct is the hematocrit concentration and the number values reflect the different time points used for sampling. Changes in plasma volume are generally calculated indirectly from values of hemoglobin and hematocrit. All samples were measured in quadruplicate. The underlying assumption when using these formulas to calculate relative changes is that peripheral circulating erythrocyte volume does not change and is comparable to total erythrocyte volume (Stewart *et al.*, 2003).

Statistics

The statistical analysis for this research was performed using SAS/Stat software, Version 9.1.3 of the SAS system for Windows (Copyright 2004 SAS Institute Inc. (SAS v9.1.3, SAS Institute, Cary, NC)). All subject characteristics are reported as means ± SD and all additional data are reported as means ± SEM. Study 1 and 2 involved three study days, where subjects acted as their own control. Subjects in Study 3 were tested only once and differences were compared between sex and training status. For the purpose of the research, repeated-measurements over time within subject, condition and sex were

analyzed with a mixed model using SAS PROC MIXED. Significance was set at $P < 0.05$ for all analyses. Each statistical plan will be discussed in the three studies (Chapter IV-IV) and additional details will be provided in the following chapters.

CHAPTER IV

EFFECTS OF THE MENSTRUAL CYCLE AND SEX ON POSTEXERCISE HEMODYNAMICS

This study could not have been completed without the contribution of Jenni L. McCord Ph.D., and John R. Halliwill, Ph.D. Jenni McCord assisted with pilot work, protocol development, data collection (and provided historic data on men to be included for analyses), and the intellectual development of the manuscript. John R. Halliwill assisted in the intellectual development and the writing of the manuscript. I completed all data collection and development of the protocol and manuscript. This study was published in the *American Journal of Physiology Regulatory Integrative Comparative Physiology* (vol. 292, pages R1260-R1270, 2007) and was reproduced with permission from the *American Journal of Physiology Regulatory Integrative Comparative Physiology* and the American Physiological Society.

Introduction

Blood pressure is typically reduced for approximately 2 hours following a single bout of dynamic exercise in sedentary and endurance-trained humans (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002b). In general, this postexercise hypotension is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing elevations in cardiac output (Halliwill, 2001). Both sedentary and endurance-trained women and sedentary men follow this typical pattern of hemodynamics following exercise but endurance-trained men exhibit postexercise

hypotension with a different hemodynamic pattern characterized by reductions in cardiac output (Senitko *et al.*, 2002).

Several mechanisms appear to underlie the sustained peripheral vasodilation following exercise. Halliwill *et al.* (1996a) demonstrated the baroreflex is reset to defend a lower pressure following exercise; thus, sympathetic vasoconstrictor outflow is reduced postexercise in humans. In addition to this reduction in sympathetic nerve activity, there is reduced vascular responsiveness to a given level of sympathetic nerve activity (Halliwill *et al.*, 1996a; Halliwill *et al.*, 2003) and a sustained histamine-receptor dependent vasodilation (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a). The overall effect on vascular conductance and arterial pressure will be determined by how these various mechanisms and pathways integrate at the level of the vascular smooth muscle. As such, other factors that modify smooth muscle tone or vascular responses may modulate the pattern of postexercise hemodynamics.

Along these lines, estrogen receptors are located in the cells of the endothelium (Deroo & Korach, 2006) and in smooth muscle in the peripheral vasculature (Karas *et al.*, 1994) and can cause vasodilation (Sarrel, 1990; Herrington, 2000; Sanada *et al.*, 2001), whereas progesterone appears to act antagonistically by opposing these actions of estrogen (Sarrel, 1990; Charkoudian, 2001). Thus, it is plausible that changing levels of endogenous hormones during the normal female menstrual cycle may alter the pattern of postexercise hemodynamics. Specifically, increased levels of estrogen around ovulation may enhance the peripheral vasodilation that underlies postexercise hypotension.

In addition to direct effects on the vasculature, estrogen and progesterone or other factors associated with the normal menstrual cycle affect arterial pressure regulation (Minson *et al.*, 2000), temperature regulation (Charkoudian, 2001), and extracellular fluid volume regulation (Oian *et al.*, 1987; Tollan *et al.*, 1990; Stachenfeld *et al.*, 2001). These three factors are affected by the normal menstrual cycle and all have effects on cardiovascular function and/or regulation that may influence postexercise hemodynamics.

First, changes in arterial baroreflex control of sympathetic neural outflow results in elevated sympathetic nerve activity to skeletal muscle vascular beds during the mid-

luteal phase of the normal menstrual cycle (Minson *et al.*, 2000). Thus, it is plausible that changes in arterial pressure regulation and sympathetic vasoconstrictor outflow associated with the menstrual cycle alter the pattern of postexercise hemodynamics. Specifically, enhanced baroreflex-mediated changes in sympathetic nerve activity in the mid-luteal phase could lead to lower overall skeletal muscle blood flow and diminished peripheral vascular conductance during recovery from exercise. This could translate into a diminished postexercise hypotension response.

Second, changes in temperature regulation during the normal menstrual cycle result in elevations of body core temperature of 0.3 to 0.5 °C after ovulation has occurred corresponding with the rise in progesterone in the luteal phase (Charkoudian, 2001). Such changes in body temperature are associated with elevations in the threshold and increased sensitivity for sweating and cutaneous vasodilation in the mid-luteal phase compared to the follicular phase during exercise and passive heating (Hessemer & Brück, 1985b, a). Thus, it is plausible that shifts in thermoregulation associated with the menstrual cycle alter the pattern of postexercise cutaneous vasodilation. Specifically, an elevation in overall cutaneous blood flow in the mid-luteal phase may hinder the maintenance of stroke volume and cardiac output during recovery from exercise. These alterations in central hemodynamics could translate into an increased postexercise hypotension response.

Third, changes in extracellular fluid volume regulation during the normal menstrual cycle result in plasma volume expansion when estrogen rises (Fortney *et al.*, 1988; Tankersley *et al.*, 1992), and volume contraction when progesterone rises (Stachenfeld *et al.*, 1999b). Thus, it is plausible that plasma volume changes associated with changing levels of endogenous hormones during the menstrual cycle alter the pattern of postexercise hemodynamics. Specifically, expanded plasma volume around ovulation may enable better maintenance of stroke volume and cardiac output during recovery from exercise. Thus, the postexercise hypotension response may be diminished during the ovulatory phase.

To date, most of the studies involving postexercise hypotension have used men as subjects, or tested women during a single phase of the menstrual cycle or oral contraceptive use. Deschenes *et al.* (2006) described the cardiovascular and metabolic responses between men and women (irrespective of menstrual phase) during submaximal exercise and recovery and showed similarities between sexes with the consistent exception of changes in plasma lactate. Currently, a comprehensive comparison of postexercise hemodynamics between sexes is lacking.

Birch *et al.* (2002) examined the effects of exogenous hormones in the form of oral contraceptives on postexercise hypotension in women. They found no differences in postexercise hemodynamics during placebo and oral contraceptive use. However, in regards to cardiovascular regulation, the impact of exogenous hormones differ from that of the normal menstrual cycle (Stephenson & Kolka, 1985; Minson *et al.*, 2000). Thus, the purpose of our study was to comprehensively describe the changes in postexercise hemodynamics and explore the potential influence of fluid balance and thermoregulation on recovery from exercise across the normal menstrual cycle in healthy women. As our study reached completion, Esformes *et al.* (2006) reported that the pattern of postexercise hypotension was largely the same during the late follicular and mid-luteal phases of the menstrual cycle in eight women, but postexercise hypotension was enhanced during the early follicular phase. However, the authors failed to measure estradiol and progesterone during this study. In order to properly characterize hormonal fluctuations of the menstrual cycle, a hormonal profile must be determined by measurement of estrogen and progesterone at each menstrual phase. In our experience, many women do not ovulate during similar protocols and this can undermine the ability to detect differences across phases of the cycle. In fact, in subject populations similar to those studied by Esformes *et al.* (2006) , between 10 and 55 % of menstrual cycles will be abnormally short, inadequate, or anovulatory (De Souza *et al.*, 1998). In addition to this limitation, Esformes *et al.* (2006) did not investigate the contribution of leg versus cutaneous blood flow to overall changes in limb blood flow. Finally, the ovulatory phase is a time of substantial changes in plasma volume and thermoregulation and postexercise

hemodynamics have not been studied during this phase. A more comprehensive study is necessary to explore many of these issues.

Thus, we hypothesized that the postexercise response in the ovulatory phase would be associated with enhanced vasodilation in skeletal muscle vascular beds and would show unchanged vasodilation in cutaneous vascular beds relative to the early follicular phase. In addition, we hypothesized that the postexercise response in the mid-luteal phase would be associated with similar vasodilation in muscle vascular beds and elevated skin blood flow relative to the early follicular phase. Further, we hypothesized that the postexercise response in the mid-luteal phase would be associated with lower stroke volume and cardiac output relative to the other phases. Finally, we hypothesized that there would be no enhanced postexercise hemodynamic response between men and the early follicular phase, but that there would be differences between men and the ovulatory and mid-luteal phase.

Methods

This study was approved by the Institutional Review Board of the University of Oregon. Each subject gave written informed consent before participating in the study.

Subjects

We recruited and screened 23 women to participate in this study. Of these, 9 were excluded from analysis because of either abnormally short, inadequate, or anovulatory cycles during the time of study. As a result, 14 women subjects between the ages of 19 and 33 yr completed all portions of this study. These subjects were healthy with no allergies, normally menstruating, non-smoking, normotensive, and taking no medications. All of these subjects had regular menstrual cycles lasting 30 ± 2 days in duration and subjects had no history of menstrual distress and had not utilized any form of hormonal contraception for a period of six months or more prior to the study. All women subjects had negative pregnancy tests on each day of participation in the study.

From data previously collected (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a; McCord & Halliwill, 2006), we matched 14 men to women subjects for age, body mass index (BMI), and relative aerobic capacity. Subjects were either sedentary or recreationally active, participating in no more than 2 h of aerobic activity each week. Subject characteristics for men and women are presented in Table 1. Men were included for sake of comparison to the women subjects but did not undergo all portions of the protocol.

Screening Visit

For each screening visit, subjects reported to the laboratory at least 2 h post-prandial and abstained from caffeine for 12 h and from exercise, alcohol, and all medications for 24 h prior to the study. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands). The test consisted of 1-min of workload increments to determine peak oxygen uptake (VO_{2peak}). Specifically, after a 2-min warm-up period of easing cycling (20-30W), workload increased at 20, 25, or 30 W every minute until volitional fatigue. Selection of the workload increment was subjective, with the goal of producing exhaustion within 8-12 min. Whole body oxygen uptake (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). All subjects reached subjective exhaustion (Borg (Borg, 1970) rating of perceived exertion = 19-20] within the 8- to 12-min period. After the subjects rested for 15-20 min, they returned to the cycle ergometer for assessment of the workload corresponding to a steady state VO_2 of 60% of VO_{2peak}. This workload was used on the study day for the 60-min exercise bout. Subjects self-reported activity levels on two questionnaires (Baecke *et al.*, 1982; Kohl *et al.*, 1988).

Study Visits

Women subjects underwent identical study visits at the same time of day on three occasions; men underwent one study visit. The three separate study days for women

were conducted in the following phases: 1) early-follicular phase (1-3 days after the start of menstruation, when estrogen and progesterone are low); 2) ovulatory phase (within 48 hr of the peak luteninzing hormone surge, when estrogen is increased but progesterone remains low); and 3) mid-luteal phase (8 to 10 days after the detection of the lutenizing hormone surge, when both estrogen and progesterone are elevated). At the end of the screening visit, women subjects gave a menstrual history and were taught how to document the start of a menstrual cycle and detect ovulation. Ovulation was determined individually with the use of an ovulation prediction kit (OvuQuick, Quidel, San Diego, CA). The menstrual phase for the study visits were randomized as five women were first tested during the early-follicular phase, five women were first tested during the ovulatory phase, and four women were first tested in their mid-follicular phase.

For each study visit, subjects reported to the laboratory at least 2 h post-prandial and abstained from caffeine for 12 h and from exercise, alcohol, and all medications for 24 h prior to the study. Subjects pre-hydrated the night before the study by drinking 7ml/kg of body weight of water. Subjects recorded all food and drink consumed after 5pm the night before and the morning of each study visit. Subjects ingested a temperature-sensing pill at least 5 h prior to each study visit; subjects reporting to the lab in the morning swallowed the pill the night before. Core temperature was not measured in the men.

Experimental Design

Upon arrival, subjects emptied their bladders and a pre-study weight measurement to the nearest 100 g was obtained. In addition to prehydration the night before the study, subjects were asked to drink 7 ml/kg body weight of water before lying supine for instrumentation. A venous catheter was inserted into the right antecubital arm region to obtain blood samples and to infuse Evans blue dye. All samples and measurements were taken after 40 min of quiet rest in the supine position. After plasma volume was determined using Evans blue dye, subjects were encouraged to empty their bladders before the start of exercise.

Venous blood samples were collected on each study visit for measurement of 17β-estradiol and progesterone to confirm menstrual cycle phase. Samples were immediately place on ice, separated, and stored at -70 °C until transport to Oregon Medical Laboratories for analysis (Eugene, OR).

Timing of the Measurements

All pre and postexercise measurements were taken after 20 min in the supine position in order to control for plasma volume shifts due to postural changes. Preexercise (baseline) measurements were obtained before and postexercise measurements were taken at 30, 60, and 90 min after the exercise bout. Preexercise and 30 min, 60 min and 90 min postexercise measurements included blood samples for plasma volume determination, cardiac output, heart rate, arterial pressure, leg blood flow, and skin blood flow. During exercise, blood pressure and heart rate were measured every 10 min. At the end of the protocol, maximum skin blood flow values were obtained through local heating to 43 °C for approximately 20 to 45min or achievement of a plateau in red blood cell flux. After the end of the complete protocol subjects were again weighed to obtain a post-study weight measurement to the nearest 100 g.

Exercise

The exercise bout consisted of a 60-min period of seated upright cycling ergometer exercise at 60% of VO_{2peak} determined on the screening day. This exercise duration and intensity has been proven to consistently produce a sustained (~2 h) postexercise hypotension in healthy normotensive subjects (Halliwill, 2001). During the entire 60 min of exercise, subjects were asked to drink 7 ml/kg body weight of water to replace water loss due to sweating. The ambient temperature of the laboratory was kept between 21 and 23 °C and the humidity ranged from 23 to 48 %.

Measurements

Plasma and Blood Volume Determination

Evans blue dye (T-1824) dilution was used to measure plasma volume prior to exercise on each study visit in the women subjects (Greenleaf *et al.*, 1979). After 40 min of quiet supine rest, a control blood sample was drawn and 15 mg of dye was injected via the indwelling catheter followed by a saline flush. Plasma dye concentration was measured in blood samples obtained at 10, 20, and 30 min post-injection by spectrophometry (Beckman Spectrophotometer DU7400, Beckman Instruments; Fullerton, CA). Although no samples were overtly turbid, we used an extrapolation of the 780-720 nm baseline absorbance to 619 nm to correct for artifact as suggested by Farjanel *et al.* (1997). Preexercise blood volume was calculated from plasma volume and hematocrit. Exercise and postexercise changes in blood and plasma volumes were calculated from changes in hematocrit and hemoglobin concentrations (Dill *et al.*, 1974). Samples were immediately measured in triplicate for hematocrit (i-Stat Portable Clinical Analyzer, Abbott Laboratories, East Windsor, NJ) and hemoglobin concentration (Hemocue AB, Angelholm, Sweden). Evans blue dye was not used in men due to limited availability; however, exercise and postexercise changes in blood and plasma volumes were determined as described above.

Heart Rate and Arterial Pressure

Heart rate and arterial pressure were monitored throughout all experimental procedures. Heart rate was monitored using a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA). Arterial pressure was measured in the arm by using an automated auscultometric device (Dinamap Pro100 vital signs monitor, Critikon, Inc., Tampa, FL).

Cardiac Output

Cardiac output was estimated using an open-circuit acetylene washin method as described previously (Johnson *et al.*, 2000; Pricher *et al.*, 2004). This method allows the

noninvasive estimation of cardiac output. We chose an open-circuit method because subjects are exposed to stable oxygen and carbon dioxide levels throughout the measurement in contrast to rebreath techniques. In brief, subjects breathed 8 to 10 breaths of a gas mixture consisting of 0.6% acetylene-9.0% helium-20.9% oxygen and balance nitrogen. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services) and tidal volume was measured via a pneumotach (model 3700, Hans Rudolf, Kansas City, MO) linearized by the technique of Yeh *et al.* (1982) and calibrated by using test gas before each study. The pneumotach and valve system had a combined dead space of 24 ml. Cardiac output calculations have been described previously (Johnson *et al.*, 2000). Stroke volume was determined from cardiac output/heart rate expressed as ml· beat $^{-1}$. Systemic vascular conductance was calculated as cardiac output/mean arterial pressure and expressed as ml· min $^{-1}$· mmHg $^{-1}$.

Leg Blood Flow

Leg blood flow was determined through measurements of femoral artery diameter and velocity using an ultrasound probe (10-Mhz linear-array vascular probe, GE Vingmed System 5, Horton, Norway). The entire width of the femoral artery was insonated with an angle of 60°. Velocity measurements were recorded immediately before diameter measurements. Leg blood flow was then calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs, and reported in ml/min. Femoral vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as ml· min $^{-1}$· mmHg $^{-1}$.

Index of Skin Blood Flow

An index of skin blood flow was derived from measuring red blood cell flux values via laser-Doppler flowmetry (DRT4, Moor Instruments LTD, Devon, England). Laser-Doppler probes were placed on the forearm and the thigh. Skin blood flows were expressed as cutaneous vascular conductance, calculated as laser-Doppler flux/mean

arterial pressure, and normalized to the maximal values achieved during local heating to 43°C at the end of the protocol (Minson *et al.*, 2001).

Core Temperature

Internal body temperature was assessed by an ingestible pill telemetry system (HQInc, Palmetto, FL) (Coyne *et al.*, 2000; Wilson *et al.*, 2004). Core temperature was not measured in the men.

Statistics

Statistical analysis was performed using SAS (SAS v9.1, SAS Institute, Cary, NC). Unpaired t-tests were used to compare subject characteristics between sexes. Plasma hormonal concentrations, plasma and blood volumes, and pre- and post-study weights were analyzed across menstrual phases using a one-way repeated-measures ANOVA. Exercise hemodynamics were analyzed across menstrual phases in women subjects using a one-way repeated-measures ANOVA and between sexes using one-way ANOVA. Repeated-measurements over time within subjects and across menstrual cycle phase and sex were analyzed with a mixed model using SAS PROC MIXED. To further explore potential postexercise differences between men and women, percent change from pre-exercise baseline values were analyzed with a mixed model using SAS PROC MIXED. Differences were considered significant when $P < 0.05$. Subject characteristics are reported as means (SD). All additional data are reported as means (SEM).

Results

Subject Characteristics

Subject characteristics for women and men are presented in Table 1. There were no differences in age ($P = 0.323$), body mass index (BMI) ($P = 0.565$), maximum heart rate ($P = 0.836$), Baecke sports index (Baecke *et al.*, 1982) ($P = 0.623$), or Index of physical activity (Kohl *et al.*, 1988) ($P = 0.915$). Men were taller ($P < 0.001$), weighed more ($P < 0.001$), had higher absolute VO_{2peak} values ($P < 0.001$) and relative VO_{2peak}

values ($P = 0.005$), and had higher maximum workloads ($P < 0.001$) compared to women.

Table 1. Subject Characteristics from Chapter IV. Values are presented as means (SD); *$P < 0.05$; VO_{2peak}, peak oxygen consumption; MET, metabolic equivalents.

	Women (n=14)	Men (n=14)
Variable		
Age, yr	24.0 (4.2)	22.5 (3.5)
Height, cm	165.5 (7.6)	180.8 (3.9) *
Weight, kg	59.4 (6.8)	72.5 (6.2) *
Body Mass Index, kg/m^2	21.7 (2.4)	22.2 (1.4)
$VO_{2\ peak}$, $ml \cdot kg^{-1} \cdot min^{-1}$	35.0 (8.5)	45.6 (9.3)*
VO_{2peak}, ml/min	2062 (577)	3307(722) *
Maximum Workload, W	214 (54)	303 (64)*
Maximum Heart Rate, beats/min	185.1 (12.0)	183.9 (11.2)
Workload at 60% of VO_{2peak}, W	92 (31)	141 (36)*
Baecke sport index, arbitrary units	10.4 (1.9)	9.9 (2.8)
Index of physical activity, $MET \cdot h^{-1} \cdot wk^{-1}$	107.4 (37.7)	109.5 (59.4)

Characterizing Menstrual Cycle Phase

Table 2 summarizes serum concentrations of estradiol and progesterone for the women across the three study visits. Estradiol concentration was increased at the ovulatory ($P = 0.002$) and mid-luteal phases ($P < 0.001$) compared with the early follicular phase; further, estradiol was increased in the mid-luteal phase compared to the ovulatory phase ($P = 0.023$). Progesterone concentration was increased at the mid-luteal phase compared to the early follicular ($P < 0.001$) and the ovulatory phases ($P < 0.001$). These values were within the range of a normal menstrual cycle, as estrogen is known to be increased during the ovulatory phase and remains elevated during the mid-luteal phase; whereas progesterone concentrations are known to increase once ovulation has

occurred (and thus are still relatively low at the ovulatory phase and are highest at the mid-luteal phase of the menstrual cycle). Typical ranges of estrogen and progesterone for healthy women range between the following values: early follicular phase (< 160 pg/ml; 0.3-2.0 ng/ml), ovulatory (34-400 pg/ml; 1.2-22 ng/ml), and mid-luteal (27-246 pg/ml; 4.4-28 ng/ml).

Table 2. Plasma Hormonal Concentration from Chapter IV. Values are presented as means (SD); [a]$P < 0.05$ vs. Early Follicular, [b]$P < 0.05$ vs. Ovulatory.

	Women (n=14)		
Variable	Early Follicular	Ovulatory	Mid-Luteal
Estradiol, pg/ml	34.6 (15.0)	82.2 (41.2)[a]	118.0 (51.1)[ab]
Progesterone, ng/ml	0.5 (0.2)	2.5 (1.4)	10.3 (5.0)[ab]

Preexercise Hemodynamics

Table 3 shows the resting (preexercise) hemodynamics for women across the three study visits and for men during their study visit.

Arterial Pressures

There were no differences in systolic blood pressure across phases of the menstrual cycle ($P = 0.143$). Men had higher systolic blood pressures than women during all phases of the menstrual cycle (all $P < 0.001$). Diastolic blood pressure in the mid-luteal phase was lower than the early follicular ($P = 0.027$) and the ovulatory phases ($P = 0.028$) which did not differ ($P = 0.753$). There were no differences in diastolic blood pressure between sexes ($P = 0.601$). Mean arterial pressure in the mid-luteal phase was lower compared to the early follicular ($P = 0.017$) and ovulatory phases ($P = 0.018$) which did not differ ($P = 0.930$). Mean arterial pressure was higher in men compared to

women during the mid-luteal phase ($P = 0.004$), and tended to be higher than the early follicular ($P = 0.051$) and ovulatory phases ($P = 0.056$).

Table 3. Preexercise Hemodynamics from Chapter IV. Values are presented as means (SEM); [a]$P < 0.05$ vs. Early Follicular, [b]$P < 0.05$ vs. Ovulatory, [c]$P < 0.05$ men vs. Early Follicular, [d]$P < 0.05$ Men vs. Ovulatory, [e]$P < 0.05$ Men vs. Mid-luteal.

Variable	Women (n=14)			Men (n=14)
	Early Follicular	Ovulatory	Mid-Luteal	
Systolic Blood Pressure, mmHg	102.7 (2.5)	103.5 (2.1)	100.6 (2.9)	116.6 (2.6)[cde]
Diastolic Blood Pressure, mmHg	64.9 (1.4)	64.5 (1.3)	62.3 (1.2)[ab]	64.6 (2.2)
Mean Arterial Pressure, mmHg	77.4 (1.7)	77.5 (1.5)	75.1 (1.5)[ab]	81.9 (2.0)[e]
Heart Rate, beats/min	59.5 (1.7)	61.9 (1.6)	64.3 (1.2)[ab]	60.9 (2.1)
Cardiac Output, l/min	4.16 (0.33)	4.31 (0.33)	4.81 (0.24)	5.46 (0.32)[cd]
Stroke Volume, ml/beat	69.9 (5.2)	70.5 (5.5)	75.4 (3.9)	91.3 (6.3)[cde]
Systemic Vascular Conductance, $ml \cdot min^{-1} \cdot mmHg^{-1}$	53.9 (4.3)	56.2 (4.6)	64.3 (3.4)[a]	67.2 (4.4)[c]
Femoral Blood Flow, ml/min	277 (46)	275 (44)	275 (38)	336 (26)
Femoral Vascular Conductance, $ml \cdot min^{-1} \cdot mmHg^{-1}$	3.19 (0.53)	3.11 (0.44)	3.20 (0.46)	4.08 (0.28)

Systemic Hemodynamics

Heart rate in the mid-luteal phase was higher compared to the early follicular ($P < 0.001$) and ovulatory phases ($P = 0.022$). Ovulatory heart rate tended to be higher than the early follicular phase ($P = 0.053$). There was no difference in heart rate between sexes ($P = 0.146$). There were no differences in cardiac output across phases of the menstrual cycle ($P = 0.067$), but cardiac output tended to be higher in the mid-luteal phase. Cardiac output was higher in men compared to women during the early follicular

($P = 0.003$) and ovulatory phases ($P = 0.008$), but not the mid-luteal phase ($P = 0.127$). There were no differences in stroke volume across phases of the menstrual cycle ($P = 0.288$). Stroke volume was higher in men compared to women across all phases (all $P < 0.05$). Systemic vascular conductance in the mid-luteal phase was higher compared to the early follicular phase ($P = 0.011$) and tended to be higher than the ovulatory phase ($P = 0.055$). Systemic vascular conductance in the ovulatory phase did not differ from early follicular ($P = 0.666$). Systemic vascular conductance was higher in the men compared to women during the early follicular ($P = 0.035$), but not the ovulatory ($P = 0.086$) or mid-luteal phases ($P = 0.603$).

Leg Hemodynamics

There were no differences in femoral blood flow across phases of the menstrual cycle ($P = 0.997$). There were no differences in femoral blood flow between sexes ($P = 0.590$). There were no differences in femoral vascular conductance across phases of the menstrual cycle ($P = 0.967$). There were no differences in femoral vascular conductance between sexes ($P = 0.340$).

Preexercise Plasma Volumes and Cutaneous Hemodynamics

Table 4 shows the resting (preexercise) plasma and blood volume measurements as well as cutaneous hemodynamics for women across the three study visits and for men during their study visit.

Plasma and Blood Volumes

Plasma volume was higher during the ovulatory phase compared to the early follicular ($P = 0.010$) and the mid-luteal phases ($P = 0.033$). Blood volume was higher during the ovulatory phase compared to the early follicular ($P = 0.002$) and mid-luteal phases ($P = 0.005$).

Core Temperature and Cutaneous Vascular Conductance

Core temperature in the mid-luteal phase was higher compared to the early follicular ($P < 0.001$) and the ovulatory phases ($P < 0.001$), which did not differ ($P = 0.466$). Core temperature was not measured in the men. There were no differences in forearm cutaneous vascular conductance across phases of the menstrual cycle ($P = 0.785$). There were no differences in forearm cutaneous vascular conductance between sexes ($P = 0.322$). There were no differences in thigh cutaneous vascular conductance across phases of the menstrual cycle ($P = 0.391$). There were no differences in thigh cutaneous vascular conductance between sexes ($P = 0.110$).

Table 4. Baseline Vascular Volumes from Chapter IV. Values are presented as means (SEM); [a]$P < 0.05$ vs. Early Follicular, [b]$P < 0.05$ vs. Ovulatory, [c]$P < 0.05$ men vs. Early Follicular, [d]$P < 0.05$ Men vs. Ovulatory, [e]$P < 0.05$ Men vs. Mid-luteal.

Variable	Women (n=14)			Men (n=14)
	Early Follicular	Ovulatory	Mid-Luteal	
Hematocrit, %	33.4 (0.9)	33.9 (0.8)	33.7 (0.8)	40.7 (0.6)[cde]
Hemoglobin, g/dl	11.7 (0.4)	12.0 (0.3)	12.0 (0.3)	14.5 (0.7)[cde]
Plasma Volume, ml	2559 (161)	2696 (170)[a]	2465 (192)[b]	--
Blood Volume, ml	3923 (259)	4106 (259)[a]	3753 (290)[b]	--
Core Temperature, °C	37.0 (0.1)	37.0 (0.1)	37.3 (0.0)[ab]	--
Forearm Cutaneous Vascular Conductance, ml·min^{-1}·mmHg^{-1}	2.59 (0.49)	2.50 (0.27)	2.28 (0.34)	3.38 (0.62)
Thigh Cutaneous Vascular Conductance, ml·min^{-1}·mmHg^{-1}	2.52 (0.40)	2.60 (0.44)	3.38 (0.80)	4.55 (0.93)

Exercise

Workload

Subjects exercised for 60 min at 60% of VO_{2peak} on all testing days. The average workload was 92.0 (31.6) W for normally menstruating women and 141.4 (36.4) W for

the men on the study days. During the early follicular, ovulatory, and mid-luteal phases, average exercising heart rates were 143.2 (4.9), 147.9 (4.9), and 147.4 (4.9) beats/min. This represented 66.7 (3.9), 68.9 (3.9), and 68.0 (3.9) % of heart rate reserve. Exercising heart rate was not different between menstrual phase ($P = 0.181$), nor was heart rate reserve ($P = 0.230$); this verifies that the exercise target was reached by all women subjects on each testing day. Men exercised at an average heart rate of 142.2 (2.2) beats/min and this was not different compared to women ($P = 0.735$). Average heart rate reserve for men was 66.6 (1.8) % and this was not different compared to women ($P = 0.926$). Mean arterial pressure during exercise (early follicular: 79.0 (1.5); ovulatory: 76.8 (1.6); mid-luteal: 79.4 (1.9) mmHg) was not different across menstrual phases ($P = 0.291$). Men had a mean arterial pressure of 93.2 (2.6) mmHg during exercise and this was higher compared to women at each phase of the menstrual cycle ($P < 0.05$). This sex difference in exercise blood pressure was reflective of preexercise values, as men had higher mean arterial pressure both prior to and during exercise.

Postexercise Hemodynamics

To simplify the presentation of results, postexercise responses are presented in figures 1 to 4 as percent change from preexercise, at baseline for each phase of the menstrual cycle.

Arterial Pressure

Figure 1A shows the percent changes in mean arterial pressure from preexercise to 30, 60, and 90 min postexercise. Mean arterial pressure was decreased following exercise in women throughout 60 min ($P = 0.002$) postexercise. The fall in pressure following exercise was not different between menstrual phase ($P = 0.878$) but the pattern tended to differ postexercise with phase ($P = 0.092$). Mean arterial pressure was reduced following exercise in men throughout 90 min ($P < 0.008$) postexercise. The fall in pressure following exercise was not different between sexes ($P = 0.560$) nor did the pattern differ postexercise with sex ($P = 0.248$).

Systemic Hemodynamics

Figure 1B shows the percent changes in heart rate from preexercise to 30, 60, and 90 min postexercise. Heart rate was elevated following exercise in women throughout 60 min ($P = 0.020$) postexercise and tended to be elevated at 90 min postexercise ($P = 0.092$). The rise in heart rate following exercise was not different between menstrual phase ($P = 0.194$) nor did the pattern differ postexercise with phase ($P = 0.391$). Heart rate tended to be elevated following exercise in men at 30 min ($P = 0.058$) postexercise. The rise in heart rate following exercise was not different between sexes ($P = 0.187$) nor did the pattern differ postexercise with sex ($P = 0.787$).

Figure 1C shows the percent changes in cardiac output from preexercise to 30, 60, and 90 min postexercise. Cardiac output was elevated following exercise in women at 30 min ($P < 0.001$) postexercise and tended to be elevated at 60 min ($P = 0.084$) postexercise. The rise in cardiac output following exercise was not different between menstrual phases ($P = 0.539$) nor did the pattern differ postexercise with phase ($P = 0.313$). Cardiac output following exercise was not different compared to preexercise in men ($P = 0.874$). The rise in cardiac output following exercise was not different between sexes ($P = 0.332$) nor did the pattern differ postexercise with sex ($P = 0.516$).

Figure 1D shows the percent changes in stroke volume from preexercise to 30, 60, and 90 min postexercise. Stroke volume following exercise was not different than preexercise in women ($P = 0.714$). This lack of change was not affected by menstrual phase ($P = 0.519$). Stroke volume following exercise was not different than preexercise in men ($P = 0.903$). This lack of change was not affected by sex ($P = 0.542$).

Figure 2A shows the percent changes in systemic vascular conductance from preexercise to 30, 60, and 90 min postexercise. Systemic vascular conductance was increased following exercise in women throughout 60 min ($P < 0.050$) postexercise and tended to be elevated at 90 min ($P = 0.063$) postexercise. The rise in systemic vascular conductance following exercise was not different between menstrual phases ($P = 0.521$) nor did the pattern differ postexercise with phase ($P = 0.255$). Systemic vascular conductance was increased following exercise in men at 30 min ($P < 0.038$) postexercise.

The rise in systemic vascular conductance following exercise was not different between sexes ($P = 0.573$) nor did the pattern differ postexercise with sex ($P = 0.476$).

Leg Hemodynamics

Figure 2B shows the percent changes in femoral blood flow from preexercise to 30, 60, and 90 min postexercise. Femoral blood flow was increased following exercise in women throughout 90 min ($P < 0.001$) postexercise. The rise in femoral blood flow following exercise was not different between menstrual phases ($P = 0.713$) nor did the pattern differ postexercise with phase ($P = 0.763$). Femoral blood flow was increased following exercise in men throughout 90 min ($P < 0.001$) postexercise. The rise in femoral blood flow following exercise was not different between sexes ($P = 0.734$) nor did the pattern differ postexercise with sex ($P = 0.534$). Figure 2C shows the percent changes in femoral vascular conductance from preexercise to 30, 60, and 90 min postexercise. Femoral vascular conductance was increased following exercise in women throughout 90 min ($P < 0.001$) postexercise. The rise in femoral vascular conductance following exercise was not different between menstrual phases ($P = 0.595$) nor did the pattern differ postexercise with phase ($P = 0.717$). Femoral vascular conductance was increased following exercise in men throughout 90 min (all $P < 0.001$) postexercise. The rise in femoral vascular conductance following exercise was not different between sexes ($P = 0.828$) nor did the pattern differ postexercise with sex ($P = 0.655$).

Core Temperature and Skin Blood Flow

Figure 3A shows the absolute changes in body core temperature from preexercise to 30, 60, and 90 min postexercise. Core temperature was increased following exercise in women at 30 min ($P < 0.001$) but not at 60 min ($P = 0.207$) postexercise. The rise in temperature following exercise was not different between menstrual phase ($P = 0.708$) nor did the pattern differ postexercise with phase ($P = 0.495$). Core temperature was not measured in men.

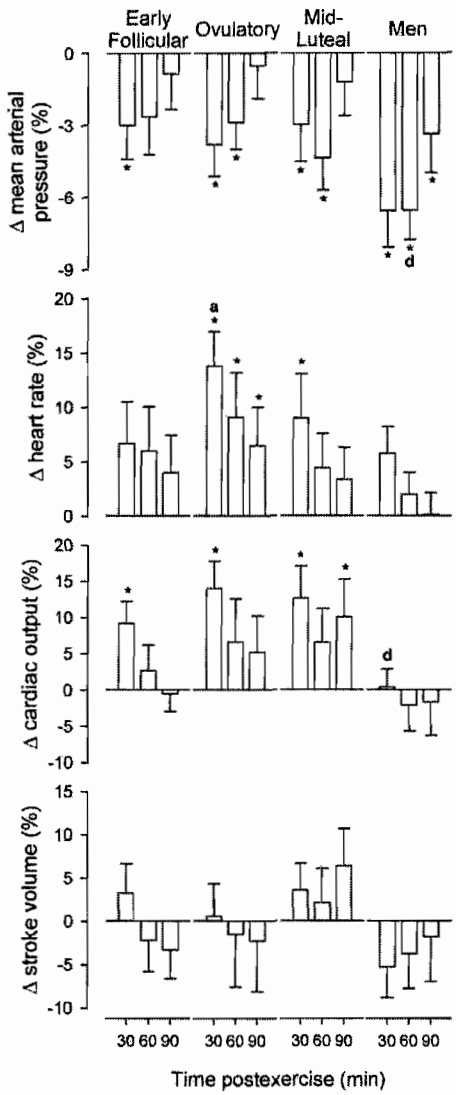

Figure 1. Mean Arterial Pressure, Heart Rate, and Cardiac Output from Chapter IV. The percent change in mean arterial pressure (A), heart rate (B), cardiac output (C), and stroke volume (D) from preexercise to 30, 60, and 90 min after exercise. Percent changes are grouped for each phase of the menstrual cycle and for men. * $P < 0.05$ vs. preexercise baseline. [a]$P < 0.05$ vs. Early Follicular, [b]$P < 0.05$ vs. Ovulatory, [c]$P < 0.05$ Men vs. Early Follicular, [d]$P < 0.05$ Men vs. Ovulatory, [e]$P < 0.05$ Men vs. Mid-luteal. Values are presented as means (SEM). n = 14 for each group.

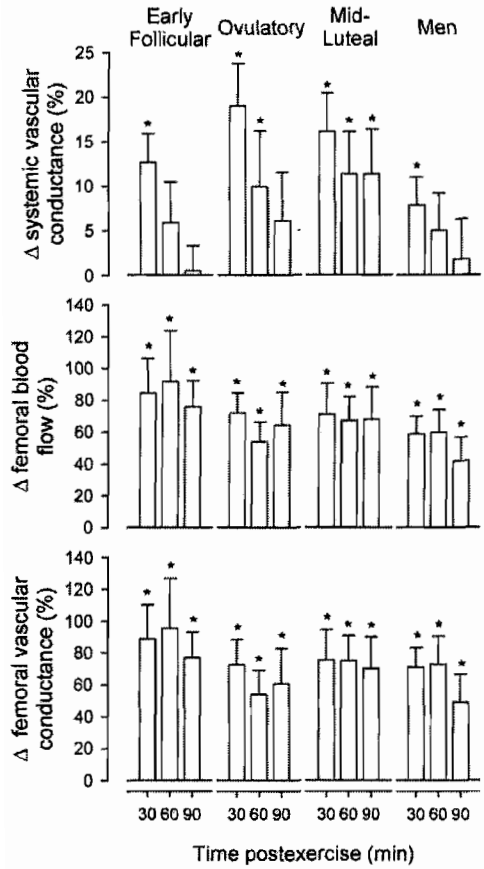

Figure 2. Postexercise Hemodynamics from Chapter IV. The percent change in systemic vascular conductance (A), femoral blood flow (B) and femoral vascular conductance (C) from preexercise to 30, 60, and 90 min after exercise from Chapter IV. Percent changes are grouped for each phase of the menstrual cycle and for men. * $P < 0.05$ vs. preexercise baseline. $^{a}P < 0.05$ vs. Early Follicular, $^{b}P < 0.05$ vs. Ovulatory, $^{c}P < 0.05$ Men vs. Early Follicular, $^{d}P < 0.05$ Men vs. Ovulatory, $^{e}P < 0.05$ Men vs. Mid-luteal. Values are presented as means (SEM). n = 14 for each group.

Figure 3B shows the absolute changes in arm cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise to 30, 60, and 90 min postexercise. Arm cutaneous vascular conductance was increased in women following exercise at 30 min ($P < 0.001$) postexercise and tended to be increased at 60 min postexercise ($P = 0.057$) but not at 90 min postexercise ($P = 0.302$). The rise in arm cutaneous vascular conductance following exercise was not different between menstrual phase ($P = 0.902$) nor did the pattern differ postexercise with phase ($P = 0.638$). Arm cutaneous vascular conductance was not increased following exercise in men ($P = 0.370$). The rise in arm cutaneous vascular conductance following exercise was not different between sexes ($P = 0.738$) nor did the pattern differ postexercise with sex ($P = 0.971$).

Figure 3C shows the absolute changes in leg cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise to 30, 60, and 90 min postexercise. Leg cutaneous vascular conductance was increased in women following exercise throughout 60 min ($P < 0.050$) postexercise but not at 90 min postexercise ($P = 0.386$). The rise in leg cutaneous vascular conductance following exercise was not different between menstrual phase ($P = 0.430$) nor did the pattern differ postexercise with phase ($P = 0.241$). Leg cutaneous vascular conductance was increased in men following exercise at 30 min ($P < 0.001$) and 60 min postexercise ($P = 0.003$) but not at 90 min postexercise ($P = 0.778$). The rise in leg cutaneous vascular conductance following exercise was greater in men than women ($P = 0.010$) but the pattern did not differ across recovery with sex ($P = 0.224$).

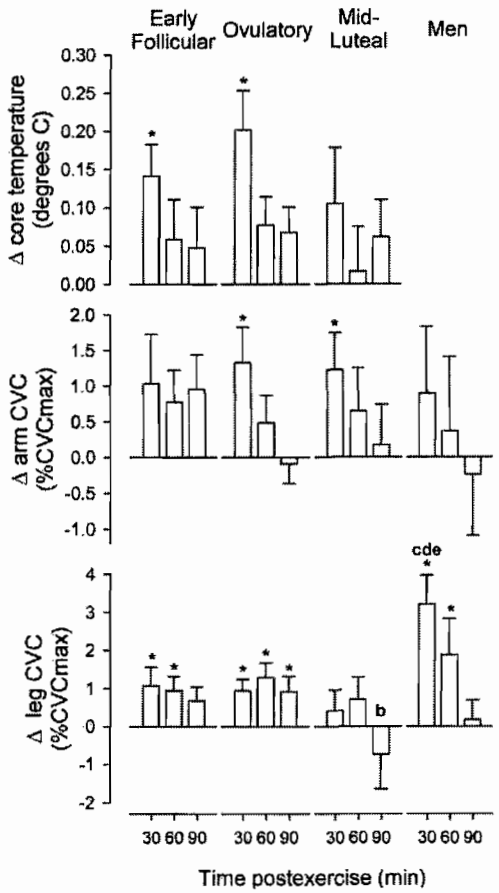

Figure 3. Core Temperature and Cutaneous Vascular Conductance from Chapter IV. The change in core temperature (A), arm cutaneous vascular conductance (B) and leg vascular conductance (C) from preexercise to 30, 60, and 90 min after exercise from Chapter IV. Percent changes are grouped for each phase of the menstrual cycle and for men. * $P < 0.05$ vs. preexercise baseline. [a]$P < 0.05$ vs. Early Follicular, [b]$P < 0.05$ vs. Ovulatory, [c]$P < 0.05$ Men vs. Early Follicular, [d]$P < 0.05$ Men vs. Ovulatory, [e]$P < 0.05$ Men vs. Mid-luteal. Values are presented as means (SEM). n = 14 for each group.

Body Weights, Plasma Volume, and Blood Volume

Total Body Water Loss

In order to maintain body weight, subjects were pre-hydrated with 7ml/kg of water the night prior to the study, the morning of the study, and during the exercise session. Subjects were weighed pre and postexercise in the same clothing before and after in order to measure the overall change in body weight. However, small losses in total body weight were seen from the beginning to the end of the study in the ovulatory [-0.29 (0.09) kg; $P < 0.001$] and mid-luteal phases [-0.21 (0.06) kg; $P < 0.001$], but not the early follicular phase [-0.06 (0.06) kg; $P = 0.427$]. Loss of weight did not differ between the ovulatory and mid-luteal phases ($P = 0.417$) but was less in the early follicular phase ($P = 0.033$ vs ovulatory; $P = 0.007$ vs mid-luteal). Weight loss in the men was 0.56 (0.14) kg, which was greater than weight loss in either the early follicular ($P = 0.004$) or mid-luteal ($P = 0.032$) phases but did not differ from the ovulatory phase ($P = 0.112$).

Plasma and Blood Volume

Figure 4A shows the percent changes in plasma volume from preexercise to 0, 30, 60, and 90 min postexercise. Plasma volume was decreased immediately following exercise ($P < 0.001$) then increased at 30, 60, and 90 min postexercise (all $P < 0.022$). The plasma volume loss and subsequent expansion following exercise was not different between menstrual phases ($P = 0.743$) but the pattern tended to differ postexercise with phase ($P = 0.099$). Plasma volume showed a similar pattern following exercise in men although it was not increased at 60 ($P = 0.293$) and 90 min postexercise ($P = 0.711$). The plasma volume loss and subsequent expansion following exercise was not different between sexes ($P = 0.237$) nor did the pattern differ postexercise with sex ($P = 0.148$).

Figure 4B shows the percent changes in blood volume from preexercise to 0, 30, 60, and 90 min postexercise. Blood volume was decreased immediately following exercise ($P < 0.001$) then increased at 30 min ($P = 0.047$) and 60 min ($P < 0.001$) postexercise and tended to remain increased at 90 min postexercise ($P = 0.055$). The blood volume loss and subsequent expansion following exercise was not different

between menstrual phases ($P = 0.344$) nor did the pattern differ across recovery with phase ($P = 0.178$). Blood volume showed a similar pattern following exercise in men although it was not increased at 60 ($P = 0.370$) and 90 min ($P = 0.441$) postexercise. The blood volume loss and subsequent expansion following exercise was not different between sexes ($P = 0.366$) nor did the pattern differ postexercise with sex ($P = 0.120$).

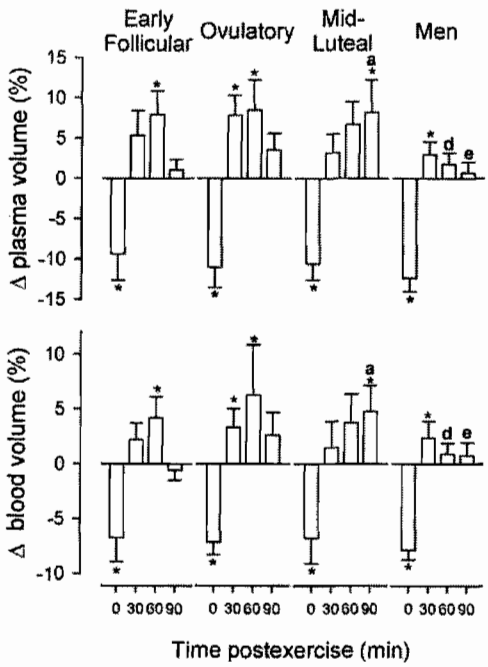

Figure 4. Vascular Volumes from Chapter IV. The percent change in plasma volume (A) and blood volume (B) from preexercise to 0 (end exercise), 30, 60, and 90 min after exercise from Chapter IV. Percent changes are grouped for each phase of the menstrual cycle and for men. * $P < 0.05$ vs. preexercise baseline. $^{a}P < 0.05$ vs. Early Follicular, $^{b}P < 0.05$ vs. Ovulatory, $^{c}P < 0.05$ Men vs. Early Follicular, $^{d}P < 0.05$ Men vs. Ovulatory, $^{e}P < 0.05$ Men vs. Mid-luteal. Values are presented as means (SEM). n = 14 for each group.

Discussion

We studied postexercise hemodynamics and explored aspects of the response related to thermoregulation and fluid balance in eumenorrheic women in three different phases of the menstrual cycle. Our main finding is that despite differences in endogenous concentrations of estrogen and progesterone and fluctuations in plasma volume and body temperature across the menstrual cycle, there was no modulation of the pattern of postexercise hypotension and postexercise hemodynamics. In contrast to our hypothesis, we found that leg vasodilation in the ovulatory phase and cutaneous vasodilation in the mid-luteal phase were not augmented compared to the early follicular phase. As well, we found no alteration in central hemodynamics following exercise in the mid-luteal phase. In addition, we observed that men have a similar postexercise hypotension pattern as the women, in agreement with prior work (Senitko *et al.*, 2002).

Preexercise Hemodynamics across the Menstrual Cycle

We found preexercise differences in resting systemic vascular conductance, mean arterial pressure, and heart rate between menstrual phases, in such that the mid-luteal phase had elevated heart rates and lower mean arterial pressure compared to the other phases. These differences are consistent with prior literature (Hessemer & Brück, 1985b) but are not always observed (Van Beek *et al.*, 1996; Minson *et al.*, 2000). These differences in resting hemodynamics carry forward as differences in postexercise hemodynamics; however, they were not associated with differences in the postexercise response (i.e., the change in hemodynamics from before to after exercise).

Postexercise Hemodynamics across the Menstrual Cycle

Postexercise hypotension is commonly characterized by a rise in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output (Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b; Halliwill, 2001). The persistent vasodilation associated with postexercise hypotension does not occur solely in the exercising muscle, as forearm, calf, and leg vascular conductances are often increased in

parallel with systemic vascular conductance (Halliwill, 2001; Pricher *et al.*, 2004). Halliwill *et al.* (1996a) demonstrated that the baroreflex is reset to defend a lower pressure after exercise which causes a decrease in sympathetic vasoconstrictor outflow. In this context, Minson *et al.* (2000) found a greater sympathetic baroreflex sensitivity and resting muscle sympathetic nerve activity in the mid-luteal phase compared to the early follicular phase. We originally thought that such enhanced baroreflex sensitivity and elevated sympathetic neural outflow would result in lower muscle blood flow and diminished peripheral vascular conductance during recovery from exercise, but this was not observed. However, since baroreflex sensitivity was not measured in this study, we can only speculate on whether or not preexercise differences in baroreflex function across the menstrual cycle continue into the postexercise period.

In addition to the reduced sympathetic outflow and impaired responsiveness to sympathetic nerve activity (Halliwill *et al.*, 1996a; Halliwill, 2001; Halliwill *et al.*, 2003), the increased vascular conductance associated with postexercise hypotension can largely be explained by histamine H_1 and H_2 receptor-mediated vasodilation (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a). We originally thought that increased levels of estrogen during the ovulatory phase would enhance vasodilation following exercise by increasing vascular responsiveness to some or all of these influences. The vasodilation seen in the presence of estrogen has been linked to enhancement of the production and release of basal nitric oxide in the endothelium (Cicinelli *et al.*, 1996; Sudhir *et al.*, 1996). Estrogen has been shown to decrease calcium entry into vascular smooth muscle cells, thus causing vascular smooth muscle relaxation (Cicinelli *et al.*, 1996). However, we saw no differences in leg vasodilation or postexercise hypotension between menstrual phases suggesting that high levels of estrogen do not enhance postexercise vasodilation. This may be due to the limited contribution of nitric oxide (a major pathway for the vascular effects of estrogen) to postexercise hemodynamics in humans (Halliwill *et al.*, 2000).

In general, our results are similar but more comprehensive than those recently reported by Esformes *et al.* (2006). However, Esformes *et al.* (2006) reported that, while the pattern of postexercise hypotension was largely the same during the late follicular and

mid-luteal phases, postexercise hypotension was enhanced during the early follicular phase. They concluded from this observation that buffering of postexercise hypotension is enhanced during the late follicular and mid-luteal phases. In contrast, we found no evidence to support this conclusion and no evidence that the postexercise response varies across the menstrual cycle. However, we documented baseline differences in arterial pressure and systemic vascular conductance that carry forward into the postexercise period. Esformes *et al.* (2006), studying only eight women, likely lacked the statistical power to demonstrate these differences and this may explain their suggestion of greater hypotension during the early follicular phase. Furthermore, they did not confirm the hormonal changes in estrogen and progesterone associated with the menstrual cycle in their subjects, so one cannot rule out the possibility that some of their subjects were anovulatory or that subjects may not have undergone the expected fluctuations in estrogen and progesterone. This is a major limitation to their study as in our experience 40 % of women may have menstrual cycles that are abnormally short, inadequate, or anovulatory at the time of study and the prevalence may be as high as 55% (De Souza *et al.*, 1998).

Similar to our current findings, Birch *et al.* (2002) found postexercise hemodynamics were not different between the active and placebo phases of oral contraceptive use. Thus, neither endogenous nor exogenous female sex hormones appear to substantially alter postexercise hemodynamics.

Carter *et al.* (2001) found that women, without consideration of menstrual cycle, had greater decreases in mean arterial pressure than men following 3 minutes of cycling at 60%VO_{2max} when studied in the seated upright position. In contrast, we did not find greater decreases in arterial pressure during recovery from exercise in women compared to men in this study or in our prior work (Senitko *et al.*, 2002) when studied in the supine position 30 to 90 min postexercise or when tilted upright at 45 min postexercise (Senitko *et al.*, 2002) and this is also consistent with work by Deschenes *et al.* (2006). The findings of Carter *et al.* (2001) in the first few minutes of recovery from exercise may be

more closely related to the overall trend for women to be less orthostatic tolerant than men (Convertino, 1998; Meendering *et al.*, 2005).

Thermoregulation and the Menstrual Cycle

Due to shifts in thermoregulatory control, core body temperature is elevated during the mid-luteal phase and this shift in control is associated with elevations in the threshold (Hessemer & Brück, 1985b, a; Stephenson & Kolka, 1985) and increased sensitivity for sweating and cutaneous vasodilation during exercise and passive heating (Hessemer & Brück, 1985b, a). In contrast, Stephenson & Kolka (1985) found no difference in the sensitivity for forearm blood flow and arm sweat rate with the menstrual phase when compared to esophageal temperature. We originally thought that these changes might result in an augmented cutaneous vasodilation during recovery from exercise in the mid-luteal phase, which could result in reductions in stroke volume and cardiac output due to a greater amount of blood pooling in the compliant cutaneous vasculature. However, we found no differences in arm or leg cutaneous vascular conductance following exercise across the menstrual cycle. One possible explanation is that exercise appears to reset the threshold for cutaneous vasodilation such that cutaneous blood flow rapidly returns to preexercise levels despite continued elevations in core temperature (Journeay *et al.*, 2005), thus minimizing the influence of skin blood flow on postexercise hemodynamics (Wilkins *et al.*, 2004).

Plasma Volume Fluctuations and the Menstrual Cycle

Plasma volume fluctuates through out the menstrual cycle (Turner & Fortney, 1984; Oian *et al.*, 1987; Fortney *et al.*, 1988; Tollan *et al.*, 1990; Fortney *et al.*, 1994) due to transcapillary fluid shifts caused by changes in estrogen and progesterone levels (Oian *et al.*, 1987; Tollan *et al.*, 1990; Stachenfeld *et al.*, 2001). Specifically, elevated estrogen in the ovulatory phase appears to increase plasma volume (Fortney *et al.*, 1988; Tankersley *et al.*, 1992; Stachenfeld *et al.*, 2001; Stachenfeld & Taylor, 2004) whereas rising progesterone in the mid-luteal phase appears to decrease plasma volume (Turner &

Fortney, 1984; Fortney *et al.*, 1994; Stachenfeld *et al.*, 1999b). In general, these changes are related to protein gain and loss from the circulation and subsequent fluid shifts in and out of the vascular space. Along these lines, plasma volume rose and fell approximately 200 ml (around 9 %) across the menstrual cycle in the current study. However, these baseline fluctuations in plasma volume did not appear to alter the plasma volume loss during exercise nor affect its restoration after exercise. This observation is consistent with one prior report in eumenorrheic trained females that changes in plasma volume at 4 and 40 minutes after submaximal exercise do not differ between the early follicular and mid-luteal phases (De Souza *et al.*, 1989).

Perspectives

In addition to direct effects on the vasculature, estrogen and progesterone or other factors associated with the normal menstrual cycle affect arterial pressure regulation, temperature regulation, and extracellular fluid volume regulation. Our results suggest that, despite known cardiovascular alterations associated with endogenous hormones both at rest and following exercise, the variables associated with postexercise hemodynamics and the overall postexercise response are largely unaffected across the menstrual cycle. Thus, it appears that the pattern of the postexercise hemodynamic response, including aspects related to thermoregulation and fluid regulation, is tightly regulated to produce the pattern of response that is consistently observed in most individuals.

Conclusion

The influence of the menstrual cycle and sex on postexercise hemodynamics, thermoregulation and fluid balance was examined in this study. Although there are preexercise differences in mean arterial pressure and heart rate, the overall pattern and magnitude of the postexercise hypotension response is unchanged through out the menstrual cycle. Our data also suggest the pattern and magnitude of postexercise hypotension does not differ between sexes. Furthermore, regardless of the menstrual cycle, postexercise hemodynamics seems to be closely regulated. However, both

menstrual cycle and sex are associated with baseline differences in resting hemodynamics that are critical to assessing postexercise responses.

The results from the experiment performed in Chapter IV entitled "Effects of the menstrual cycle and sex on postexercise hemodynamics" suggest that postexercise hemodynamics are largely unaffected by sex or factors associated with the menstrual cycle. This study observed differences in resting hemodynamics as systemic vascular conductance, heart rate, and mean arterial pressure were altered during mid-luteal phase. Despite differences in hormonal concentrations and fluctuations in the plasma volume and body temperature across the menstrual cycle these factors did not affect postexercise hemodynamics. In contrast to the hypothesis we observed no interaction of estrogen at the level of the vascular smooth muscle and thus, no augmented postexercise hypotension during the ovulatory phase. Therefore, it appears that postexercise hemodynamics seem to be tightly regulated as women showed the same drop in mean arterial pressure as men. In this study plasma volume was measured over the normal menstrual cycle with Evans blue dye. The studies outlined in Chapter V and VI will further investigate training status and fluid regulation on postexercise hypotension and specifically address the role of plasma volume and body fluid balance (i.e., fluid replacement and sweat losses) affect postexercise hemodynamics in endurance-exercise trained and sedentary individuals.

CHAPTER V

FLUID REPLACEMENT DURING EXERCISE ALTERS POSTEXERCISE CARDIAC HEMODYNAMICS IN EXERCISE ENDURANCE-TRAINED MEN

This study could not have been completed without the help of John R. Halliwill, Ph.D. John Halliwill assisted in the intellectual development and writing of the manuscript. I completed all data collection and developed the protocol and manuscript.

Introduction

Blood pressure is typically reduced ~5 to 10 mmHg following a single bout of dynamic exercise in sedentary and endurance exercise-trained humans (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002a). Although the mechanisms underlying postexercise hypotension remain unclear, the response in the general population (healthy sedentary and normally active individuals) is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing elevations in cardiac output (Halliwill, 2001). Several mechanisms appear to underlie the sustained peripheral vasodilation following exercise. Halliwill *et al.* (1996a) demonstrated that the baroreflex is reset to defend a lower pressure following exercise; thus, sympathetic vasoconstrictor outflow is reduced postexercise in humans. In addition to this reduction in sympathetic nerve activity, there is reduced vascular responsiveness to a given level of sympathetic nerve activity (Halliwill *et al.*, 1996a; Halliwill *et al.*, 2003) and a sustained

histamine-receptor dependent vasodilation (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a).

The mechanisms underlying this postexercise hypotension are still under investigation; however, the overall hemodynamic response seen in endurance-trained men may be different compared to the general population. In contrast to this sustained postexercise peripheral vasodilation, two reports in the literature suggest that postexercise hypotension in exercise-trained men is secondary to altered cardiac hemodynamics. First, Senitko *et al.* (2002) found that cardiac output was reduced and systemic vascular conductance was unchanged in a group of trained men. Second, Dujic *et al.* (2006) confirmed these results in elite male Croatian soccer players.

The reasons for these hemodynamic differences remain unclear and may be due to plasma volume losses secondary to greater sweat rates in trained men (Frye & Kamon, 1981; Myhre *et al.*, 1985; Sawka *et al.*, 2007) that may ultimately reduce central blood volume and ventricular filling (i.e., preload) during recovery from exercise. However, these studies failed to document hydration status (i.e., pre to postexercise changes in body weight) and the amount of oral fluid during exercise was ad libitum (Senitko *et al.*, 2002) or completely restricted (Dujic *et al.*, 2006). It is known that ab libitum drinking fails to match sweat loss, resulting in incomplete fluid replacement (Bean & Eichna, 1943; Adolph, 1947; Wolf, 1958; Sawka & Montain, 2000; Maresh *et al.*, 2004). In addition, this trained population is at greater risk of dehydration due to greater sweat rates (Costill, 1977; Sawka *et al.*, 2007). The interpretation of these previous results is limited to a relatively small sample of moderately trained subjects as maximal oxygen uptake values were not reflective of a highly trained population. Currently, limited studies exist on the role that fluid replacement plays in postexercise hemodynamics in a group of endurance exercise-trained men.

Cardiovascular responses during exercise and heat-stress have been studied extensively (Rowell, 1974; Nadel *et al.*, 1979; Montain & Coyle, 1992b); however, little is known about the influence of metabolic and environmental heat production on postexercise hemodynamics in a trained population. Early studies have observed

elevated heart rates following heat-stress (Mathews *et al.*, 1969). Postexercise hypotension is a highly regulated physiological phenomenon (Raine *et al.*, 2001) and despite differing pressor results following heat-stress (Brouha, 1960; Kilgour *et al.*, 1993) the postexercise pressure response is proposed to be well regulated.

Therefore the goal of this study was to assess the influence of fluid replacement on postexercise arterial pressure responses and cardiac and peripheral hemodynamics in a group of highly fit endurance exercise-trained men to determine if cardiac output is maintained with adequate fluid replacement during exercise. We hypothesized that first, adequate fluid replacement during exercise in a group of trained men would prevent the postexercise fall in cardiac output and second, under conditions of no fluid replacement and exercise in a warm condition, further decreases in cardiac output would be seen in a group of trained men compared to fluid replacement. Lastly, we hypothesized that the magnitude of postexercise hypotension would not be different between conditions during recovery from exercise.

Methods

This study was approved by the Institutional Review Board of the University of Oregon. Each subject gave written informed consent before participating in the study.

Subjects

Due to the specific research objectives of this study, only men were studied. A total of fourteen subjects (between 20 and 40 yr) participated in this study. On the basis of their exercise habits over the previous 12 mo, subjects were classified as "endurance-exercise trained" defined as performing endurance type exercises (running, cycling, or swimming) on at least 4 days/wk and averaging at least 1 h/day that they exercise. All subjects were primarily cyclists and runners performing between 7 and 12 h of endurance exercise per week.

Subjects were healthy with no allergies, non-smoking, normotensive, and taking no medications. The experimental design consisted of four separate testing days: a screening visit and three study visits. Subjects were scheduled for the first study visit within 7 to 10 days after the screening visit and the second and third visits were separated by 3 to 7 days. All data was collected in Eugene, Oregon, during the winter months (January and February) when subjects were naturally unacclimated to heat.

Screening Visit

Subjects reported to the laboratory for a screening visit and cycle ergometer test at least 2 h post-prandial and abstained from caffeine, alcohol, and exercise for 24 h prior to the screening visit. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands). Subjects were attached to a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA) in order to determine heart rate through the protocol. Seat height was adjusted and recorded and subjects were instructed to do a self-selected warm-up for approximately 5 min before the start of the test. The test consisted of 1-min of workload increments to determine peak oxygen uptake (VO_{2peak}). Specifically, after a 5-min warm-up period of light cycling (~100-150 W), workload increased at 25 or 30 W every minute until volitional fatigue. Selection of the workload increment was subjective based on the warm-up and the overall size of the subject, with the goal of producing exhaustion within 9-12 min. Whole body oxygen uptake (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). All subjects reached subjective exhaustion (Borg, 1970) rating of perceived exertion = 19-20] within a 9- to 15-min period and maximal heart rate was determined just before termination of exercise. After the subjects rested for 15-20 min, they returned to the cycle ergometer for assessment of the workload corresponding to a steady-state VO_2 of 60% of VO_{2peak}. This workload was used on each of the study days for the 60-min exercise bout. Subjects self-reported activity levels on two questionnaires (Baecke *et*

al., 1982; Kohl *et al.*, 1988) during the 15-20 min rest period before the start of the 60% of VO_{2peak} verification test.

Study Visits and Experimental Protocol

Subjects were tested on three separate study days. Subjects reported to the laboratory at least 2 h post-prandial and abstained from caffeine for 12 h and from exercise and all medications for 24 h prior to the study. Subjects pre-hydrated the night before each study day by drinking 7 ml per kg of body weight of water. Subjects recorded all food and drink consumed after 5 pm the night before and the morning of each of the study visits in a dietary log that was given to the subjects after the screening visit. Subjects ingested a temperature-sensing pill at least 5 h prior to the study visits and reported to the lab in the morning having swallowed the pill the night before. Each study day was conducted at approximately the same time of day.

Upon arrival, subjects voided their bladders into a urine collection container for measurement of urine specific gravity in order to determine baseline hydration status. Urine specific gravity was measured in duplicate at baseline. If results of urine specific gravity showed subjects were dehydrated, they were asked to rest and drink 500 ml of water and then provide a urine sample for re-testing. After providing a urine sample, subjects were instructed to undress to their underwear behind a privacy screen and were weighed to the nearest 5 g. The subjects were then laid in a supine position for instrumentation. A venous catheter was inserted into the right antecubital arm region to obtain blood samples throughout the protocol. All preexercise samples and measurements were taken after 40 min of quiet rest.

Timing of the Measurements

All pre and postexercise measurements were taken in a supine position and subjects rested in this position between measurements. Preexercise (baseline) measurements were obtained after 30 min of quiet supine rest. Postexercise measurements were taken every 7 min for 90 min after completion of the exercise bout,

with the exception of femoral blood flow which was taken preexercise and then every 30 minutes following exercise. Preexercise and postexercise measurements included heart rate, arterial pressure, cardiac output, core body temperature, femoral blood flow, and skin blood flow. Blood samples of hematocrit and hemoglobin were taken once preexericse and after 45 min of exercise and then every 30 min postexercise. Nude body weights were obtained at baseline, before exercise, after exercise, and at the end of the study. During exercise blood pressure (measured by manual sphygmomanometer on the arm), core body temperature, and heart rate were measured every 10 min. At the end of the protocol, maximum skin blood flow values were obtained through local heating to 43 °C. The ambient temperature of the laboratory for pre and postexercise measurements was controlled between 21 and 23 °C. All urine excreted during the study days was collected and weighed to the nearest 5g.

Exercise

On each study day the exercise bout consisted of a 60-min period of seated upright cycling exercise at 60% of VO_{2peak} as determined during the screening visit. This exercise duration and intensity has been proven to consistently produce a sustained (~2 h) postexercise hypotension in healthy normotensive subjects (Halliwill, 2001).

Oral Ingestion of Fluid

The collection of urine and the measurement of body weight throughout the study allowed for the calculation of total body water losses and volume of sweat loss during exercise and through recovery for each condition. The amount of fluid given was calculated based on sweat loss (change in body weight minus urine production) from directly before exercise to the end of the study during the individual subject's participation in the no fluid condition (i.e., difference in preexercise and postexercise nude weight) corrected for urine loss. Fluid replacement was given during the exercise bout during a single study condition (fluid condition). During the no fluid and no fluid

and exercise in a warm condition, subjects were given no oral fluid replacement during the exercise period.

Experimental Conditions during the Exercise Bout

The three study visits were identical in measurements and procedures, with the exception of temperature of the room and if fluids were given to each subject during the exercise session. The exercise bout was conducted in an environmental chamber; this allowed for the tight control of ambient temperature and relative humidity for the entire exercise period. The environmental temperatures were either set at thermoneutral (22 °C) or warm (30 °C) and the relative humidity was controlled at 30 % for exercise during each of the study conditions. Subjects either exercised with or without fluid replacement. The three conditions included the following specific fluid conditions and temperature settings: 1) fluid (exact amount of water given equal to the sweat loss through the entire study from the no fluid condition) during exercise in a thermoneutral environment (22 °C and 30 % humidity), 2) no fluid (no water given during exercise) and exercise in a thermoneutral environment (22 °C and 30 % humidity), and 3) no fluid (no water given during exercise) and exercise in a warm environment (30 °C and 30 % humidity).

Experimental Design

Each of the study days were randomized prior to the screening visit and order was divided and assigned to each subject. The no fluid conditions had to precede the fluid condition in order to calculate the exact amount of oral fluids. Thus, for randomization purposes the no fluid and exercise in a warm condition was ordered between the no fluid and the fluid condition. The possible order of the conditions was as followed: 1) no fluid condition, fluid condition, and no fluid and exercise in a warm environment; 2) no fluid condition, no fluid and exercise in a warm environment, and fluid condition or; 3) no fluid condition, no fluid and exercise in a warm environment, and fluid condition. Five subjects were selected to order 1, four subjects were selected to order 2, and five subjects were selected to order 3.

Measurements

Heart Rate and Arterial Pressure

Heart rate and arterial pressure were monitored throughout all experimental procedures. Heart rate was monitored using a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA). Resting arterial pressure was measured in the arm by using an automated oscilometric device (Dinamap Pro100 vital signs monitor, Critikon, Inc., Tampa, FL).

Cardiac Output

Cardiac output was estimated using an open-circuit acetylene washin method as described previously (Johnson *et al.*, 2000; Pricher *et al.*, 2004). This method allows for the noninvasive estimation of cardiac output. We chose an open-circuit method because subjects are exposed to stable oxygen and carbon dioxide levels throughout the measurement in contrast to rebreathing techniques. In brief, subjects breathed 8 to 10 breaths of a gas mixture consisting of 0.6% acetylene-9.0% helium-20.9% oxygen, and balance nitrogen. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services) and tidal volume was measured via a pneumotach (model 3700, Hans Rudolf, Kansas City, MO) linearized by the technique of Yeh *et al.* (1982) and calibrated by using test gas before each study. The pneumotach and valve system had a combined dead space of 24 ml. Cardiac output calculations have been described previously (Johnson *et al.*, 2000). Stroke volume was determined from cardiac output/heart rate. Systemic vascular conductance was calculated as cardiac output/mean arterial pressure and expressed as ml$\cdot$ min$^{-1}\cdot$ mmHg^{-1}.

Core Temperature

Internal body temperature was assessed by an ingestible pill telemetry system (HQInc, Palmetto, FL) (Coyne *et al.*, 2000; Wilson *et al.*, 2004).

Femoral Blood Flow

Femoral blood flow was determined through measurements of femoral artery diameter and velocity using an ultrasound probe (10-Mhz linear-array vascular probe, GE Vingmed System 5, Horton, Norway). The entire width of the femoral artery was insonated with an angle of 60°. Velocity measurements were recorded immediately before diameter measurements. Femoral blood flow was then calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs, and reported in ml/min. Femoral vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$.

Blood Sampling

A 2.5-cm, 22-gauge flexible catheter was placed in the antecubital area in the right arm using sterile techniques at the beginning of the study. Samples were collected in 3 ml heparizined arterial blood gas syringes (Smiths Medical ASD, Inc., Keene, NH). Hemoglobin and hematocrit were measured in quadruplicate. Hemoglobin was measured spectrophotometrically with a diode-array spectrophotometer (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary method after 5 min of centrifuging at 9,500 *g* (Autocrit Ultra 3, Becton Dickson, USA). Throughout the study visit, changes in blood and plasma volume were calculated from hemoglobin concentration and hematocrit using the method of Dill & Costill (1974).

Index of Skin Blood Flow

An index of skin blood flow was derived from measuring red blood cell flux values via laser-Doppler flowmetry (DRT4, Moor Instruments LTD, Devon, England). Laser-Doppler probes were placed on the skin of the right ventral forearm and the anterior right thigh. Skin blood flows were expressed as cutaneous vascular conductance, calculated as laser-Doppler flux/mean arterial pressure, and normalized to the maximal

values achieved during local heating to 43 °C at the end of the protocol (Minson *et al.*, 2001).

Urine Specific Gravity and Body Weight

Baseline hydration status was estimated in duplicate from urine specific gravity (Hand refractometer, NSG Precision Cells, Inc., Farmingdale, NY, USA). Nude body weights were obtained four separate times throughout the study (Sartorius EB6CE-I, Precision Weighing Balances, Bradford, MA, USA). After exercise, subjects toweled dry before body weight was measured. The extent of dehydration was calculated as the change in body weight from preexercise to the end of the study (total body water loss). The overall sweat loss (ml) (from directly before exercise to the end of the study) was calculated with the following formula: [(total body water loss – volume of urine loss) – (oral fluid replacement)].

Statistics

The statistical analysis was generated using SAS/Stat software, Version 9.1.3 of the SAS system for Windows (Copyright 2004 SAS Institute Inc. (SAS v9.1.3, SAS Institute, Cary, NC)). Subject characteristics are reported as means ± SD and all additional data are reported as means ± SEM. Subject characteristics and baseline exercise and hemodynamic values were analyzed across all conditions using one-way repeated measures analysis of variance (RMANOVA). When significance was detected least squared means (LSMeans) were used to compare group means. Differences in the postexercise hemodynamic variables [dependent variables] were analyzed with a three condition level longitudinal analysis of variance (ANOVA) with a quadratic fit mixed linear model with SAS PROC MIXED. Significance was set at a p-value less than or equal to 0.05.

Results

Subject Characteristics

Subject characteristics are presented in Table 5. VO_{2peak} values (63.5 ± 4.7 ml·kg^{-1}·min^{-1}) and maximal power output (406 ± 60 W) for men were reflective of a highly fit endurance exercise-trained population.

Table 5. Subjects Characteristics from Chapter IV. Values are presented as means ± SD; VO_{2peak}, peak oxygen consumption; MET, metabolic equivalents.

Variable	Endurance-trained men (n = 14)
Age, yr	26.5 ± 6.9
Height, cm	178.5 ± 8.7
Weight, kg	73.5 ± 9.9
Body Mass Index, kg/m^2	23.0 ± 1.9
VO_{2peak}, ml/min	4661 ± 617
Maximum Heart Rate, beats/min	194.5 ± 7.8
Workload at 60% of VO_{2peak}, W	206 ± 32
Baecke Sport Index, arbitrary units	15.3 ± 1.6
Index of Physical Activity, MET·h^{-1}·wk^{-1}	171 ± 56

Preexercise Hemodynamics

Table 6 shows the resting (preexercise) hemodynamics for subjects across the three study visits. Supine resting heart rate (P = 0.881) and mean arterial pressure (P = 0.943) were not different between conditions

Table 6. Preexercise Hemodynamics from Chapter V. Values are presented as means ± SEM.

Variable	Endurance-trained men (n = 14)		
	No Fluid and Exercise in a Thermoneutral Environment	Fluid and Exercise in a Thermoneutral Environment	No Fluid and Exercise in a Warm Environment
Systolic Blood Pressure, mmHg	110.7 ± 2.5	111.0 ± 2.8	109.3 ± 2.0
Diastolic Blood Pressure, mmHg	61.3 ± 2.1	60.2 ± 2.3	60.7 ± 1.9
Cardiac Output, l/min	5.04 ± 0.32	4.95 ± 0.31	5.06 ± 0.26
Stroke Volume, ml/beat	88.4 ± 5.4	88.7 ± 4.9	89.7 ± 3.5
Systemic Vascular Conductance, $ml \cdot min^{-1} \cdot mmHg^{-1}$	65.6 ± 4.6	64.7 ± 4.3	66.1 ± 3.4
Femoral Blood Flow, ml/min	255 ± 27	264 ± 22	295 ± 19
Femoral Vascular Conductance, $ml \cdot min^{-1} \cdot mmHg^{-1}$	3.3 ± 0.4	3.5 ± 0.3	3.9 ± 0.2
Core Temperature, °C	36.9 ± 0.1	36.9 ± 0.0	36.9 ± 0.1
Forearm Cutaneous Vascular Conductance, % CVCmax	1.2 ± 0.3	1.6 ± 0.4	1.2 ± 0.2
Thigh Cutaneous Vascular Conductance, % CVCmax	6.3 ± 0.8	6.7 ± 0.9	5.5 ± 0.6
Hematocrit, %	40.6 ± 0.7	40.9 ± 0.5	40.7 ± 0.6
Hemoglobin, g/dl	14.2 ± 0.2	14.1 ± 0.1	14.0 ± 0.2
Body Weight, kg	72.7 ± 10.1	72.8 ± 10.2	72.8 ± 10.3
Urine Specific Gravity	1.019 ± 0.001	1.018 ± 0.001	1.019 ± 0.001

Exercise

Subjects exercised for 60 min at 60% of VO_{2peak} on all study days. Average workloads are shown in Table 5 for each condition. Exercise mean arterial pressure was

80.7 ± 1.6 mmHg (fluid), 82.3 ± 0.9 mmHg (no fluid), and 81.1 ± 1.3 mmHg (no fluid and exercise in a warm environment) and this was not different between conditions (P = 0.646).

Table 7. Exercise Hemodynamics from Chapter V. Values are presented as means ± SEM; [a]P < 0.05 vs. No Fluid and Thermoneutral Environment, [b]P < 0.05 vs. Fluid and Thermoneutral Environment.

Variable	Endurance-trained men (n = 14)		
	No Fluid and Exercise in a Thermoneutral Environment	Fluid and Exercise in a Thermoneutral Environment	No Fluid and Exercise in a Warm Environment
Heart Rate, bpm	160.1 ± 2.5	155.9 ± 2.8 [a]	168.8 ± 2.3 [ab]
Core Temperature, °C	38.0 ± 0.1	37.9 ± 0.4	38.2 ± 0.1
Heart Rate Reserve, %	75.2 ± 2.1	72.4 ± 2.3 [a]	81.4 ± 1.6 [ab]

Postexercise Hemodynamics

Postexercise hemodynamic responses are presented in figures 5 to 9 for each condition.

Arterial Pressure

Figure 5A shows mean arterial pressure from preexercise through 90 min postexercise. There was an initial elevation in mean arterial pressure 7 min postexercise compared to preexercise (P < 0.001) across all 90 min of recovery. Mean arterial pressure was not different across all 90 min of recovery between conditions (P = 0.453), but was decreased postexercise compared to preexercise (P = 0.001). Mean arterial pressure in the fluid condition was not different throughout 28 min postexercise (P = 0.185), but was decreased until 84 min postexercise (P = 0.033) and returned to baseline

at 91 min postexercise ($P = 0.150$). Mean arterial pressure in the no fluid condition was not different throughout 28 min postexercise ($P = 0.324$), but was decreased until 77 min postexercise ($P = 0.049$) and returned to baseline at 84 min postexercise ($P = 0.170$). Mean arterial pressure in the no fluid and warm condition was not different throughout 28 min postexercise ($P = 0.130$), but was decreased until 84 min postexercise ($P = 0.011$) and returned to baseline at 91 min postexercise ($P = 0.061$).

Systemic Hemodynamics

Figure 5B shows cardiac output from preexercise through 90 min postexercise. Cardiac output was different between conditions ($P = 0.027$). The fluid condition had cardiac outputs that were 0.414 L/min higher than the no fluid condition ($P = 0.017$) across all 90 min of recovery. There was no difference in the fluid condition compared to the no fluid and warm condition ($P = 0.896$) across all 90 min of recovery. The cardiac output was 0.392 L/min higher in the no fluid and warm condition ($P = 0.023$) compared to the no fluid condition across all 90 min of recovery. Cardiac output was different postexercise ($P = 0.003$) compared to preexercise. Cardiac output in the fluid condition was elevated until 21 min postexercise compared to preexercise ($P = 0.049$), but after this time point showed no differences throughout the entire recovery period compared to preexercise (all $P > 0.05$). Cardiac output in the no fluid condition was elevated 14 min postexercise compared to preexercise ($P = 0.001$), but then was reduced at 35 min postexercise and did not return to baseline for the entire recovery period compared to preexercise (all $P = 0.001$). Cardiac output in the no fluid and warm condition was elevated 14 min postexercise (all $P = 0.001$), but after this time point showed no differences throughout the entire recovery period compared to preexercise (all $P > 0.05$).

Figure 5C shows systemic vascular conductance from preexercise through 90 min postexercise. Systemic vascular conductance was different between conditions ($P = 0.001$) across all 90 min of recovery. Systemic vascular conductance in the fluid condition was 5.97 $ml \cdot min^{-1} \cdot mmHg^{-1}$ higher than the no fluid condition ($P = 0.001$) and there was no difference in systemic vascular conductance in the fluid condition compared

to the no fluid condition and warm condition ($P = 0.896$) across all 90 min of recovery. The systemic vascular conductance was 5.92 ml·min^{-1}·mmHg^{-1} higher in the no fluid and warm condition ($P = 0.008$) compared to the no fluid condition across all 90 min of recovery. Systemic vascular conductance was different postexercise ($P < 0.001$) compared to preexercise. Systemic vascular conductance in the fluid condition was elevated until 21 min postexercise compared to preexercise ($P = 0.049$), but after this time point showed no differences throughout the entire recovery period compared to preexercise (all $P > 0.05$). Systemic vascular conductance in the no fluid condition was elevated 14 min postexercise compared to preexercise ($P = 0.044$), but then was reduced at 42 min postexercise for the entire recovery period compared to preexercise (all $P = 0.001$). Systemic vascular conductance in the no fluid and warm condition was elevated 14 min postexercise (all $P = 0.001$), but then was not different from baseline throughout the entire recovery period (all $P > 0.080$).

Figure 6A shows heart rate from preexercise through 90 min postexercise. Heart rate was different between conditions ($P < 0.001$) across all 90 min of recovery. The fluid condition had no difference in heart rate compared to the no fluid condition ($P = 0.101$) across all 90 min of recovery. The heart rate response in the no fluid and warm condition was 7.1 beats/min higher compared to the fluid condition and 5.4 beats/min higher compared to the no fluid condition (both $P < 0.001$) across all 90 min of recovery. Heart rate was different postexercise ($P < 0.001$) compared to preexercise. Heart rate was elevated in the fluid, no fluid, and no fluid and warm condition at every time point postexercise compared to preexercise (all $P = 0.001$).

Figure 6B shows stroke volume from preexercise through 90 min postexercise. Stroke volume was different between conditions ($P < 0.001$) across all 90 min of recovery. The fluid condition had stroke volumes that were 9.1 ml/beat higher than the no fluid condition ($P < 0.001$) and 8.4 ml/beat higher than the no fluid and warm conditions ($P < 0.001$) across all 90 min of recovery. Stroke volume was not different between the no fluid and the no fluid and warm condition ($P = 0.753$) across all 90 min of recovery. Stroke volume was different postexercise ($P > 0.001$) compared to

preexercise. Stroke volume was reduced in the fluid, no fluid, and no fluid and warm condition at every time point postexercise compared to preexercise (all $P = 0.001$).

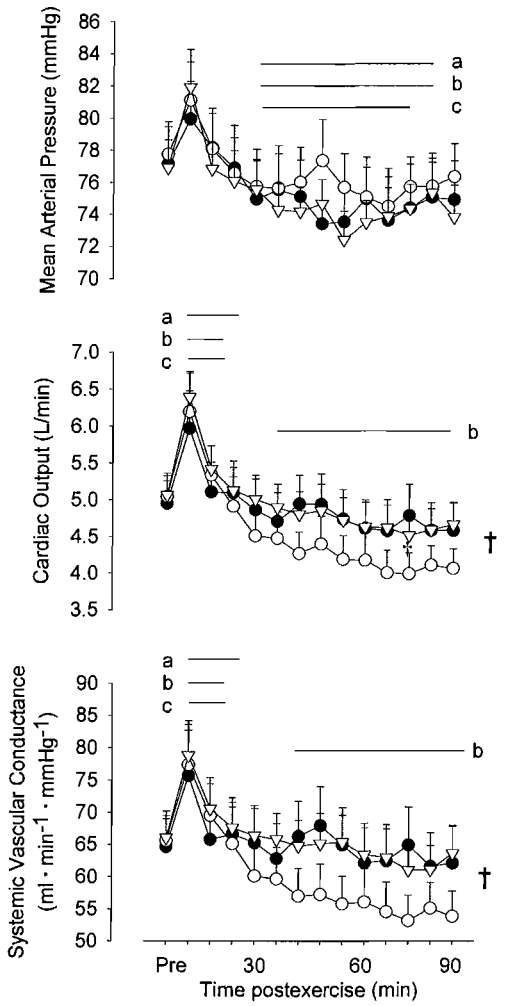

Figure 5. Postexercise Cardiac Hemodynamics from Chapter V. Mean arterial pressure (A), cardiac output (B), and systemic vascular conductance (C) from preexercise through 90 min postexercise from Chapter V. Open circles, No fluid; Filled circles, Fluid; Open upside down triangles, No fluid and warm environment. Values are presented as means ± SEM. n = 14 for each group. † Main variable effect between conditions. Fluid condition; $^{a}P < 0.05$ vs. preexercise baseline; No fluid condition; $^{b}P < 0.05$ vs. preexercise baseline; No fluid and warm environment; $^{c}P < 0.05$ vs. preexercise baseline.

Leg Hemodynamics

Figure 7A shows the percent changes in femoral blood flow from preexercise through 90 min postexercise. Femoral blood flow was not different between condition ($P = 0.608$) across all 90 min of recovery, but was increased postexercise ($P < 0.001$) compared to preexercise. Femoral blood flow was elevated throughout the entire postexercise recovery period compared to preexercise for each condition (all $P = 0.001$) across all 90 min of recovery.

Figure 7B shows the percent changes in femoral vascular conductance from preexercise through 90 min postexercise. Femoral vascular conductance was not different between condition ($P = 0.424$) across all 90 min of recovery, but was increased postexercise ($P < 0.001$) compared to preexercise. Femoral vascular conductance was elevated throughout the entire postexercise recovery period compared to preexercise for each condition (all $P = 0.001$).

Core Temperature and Skin Blood Flow

Figure 8A shows core temperature from preexercise through 90 min postexercise. Core temperature was not different between conditions ($P = 0.065$), but tended to be elevated in the no fluid and warm condition across all 90 min of recovery. Core temperature was different postexercise ($P < 0.001$) compared to preexercise. Core temperature in the fluid condition was elevated until 42 min postexercise compared to preexercise ($P = 0.020$), but after this time point showed no differences throughout the entire recovery period compared to preexercise (all $P > 0.05$). Core temperature in the no fluid condition was elevated 49 min postexercise compared to preexercise ($P = 0.013$), but after this time point showed no difference throughout the entire recovery period compared to preexercise (all $P > 0.05$). Core temperature in the no fluid and warm condition was elevated 49 min postexercise compared to preexercise (all $P = 0.001$), but then was not different from baseline throughout the entire recovery period (all $P > 0.172$).

Figure 8B shows the absolute changes in thigh cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise through 90 min postexercise. Thigh cutaneous vascular conductance was not different between conditions ($P = 0.274$) across all 90 min of recovery, but was different postexercise ($P < 0.001$) compared to preexercise. Thigh cutaneous vascular conductance in the fluid condition was elevated until 56 min postexercise compared to preexercise ($P = 0.049$), but after this time point showed no differences throughout the entire recovery period compared to preexercise (all $P > 0.05$). Thigh cutaneous vascular conductance in the no fluid condition was elevated for the entire postexercise recovery period compared to preexercise ($P = 0.001$). Thigh cutaneous vascular conductance in the no fluid and warm condition was elevated 42 min postexercise ($P = 0.032$), but then was not different from baseline throughout the entire recovery period (all $P > 0.236$).

Figure 8C shows the absolute changes in forearm cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise through 90 min postexercise. Forearm cutaneous vascular conductance was not different between conditions ($P = 0.121$) across all 90 min of recovery, but was different postexercise ($P < 0.001$) compared to preexercise. Forearm cutaneous vascular conductance in the fluid condition tended to be elevated ($P = 0.059$) in the postexercise recovery period; however, forearm cutaneous vascular conductance was different from baseline at 70 min postexercise until the end of 91 min ($P = 0.004$). Forearm cutaneous vascular conductance in the no fluid condition tended to be elevated ($P = 0.064$) in the postexercise recovery period; however, forearm cutaneous vascular conductance was different from baseline at 70 min postexercise until the end of 91 min ($P = 0.019$). Forearm cutaneous vascular conductance in the no fluid and warm condition was not different from preexercise at any time point during exercise recovery (all $P > 0.225$).

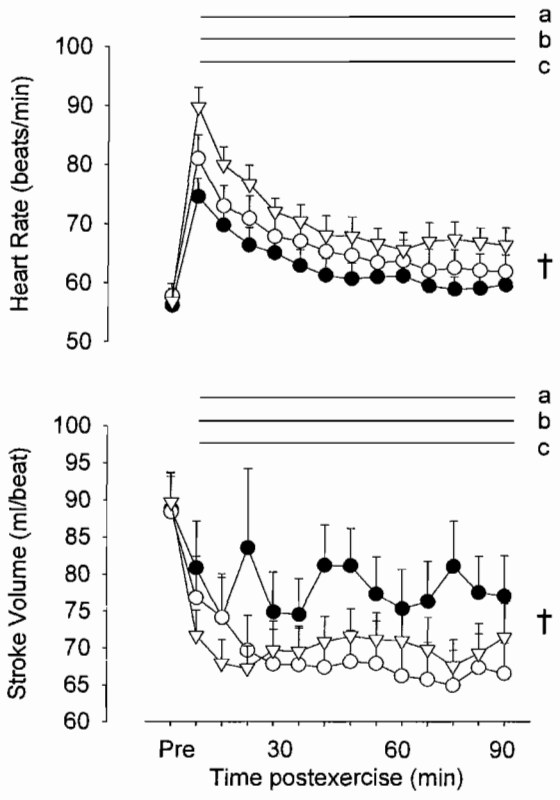

Figure 6. Postexercise Heart Rate and Stroke Volume from Chapter V. Heart rate (A) and stroke volume (B) from preexercise through 90 min postexercise from Chapter V. Open circles, No fluid; Filled circles, Fluid; Open upside down triangles, No fluid and warm environment. Values are presented as means ± SEM. n = 14 for each group. † Main variable effect between conditions. Fluid condition; $^{a}P < 0.05$ vs. preexercise baseline; No fluid condition; $^{b}P < 0.05$ vs. preexercise baseline; No fluid and warm environment; $^{c}P < 0.05$ vs. preexercise baseline.

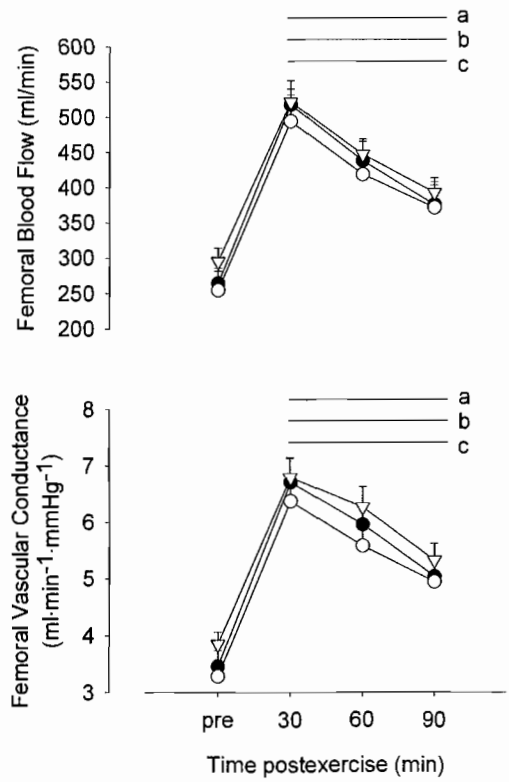

Figure 7. Postexercise Femoral Blood Flow and Femoral Vascular Conductance from Chapter V. Femoral blood flow (A) and femoral vascular conductance (B) from preexercise through 90 min postexercise from Chapter V. Open circles, No fluid; Filled circles, Fluid; Open upside down triangles, No fluid and warm environment. Values are presented as means ± SEM. n = 14 for each group. Fluid condition; $^{a}P < 0.05$ vs. preexercise baseline; No fluid condition; $^{b}P < 0.05$ vs. preexercise baseline; No fluid and warm environment; $^{c}P < 0.05$ vs. preexercise baseline.

Total Body Water, Plasma Volume, and Blood Volume

Based on the calculation of overall sweat loss (total body water loss minus urine volume) during the no fluid condition, subjects were given 1022 ± 45 ml to match the amount of sweat lost during the fluid replacement condition. In order to measure total body water loss over the course of the study, nude body weights were taken four times (baseline, before exercise, after exercise, and at the end of the study). Total body water, urine, and sweat losses were calculated for each condition, and comparisons were made

from directly before exercise to directly after exercise and directly before exercise to the end of the study.

Total Body Water Loss

The change in total body water loss was different between conditions ($P = 0.024$) and over time ($P < 0.001$). The goal of the study was to either maintain (fluid condition) or decrease body weight (no fluid and no fluid and warm environment) postexercise; thus, there was a difference in slope in the change in total body water loss over time between conditions postexercise (condition*time interaction) ($P < 0.001$). The fluid replacement condition produced a not significant increase of 0.008 kg in total body water (0.0 ± 0.0 %) ($P = 0.911$) from before exercise to the end of exercise, whereas at the same time point the no fluid condition caused a decrease of 0.852 kg in total body water (-1.2 ± 0.1 %) ($P < 0.001$), and the no fluid and warm condition caused a 1.027 kg loss in total body water (-1.4 ± 0.1 %) ($P < 0.001$). The fluid replacement condition caused a decrease of 0.331 kg in total body water loss (-0.5 ± 0.1 %) ($P < 0.001$) from before exercise to the end of the study, whereas at the same time point the no fluid condition caused a decrease of 1.078 kg in total body water (-1.5 ± 0.1 %) ($P < 0.001$), and the no fluid and warm condition caused a decrease of 1.352 kg in total body water (-1.8 ± 0.2 %) ($P < 0.001$).

Urinary Loss

Urine losses from before exercise to the end of the study were different between conditions ($P < 0.001$) [(0.313 ± 0.039 ml (fluid replacement condition), 0.121 ± 0.027 ml (no fluid condition), and 0.090 ± 0.028 ml (no fluid and warm condition)]. There was a difference between urine losses between the fluid and the no fluid condition ($P < 0.001$), and the fluid condition and the no fluid and warm condition ($P < 0.001$) but, there was no difference between urine loss between the no fluid condition and the no fluid and warm condition ($P = 0.483$) from before exercise to the end of the study. This confirms that urinary water loss is decreased during heat-stress and conditions of inadequate fluid intake, which is consistent with prior research (Pivarnik *et al.*, 1984).

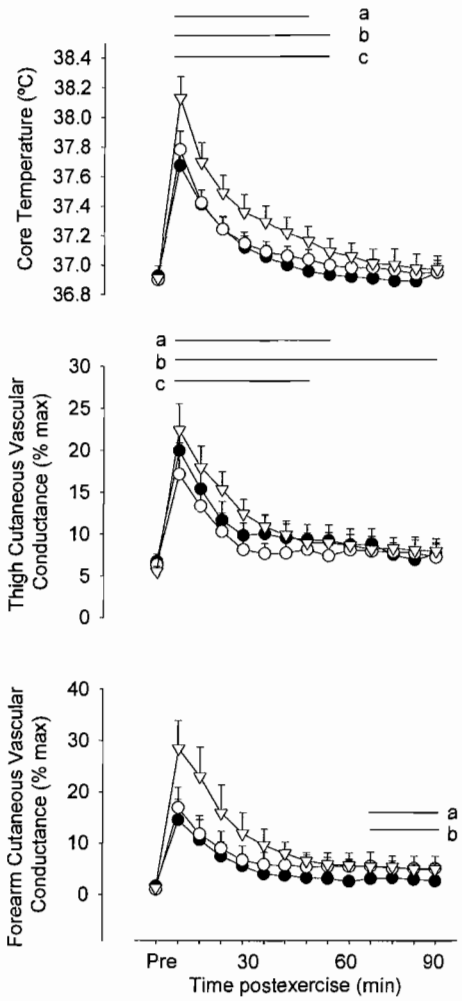

Figure 8. Core Temperature and Cutaneous Vascular Conductance from Chapter V. Core temperature (A), thigh cutaneous vascular conductance (B), and forearm cutaneous vascular conductance (C) from preexercise through 90 min postexercise from Chapter V. Open circles, No fluid; Filled circles, Fluid; Open upside down triangles, No fluid and warm environment. Values are presented as means ± SEM. n = 14 for each group. Fluid condition; $^{a}P < 0.05$ vs. preexercise baseline; No fluid condition; $^{b}P < 0.05$ vs. preexercise baseline; No fluid and warm environment; $^{c}P < 0.05$ vs. preexercise baseline.

Sweat Loss

Total sweat loss from before exercise to the end of the study were different between conditions ($P = 0.040$) [1004 ± 105 ml (fluid replacement condition), 957 ± 44 ml (no fluid condition), and 1262 ± 101 ml (no fluid and warm condition)]. There was no difference between sweat rates between the fluid and the no fluid condition ($P = 0.706$), but there was a difference between the fluid condition and the no fluid and warm condition ($P = 0.044$), and the no fluid condition and the no fluid and warm condition ($P = 0.019$) from before exercise to the end of the study.

Plasma and Blood Volumes

Figure 9A shows the percent changes in plasma volume from preexercise to end of exercise, 30, 60, and 90 min postexercise. Across recovery, plasma volume was different between conditions ($P = 0.042$). Plasma volume in the fluid condition was 4.51 and 5.97 % higher compared to the no fluid condition ($P = 0.027$), and no fluid and warm condition ($P = 0.005$). There was no difference in plasma volume between the no fluid condition and the no fluid and warm condition (0.96 %) ($P = 0.640$). Plasma volume was different over time ($P < 0.001$). The loss in plasma volume at the end of exercise was not different between conditions (all $P > 0.05$). Plasma volume was different at all time points for all conditions compared to end of exercise ($P < 0.001$); however, plasma volume recovered faster in the fluid and no fluid condition as no differences in plasma volume were seen between 30 and 90 min postexercise (all $P > 0.131$). The no fluid and warm condition showed a slower recovery to baseline values, as difference in plasma volume were seen between 30 and 90 min postexercise (all $P = 0.030$).

Figure 9B shows the percent changes in blood volume from preexercise to end of exercise, 30, 60, and 90 min postexercise. Across recovery, blood volume was different between conditions ($P = 0.039$). Blood volume in the fluid condition was 3.61 and 2.62 % higher compared to the no fluid condition ($P = 0.038$) and no fluid and warm condition ($P = 0.006$). There was no difference in blood volume between the no fluid condition and the no fluid and warm condition (0.99 %) ($P = 0.440$). Blood volume was

different over time ($P < 0.001$). The loss in blood volume at the end of exercise was not different between conditions (all $P > 0.05$). Blood volume was different at all time points for all conditions compared to end of exercise (all $P < 0.001$). However, blood volume recovered in the same manner for each condition as no difference was seen between 30 and 90 min postexercise (all $P > 0.154$).

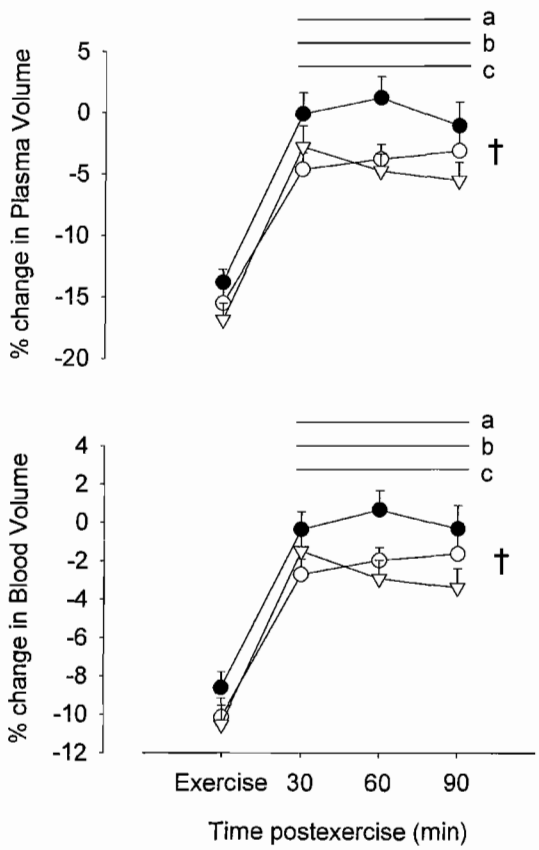

Figure 9. Change in Vascular Volume from Chapter V. The percent change in plasma volume (A) and blood volume (B) from preexercise to 0 (end exercise), 30, 60, and 90 min after exercise from Chapter V. Open circles, No fluid; Filled circles, Fluid; Open upside down triangles, No fluid and warm environment. Values are presented as means ± SEM. n = 14 for each group. Fluid condition; $^{a}P < 0.05$ vs. preexercise baseline; No fluid condition; $^{b}P < 0.05$ vs. preexercise baseline; No fluid and warm environment; $^{c}P < 0.05$ vs. preexercise baseline.

Discussion

We studied the influence of oral fluid replacement and a warm environment during exercise on postexercise central and peripheral hemodynamics and explored aspects of the response related to thermoregulation and fluid balance in a group of endurance exercise-trained men. We tested the hypothesis that fluid replacement during exercise in a group of trained men would prevent the postexercise decreases in cardiac output compared to no fluid replacement and further, that there would be greater decreases in cardiac output with greater plasma volume losses (due to increased sweat loss) in the condition of no fluid replacement and exercise in a warm environment. In other words, we expected fluid replacement to correct the previously reported decreases in cardiac output postexercise in endurance exercise-trained men. Our results suggest that fluid replacement partially mitigates the postexercise decrease in cardiac output in trained men as cardiac output was ~0.4 L/min higher compared to the no fluid replacement condition. Also, contrary to our hypothesis, exercise in a warm environment in the absence of fluid replacement did not produce the lowest cardiac output postexercise compared to the other conditions. Lastly, we hypothesized that mean arterial pressure during exercise recovery would not be different between conditions. In agreement with this hypothesis, we observed that the magnitude of postexercise hypotension was not different between conditions; which confirms that postexercise hypotension is a highly regulated physiological response, in that manipulation of cardiac output is counteracted by vascular changes such that pressure remains reduced compared to preexercise.

The Magnitude of Postexercise Hypotension

In the present study, we examined the magnitude of decrease in arterial pressure following exercise across three different fluid replacement and environmental conditions. We originally hypothesized that mean arterial pressure would be the same following exercise in these conditions. We confirmed the occurrence of postexercise hypotension in endurance-trained men (Senitko *et al.*, 2002; Dujic *et al.*, 2006; McCord & Halliwill,

2006), but found the decrease in magnitude was not different between conditions. Therefore, we report arterial pressure to be tightly regulated postexercise across all conditions and our result is consistent with results from Chapter IV and Brouha (1960) found that postexercise pressure response was not altered by environmental temperature during exercise. When fluids were given orally during exercise or intravenously during recovery in two different studies, the drop in mean arterial pressure was partially attenuated (Davis & Fortney, 1997; Charkoudian *et al.*, 2003). Charkoudian *et al.* (2003) found that an intravenous infusion postexercise increased plasma volume by 7 % above baseline values, whereas in our study, the oral fluid replacement given during exercise only increased plasma volume ~2 % above baseline; thus, the timing of rehydration and the over volumization in the study by Charkoudian *et al.* (2003) may explain the differences observed between studies. The persistence of a sustained peripheral vasodilation, and not postexercise hypovolemia, has been shown to be the underlying mechanism of postexercise hypotension (Piepoli *et al.*, 1993a; Halliwill *et al.*, 1996a). Despite the fact that postexercise hypotension is not the result of decreases in vascular volumes, the role of fluid replacement and exercise in a warm environment (i.e., plasma volume and sweat loss) on postexercise hemodynamics has not been addressed in an endurance-trained population prior to the present study.

Postexercise Hemodynamics in Sedentary Healthy Subjects

In the general populations, postexercise hypotension is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output (Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b; Halliwill, 2001). After exercise, the reduction in arterial pressure is sustained due to a rise in systemic vascular conductance and this causes a rise in blood flow through the vasodilated regions. The inactive muscle pump causes an increase in venous pooling and in combination with plasma volume loss from sweating during exercise, preload is reduced. However, stroke volume is maintained due to reduction in afterload and an increase in cardiac contractility, and the result is in an elevation in cardiac output

(increased heart rate) and vascular conductance following exercise. This was the model that was used to describe the hemodynamic response in the general population (Halliwill, 2001); however, this model could be an oversimplification of the response in trained subjects, as two recent studies have suggested that trained men mediate postexercise hypotension through a different hemodynamic balance (Senitko *et al.*, 2002; Dujic *et al.*, 2006). In addition, plasma volume in the sedentary individual is known to recover quicker (Gillen *et al.*, 1991; Hayes *et al.*, 2000) and increased plasma volume loss in the trained athlete may exacerbate the postexercise hemodynamic response. We originally thought that inadequate fluid replacement could explain the previously reported alteration in central hemodynamics (Senitko *et al.*, 2002; Dujic *et al.*, 2006) in contrast to the general population (Coats *et al.*, 1989; Halliwill *et al.*, 1996a).

Postexercise Hemodynamics in an Endurance-Trained Population

In previous studies endurance exercise-trained men showed altered vascular responses as systemic vascular conductance remained unchanged and cardiac output fell during recovery from exercise (Senitko *et al.*, 2002; Dujic *et al.*, 2006); however, these studies failed to standardize hydration. Thus, we report that when hydration is maintained (i.e., maintenance of total body water in the fluid replacement condition) in a group of endurance-trained men the postexercise decrease in cardiac hemodynamics is lessened. In the present study, hydration status (measured by urine specific gravity) was not different at baseline between conditions and body weight was maintained during the postexercise period in the fluid condition compared to the other conditions. Oral fluid replacement was chosen as the method for hydration for the present study. Fluids (~1020 ml) were given to match sweat loss over the course of the study and were consumed over a constant period during exercise and completely finished with 15 minutes to the end of exercise, which maximizes time for gastric emptying and intestinal absorption (Gisolfi *et al.*, 1998). Oral fluids have been shown to be equally effective for rehydration compared to intravenous fluids (Castellani *et al.*, 1997). Oral fluid replacement after exercise-induced dehydration restores plasma volume and normalizes heart rate to baseline values

(Costill & Sparks, 1973). This is the first study to show that inadequate fluid replacement may explain the previous reported altered central hemodynamics and we report that fluid replacement improves cardiac output postexercise in trained men compared to no fluid replacement. Despite maintaining hydration in the fluid replacement condition, we still did not observe an elevation in cardiac output postexercise (typically seen in the general population) (Halliwill, 2001). This may be due to the small overall loss in total body water from the end of exercise to the end of the study (~ loss of 0.330 kg) in the fluid condition. The possibility exists that cardiac output may have shown further rises with a greater amount of oral fluid replacement; however, the amount of water loss during exercise in the fluid condition was well-matched. Further, individual data shows that cardiac output was not better maintained when there was no change in total body water from before exercise to the end of the study during the fluid replacement condition. Thus, the decrease in central cardiac hemodynamics in trained men may also be dependent first on alterations in heart structure and filling capacity, and second on increased thermal strain which may cause increased distribution of blood flow to compliant vascular beds (i.e., cutaneous circulation) during recovery from exercise.

Mechanisms for Postexercise Fall in Cardiac Output in Endurance-Trained Men

Our results show that inadequate hydration cannot completely account for the altered postexercise cardiac hemodynamics; therefore, the question arises: What mechanisms might explain the postexercise decrease in cardiac output in trained men? Chronic endurance-training is known to alter vascular conductance (Snell *et al.*, 1987), vascular function (Martin *et al.*, 1991), arterial pressure regulation (Seals & Chase, 1989; Raven & Pawelczyk, 1993), and induce cardiac hypertrophy (Fagard, 2003; Sharma, 2003). The following mechanisms have been suggested to explain the altered postexercise cardiac hemodynamics in trained men: 1) a greater loss of plasma volume (i.e., reduction in preload from increase sweating), 2) shunting of blood flow to more compliant vascular beds (i.e., skin), 3) enhanced baroreflex-mediated changes in

sympathetic vasoconstrictor outflow that opposes peripheral vasodilation, and 4) greater sensitivity to reductions in cardiac preload (Frank-Starling pressure volume relationship).

Originally, enhancement of plasma volume was thought to improve preload and cardiac output postexercise. The role of plasma volume loss has been addressed in the current study as results showed that maintaining hydration partially improves cardiac output postexercise; however, we did not observe an elevation in cardiac output during recovery from exercise. Thus, alterations in plasma volume cannot completely explain the previously reported fall in cardiac output postexercise in trained-men (Senitko *et al.*, 2002; Dujic *et al.*, 2006).

The reduction in preload could be due to a redistribution of cardiac output from less compliant to more compliant vascular beds, such as the skin (Rowell, 1986), in the face of elevated core temperature and increased thermal load in this trained population. Additionally, the fall in preload may be due to an increased thermal load in trained men, and thus an increase in cutaneous blood flow may be needed in this scenario to dissipate heat postexercise. In other words, the augmented cutaneous vasodilation during recovery from exercise could result in reductions in stroke volume and cardiac output due to a greater amount of blood pooling in the compliant cutaneous vasculature.

Franklin *et al.* (1993) assessed postexercise hypotension under three different environmental conditions during recovery from upright cycling exercise. Recovery in the warm condition caused elevations in core and skin temperature at 60 min postexercise which worsened postexercise hypotension. In the thermoneutral and cold conditions core temperature and mean arterial pressure returned to baseline at 60 min postexercise. Franklin *et al.* (1993) concluded that the persistence of vasodilation during exercise recovery and magnitude of postexercise hypotension was dependent on elevations in skin and core temperature. It seems that thermoregulatory mechanisms might play a role in postexercise hypotension. However, Wilkins *et al.* (2004) showed that during exercise recovery mean arterial pressure was reduced when cutaneous blood flow returned to preexercise values. Thus, it appears that the contribution of the cutaneous circulation cannot account for the reductions in mean arterial pressure after exercise. However,

healthy normally active subjects were studied in the two previously mentioned studies (Franklin *et al.*, 1993; Wilkins *et al.*, 2004). Despite alteration in fluid intake and environmental temperature we found no difference following exercise in cutaneous vascular conductance (both forearm and thigh), leg blood flow, and core temperature between conditions. Thus, we report that the distribution of blood to the skin during recovery from exercise seems unlikely to explain the fall in preload and cardiac output postexercise in the trained men.

To compensate for the fall in cardiac output, it is plausible that trained men have increased sympathetic vasoconstrictor outflow during recovery from exercise that would oppose peripheral vasodilation. Endurance exercise-trained men show no change in systemic vascular conductance; however, they demonstrate skeletal muscle vasodilation postexercise (McCord & Halliwill, 2006) and this vasodilation is consistent with results from the present study. These data suggest that there may be vasoconstriction in other areas; specifically, vasoconstriction in the splanchnic or renal vascular beds may offset the vasodilation in the skeletal muscle. Pricher *et al.* (2004) found that there was no change in renal or splanchnic blood flow following exercise in a group of normally active subjects. However, no endurance-trained athletes were tested in this study (subject's mean VO_{2peak} was ~41 ml·kg $^{-1}$·min $^{-1}$). It is possible that these vascular beds undergo baroreflex-mediated sympathetic vasoconstriction in response to leg vasodilation; however, blood flow to these vascular beds was not measured in our study.

Training is thought to alter baroreflex control of heart rate (Seals & Chase, 1989; Shi *et al.*, 1993) and alter vascular resistance in response to arterial baroreflex perturbations (Mack *et al.*, 1987). Baroreflex and vasoconstrictor responsiveness to α-adrenergic receptor stimulation has been reported to be increased, remain unchanged (Mack *et al.*, 1987; Vroman *et al.*, 1988), and be attenuated (Smith *et al.*, 1988) in the endurance-trained subject compared to the sedentary individual. Physically fit individuals may be better able to induce an effective baroreceptor reflex, decreasing the slope of skin blood flow to esophageal temperature and thus, at higher temperatures, the rate at which the dependent veins fill is reduced (Nadel *et al.*, 1979). In the present

study, if cardiopulmonary and arterial baroreflexes were altered following exercise in endurance-trained men, there would have been observable differences in cutaneous vascular conductance and leg blood flow; however, this was not the case and these assumptions are based on the ability to assess baroreflex function from altered vascular tone. However, because baroreflex sensitivity was not measured in this study, we can only infer on whether or not differences in baroreflex function accounted for the altered postexercise hemodynamics.

It is possible that the same fall in preload produces a greater fall in stroke volume due to cardiac hypertrophy present in trained men (Frank-Starling cardiac pressure-volume curve). Levine *et al.* (1991) proposed orthostatic intolerance was due to structural and mechanical adaptations of the heart due to chronic training and showed that endurance exercise-trained men had increased ventricular compliance in response to lower body negative pressure. Left ventricular performance has been assessed during recovery from exercise in healthy and moderately trained subjects (Stein *et al.*, 1978; Plotnick *et al.*, 1986; Douglas *et al.*, 1987; Seals *et al.*, 1988; Goodman *et al.*, 2001). Decreases in left ventricular function, diameter/dimension, and end-diastolic volume (Stein *et al.*, 1978; Plotnick *et al.*, 1986; Douglas *et al.*, 1987; Seals *et al.*, 1988) have been reported postexercise. The effects of left ventricular compliance and cardiac function may partially explain the fall in cardiac output postexercise. However, we cannot speculate on the contribution of increased left ventricular compliance on the fall in preload during exercise recovery, as it was not directly measured in this study. Thus, a study examining cardiac function in high-level endurance-trained athletes is warranted and has yet to be evaluated.

Cardiovascular and Thermoregulatory Responses to a Warm Environment

Much is known about cardiovascular compensation during exercise in the heat (Rowell, 1974; Nadel *et al.*, 1979; Montain & Coyle, 1992b); however, little is known about the influence of metabolic and environmental heat production on postexercise hemodynamics. The impact of environmental factors such as heat and dehydration are

thought to worsen postexercise hypotension and potentially reduce orthostatic tolerance (Halliwill, 2001) through increased sweat and plasma volume loss and blood distribution to the cutaneous circulation for heat loss mechanisms. Early studies observed elevations in heart rate and core and skin temperature (Brouha, 1960; Mathews *et al.*, 1969) and pressor responses (Brouha, 1960) during recovery from heat-stress, but did not observe the hemodynamics associated with a thermal exercise stress postexercise .

In the present study, thermal strain imposed from exercise in a warm environment in the absence of fluid intake resulted in typical drift in heart rate (cardiovascular drift) and elevation in core temperature compared to the other two conditions (Rowell *et al.*, 1966; MacDougall *et al.*, 1974). Higher heart rates and core temperatures during exercise with heat have been well documented in the literature (e.g. Greenleaf & Castle, 1971). In the present study, exercising heart rate, urine production, sweat rate, and total body water was markedly affected by fluid replacement and environmental conditions.

To our knowledge, this is the first study that has assessed postexercise (central and peripheral) hemodynamics in supine recovery in a group of endurance-trained men following exercise in a warm environment. Kilgour *et al.* (1993) examined the effect of thermal stress due to exercise on hemodynamics during 15 min of upright passive recovery. Eight male normally active subjects performed cycling exercise at 60 % of VO_{2max} for 30 min under control and a heat-stress condition (thermal suit worn during exercise and the recovery period). Kilgour *et al.* (1993) found a decline in stroke index to below preexercise values in both control and the heat condition, but there was no difference between conditions; however, heart rate recovery was slower in the heated condition which accounted for the increase in cardiac index above preexercise levels. We too saw a decline in stroke volume; however, there was a difference between conditions, in that no fluid and exercise in a warm environment produced higher central hemodynamic responses compared to the no fluid condition. The overall decline in stroke volume observed in our study in the no fluid and warm condition may be due to a greater blood distribution to the compliant circulation; however, we found no difference in skin blood flow, leg blood flow, and core temperature between conditions. Kilgour *et*

al. (1993) studied normally active men and did not evaluate the effects in the endurance-trained individual and failed to characterize the peripheral hemodynamics during recovery from heat-stress, as only cardiac hemodynamics were examined. As well, in the Kilgour *et al.* (1993) study heat-stress was continued into the recovery period and this design differs from our study. Thus, the complete hemodynamic profile during supine recovery after exercise in a warm environment and the impact that exercise-induced dehydration had on an extended time period of exercise recovery was evaluated in the present study.

We observed an elevation in cardiac output postexercise in the no fluid and warm condition, and this was seemingly due to inotropic response (e.g. contractility) (Johnson & Proppe, 1996). Cardiac output is known to increase ~6.6 L/min in humans during passive heating (Rowell, 1974) and heart rate increases ~30 beats/min for every degree rise in core temperature (el-Sherif *et al.*, 1970). The increased temperature has a direct effect on the sinoatrial node (which accounts for a 7-8 beats/min rise per °C) (Johnson & Proppe, 1996). Myocardial contractility was thought to be responsible for the overall elevation in cardiac output during heat-stress (Kilgour *et al.*, 1993) and this response may be due to increased adrenergic sensitivity (Dimsdale *et al.*, 1984). It is known that the hyperthermia causes elevated sympathetic activity and parasympathetic withdraw (Johnson & Proppe, 1996). However, in our study, in addition to the contribution of contractility, the increase in postexercise cardiac output after exercise in a warm environment compared to the no fluid condition may also be due to an increase in preload; however, our data cannot differentiate between the contributions of these two factors. As well, catecholamines were not measured in our study. Thus, we document that the maintenance of central hemodynamics following the exercise in a warm environment was mainly a result of an elevation in heart rate which is consistent with past data (Kilgour *et al.*, 1993).

It has been suggested that the ability to dissipate heat is directly related to training status (Astrand *et al.*, 1960; Saltin & Hermansen, 1966; MacDougall *et al.*, 1974). If heat loss mechanisms are not effective at removing heat (i.e., due to the environment or

changes in fluid balance) then core temperature will continue to rise. Although speculative, we propose that ineffective heat loss mechanism in the general population may explain the typically observed elevations in postexercise cardiac output (similar to passive heating) and trained men may show no rise in cardiac output postexercise due to better heat loss ability during recovery from exercise. Thus, even though trained men produced more heat during exercise due to higher absolute workloads relative to a sedentary population, they may be better able to dissipate heat postexercise and thus, do not show elevated cardiac output during recovery from exercise. Despite no difference between core temperatures between conditions postexercise there was a trend for elevated core temperature in the warm condition (~0.2 °C); however, this overall core body temperature is not reflective of temperature in the active muscle or at the level of the heart. Higher cardiac output in the no fluid and warm condition compared to the no fluid condition may be a result of higher muscle temperature and greater heat storage. However, in the present study, we did not measure intramuscular temperature or heat storage between conditions.

Perspective

Our results suggest that there are cardiovascular alterations during recovery from exercise that are associated with oral fluid replacement and exercise in a warm environment. Thus, even though mean arterial pressure appears to be tightly regulated, postexercise hypotension in endurance exercise-trained men is mediated by decreases in cardiac output and unchanged systemic vascular resistance; however, the fall in cardiac output is attenuated when adequate oral fluids are given during exercise. It should be mentioned that four of the fourteen subjects showed a rise in cardiac output postexercise (similar to the general population) with oral fluid replacement compared to the other two conditions. Thus, we report individual variations in postexercise hemodynamics across our population. In comparison to the previously reported data (Senitko *et al.*, 2002; Dujic *et al.*, 2006), our subjects were highly trained as reported activity levels and maximal oxygen uptake values were significantly greater (63 vs. 47 and 56 ml·kg $^{-1}$·min $^{-1}$). It is

important to assess the hemodynamics during recovery from exercise superimposed on heat-stress, as these alterations in preload and afterload could increase orthostatic intolerance and heat-related syncope in the general and trained populations.

Conclusion

The influence of fluid replacement on postexercise hemodynamics, thermoregulation and fluid balance in a group of trained men was evaluated in this study. In the no fluid and warm condition there seems to be a sympathoexcitory effect on cardiac output and heart rate and myocardial contractility may be responsible for these increases in cardiac output during recovery from exercise compared to the thermoneutral conditions. We found that fluid replacement mitigates the decrease in cardiac output in endurance-trained men after exercise, as cardiac output was higher in the fluid replacement condition compared to the no fluid condition postexercise. Thus, even though inadequate fluid replacement (plasma volume losses) may account for some of the altered cardiac hemodynamics, endurance-trained men may have cardiac structural alterations that may partially explain the decrease in cardiac output following exercise. Controlling hydration (water to match sweat loss) is critical in assessing postexercise responses in an endurance exercise-trained population. A large study (taking hydration into account) is needed to examine if there is a sex by training status interaction on postexercise hemodynamics and, further, if alterations are noted, what mechanisms explain the responses. Currently, limited studies exist on the mechanisms associated with postexercise hemodynamics in a large sample of highly endurance exercise-trained and sedentary individuals and further investigation is needed.

The results from the experiment performed in Chapter V entitled "Fluid replacement during exercise alters postexercise cardiac hemodynamics in enduranceexercise-trained men" suggest that fluid replacement corrects the previously reported decrease in cardiac output and stroke volume in endurance-trained men. We observed that postexercise hypotension was not different between exercise in a warm environmental and fluid replacement and thus, postexercise hypotension is a tightly

regulated physiological phenomenon. Following exercise in a warm environment we observed an increase in heart rate and cardiac output compared to the no fluid condition, possibly due to an increase in contractility. This is the first study to examine the effects of a warm environment on the recovery hemodynamics in endurance-trained men. The importance of fluid replacement during exercise in these endurance-trained men was a novel finding in this study. With this knowledge, the study in Chapter VI was designed to examine the postexercise hemodynamics of a group of trained and sedentary individuals when fluid replacement during exercise is standardized.

CHAPTER VI

VARIATIONS IN POSTEXERCISE HEMODYNAMICS ACROSS SEDENTARY AND ENDURANCE EXERCISE-TRAINED MEN AND WOMEN

This study could not have been completed without contributions by Zachary Barrett-O'Keefe, Santiago Lorenzo, M.S., and John R. Halliwill, Ph.D. Zachary Barrett-O'Keefe assisted with data collection and intellectual development of the protocol. Santiago Lorenzo performed the DEXA scans. John Halliwill assisted in the intellectual development and writing of the manuscript. I completed all data collection and developed the protocol and manuscript. This study could not have been completed without the statistical help of Robin High.

Introduction

Mean arterial pressure is typically reduced ~5 to 10 mmHg following a single bout of dynamic exercise in sedentary and endurance exercise-trained humans (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002a). Although the mechanisms underlying postexercise hypotension remain unclear, in the general population (sedentary and normally active) the response is characterized by an elevation in systemic vascular conductance and not from a fall in cardiac output, but exceptions have been found (Halliwill, 2001). Several mechanisms appear to underlie the sustained peripheral vasodilation following exercise. Halliwill *et al.* (1996a) demonstrated that the baroreflex is reset to defend a lower pressure following exercise; thus, sympathetic vasoconstrictor

outflow is reduced postexercise in humans. In addition to this reduction in sympathetic nerve activity, there is reduced vascular responsiveness to a given level of sympathetic nerve activity (Halliwill *et al.*, 1996a; Halliwill *et al.*, 2003) and a sustained histamine-receptor dependent vasodilation (Lockwood *et al.*, 2005b; McCord *et al.*, 2006a).

Variations in postexercise hemodynamics across populations and individuals are known to exist (Halliwill, 2001). Older hypertensives mediate postexercise reductions in arterial pressure through decreases in cardiac output with no change in systemic vascular conductance (Hagberg *et al.*, 1987; Floras & Wesche, 1992). In line with this altered postexercise vascular response, initial observations in a small group of moderately fit exercise-endurance trained men have shown decreases in cardiac output with no change in systemic vascular conductance during postexercise hypotension (in contrast to other populations) (Senitko *et al.*, 2002) and this observation was confirmed in elite soccer players (Dujic *et al.*, 2006).

Recently, we studied the influence of hydration and a warm condition on cardiac hemodynamics during recovery from exercise and found that fluid replacement and exercise in a warm environment mitigated the decrease in postexercise cardiac output in endurance-trained males, as cardiac output was higher in these conditions compared to no fluid replacement (Chapter V). Despite standardizing hydration in this previously mentioned study, cardiac output did not increase above baseline values and the reasons for these hemodynamic differences remain unclear and may be due to plasma volume losses that may ultimately reduce central blood volume and ventricular filling during recovery from exercise. As well, the mitigated fall in postexercise cardiac output following exercise in a warm environment (Chapter V) was seemingly due in part to thermal strain as increased temperature is known to have direct effects on the sinoatrial node (Johnson & Proppe, 1996) and contractility (Kilgour *et al.*, 1993). Currently, limited studies exist on the mechanisms associated with postexercise hemodynamics in a large sample population of highly fit endurance exercise-trained and sedentary individuals.

Therefore the goal of this study was to investigate central (i.e., heart structure and filling capacity) and peripheral (i.e., skin and leg blood flow) hemodynamics in well-hydrated sedentary and trained men and women during recovery from exercise. While our previous study (Chapter V) has focused on fluid replacement and exercise in a warm environment in trained men, the present study extends our understanding of the mechanisms of postexercise hemodynamics in sedentary and trained men and women when hydration is standardized. We hypothesized that there would be variation in the postexercise hemodynamics across groups, but that the magnitude of postexercise hypotension would remain the same. We also evaluated the hypothesis that differences in postexercise cardiac output response across subjects would be correlated to plasma volume, core temperature, central venous pressure, and left ventricular end-diastolic diameter.

Methods

This study was approved by the Institutional Review Board of the University of Oregon. Each subject gave written informed consent before participating in the study.

Subjects

A total of sixty-four subjects (34 men and 30 women (between 18 and 40 yr) participated in this study. On the basis of their exercise habits over the previous 12 mo, subjects were classified as "sedentary" (no regular physical exercise) or "endurance exercise-trained" (strenuous endurance exercise > 5 days/wk). Thirty-one subjects (16 men and 15 women) were sedentary, performing < 30 min of aerobic exercise per week. Thirty-three subjects (18 men and 15 women) were endurance exercise-trained, performing >5 h of endurance exercise per week. The endurance trained athletes were primarily runners, cyclists, and triathletes performing between 7 and 20 h of endurance exercise per week.

Subjects were healthy with no allergies, normally menstruating, non-smoking, normotensive, and taking no medications other than oral contraceptives. All women

subjects had negative pregnancy tests on each day of participation in the study. The experimental design consisted of three separate testing days: a screening visit, a DEXA scan, and a study visit.

Screening Visit

Subjects reported to the laboratory for a screening visit and cycle ergometer test at least 2 h post-prandial and abstained from caffeine, alcohol, and exercise for 24 h prior to the screening visit. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands). Subjects were attached to a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA) in order to determine heart rate through the protocol. Seat height was adjusted and recorded and subjects were instructed to do a self-selected warm-up for approximately 5 min before the start of the test. The test consisted of 1-min of workload increments to determine peak oxygen uptake (VO_{2peak}). Specifically, after a 2-min warm-up period of easy cycling (40-120 W), workload increased at 20, 25, or 30 W every minute until volitional fatigue. Selection of the workload increment was subjective base on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen uptake (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). All subjects reached subjective exhaustion (Borg, 1970) [rating of perceived exertion = 19-20] within the 9- to 12-min period and max heart rate was determined immediately before termination of exercise. After the subjects rested for 15-20 min, they returned to the cycle ergometer for assessment of the workload corresponding to a steady-state VO_2 of 60% of VO_{2peak}. This workload was used on the study day for the 60-min exercise bout. Subjects self-reported activity levels on two questionnaires (Baecke *et al.*, 1982; Kohl *et al.*, 1988) during the 15-20 min rest period before the start of the 60% of VO_{2peak} verification test.

Determination of Body Composition

On a subsequent testing day that was within one week of the study day, subjects reported to the lab for a dual-energy x-ray absorptiometry (DEXA) scan. A DEXA (Total Body Analysis, ver. 3.6y, Lunar, Madison, WI) was used to estimate regional (lower body) muscle mass. This technique, which has been long recognized for its effectiveness in measuring bone density, has also demonstrated excellent precision in the measurement of bone-free lean tissue (i.e., muscle) and fat. Bony landmark sites described by Heymsfield *et al.* (1990) were used to obtain the summed lower extremity (appendicular) muscle mass (i.e., bone-free lean tissue for both legs, in kilograms).

Study Visit

Subjects reported to the laboratory at least 2 h post-prandial and abstained from caffeine for 12 h and from exercise and all medications for 24 h prior to the study. Subjects pre-hydrated the night before the study by drinking 7 ml per kg of body weight of water. Subjects recorded all food and drink consumed after 5 pm the night before and the morning of the study visit. Subjects ingested a temperature-sensing pill at least 5 h prior to the study visit; subjects reporting to the lab in the morning swallowed the pill the night before.

Upon arrival, subjects voided their bladders and a pre-study weight measurement to the nearest 5 g was obtained. The subjects were laid in a supine position for instrumentation. A venous catheter was inserted into the right antecubital arm region to obtain blood samples throughout the protocol. All samples and measurements were taken after 40 min of quiet rest. Male subjects were scheduled for the study visit within 7 to 10 days after the screening visit and women were studied during the early follicular phase (1 to 4 days after the onset of menstruation) or during the placebo phase of oral contraceptive use, which was no more than one month after the screening visit.

Timing of the Measurements

All pre- and postexercise measurements were taken in a supine position with the exception of two measurements: 1) left-ventricular end-diastolic diameter (echocardiography) was performed with subjects lying on their left side and 2) estimated central venous pressure (Gauer method) where subjects were moved to a right lateral decubitus position with the right arm dependent. Subjects were always returned to the supine position between set of measurements and rested in this position. Due to these body position changes, heart rate was recorded through out the entire study visit to ensure that the alteration in body positioning did not physically stress the subjects. Preexercise (baseline) measurements were obtained before and postexercise measurements were taken at 40, 80, and 120 min after the exercise bout. Preexercise and 40 min, 80 min, and 120 min postexercise measurements included heart rate, arterial pressure, cardiac output, core body temperature, femoral blood flow, left-ventricular end-diastolic diameter, estimated central venous pressure, blood samples for hematocrit and hemoglobin to determine changes in blood and plasma volume, and skin blood flow. During exercise, blood pressure, core body temperature, and heart rate were measured every 10 min. At the end of the protocol, maximum skin blood flow values were obtained through local heating to 43 °C. During the local heating protocol, the carbon monoxide (CO) uptake method was used to determine absolute blood and plasma volumes. After completion of the protocol, subjects were again weighed to obtain a post-study weight measurement to the nearest 5 g.

Exercise

The exercise bout consisted of a 60-min period of seated upright cycling exercise at 60% of VO_{2peak} as determined during the screening visit. This exercise duration and intensity has been proven to consistently produce a sustained (~2 h) postexercise hypotension in healthy normotensive subjects (Halliwill, 2001). During exercise, subjects were asked to drink 15 ml per kg body weight of water to replace water loss due to sweating. The ambient temperature of the laboratory was kept between 20 and 23 °C.

Measurements

Heart Rate and Arterial Pressure

Heart rate and arterial pressure were monitored throughout all experimental procedures. Heart rate was monitored using a 5-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA). Arterial pressure was measured in the arm by using an automated auscultometric device (Dinamap Pro100 vital signs monitor, Critikon, Inc., Tampa, FL).

Cardiac Output

Cardiac output was estimated using an open-circuit acetylene washin method as described previously (Johnson *et al.*, 2000; Pricher *et al.*, 2004). This method allows the noninvasive estimation of cardiac output. We chose an open-circuit method because subjects are exposed to stable oxygen and carbon dioxide levels throughout the measurement in contrast to rebreath techniques. In brief, subjects breathed 8 to 10 breaths of a gas mixture consisting of 0.6% acetylene-9.0% helium-20.9% oxygen and balance nitrogen. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services) and tidal volume was measured via a pneumotach (model 3700, Hans Rudolf, Kansas City, MO) linearized by the technique of Yeh *et al.* (1982)and calibrated by using test gas before each study. The pneumotach and valve system had a combined dead space of 24 ml. Cardiac output calculations have been described previously (Johnson *et al.*, 2000). Stroke volume was determined from cardiac output/heart rate. Systemic vascular conductance was calculated as cardiac output/mean arterial pressure and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$.

Core Temperature

Internal body temperature was assessed by an ingestible pill telemetry system (HQInc, Palmetto, FL) (Coyne et al. 2000, Wilson et al. 2004).

Femoral Blood Flow

Femoral blood flow was determined through measurements of femoral artery diameter and velocity using an ultrasound probe (10-Mhz linear-array vascular probe, GE Vingmed System 5, Horton, Norway). The entire width of the femoral artery was insonated with an angle of 60°. Velocity measurements were recorded immediately before diameter measurements. Leg blood flow was then calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs, and reported in ml/min. Femoral vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$.

Left Ventricular End-Diastolic Diameter (LVEDD)

Two-dimensional guided M-mode transthoracic images was obtained from the left parasternal position by using a cardiac ultrasound machine (GE Vingmed) equipped with a 2.5 Mhz vector array cardiac probe, as performed previously by Lawler *et al.* (1998). Transducer positioning was optimized to obtain the least oblique image at the mid-ventricular short-axis papillary muscle level. Left-ventricular end-diastolic diameter was measured at the peak of the R wave. Measurements over five to ten consecutive cardiac cycles were used to estimate left ventricular end-diastolic diameter for a given experimental time point. This measurement was used as an indication of ventricular volume as a surrogate for ventricular distensibility and compliance and has been used previously for the measurement of left ventricular end-diastolic diameter in hypertensive and normotensive individuals following exercise (Cleroux *et al.*, 1989; Cleroux *et al.*, 1992b).

Central Venous Pressure

At the beginning of the study visit, the subjects underwent the placement of an intravenous catheter for blood sampling and measurement of peripheral venous pressure. A 2.5-cm, 22-gauge flexible catheter was placed in the antecubital area in the right arm

using sterile techniques. The catheter was attached to a single-use sterile pressure transducer and continuously flushed with pressurized heparinized saline to prevent clotting (3 ml h^{-1}, 2 units ml^{-1}). Using the attached pressure transducer to measure peripheral venous pressure, central venous pressure was estimated by the method of Gauer & Sieker (1956). This technique has been used previously for the measurement of postexercise hemodynamics in hypertensive and normotensive individuals (Cleroux *et al.*, 1992b). This method required the subject to lie in the right lateral decubitus position with the right arm dependent. This positioning established a continuous hydrostatic gradient between the vena cava and the peripheral venous catheter such that changes in peripheral venous pressure accurately reflect change in central venous pressure (Convertino *et al.*, 1991). Resting peripheral venous pressure was monitored continuously for 2 min with a pressure transducer (Edwards Lifesciences LLC, Irvine, CA) and was recorded onto a chart recorder. With the subject in the lateral decubitus position, the transducer was positioned and marked at the level of the midsternum (xiphoid process). Care was taken to ensure accurate repeatable location of the transducer for pre and postexercise central venous pressure measurements.

Blood Sampling

Throughout the study visit, changes in blood and plasma volume were estimated from changes in hematocrit and hemoglobin using the method of Dill & Costill (1974). Hemoglobin and hematocrit were measured in quadruplicate. Hemoglobin was measured spectrophotometrically with the diode-array spectrophotometer OSM3 hexoximeter (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary method after 5 min of centrifuging at 9,500 *g* (Autocrit Ultra 3, Becton Dickson, USA). Samples were collected in 3 ml heparizined arterial blood gas syringes (Smiths Medical ASD, Inc., Keene, NH).

Index of Skin Blood Flow

An index of skin blood flow was derived from measuring red blood cell flux values via laser-Doppler flowmetry (DRT4, Moor Instruments LTD, Devon, England). Laser-Doppler probes were placed on the skin of the right ventral forearm and the anterior right thigh. Skin blood flows were expressed as cutaneous vascular conductance, calculated as laser-Doppler flux/mean arterial pressure, and normalized to the maximal values achieved during local heating to 43 °C at the end of the protocol (Minson *et al.*, 2001).

Carbon Monoxide Uptake Method

We estimated absolute blood and plasma volume by measuring total body hemoglobin via a CO uptake method as previously described (Burge & Skinner, 1995). This was done at the end of the protocol with the subjects in a stable body (supine) position. The dosage of CO administered in this study was 1.25 ml per kg body weight (up to a maximum dose of 100 ml). This dosage is predicted to raise HbCO by ~ 6.5 % to levels around 8 %. Subjects breathed 100 % O_2 from a Douglas bag for 4 min on an open circuit to flush N_2 from their lungs and then were switched to a rebreathing circuit with a 3 L anesthesia bag that was pre-filled with 100 % O_2. The rebreathing circuit was equipped with a soda-lime CO_2 scrubber (to prevent the buildup of CO_2 in the circuit) and had a fresh O_2 supply so that subjects stayed hyper-oxygenated throughout the procedure. After becoming accustomed to the rebreathing circuit (2 to 4 min), a calibrated volume of ultra high purity (> 99.9 % pure) CO was injected into the rebreathing circuit with a glass syringe over a 15 to 30 second period. Subjects continued to breathe on the circuit for an additional 10 min. Blood samples were obtained before and 10 minutes after the CO was added to the rebreathing circuit in order to measure the percent of hemoglobin that is bound to CO (%HbCO). This method has been in use as a reliable method for measuring blood and plasma volume (Gore *et al.*, 2005). Hemoglobin, hematocrit, and CO-hemoglobin were measured for the purpose of estimating blood volume and plasma volume. %HbCO and hemoglobin were measured

spectrophotometrically with the diode-array spectrophotometer OSM3 hexoximeter (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary centrifuge method (Autocrit Ultra 3, Becton Dickson, USA). Samples were collected in 3 ml heparizined arterial blood gas syringes (Smiths Medical ASD, Inc., Keene, NH).

Statistics

The statistical analysis for this paper was generated using SAS/Stat software, Version 9.1.3 of the SAS system for Windows [Copyright 2004 SAS Institute Inc. (SAS v9.1.3, SAS Institute, Cary, NC)]. Subject characteristics are reported as means ± SD and all additional data are reported as means ± SEM.

To compare group differences in the hemodynamic variables [dependent variables], percent change from preexercise baseline values were analyzed with a mixed linear model with SAS PROC MIXED evaluating the between-subject class factors (sex and training status) and the within-subjects factors (3 postexercise time points (percent difference compared to preexercise)). PROC MIXED allows one to account for random effects due to differences between-subjects and correlations among the observations over time in the modeling process with a variety of covariance structures. Since there was equally spaced time points during exercise recovery the auto regressive correlation structure was applied for the within subject variable (time).

Following this conventional class analyses, alternative models were developed based on a combination of class variables and covariates and used a pseudo R-squared to explain the variance. Specifically, the analysis explored whether the effects attributed to training status could be better explained by covariates associated with training status (workload and oxygen uptake). The pseudo R-squared method is used to explain how each model accounts for a percentage of the overall ratio variance. This pseudo R-squared method can be used for multiple and independent observations for complex modeling and is analogous to R-squared. The pseudo R-squared gives an indication of whether adding the covariate can improve the model and better explain the ratio variance

of the dependent variable. Training status, workload at 60% (VO_{2peak}), and absolute maximal oxygen uptake at 60% served as subject-specific covariates for the models. Final models consisted of the main effects and interactions with time dependent covariates added. Backward elimination of non-significant terms was used to produce the final model for each variable of interest. The pseudo R-squared was computed for each model including the following covariates: 1) 60% workload (watts), 2) absolute oxygen uptake (l/min), 3) status (trained vs. sedentary), and 4) time were compared to the null model (model with no covariates).

To determine the effects of sex, time, and the covariates (status, workload at 60%, and absolute oxygen uptake at 60%) Type-3 F-tests were computed for all main effects and interactions. Type-3 F-tests are specifically used for unbalanced data and improvement of the model (based on the covariates) can be analyzed irrespective of sequential order (unlike a Type-1 F-test). For significant interactions simple effects for factors of interest in the interaction were extracted. The mean differences were computed when the between- and within-subjects factors were significant at p-value less than or equal to 0.05. A main effect or interaction was considered to be "of interest" when the p-value was between 0.05 and 0.15. The full models consisted of main effects two-way and three-way interactions of sex, time, and a covariate (training status, workload at 60%, and absolute oxygen uptake at 60%).

A contrast in the means compared the differences due to the main effect of status or the simple effect of status over time. In the post hoc analyses, multiple comparisons with the Bonferonni adjustment established the critical p-values to maintain an overall of Type I error probability of $\alpha = 0.05$. For example, for the simple effect of status in a status*time effect, the p-value was declared significant when it was less than 0.0167 (0.05/3). When simple comparisons were made the Bonferonni adjustment is known to be too conservative thus, significance was set at a p-value less than or equal to 0.05.

Results

Anthropometric Data

Subject anthropometric data for all subjects are presented in Table 8. Body mass index was higher in sedentary men compared to sedentary women ($P = 0.011$) and in sedentary men compared to trained men ($P = 0.018$); however, body mass index was not different between trained and sedentary women ($P = 0.327$) or trained women and trained men ($P = 0.191$).

Table 8. Anthropometric Data from Chapter VI. Values are means ± SD; *N*, no. of subjects. * $P < 0.05$ vs. sedentary within same sex. † $P < 0.05$ vs. women within same training status.

	Sedentary Women	Trained Women	Sedentary Men	Trained Men
N	15	15	16	18
Age, yr	21.8 ± 3.7	27.3 ± 8.1*	21.8 ± 1.6	26.1 ± 6.9*
Height, cm	162 ± 8	166 ± 6	179 ± 9†	183 ± 8†*
Weight, kg	59.3 ± 10.1	60.2 ± 8.2	80.3 ± 17.0†	76.9 ± 10.6†
Lean Mass, kg	38.0 ± 5.4*	43.5 ± 5.0*	59.5 ± 13.1†	64.9 ± 8.0†
Lean Leg Mass, kg	12.7 ± 2.0	14.7 ± 1.8*	20.1 ± 4.8†	22.5 ± 2.8*†
Body Fat, %	30.3 ± 7.0	22.7 ± 5.5*	21.0 ± 6.7†	10.7 ± 5.2*†

Exercise

Subject baseline exercise parameters are presented in Table 9. VO_{2peak} values were within the expected range for the sedentary population [sedentary men: 44.9 ± 6.6 $ml \cdot kg^{-1} \cdot min^{-1}$ (mean ± SD); sedentary women: 34.1 ± 5.5 $ml \cdot kg^{-1} \cdot min^{-1}$] and for the endurance-trained population (trained men: 63.8 ± 8.7 $ml \cdot kg^{-1} \cdot min^{-1}$; trained women: 52.2 ± 4.6 $ml \cdot kg^{-1} \cdot min^{-1}$). The trained groups had higher relative VO_{2peak} values compared to their sedentary counterparts (both $P < 0.001$). Men had higher VO_{2peak} values compared to women (both $P < 0.001$).

Table 9. Subject Baseline Exercise Data from Chapter VI. Values are means ± SD; *N*, no. of subjects, VO_{2peak}, peak O_2 consumption; MET, metabolic equivalents. * $P < 0.05$ vs. sedentary within same sex. † $P < 0.05$ vs. women within same training status.

	Sedentary Women	Trained Women	Sedentary Men	Trained Men
N	15	15	16	18
VO_{2peak}, ml/min	2022 ± 451	3157 ± 580*	3572 ± 731†	4846 ± 559*†
Max Workload, W	180 ± 40	289 ± 54*	303 ± 62†	425 ± 58*†
Workload at 60% of VO_{2peak}, W	78 ± 23	135 ± 28*	154 ± 36†	221 ± 59*†
Max Heart Rate, bpm	186.8 ± 12.2	189.1 ± 9.5	191.1 ± 7.1	185.7 ± 9.2
Baecke Sport Index, arbitrary units	7.9 ± 1.2	13.3 ± 2.2*	8.7 ± 1.1	14.9 ± 3.5*
Index of Physical Activity, MET·h·wk	44 ± 30	162 ± 62*	59 ± 26	181 ± 53*

Figure 10 compares peak oxygen uptake (l/min) and workload at 60 % (W) plotted as a function of body weight (kg) and lean leg mass (kg) across all subjects. The relationship between body weight and peak oxygen uptake (see Fig. 10, *top left*) was highly correlated ($r^2 = 0.74, P < 0.001$) as was the relationship between body weight and relative 60% workload (see Fig. 10, *bottom left*) ($r^2 = 0.78; P < 0.001$). The relationship between lean leg mass and peak oxygen uptake was highly correlated (see Fig. 10, *top right*) ($r^2 = 0.89, P < 0.001$) as was the relationship between lean leg mass and relative 60% workload (see Fig. 10, *bottom left*) ($r^2 = 0.90; P < 0.001$). Thus, we found body weight to be a predictor of peak oxygen uptake and relative workload at 60%, but in comparison lean leg mass was a better predictor of peak oxygen uptake and relative workload at 60% during aerobic cycling and this was independent of training status.

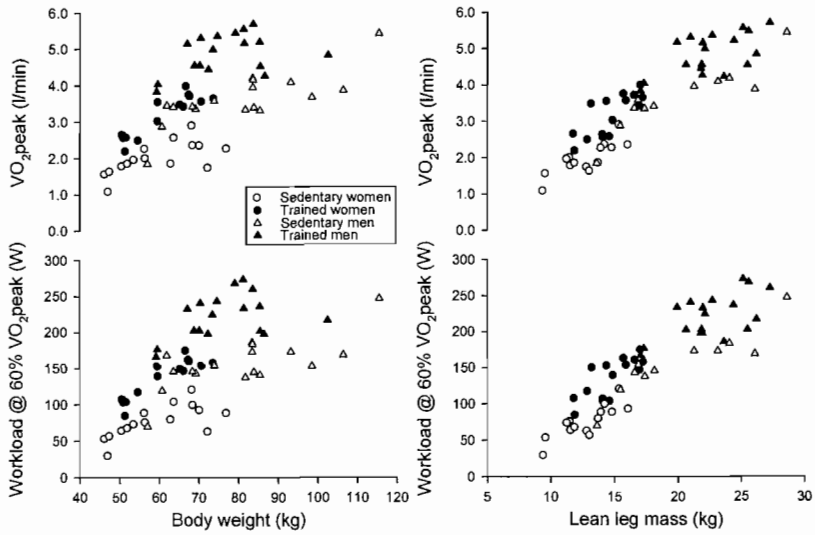

Figure 10. Training Status as a Function of Lean Leg Mass. *Top left:* Peak oxygen uptake (l/min) as a function of body weight (kg) from Chapter V. *Bottom left:* Workload at 60 % as a function of body weight (kg). *Top right:* Peak oxygen uptake (l/min) as a function of lean leg mass (kg). *Bottom right:* Workload at 60 % as a function of lean leg mass (kg).

The goal was to have subjects exercise for 60 min at 60% of VO_{2peak} on the study day. The percentage of heart rate reserve (heart rate reserve is defined as maximal heart rate achieved during VO_{2peak} testing minus the resting supine heart rate) reached during exercise by sedentary women (68.9 ± 8.3 %) and men (76.5 ± 8.2 %) and trained women (67.0 ± 6.3 %) and men (76.5 ± 7.6 %) did not differ between the sedentary and trained subjects for the same sex ($P = 0.173$ (women); $P = 0.979$ (men)), but was different between men and women for the same training status ($P = 0.012$ (sedentary); $P < 0.001$ (trained)). The core temperature during exercise by sedentary women (37.7 ± 0.4 °C) and men (37.9 ± 0.3 °C) and trained women (38.2 ± 0.6 °C) and men (38.2 ± 0.4 °C) was not different between groups ($P = 0.979$). This verifies that the exercise target was reached by the subjects on the study day.

Preexercise Hemodynamics

Preexercise (resting) hemodynamics for the subject groups are presented in Table 10.

Table 10. Preexercise Hemodynamics from Chapter VI. Values are means ± SEM; *N*, no. of subjects. *$P < 0.05$ vs. sedentary within same sex. † $P < 0.05$ vs. women within same training status.

	Sedentary Women	Trained Women	Sedentary Men	Trained Men
N	15	15	16	18
Stroke Volume, ml/beat	58.8 ± 5.4	80.6 ± 7.3*	84.6 ± 6.1†	125.1 ± 7.9*†
Blood Volume, ml	4216 ± 725	5454 ± 1198*	5420 ± 1169†	7054 ± 1101*†
Plasma Volume, ml	2552 ± 434	3257 ± 684*	3297 ± 746†	3979 ± 782*†
Mean arterial pressure, mmHg	78.0 ± 2.1	80.5 ± 1.7	81.6 ± 1.7	80.9 ± 1.7
Systemic Vascular Conductance, ml·min^{-1}·mmHg^{-1}	51.8 ± 4.1	57.2 ± 6.8	64.0 ± 4.3	76.3 ± 4.8†
Femoral Blood Flow, ml/min	233 ± 32	352 ± 81	286 ± 31	316 ± 44
Femoral Vascular Conductance, ml·min^{-1}·mmHg^{-1}	3.0 ± 0.4	4.4 ± 1.1	3.5 ± 0.4	3.9 ± 0.5
Hematocrit, %	36.0 ± 0.7	36.0 ± 0.7	40.7 ± 0.7†	39.1 ± 0.8†
Hemoglobin, g/dl	12.5 ± 0.3	12.3 ± 0.3	14.5 ± 0.2†	13.5 ± 0.3*†
Core Temperature, °C	36.9 ± 0.1	37.1 ± 0.1	37.0 ± 0.0	36.9 ± 0.1
Forearm Cutaneous Vascular Conductance, ml·min^{-1}·mmHg^{-1}	1.1 ± 0.2	3.5 ± 1.1*	2.4 ± 0.5	3.6 ± 0.8
Thigh Cutaneous Vascular Conductance, ml·min^{-1}·mmHg^{-1}	5.7 ± 1.9	3.5 ± 1.0	4.8 ± 0.5	7.3 ± 0.1†

As expected, resting heart rate was lower in men compared to women of the same training status ($P = 0.013$ (trained); $P = 0.009$ (sedentary)) and lower in trained versus sedentary subjects (both $P < 0.001$). There was no difference in resting diastolic pressure between groups ($P = 0.574$); however, systolic blood pressure was higher in sedentary men compared to sedentary women ($P = 0.029$) and not different between the trained and sedentary men ($P = 0.396$) or between trained and sedentary women ($P = 0.654$). Cardiac output was higher in the trained men compared to the trained women ($P = 0.006$), but was not higher in the sedentary men compared to the sedentary women ($P = 0.421$), trained women compared to the sedentary women ($P = 0.265$) and the trained men compared to the sedentary men ($P = 0.085$). Subject characteristics (with the exception of higher VO_{2peak} values and workloads in the trained men and women) (see Table 9) are consistent with previously work where the groups were separated by training status and sex (Senitko *et al.*, 2002; McCord *et al.*, 2006a; Lynn *et al.*, 2007).

Pre and Postexercise Body Weight

In order to standardize hydration at baseline, subjects were pre-hydrated with ~7ml/kg of water the night prior to the study and this amount was kept identical for each subject. Nude body weight was taken two times over the course of the study [baseline (1) and at the end of the study (2)]. Despite administration of water during the exercise bout (15 ml/kg) all subjects lost body weight over the course of the study. Trained men lost 0.821 ± 0.10 kg, trained women lost 0.507 ± 0.090 kg, sedentary men lost 0.430 ± 0.060 kg, and sedentary women lost 0.113 ± 0.060 kg from pre to postexercise. The change in body weight was different between sex ($P = 0.039$) as men lost 0.636 ± 0.407 kg and women lost 0.310 ± 0.338 kg, but this was not different between status ($P = 0.507$) as the trained subjects lost 0.677 ± 0.412 kg and sedentary subjects lost 0.276 ± 0.287 kg. There was no significant sex difference by training status interaction ($P = 0.561$) for total body water loss. Since urine was not collected during the study, sweat loss cannot be seperated from urine loss of body water.

Postexercise Hemodynamics

Figures 11 to 15 show the postexercise hemodynamic responses in each group. To simplify the presentation of results, postexercise hemodynamic responses are displayed as percent change from preexercise to postexercise (40, 80, and 120 min) for most variables.

Mean Arterial Pressure

Figure 11A shows the percent change in mean arterial pressure from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, mean arterial pressure was reduced at 40 and 80 min postexercise compared to preexercise (-3.0 and -3.7 %) (both $P < 0.001$), but was not different at 120 min postexercise compared to preexercise (-0.97 %) ($P = 0.111$). The conventional model that showed a sex*status interaction ($P = 0.034$) had a pseudo R-squared of 0.109. In this model, mean arterial pressure in sedentary men was decreased more from preexercise to postexercise compared to trained men (3.1 %) ($P = 0.045$) and in sedentary women mean arterial pressure was decreased less from preexercise to postexercise compared to trained women (2.5 %) ($P = 0.090$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Heart Rate

Figure 11B shows the percent change in heart rate from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, heart rate was higher throughout postexercise (16.7, 7.6, and 5.6 %) (all $P < 0.001$) compared to preexercise. In addition, the percent difference in heart rate was higher in men from preexercise to postexercise compared to women (11.3 %). Substitution of VO_2 60% and workload 60% for status improved the model. The conventional model that showed a workload 60%*time interaction ($P < 0.001$) had a pseudo R-squared of 0.295. In this model, heart rate was higher in those with the lowest range 60% workload (e.g. ~100 watts 13.2 %) ($P < 0.001$) at 40 min postexercise compared to preexercise, but no

differences in heart rate were seen at 80 and 120 min postexercise compared to preexercise (4.0 and 2.0 %) ($P = 0.126$; $P = 0.440$). Heart rate was higher in those at the middle range for 60% workload (e.g. ~200 watts 19.5, 10.3, and 8.3 %) throughout postexercise compared to preexercise ($P < 0.001$; $P = 0.006$; $P = 0.026$). Heart rate was higher in those at the highest range for 60% workload (e.g. ~280 watts 25.7 and 16.6 %) at 40 and 80 min postexercise compared to preexercise ($P = 0.001$; $P = 0.034$) but heart rate tended not to be elevated at 120 min postexercise compared to preexercise (14.6 %) ($P = 0.064$). A model with VO_2 60%*time interaction ($P < 0.001$) had a pseudo R-squared of 0.298. In this model, there was no difference in heart rate in those with the lowest VO_{2peak} values (e.g. ~1200 ml/min 9.4, 0.24, and 1.8 %) throughout postexercise compared to preexercise ($P = 0.058$; $P = 0.961$; $P = 0.718$). Heart rate was higher in those with the middle range VO_{2peak} values (e.g. ~3470 ml/min 16.5, 7.3, and 5.3 %) throughout postexercise compared to preexercise ($P < 0.001$; $P = 0.002$; $P = 0.025$). Heart rate was higher in those with the highest VO_{2peak} values (e.g. ~5500 ml/min 22.7, 13.6, and 11.6 %) throughout postexercise compared to preexercise ($P < 0.001$; $P = 0.018$; $P = 0.043$).

Cardiac Output

Figure 11C shows the percent change in cardiac output from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, cardiac output was higher 40 min postexercise compared to preexercise (6.7 %) ($P < 0.001$), but was not different at 80 and 120 min postexercise compared to preexercise (2.9 and 1.2 %) ($P = 0.105$; $P = 0.495$). In addition, the percent difference in cardiac output was higher in men from preexercise to postexercise compared to women (7.3 %). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Stroke Volume

Figure 11D shows the percent change in stroke volume from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups,

stroke volume was reduced throughout postexercise compared to preexercise (-7.7, -4.0, and -3.7 %) ($P < 0.001$; $P = 0.008$; $P = 0.014$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Systemic Vascular Conductance

Figure 12A shows the percent change in systemic vascular conductance from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, systemic vascular conductance was higher at 40 and 80 min postexercise compared to preexercise (10.3 and 7.1 %) ($P = 0.001$; $P = 0.003$), but was not different at 120 min postexercise compared to preexercise (2.6 %) ($P = 0.179$). In addition, the percent difference in systemic vascular conductance was higher in men from preexercise to postexercise compared to women (10.4 %). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Femoral Blood Flow

Figure 12B shows the percent change in femoral blood flow from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, femoral blood flow was higher throughout postexercise compared to preexercise (108.8, 85.6, and 60.5 %) (all $P < 0.001$). In addition, the femoral blood flow was higher in men from preexercise to postexercise compared to women (45.7, 31.7, and 24.4 %) (all $P < 0.001$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

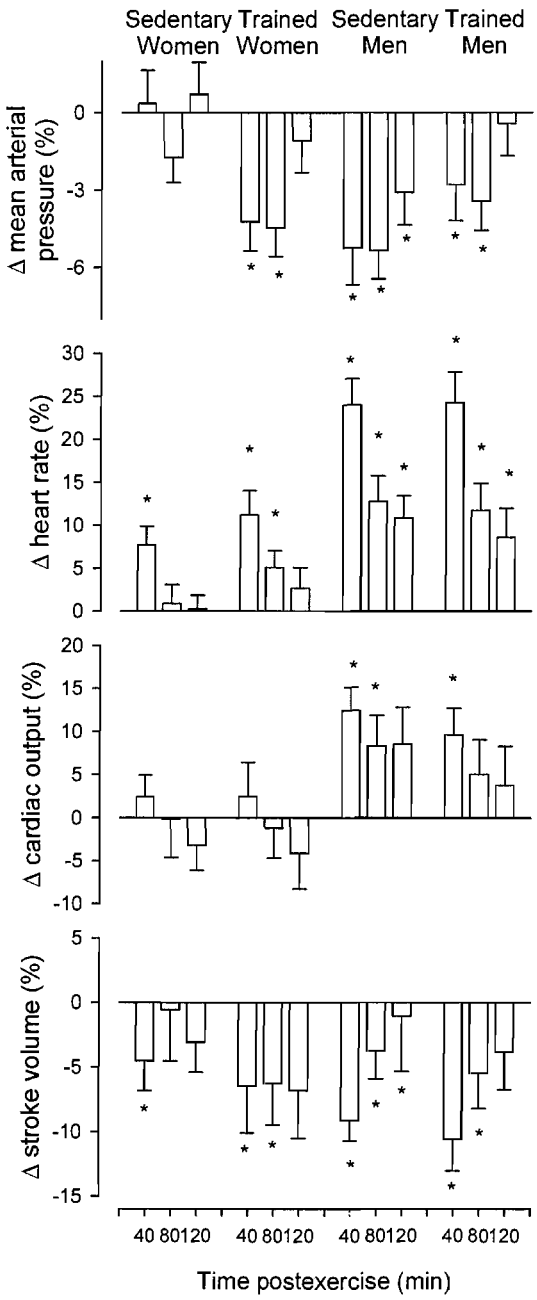

Figure 11. Postexercise Cardiac Hemodynamics from Chapter VI. The percent change in mean arterial pressure (A), heart rate (B), cardiac output (C), and stroke volume (D) from preexercise to 40, 80, and 120 min after exercise from Chapter VI. Percent changes are grouped for sedentary women, trained women, sedentary men and trained men. * $P < 0.05$ vs. preexercise baseline. Values are presented as means ± SEM.

Femoral Vascular Conductance

Figure 12C shows the percent change in femoral vascular conductance from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, femoral vascular conductance was higher throughout postexercise compared to preexercise (116.0, 93.0, and 62.3 %) (all $P < 0.001$). In addition, the percent difference in femoral vascular conductance was higher in men from preexercise to postexercise compared to women (26.7, 18.5, and 28.0 %) (all $P < 0.001$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Central Venous Pressure

Figure 13A shows an absolute change in estimated central venous pressure from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, central venous pressure was reduced throughout postexercise compared to preexercise (-1.2, -1.2, and -1.1 mmHg) ($P < 0.001$; $P < 0.008$; $P = 0.001$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Left Ventricular End-Diastolic Diameter.

Figure 13B shows an absolute change in left ventricular end-diastolic diameter from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, left ventricular end-diastolic diameter was reduced through postexercise compared to preexercise (-0.06, -0.05, and -0.03 cm) (all $P < 0.001$). The conventional model that showed a sex*time interaction ($P = 0.001$) and VO_2 60% ($P = 0.019$) had a pseudo R-squared of 0.099. In this model, the percent difference in left ventricular end-diastolic diameter was reduced in men from preexercise to postexercise compared to women (-0.02 cm) ($P < 0.001$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

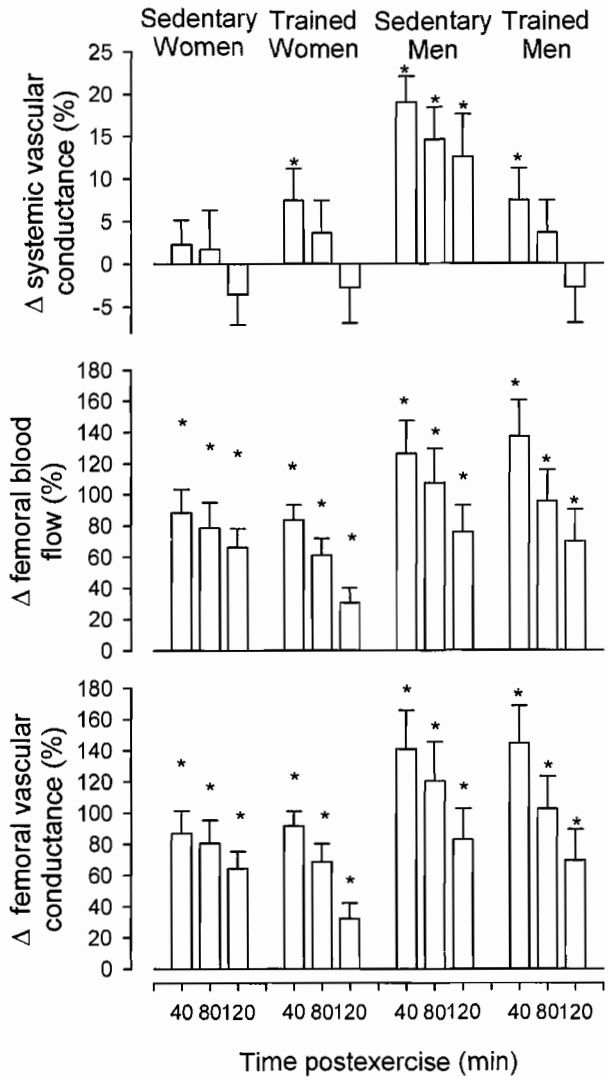

Figure 12. Systemic and Femoral Vascular Conductance from Chapter VI. The percent change in systemic vascular conductance (A), femoral blood flow (B), and femoral vascular conductance (C) from preexercise to 40, 80, and 120 min after exercise from Chapter VI. Percent changes are grouped for sedentary women, trained women, sedentary men and trained men. * $P < 0.05$ vs. preexercise baseline. Values are presented as means ± SEM.

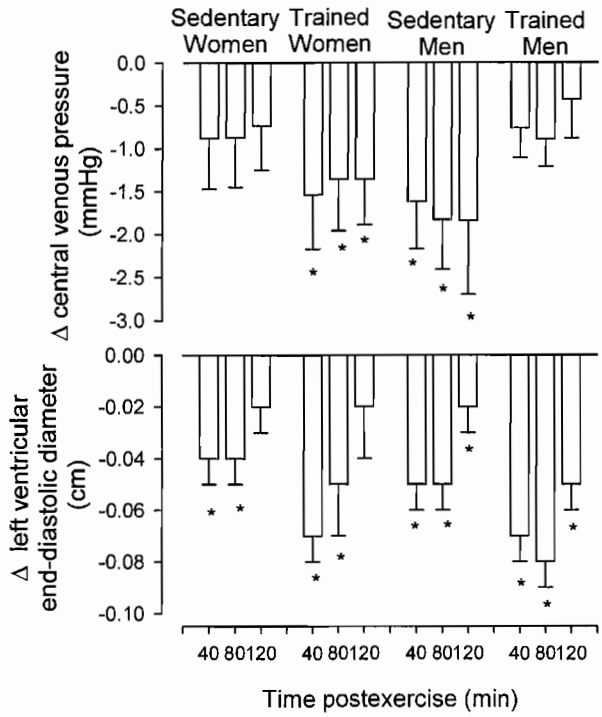

Figure 13. Postexercise Changes in Preload. The percent change in central venous pressure (A) and left ventricular end-diastolic diameter (B) from preexercise to 40, 80, and 120 min after exercise from Chapter VI. Percent changes are grouped for sedentary women, trained women, sedentary men and trained men. * $P < 0.05$ vs. preexercise baseline. Values are presented as means ± SEM.

Core Temperature

Figure 14A shows an absolute change in core temperature from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, core temperature was higher at 40 min postexercise compared to preexercise (0.2 °C) ($P = 0.009$), but was not different at 80 and 120 min postexercise compared to preexercise (0.1 and 0.1 °C) ($P < 0.097$; $P = 0.231$). A model with status*time ($P =$

0.052) had a pseudo R-squared of 0.034. Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Arm Cutaneous Vascular Conductance

Figure 14B shows an absolute change in arm cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, arm cutaneous vascular conductance tended to be higher postexercise compared to preexercise ($P = 0.060$). The conventional model that showed a sex*time interaction ($P = 0.033$) with the covariate workload at 60% ($P = 0.001$) had a pseudo R-squared of 0.103. In this model, arm cutaneous vascular conductance was higher in men from prexercise to postexercise compared to women (0.85, 2.3, and 2.1 %) ($P = 0.001$; $P < 0.001$; $P < 0.001$). A model with a VO_2 60%*sex interaction ($P = 0.021$) had a pseudo R-squared of 0.074. In this model, men with the lowest VO_{2peak} values (~1200 ml/min 0.32%) showed no difference in arm cutaneous vascular conductance from preexercise to postexercise compared to women ($P = 0.634$). Men with a middle range VO_{2peak} value (~3470 ml/min 1.4 %) had higher arm cutaneous vascular conductance from preexercise to postexercise compared to women ($P = 0.004$). Men with the highest VO_{2peak} value (~5500 ml/min 2.9 %) had higher arm cutaneous vascular conductance from preexercise to postexercise compared to women ($P = 0.000$). Substitution of workload 60% for status did not improve the model.

Leg Cutaneous Vascular Conductance

Figure 14C shows an absolute change in leg cutaneous vascular conductance (scaled as a percentage of maximal cutaneous vascular conductance, or CVCmax) from preexercise through 120 min postexercise. Conventional analysis showed that averaged across all groups, leg cutaneous vascular conductance was higher throughout postexercise compared to preexercise (3.8, 4.0, and 2.6 % ($P = 0.000$; $P = 0.000$; $P = 0.013$). The conventional model showed a sex*time interaction ($P = 0.086$) that was of interest for leg

cutaneous vascular conductance as men tended to have higher leg cutaneous vascular conductance compared to women. Substitution of either VO_2 60% or workload 60% for status did not improve the model.

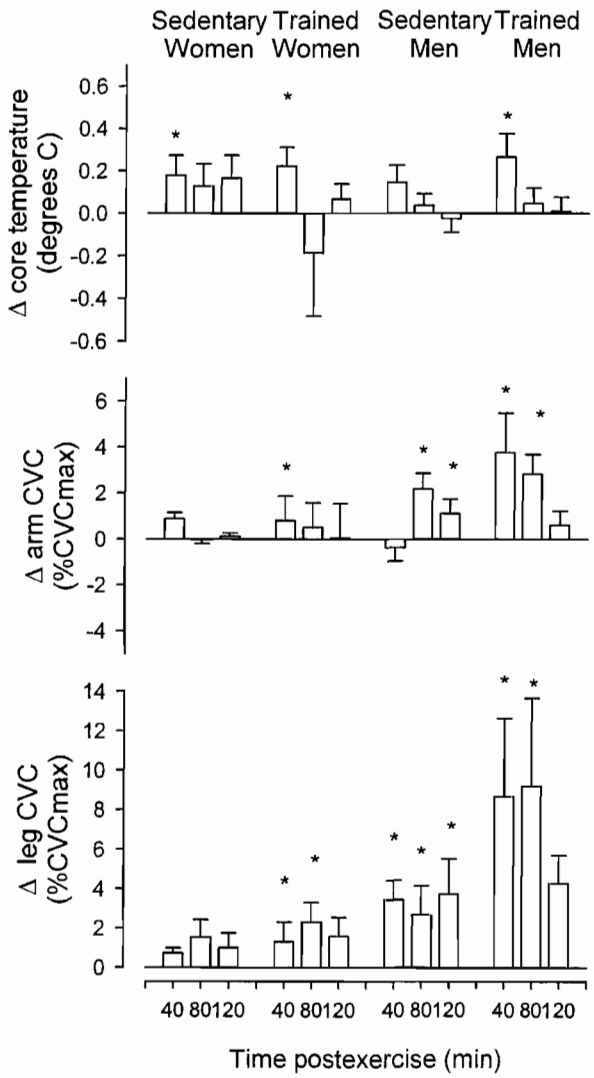

Figure 14. Postexercise Core Temperature and Cutaneous Vascular Conductance from Chapter VI. The absolute change in core temperature (A), arm cutaneous vascular conductance (B), and leg vascular conductance (C) from preexercise to 40, 80, and 120 min after exercise from Chapter VI. Percent changes are grouped for sedentary women, trained women, sedentary men and trained men. * $P < 0.05$ vs. preexercise baseline. Values are presented as means ± SEM.

Plasma Volume

Figure 15A shows an absolute change in plasma volume from preexercise to end of exercise, through 120 min postexercise. Conventional analysis showed that averaged across all groups, plasma volume was reduced at the end of exercise compared to preexercise (-18.1 %) ($P < 0.001$), but was not different at 40, 80, and 120 min postexercise and at the end of the study compared to preexercise (-0.7, 1.0, -0.8, and -1.2 %) ($P = 0.475$; $P = 0.318$; $P = 0.434$; $P = 0.221$). In addition, plasma volume was not different preexercise compared to postexercise between men and women (-4.9 and -3.0 %) ($P = 0.238$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Blood Volume

Figure 15B shows an absolute change in blood volume from preexercise to end of exercise, through 120 min postexercise. Conventional analysis showed that averaged across all groups, blood volume was reduced at the end of exercise compared to preexercise (-11.9 %) ($P < 0.001$), but was not different at 40, 80, and 120 min postexercise and at the end of the study compared to preexercise (-0.4, 0.1, -1.1, and -0.3 %) ($P = 0.574$; $P = 0.858$; $P = 0.104$; $P = 0.714$). In addition, blood volume was not different preexercise compared to postexercise between men and women (-2.6 and -2.8 %) ($P = 0.811$). Substitution of either VO_2 60% or workload 60% for status did not improve the model.

Figure 16 compares the relationship between postexercise cardiac output responses plotted as function of left ventricular end-diastolic diameter, central venous pressure, and core temperature at 40 min postexercise across all subjects. The relationship between cardiac output and left ventricular end-diastolic diameter (see Fig. 16A) was weakly correlated ($r^2 = 0.02$; $P > 0.05$). The relationship between cardiac output and central venous pressure (see Fig. 16B) was weakly correlated ($r^2 = 0.11$; $P > 0.05$). The relationship between cardiac output and core temperature (see Fig. 16C) was weakly correlated ($r^2 = 0.03$; $P > 0.05$).

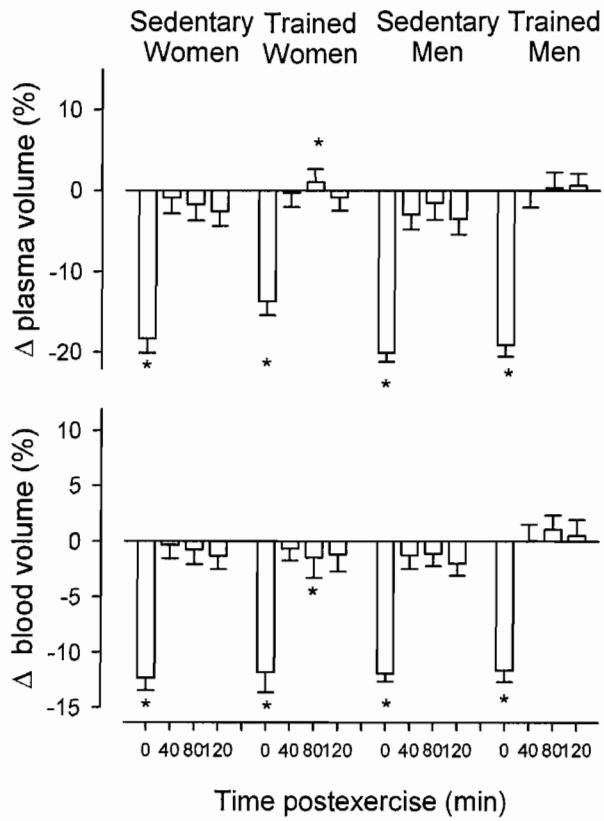

Figure 15. Postexercise Change in Vascular Volumes. The percent change in absolute plasma volume (A) and absolute blood volume (B) from preexercise to end of exercise, 40, 80, 120 min postexercise from Chapter VI. Percent changes are grouped for sedentary women, trained women, sedentary men and trained men. * $P < 0.05$ vs. preexercise baseline. Values are presented as means ± SEM.

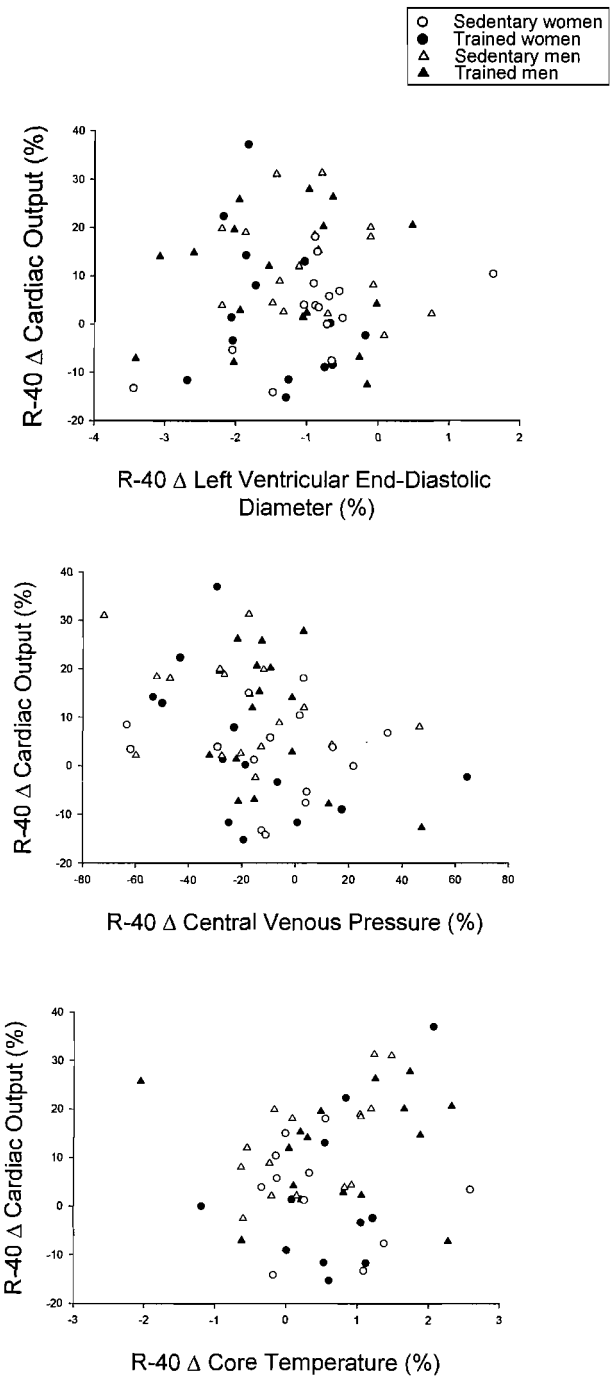

Figure 16. Postexercise Cardiac Responses from Chapter VI. The percent change in cardiac output (40 min postexercise) as a function of percent change in left ventricular end-diastolic diameter (40 min postexercise) (A), percent change in central venous pressure (40 min postexercise) (B), and percent change in core temperature (40 min postexercise) (C).

Discussion

Senitko *et al.* (2002) indicated that a sex by training status interaction could affect the postexercise hemodynamics as endurance-trained men achieved a fall in pressure through altered cardiac hemodynamics postexercise in contrast to trained women and sedentary men and women. The trained men in the Senitko *et al.* (2002) study showed no change in systemic vascular conductance and cardiac output was decreased following exercise an observation which was later confirmed (Dujic *et al.*, 2006). In the present study, workload and oxygen uptake were used as a surrogate for the training effect to potentially explain the variation in each hemodynamic variable across time (postexercise) between groups. We tested whether status, 60% VO_{2peak}, and workload at 60% could explain the variance in our basic model with a stepwise procedure. In the present study, we saw no interaction of training status (or covariates related to training) on postexercise hypotension (e.g. cardiac output, stroke volume, systemic vascular conductance, central venous pressure, and left ventricular end-diastolic volume). We hypothesized that there would be variation in the postexercise hemodynamics across groups, but that the magnitude of postexercise hypotension would remain the same. We also evaluated the hypothesis that differences in postexercise cardiac output response in trained men would be correlated to plasma volume, core temperature, central venous pressure, and left ventricular end-diastolic diameter; however, we saw a weak correlation between these factors (see Fig. 16). Additionally, in contrast to previous findings, trained men in the present study did not demonstrate a fall in cardiac hemodynamics during exercise recovery.

Postexercise Hemodynamics in Sedentary Healthy Subjects

In the general populations (sedentary and normally active), postexercise hypotension is characterized by a sustained increase in systemic vascular conductance that is not completely offset by ongoing increases in cardiac output (Halliwill *et al.*, 1996a; Halliwill *et al.*, 1996b; Halliwill, 2001). After exercise, the reduction in arterial pressure is sustained due to a rise in systemic vascular conductance (Halliwill, 2001) and this causes a rise in blood flow through the vasodilated regions. The combination of an

increase in venous pooling (inactive muscle pump) and loss of plasma volume (from sweating during exercise), reduce cardiac preload. However, stroke volume is maintained due to reduction in afterload and an increase in cardiac contractility, and the result is in an elevation in cardiac output (increased heart rate) and vascular conductance following exercise. This was the model that was used to describe the hemodynamic response in the general population but variations are known to exist (see review (Halliwill, 2001)). We previously reported that this model may be an oversimplification for the hemodynamic response in trained subjects in light of two recent findings suggesting that trained men mediate postexercise hypotension through different hemodynamic balance (Senitko *et al.*, 2002; Dujic *et al.*, 2006). We recently showed that fluid replacement partially mitigated the fall in postexercise cardiac output in trained men (Chapter V); however, in this study, despite standardizing hydration, cardiac output was not elevated following exercise. In order to elucidate mechanisms involved in previously observed postexercise responses (Senitko *et al.*, 2002; Dujic *et al.*, 2006) (Chapter V), our current protocol included methods to measure central and peripheral hemodynamics of trained and sedentary individuals. Surprisingly, we observed no sex and training interaction for cardiac output and systemic vascular conductance postexercise in the present study.

The Effect of Sex on Postexercise Hypotension and Hemodynamics

Postexercise hypotension has been found in men and women. Studies specifically addressing the role of sex on postexercise hypotension have shown reductions in arterial pressure to be of similar magnitude between men and women (Senitko *et al.*, 2002; Deschenes *et al.*, 2006; Journeay *et al.*, 2007); whereas, women were found to have a more pronounced decrease in mean arterial pressure following exercise compared to men (Fisher *et al.*, 1999; Kenny & Jay, 2007). Carter *et al.* (2001) found that women, without consideration of the menstrual cycle, had greater decreases in mean arterial pressure than men following 3 min of moderate cycling; however, we recently observed that postexercise hypotension was largely unaffected by sex when women were tested during

three separate phases of the menstrual cycle and compared to a group of men over a 90 min postexercise supine recovery period (Chapter IV) (Lynn *et al.*, 2007). The difference in experimental design may explain the conflicting results between our study (Chapter IV) (Lynn *et al.*, 2007) and the study by Carter *et al.* (2001). In the current study, we observed a sex by training status interaction as the sedentary men showed a greater pressure reduction compared to trained men and sedentary women showed no reduction in mean arterial pressure following exercise compared with the other groups. The lack of reduction in mean arterial pressure in the sedentary women is perplexing and will be discussed in an upcoming section. However, in the present study we observed no difference in postexercise hypotension between trained men and women as both showed a reduction in arterial pressure following exercise which is consistent with past data (Senitko *et al.*, 2002; Deschenes *et al.*, 2006).

Interestingly, the hemodynamic changes (percent change from preexercise) that underlie postexercise hypotension were (in most cases) elevated in men compared to women. Men had higher heart rates, cardiac output, systemic vascular conductance, femoral blood flow, femoral vascular conductance, and cutaneous vascular conductance compared to women postexercise. The reasons for these differences are unknown; however, Senitko *et al.* (2002) showed a trend for higher postexercise heart rates in men compared to women, which is consistent with results from the present study.

Proposed Mechanisms for Altered Cardiac Output in Endurance-Trained Men

Chronic endurance-training is known to alter vascular conductance (Snell *et al.*, 1987) and vascular function (Martin *et al.*, 1991; Schmidt-Trucksass *et al.*, 2000), arterial pressure regulation (Seals & Chase, 1989; Raven & Pawelczyk, 1993), and induce cardiac hypertrophy (Fagard, 2003; Sharma, 2003). The following mechanisms have been suggested to explain the altered postexercise cardiac hemodynamics in trained men (Senitko *et al.*, 2002): first, a greater loss of plasma volume (i.e., reduction in preload from increase sweating), second, shunting of blood flow to more compliant vascular beds (i.e., skin), third, enhanced baroreflex-mediated changes in sympathetic vasoconstrictor

outflow that opposes peripheral vasodilation, and finally, greater sensitivity to reductions in cardiac preload (Frank-Starling pressure volume relationship). The current study was designed to explore the central and peripheral mechanisms in trained and sedentary subjects to determine the mechanisms involved in the variation in stroke volume postexercise.

Our previous results (Chapter V) showed that inadequate hydration (i.e., greater loss of plasma volume) could account for some the previously reported altered postexercise cardiac hemodynamics in the trained men (Senitko *et al.*, 2002; Dujic *et al.*, 2006). It has previously been reported that central venous pressure falls ~2 mmHg postexercise in normotensive subjects (Halliwill *et al.*, 2000; Halliwill, 2001). In the current study, there was no status interaction for postexercise plasma volume and central venous pressure. We did not find a decrease in cardiac output in the trained men, but it is worth noting that trained men had the smallest postexercise reductions in central venous pressure (-0.7 mmHg) compared to other groups.

The reduction in preload (i.e., central venous pressure) could be due to a redistribution of cardiac output from less compliant to more compliant vascular beds, such as the skin (Rowell, 1986), in the face of elevated core temperature and increased thermal load in this trained population. In other words, the augmented cutaneous vasodilation during recovery from exercise could result in reductions in stroke volume and cardiac output due to a greater amount of blood pooling in the compliant cutaneous vasculature and this would ultimately manifest as a decrease in central venous pressure. Franklin *et al.* (1993) concluded that the magnitude of postexercise hypotension was dependent on elevations in skin and core temperature postexercise. From this data, it seems that thermoregulatory mechanisms could potentially play a role in postexercise hypotension. However, Wilkins *et al.* (2004) showed that postexercise mean arterial pressure was reduced when cutaneous blood flow returned to preexercise values. Thus, it appears that the contribution of the cutaneous circulation cannot account for the reductions in mean arterial pressure after exercise. However, healthy normally active subjects were studied in the two previously mentioned studies (Franklin *et al.*, 1993;

Wilkins *et al.*, 2004). We recently found no difference following exercise in cutaneous vascular conductance (both forearm and thigh), leg blood flow, and core temperature between environmental conditions in trained men (Chapter V); thus, it seems unlikely that these factors could explain the fall in cardiac output in trained men. In the current study, there was no status interaction for postexercise thigh cutaneous vascular conductance, leg blood flow, plasma volume, and central venous pressure, but subjects with higher peak oxygen uptake had higher forearm cutaneous vascular conductance; however, we report no decrease in cardiac output postexercise in the trained men. Thus, the decrease in cardiac output in trained men in past studies (Senitko *et al.*, 2002; Dujic *et al.*, 2006) could be due to an exaggerated decrease in central venous pressure due to a lack of hydration.

Training is thought to alter baroreflex control of heart rate (Seals & Chase, 1989; Shi *et al.*, 1993) and alter vascular resistance in response to arterial baroreflex perturbations (Mack *et al.*, 1987). Baroreflex and vasoconstrictor responsiveness to α-adrenergic receptor stimulation has been reported to be increased (Vroman *et al.*, 1988), remain unchanged (Mack *et al.*, 1987), and be attenuated (Smith *et al.*, 1988) in the endurance-trained subject compared to the sedentary individual. Physically fit individuals may be better able to induce an effective baroreceptor reflex, decreasing the slope of skin blood flow to esophageal temperature and thus, at higher temperatures, the rate at which the dependent veins fill is reduced (Nadel *et al.*, 1979). In the present study, if cardiopulmonary and arterial baroreflexes were altered following exercise in endurance-trained men, there would have been observable differences in cutaneous vascular conductance and leg blood flow; however, this was not the case and these assumptions are based on the ability to assess baroreflex function from altered vascular tone. However, because baroreflex sensitivity was not measured in this study, nor were altered central hemodynamics observed, we can only speculate on the importance of baroreflex function in the trained subjects as an explanation for the previously altered postexercise hemodynamics. To compensate for the fall in cardiac output, it is plausible that trained men that show decreased cardiac output postexercise have increased

sympathetic vasoconstrictor outflow during recovery from exercise that would oppose peripheral vasodilation. Endurance exercise-trained men show no change in systemic vascular conductance; however, they demonstrate skeletal muscle vasodilation postexercise (McCord & Halliwill, 2006) and this vasodilation is consistent with results from the present study as femoral blood flow and femoral vascular conductance was elevated postexercise across all populations. These data suggest that there may be vasoconstriction in other areas; specifically, vasoconstriction in the splanchnic or renal vascular beds may offset the vasodilation in the skeletal muscle. Pricher et al. (Pricher *et al.*, 2004) found that there was no change in postexercise renal and splanchnic blood flow in a group of normally active subjects. It is possible that these vascular beds undergo baroreflex-mediated sympathetic vasoconstriction in response to leg vasodilation; however, splanchnic and renal blood flow was not measured in the current study, nor did we find a difference in systemic vascular conductance in any of the groups following exercise, in fact, men had elevated postexercise systemic vascular conductance compared to women. This study shows an increase in cardiac output and systemic vascular conductance postexercise in the trained men. Past studies (Senitko *et al.*, 2002; Dujic *et al.*, 2006) have shown that trained men have unchanged systemic vascular conductance following exercise and this demonstrates in both cases that the baroreflex is functioning and controlling arterial pressure at a lower level after exercise.

It is possible that the same fall in preload produces a greater fall in stroke volume due to cardiac hypertrophy present in trained men (Graettinger, 1984; Cohen & Segal, 1985; Roy *et al.*, 1988; Fagard, 2003; Sharma, 2003) (Frank-Starling cardiac pressure-volume curve). Levine *et al.* (1991) proposed orthostatic intolerance was due to structural and mechanical adaptations of the heart due to chronic training and showed that endurance exercise-trained men had increased ventricular compliance in response to lower body negative pressure. Left ventricular performance has been assessed during recovery from exercise in healthy and moderately trained subjects (Douglas *et al.*, 1987; Seals *et al.*, 1988), (Stein *et al.*, 1978; Plotnick *et al.*, 1986; Goodman *et al.*, 2001). Decreases in left ventricular function, diameter/dimension, and end-diastolic volume

have been reported postexercise and in response to prolonged exercise (Stein *et al.*, 1978; Plotnick *et al.*, 1986; Douglas *et al.*, 1987; Seals *et al.*, 1988). The changes in left ventricular end-diastolic diameter may partially explain the fall in cardiac output postexercise in previously studies (Senitko *et al.*, 2002; Dujic *et al.*, 2006) (Chapter V). In the current study, we measured left ventricular end-diastolic diameter as a surrogate of ventricular compliance and disensibility in attempt to potentially explain the fall in preload. Our data shows that men had greater decreases in left ventricular end-diastolic diameter postexercise compared to women; however, we report no sex and status interaction on left ventricular end-diastolic diameter and subsequently, no fall in cardiac output postexercise in the trained subjects. It should be noted that the large decreases in left ventricular end-diastolic in trained men occur in the presence of the smallest decreases in central venous pressure across subjects. This observation is consistent with past results indicating that trained men have more compliant hearts (Levine *et al.*, 1991).

Thermal Strain in Athletes

We originally hypothesized that cardiac output responses would be correlated to core temperature. It is known that hyperthermia causes elevated sympathetic activity and parasympathetic withdraw. The increased temperature has a direct effect on the sinoatrial node (Johnson & Proppe, 1996), myocardial contractility, and increased adrenergic sensitivity (Dimsdale *et al.*, 1984) which are thought to be responsible for the overall elevation in cardiac output during heat-stress (Kilgour *et al.*, 1993).

Kilgour *et al.* (1993) showed recovery heart rate to be highly dependent upon the thermal state or core temperature of the individual. It has been suggested that the amount of total heat produced is a function of the absolute metabolic rate thus, the highly trained athlete must be producing more heat (MacDougall *et al.*, 1974) compared to the sedentary individual. However, it is believed that athletes have better heat dissipating ability (Astrand *et al.*, 1960; Saltin & Hermansen, 1966; MacDougall *et al.*, 1974) than sedentary subjects. If heat loss mechanisms are not effective at removing heat (i.e., due

to elevated metabolic heat production and impaired removal) then core temperature will continue to rise (Reilly *et al.*, 2006).

In the present study, the increased cardiac responses in the trained men may be a function of higher absolute work intensity (~221 W) for the 60 min exercise bout compared to the other groups and past research on trained subjects (~153 W) (Senitko *et al.*, 2002). As evidence for increased thermal strain, the subjects in the present study had higher skin blood flow, core temperature (~0.2 °C), and recovery heart rate (~12 beats/min) at the same postexercise time point compared to subjects from Chapter V (thermoneutral and fluid replacement condition). McCord & Halliwill (2006) showed that trained men had higher brachial vascular conductance compared to trained women and sedentary men and women as further evidence for thermal dynamic load. In addition, the trained men in the current study had postexercise core temperature, skin blood flow, and heart rates (following exercise in a thermoneutral environment) that were similar to hemodynamics following exercise in a warm environment (with no fluid replacement) (Chapter V). It is unclear whether the conflicting results in the present study compared to Chapter V are due to subject differences as trained men in the present study exercised at higher absolute workload compared to trained men from Chapter V (221 vs. 205 W) and subjects in the current study weighed more compared to subjects from Chapter V (76.9 vs. 73.5 kg). Perhaps the differences were due to laboratory condition [as subjects exercised in an environmental chamber in Chapter V and in the present study, subjects exercised in a laboratory (both kept at a constant thermoneutral (~20-22 °C))]. The total body water loss for the same given exercise intensity and temperature are less in the environmental chamber in comparison. However past research that has shown a sex and training status interaction on postexercise decreases in cardiac output did not use an environmental chamber (Senitko *et al.*, 2002), so it unlikely that this explains the difference.

We hypothesized that differences in postexercise cardiac output response across subjects would be correlated to plasma volume, core temperature, central venous pressure, and left ventricular end-diastolic diameter. To investigate this notion, Figure 16

shows a weak relationship between postexercise cardiac output responses and left ventricular end-diastolic diameter, central venous pressure, and core temperature at 40 min postexercise across all subjects ($r^2 = 0.02$, 0.11, and 0.03; $P > 0.05$). Although not significant, there is an interesting positive relationship between postexercise cardiac output responses and core temperature at 40 min postexercise as the subjects with the greater change in core temperature show a trend for greater increases in cardiac output (see Fig. 16C). However, in the present study, we did not measure intramuscular temperature or heat storage. Despite the likelihood that muscle temperature was slightly greater during recovery from exercise in the trained men, we did not document any difference in core body temperature between groups. This contradiction limits the interpretation of the core body temperature data as it relates to the measurement of muscle temperature.

We observed a trend for higher core temperatures in trained subjects and higher postexercise heart rates and arm cutaneous vascular conductance in those subjects with higher oxygen uptake values and in men compared to women. We previously proposed that ineffective heat loss mechanisms in the general population may explain the typically observed elevations in postexercise cardiac output. In the current study, those subjects with elevated core temperatures (trained and sedentary men) had elevated cardiac output postexercise (See Fig. 14A and 11C). Thus, thermal load may partly explain the elevation in postexercise cardiac output in the trained men and sedentary men. However, the fact that there is a trend for higher core temperature does not explain why the trained women did not show elevations in cardiac output postexercise.

Postexercise Hemodynamics in Sedentary Women

The hemodynamic responses in the sedentary women are somewhat puzzling. This group of sedentary women showed a lack of response following exercise as mean arterial pressure, systemic vascular conductance, heart rate, cardiac output, stroke volume, central venous pressure, and arm and leg cutaneous vascular conductance remained relatively unchanged following exercise. Postexercise hypotension was absent

in this group of sedentary women and this may be due to lower baseline mean arterial pressure in the sedentary women (~2 mmHg) compared to the sedentary men, trained women, and trained men; however, baseline mean arterial pressure was not different between subjects (see Table 10).

Recommendations for a Fluid Replacement Model

In the current study, despite water being given during the exercise bout (15 ml/kg) all subjects lost body weight over the course of the study. Controlling hydration (water to match sweat loss and overall total body water loss) is critical in assessing postexercise responses across populations. In light of the observations from this study and Chapter V, we suggest a model of fluid replacement for trained and sedentary subjects in order to standardize hydration. The relationship between the loss of total body water (assessed by overall pre to postexercise weight change) and training status was examined. In this fluid replacement model (see Fig. 17), we compiled data from the subjects in the current study and previously collected data from our lab (McCord *et al.*, 2006a; McCord & Halliwill, 2006) and in total 92 subjects were studied (49 men and 43 women; between the ages of 18 and 40 yr) in which 40 were endurance-trained, (>5 hr of aerobic exercise per wk) (57.3 ± 9.6 ml·kg^{-1}·min^{-1} VO_{2peak}) and 52 were sedentary (< 30 min of aerobic exercise per wk) (38.8 ± 8.4 ml·kg^{-1}·min^{-1} VO_{2peak}). Linear regression produced a final model ($r^2 = 0.534$) with workload at 60% (surrogate for training status). Workload at 60% was an excellent predictor of total body water loss ($P < 0.001$). There was no interaction between workload at 60% and status ($P = 0.8713$). The trained subjects had a slope of (531.29 ± 158.2 ml of total body water loss/Watts), while the sedentary subjects had a slope (640.41 ± 404.1 ml of total body water loss/Watts).

Therefore, we recommend using the following equations to calculate oral fluid replacement (water) for trained and sedentary subjects for 60 min of exercise at 60% of VO_{2peak} in a thermoneutral environment (22 °C):

Trained subjects (ml) = 531 + 6.21 x workload at 60%

Sedentary subjects (ml) = 640 + 5.92 x workload at 60%

Study Limitations

In the present study, (due to the large number of measurements) data was collected at 40, 80, and 120 min postexercise thus, the timing and time spread between measurements is not in line with prior postexercise hypotension studies from our lab (McCord *et al.*, 2006a; Lynn *et al.*, 2007). Heart rate, cardiac output, and mean arterial pressure were sampled at the beginning of the postexercise time interval followed by ultrasound (in the supine position), central venous pressure (right side), and finally, left ventricular end-diastolic diameter (left side). This was designed to limit subject movement. Thus, supine measurements were collected on average 5 to 10 min before central venous pressure and left ventricular end-diastolic diameter. However, the timing of the measurements was kept the same for all subjects.

We enrolled subjects with a range of body sizes in all groups to investigate issues related to metabolic heat production and fluid replacement. In retrospect, this may have contributed to the variance in postexercise hemodynamics across groups (e.g. sedentary women).

Summary

The influence of training status and sex on postexercise hemodynamics (central and peripheral mechanisms), thermoregulation, and fluid balance was examined in this study. Our results suggest that there was no sex and training interaction on recovery hemodynamics due to the overall variation in the individual hemodynamic response and, thus, we observed no decrease in cardiac output in trained men. We found that sedentary women did not display a reduction in arterial pressure following exercise but postexercise hypotension was present in the other groups and was not different between trained men and women. A fall in preload, alterations in heart function, and increased distribution of blood flow to compliant vascular beds were not observed in this study as a function of training status, as we, surprisingly, saw increased postexercise cardiac output in trained men. The elevation in postexercise cardiac output may be due, in part, to an increased thermal strain which is manifested as higher heart rates and a trend for elevated core

temperature during recovery from exercise. Our data showed that when trained men are adequately hydrated the primary hemodynamic response associated with postexercise hypotension is unchanged and that fluid replacement shifts the response upwards to reflect hemodynamics that are observed postexercise in the general population. Controlling hydration (water to match sweat loss) is critical across all subjects in assessing postexercise responses.

The results from the experiment performed in Chapter VI entitled "Variations in postexercise hemodynamics across sedentary and endurance exercise-trained men and women" suggest that there was no interaction of training status on postexercise hemodynamics (e.g. cardiac output, systemic vascular conductance, and stroke volume). A fall in preload, alterations in heart structure, and increased distribution of blood flow to cutaneous vascular beds was surprisingly not observed in the trained men in this study. There was a sex training status interaction on mean arterial pressure as sedentary women showed a lack of a reduction in mean arterial pressure; however, the reduction in postexercise arterial pressure did not differ between trained men and women. There was a trend for elevated core temperatures, higher heart rates and forearm cutaneous vascular conductance in the subjects with higher oxygen uptake values. The subjects with elevated core temperature had elevated cardiac output, thus thermal load may partially explain the elevation of postexercise cardiac output in trained and sedentary men. A fluid replacement model is suggested to standardized hydration in studies of this nature (see Chapter VII, Fig. 17).

CHAPTER VII

CONCLUSIONS

The purpose of the studies outlined in this dissertation was to determine how sex, training status, and fluid regulation influence postexercise hypotension. The role of the normal menstrual cycle, the effects of fluid replacement and exercise in a warm environment, and the hemodynamics in a trained and sedentary population were studied to determine how each of these factors affects postexercise hemodynamics. The role of fluid regulation and notably plasma volume (fluctuation with the menstrual cycle and sweat loss) was a theme in each of these studies. This chapter will provide a brief synopsis of the main findings of each study in order to understand how these results contribute to the further understanding of postexercise hypotension. This chapter is not intended to be a detailed review of the studies in the previous chapters. Rather, the purpose of this chapter is to provide an overview of the findings for the experiments from the preceding chapters. Finally, a fluid replacement model will be proposed in order to provide a method for calculating the amount of water needed for studies in this area. This chapter will conclude with questions that still need to be answered and areas for future research.

Chapter IV: Effects of the Menstrual Cycle and Sex on Postexercise Hemodynamics

Role of the Menstrual Cycle

The major new finding of this study was that despite differences in hormonal concentrations that altered plasma volume and body temperature across the menstrual cycle these factors did not affect postexercise hemodynamics (see Fig. 1). There were alterations in preexercise resting hemodynamic differences during the mid-luteal phase; however, the overall postexercise hemodynamic response was largely unaffected by endogenous hormones or factors associated with the normal menstrual cycle.

Role of Sex

The results of this experiment suggest that the pattern and magnitude of postexercise hypotension does not differ between sexes. However, there are baseline hemodynamic differences between men and women that are critical to assessing postexercise hypotension (see Table 3).

Chapter V: Fluid Replacement during Exercise alters Postexercise Cardiac Hemodynamics in Endurance Exercise-Trained Men

The Role of Fluid Replacement

The rationale for this study was based on data from two studies (Senitko *et al.*, 2002; Dujic *et al.*, 2006) who suggested that endurance exercise-trained men achieve postexercise hypotension through a different hemodynamic balance compared to other populations as this group of trained men show reduced cardiac output postexercise. We believe that inadequate hydration could explain these differences in postexercise hemodynamics.

The new major finding from this study is that fluid replacement mitigates the decrease in cardiac output in trained men. This was evidenced by higher postexercise cardiac output in the fluid replacement condition compared to the no fluid replacement condition (see Chapter V, Fig. 7). Futhermore, the data from this study suggests that

inadequate fluid replacement may account for some of the previously reported altered postexercise cardiac hemodynamics in trained men. However, even though fluid replacement corrected a portion of the cardiac hemodynamics, this trained group may have cardiac structural changes that may potentially explain the decreased cardiac output following exercise.

Based on the data from this study, we suggest that controlling hydration (matching sweat and total body water loss) is critical to assessing postexercise responses in trained and sedentary populations. Three questions remained after this study: Do endurance exercise-trained men have differences in postexercise cardiac function? Is there a sex and training status interaction on postexercise hemodynamics when sedentary and trained subjects are well hydrated? Are there individual variations in postexercise hemodynamics across populations?

The Role of Exercise in a Warm Environment

This is the first study to examine the effects of exercise in a warm environment on the recovery hemodynamics in endurance-trained men. Surprisingly, exercise in a warm environment also mitigated the fall cardiac output postexercise compared to a thermoneutral environment (see Chapter V, Fig. 7). The data suggest that the elevated postexercise cardiac output following exercise in a warm environment was associated with increased recovery heart rate, via increased contractility. This finding leaves the following questions: Do well hydrated trained men show further improvement in postexercise cardiac output if exercise is performed in a warm environment? In other words, is there an impact of thermal strain on postexercise hemodynamics in an endurance-trained population?

Chapter VI: Variations in Postexercise Hemodynamics across Sedentary and Endurance Exercise-Trained Men and Women

Endurance Exercise-Trained Population

The rationale for this study was based on previous research showing that an endurance-exercise trained population showed reductions in arterial pressure postexercise, but with an altered hemodynamic balance compared to other groups. There was a sex training status interaction on mean arterial pressure as sedentary women showed a lack of a reduction in mean arterial pressure; however, the reduction in postexercise arterial pressure did not differ between trained men and women. In addition, the sedentary women had a lack of response postexercise as most hemodynamic variables remained relatively unchanged. The analysis attempted to prove that variables related to training status could explain the variance in postexercise hemodynamic variables for each group. The novel main finding is the absence of the previously reported sex and training interaction on the postexercise hemodynamic response variables cardiac output, systemic vascular conductance, and stroke volume. These did not differ across well-hydrated trained and sedentary men and women in this study. There was no status interaction for measures related to preload (plasma volume and central venous pressure); however, the trained men showed the smallest decrease in postexercise central venous pressure compared to other groups which may explain the surprising increase in postexercise cardiac output in this group of trained subjects. In past studies, the decreased cardiac hemodynamics may be attributed to greater decreases in central venous pressure due in inadequate hydration in the trained men. There was a weak positive relationship between core temperature and cardiac output as subjects with the greater increase in core temperature showed an overall trend for higher postexercise cardiac output (status*time; $P = 0.052$). Thus, thermal load may explain some of the elevation in cardiac output in the trained men and sedentary men, but does not explain why the trained women had higher core temperature postexercise, but showed an overall decrease in cardiac output during recovery from exercise. A greater fall in preload or clear evidence of alterations in cardiac structure/function in trained men were not observed in this study as trained

men showed an increase in cardiac output postexercise. The data suggest that hydration may possibly shift the cardiac output response upward in trained men to reflect typical postexercise hemodynamics observed in the general population. Fluid replacement (water) during exercise is crucial when assessing postexercise hemodynamics.

Proposed Model of Fluid Replacement

The model of fluid replacement presented in Figure 17 is based on total body water loss. This model of fluid replacement will aid to standardize hydration across trained and sedentary subjects in studies examining postexercise hemodynamics. Data from 92 subjects (49 men and 43 women; between the ages of 18 and 40 yr) in which 40 were endurance-trained, (>5 hr of aerobic exercise per wk) (57.3 ± 9.6 $ml \cdot kg^{-1} \cdot min^{-1}$ VO_{2peak}) and 52 were sedentary (< 30 min of aerobic exercise per wk) (38.8 ± 8.4 $ml \cdot kg^{-1} \cdot min^{-1}$ VO_{2peak}) was complied. Linear regression produced a final model ($r^2 = 0.534$) with workload at 60% (surrogate for training status). Workload at 60% was an excellent predictor of total body water loss ($P < 0.001$). There was no interaction between workload at 60% and status ($P = 0.871$). The trained subjects had a slope of (531.29 ± 158.2 ml of total body water loss/Watts), while the sedentary subjects had a slope (640.41 ± 404.1 ml of total body water loss/Watts). The analysis reveals that for sedentary subjects with a 60% workload at the lower ranges (~90 W) that over the course of an hour long cycle bout at 60% of VO_{2peak}, these subjects will lose 1090 ml of water, whereas, the trained subjects at the same lower ranges will lose 1173 ml. Sedentary subjects with a 60% workload at the median ranges (~158 W) will lose 1398 ml of water, whereas, the trained subjects at the same median workload ranges will lose 1467 ml. Sedentary subjects with a 60% workload at the higher ranges (~250 W) will lose 2084 ml of water, whereas, the trained subjects at the same higher workload ranges will lose 2121 ml.

Therefore, it is recommended to use the following equations to calculate oral fluid replacement (water) for trained and sedentary subjects for 60 min of exercise at 60% of VO_{2peak} in a thermoneutral environment (22 °C):

Trained subjects (ml) = 531 + 6.21 x workload at 60%

Sedentary subjects (ml) = 640 + 5.92 x workload at 60%

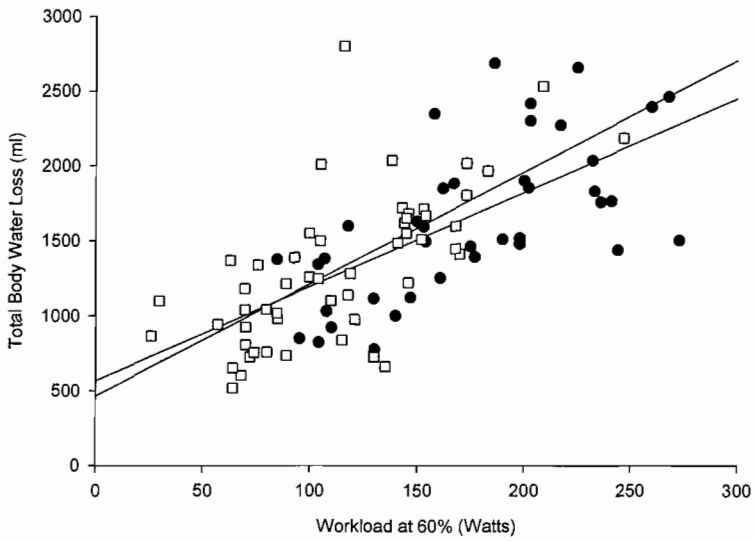

Figure 17. Total Body Water Loss as a Function of Workload. Open squares are sedentary subjects; filled circles are trained subjects.

Clinical Significance

The long-term goal of this research is to explore the link between postexercise hypotension and alterations in cardiovascular regulation following exercise, and its implications for the use of exercise as an anti-hypertensive intervention. To date, postexercise hypotension is not a well understood physiological phenomenon. The immediate goal of this research is to give insight into the clinical implications for postexercise hypotension and to further explore the mechanisms of reduction in arterial

pressure following exercise in humans. This research may ultimately contribute to the overall understanding of the physiology associated with hypertension.

Future Studies

The studies outlined in this dissertation examine the role of sex, training status, and fluid regulation on postexercise hypotension and increase the knowledge and understanding of blood pressure regulation following exercise in humans. These studies shed light on the mechanisms of postexercise hemodynamics, but unanswered questions remain:

1. We have documented the importance of fluid replacement in a group of endurance-trained men. However, the question remains, what is the impact of dehydration on postexercise hemodynamics in healthy recreationally trained and sedentary individuals? Fluid replacement mitigates the fall in cardiac hemodynamics postexercise in this endurance-trained population. A study addressing the effects of dehydration on postexercise hemodynamics in lesser fit and healthy populations needs to be completed

2. The postexercise hemodynamics (with adequate fluid replacement) under conditions of a warm environment in healthy, sedentary, and endurance-trained women has yet to be addressed. This study will shed light on the importance of exercise in a warm environment (i.e., contractility) on the postexercise recovery profile in different populations.

3. Past results show unchanged or decreased systemic vascular conductance in endurance exercise-trained men postexercise. This could indicate other vascular beds are vasoconstricting and contributing to the lack of increase in systemic vascular conductance (i.e., renal and splanchnic circulation) to maintain arterial pressure in the face of decrease cardiac output postexercise. A prior study

showed no change in splanchnic or renal circulation postexercise in healthy subjects; however, no endurance-trained individuals were tested in this study. Thus, the contribution of the splanchnic and renal circulation to postexercise hypotension needs to be addressed in endurance-trained men. This leads to the question of whether vasoconstriction is present in the inactive vascular bed (i.e., gut, kidney, and spleen) postexercise and if intestinal absorption is hindered during recovery from exercise. Although these vascular beds constrict during exercise and there seems to be no diminished effect on fluid absorption during this time. To our knowledge, the effect on postexercise fluids and nutrient absorption has not been studied in those individuals that show decreased systemic vascular conductance after exercise.

4. Fluid replacement partially corrected the fall in cardiac output postexercise in Chapter V; however, in Chapter VI, increases in cardiac output in the endurance-trained men were seen. The reason for these differences is not clear. Future rigorous studies are needed on those subjects that demonstrate decreased cardiac output postexercise in order to elucidate the mechanisms. Dehydrating trained subjects during exercise may reproducibly reduce postexercise cardiac hemodynamics in trained men and thus, may be used as a model for altering hemodynamics and studying the mechanisms related to this response.

Limitations

In the studies listed in this dissertation there are limitations in the techniques, measurements, and the interpretation of the results that need to be discussed. The limitations include:

1. In the Study 1 in Chapter IV subjects were tested during the early follicular, ovulatory, and mid-luteal phase of the menstrual cycle. Subjects were not tested during their preovulatory phase when estrogen is known to be the highest and

progesterone is absent. It is plausible that during the preovulatory phase there would be increased effects of estrogen on the peripheral vasculature and on plasma volume expansion, thus causing greater differences in postexercise hemodynamics. The ovulatory phase was chosen because it could be accurately predicted compared to the preovulatory phase.

2. In the Study 2 from Chapter V maintaining fluid levels (based on sweat loss) during the euhydration condition while also preventing over and under volumization of the subject was difficult as subjects lost ~330g from before exercise to the end of the study. The protocol could only be performed during the fall and winter months in order to control for differences in heat acclimation.

3. In Study 3 in Chapter VI, our results are limited by the utilization of the Gauer method and echocardiography instead of direct measures of central venous pressure and left ventricular compliance. The timing of the measurements was not in line with past work on postexercise hypotension.

APPENDIX A

INFORMED CONSENT FORM A

TITLE: "Mechanisms of Post-exercise Hypotension in Humans (Protocol C)"
INVESTIGATOR: Dr. J. R. Halliwill and Colleagues
APPROVED BY INSTITUTIONAL REVIEW BOARD: November 14, 2003

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this study being done?

When humans exercise, there is an increase in blood flow to the exercising muscle. This increase in blood flow lasts for several hours after exercise and is associated with a lowering of blood pressure. The lowering of blood pressure is called "post-exercise hypotension". The purpose of this study is to provide more information on what causes blood flow increases to occur and to explain why blood pressure is lowered after exercise and how this relates to hormonal fluctuations associated with the female menstrual cycle.

What will happen in the study?

Initial visit

1. You will arrive at Dr. Halliwill's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately two hours. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Prior to starting the study, you will need to have a negative pregnancy test (meaning that you are not pregnant). This will require you to collect a sample of urine and have it tested by one of the investigators. If the test is positive (meaning that you may be pregnant), you will not be allowed to participate in the study. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will be given a physical activity questionnaire, which will allow investigators to determine your activity level. The questionnaire will take approximately 15 minutes to complete. You will need to wear a t-shirt, shorts, and refrain from eating at least three hours prior to arrival. We

will also discuss when you need to swallow a temperature sensing pill so that we can accurately measure your body temperature during the study days.

2. Exercise bicycle test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor). After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the three study days. We will then teach you how to use an ovulation prediction kit so that we can determine the different phases of your menstrual cycle.

3. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, and nausea.

Study visit

1. You will then return to Dr. Halliwill's laboratory to participate in the experimental protocol on three separate days. The three days will be scheduled according to the timing of your menstrual cycle. Each of these three testing sessions will take approximately four hours. You will need to wear a t-shirt, shorts, and refrain from eating or ingesting caffeine at least three hours prior to arrival.

2. You will need to swallow the temperature pill at least 4 hours before the start of each study day, or the night before a study that starts in the early morning so that we can accurately measure your body temperature during the study days.

3. During the study visits, your heart rate will be monitored by electrocardiogram electrodes placed on your skin. Your blood pressure will be measured at periodic intervals by the inflation` of a blood pressure cuff around your arm. Periodically, you will breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people. This is used to study how much work the heart is performing. A small probe (laser-Doppler probe) will be placed over an area of skin on your forearm and another on your thigh. The laser-Doppler probe uses light to measure skin blood flow in these areas, and is taped in place. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your elbow and an artery at your groin-hip intersection. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in these arteries.

4. Venous Catheter: You will have one small flexible needle ("intravenous catheter")

placed into a vein near your elbow. The skin will be sterilized before this procedure. This catheter will remain in your skin throughout the four hour study. Several blood samples will be collected from the catheter during each of the four hour studies. In total, 5 ounces of blood (150 ml) will be withdrawn during the study visits (50 ml on each study visit). This is about half the amount withdrawn when you volunteer to "give blood" for American Red Cross or similar blood donation programs. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin.

5. Infusion of Evans Blue Dye: During the study, a dye called Evans Blue will be given through the catheter at your elbow. Evans Blue is used to measure the amount of blood in your body. Several blood samples will be taken from the venous catheter before and after the infusion of the dye to complete the analysis.

6. Bicycle exercise session: During each of the study visits, you will pedal on a bicycle at a moderate rate for 1 hour.
7. Local heating: Towards the end of each study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 103°F for a period of 40 minutes. This will feel warm but should not cause discomfort.

8. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin. You will then be sent home.

9. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the study.

How long will I be in the study?

You will be in the study for four days (initial visit and three study visits). The initial visit will last two hours. The study visits will last four hours each.

What are the risks of the study?

1. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000.

2. Intravenous catheters: There may be some discomfort during the insertion of the

small flexible needle into your vein. Once the needle is in place, the pain should subside. Blood sampling through the needle should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needle is sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein are a small bruise and pain at the site of the needle location which may last several days after removal of the catheter.

3. Evans Blue Dye: There is the possibility that you are allergic to Evans Blue and may have an allergic reaction to this dye including changes in blood pressure and difficulty breathing. There is the possibility that some of the dye will leak into the skin near the location of the intravenous catheter and stain the skin. If this happens, the skin may have a blue colored stain in that area for several weeks.

4. Blood withdrawal: In total, 150 ml will be withdrawn during the study visit. This is about half of the amount withdrawn when you volunteer to "give blood" for American Red Cross or similar blood donation programs. You will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before taking part in the graded exercise test on the initial visit. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not need to pay for any tests or procedures that are done just for this research study. You will get **$140** for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated to how much of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Exercise and Movement Science at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The University of Oregon is not able to offer financial compensation nor absorb the costs of medical treatment should you be injured as a result of participation in this research. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment that will be provided at the usual charge.
The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped. You will be told of important new findings or any changes in the study or procedures that may happen.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel
Office of the President
University of Oregon
Eugene, OR 97403

Office of Human Subjects Compliance
University of Oregon
Eugene, OR 97403
(541) 346-2510 or (541) 346-3082

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be $100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive $500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a "subject identification number". The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill's office.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Human Subjects Compliance, 5219 University of Oregon, Eugene, OR 97403, 541-346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

_____ _____

(Date) (Signature of Participant)

_____ _____

(Date) (Signature of Individual Obtaining Consent)

APPENDIX B

INFORMED CONSENT FORM B

TITLE: "Cardiac Function during Postexercise Hypotension"
INVESTIGATOR: Dr. J. R. Halliwill and Colleagues
APPROVED BY INSTITUTIONAL REVIEW BOARD: December 14, 2005

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

When humans exercise, there is an increase in blood flow to the exercising muscle. This increase in blood flow lasts for several hours after exercise and is associated with a lowering of blood pressure called "postexercise hypotension". The cause of this postexercise hypotension seems to differ across different groups of people, as some people have postexercise hypotension because of the increase in blood flow to the exercising muscle but some people also have postexercise hypotension because of changes in heart function. In this research study, we are trying to understand the changes in heart function that occur in some people during recovery from exercise. The changes in heart function may be due to an increased amount of sweating during exercise in those individuals. It may also be due to a rise in blood flow to the skin or other areas. Or it may be due to differences in how the heart responds to exercise in very fit people. Some of these factors may also be related to body composition and how much physical exercise an individual can perform. The purpose of this study is to provide more information on how factors such as fitness level, body composition, sweat loss, and heart function affect blood pressure during recovery from exercise in groups of people with varying levels of fitness and body size.

What will happen in the study?

<u>Initial Visit</u>

1. You will arrive at Dr. Halliwill's laboratory in room 166 in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately three hours. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. At this time you will be asked to take a pregnancy test (females only). You will be given a physical activity questionnaire, which will allow investigators to determine your activity level. The questionnaire will take approximately 15 minutes to complete. You will need to wear a t-shirt, shorts, running shoes and refrain from eating at least three hours prior to arrival. Before leaving Dr. Halliwill's laboratory on the screening day, we will discuss with you how and when to take a temperature sensing pill. This pill enables the accurate measurement of core body temperature during the study day. At this time you at reminded not to donate blood or to participate in other research studies where they will be drawing blood for 8 weeks.

2. Body Composition Scan: One of the investigators will take you to the Bowerman Building where the amount of fat and muscle in your body will be measured using a DEXA PRODIGY scanner. The DEXA scanner is a specialized x-ray machine that is used for this purpose. You will lie on a table while a scan is performed. The scan takes about 6 minutes.

3. Graded Exercise Test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor) (if you are a female subject this will be attached to your body by a female lab personal). After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study day.

You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, and nausea.

Study Visit

1. You will then return to Dr. Halliwill's laboratory to participate in the study visit, which will be between 7 and 10 days after the initial visit. This testing session will take approximately five hours each. You will need to wear a t-shirt, shorts, and refrain from eating at least three hours prior to arrival. At this time you will be asked to take a second pregnancy test (females only).

2. During the study visits, your heart rate will be monitored by electrocardiogram electrodes placed on your skin (if you are a female subject this will be attached to your body by a female lab personal). Your blood pressure will be measured at periodic intervals by the inflation` of a blood pressure cuff around your arm. Periodically, you will breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people. This is used to study how much work the heart is performing. A small probe (laser-Doppler probe) will be placed over an area of skin on your forearm and another on your thigh. The laser-Doppler probe uses light to measure skin blood flow in these areas, and is taped in place. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your elbow and an artery at your groin-hip intersection and also over your heart. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in these arteries and also to determine to size of your heart (left ventricle diameter). Your core temperature will be measured with a core temperature pill that you swallowed the night before the study. Core Temp pill is the size of a multivitamin and this pill will harmlessly pass through your system within 2 days.

3. Bicycle Exercise Session: During the study visit, you will pedal on a bicycle at a moderate rate for 1 hour.

4. Venous Catheter: You will have one small flexible needle ("intravenous catheter") placed into a vein near your elbow. The skin will be sterilized before this procedure. This catheter will remain in your skin throughout the five hour study. The catheter will be attached to a pressure transducer in order to measure the pressure of blood in the veins in your arm. At times, you will be asked to lie on your side with your arm hanging down to assist with this measurement.

5. Blood Sampling: Several blood samples will be collected from the catheter during the five hour study. In total, 50 ml will be withdrawn. This is about a quarter of the amount withdrawn when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin.

6. Local Heating: Towards the end of the study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 103°F for a period of 40 minutes. This will feel warm but should not cause discomfort.

7. Blood Volume Measurement: The amount of blood in your body will be measured at the end of the study visit with a carbon monoxide uptake test. For this test, you will breathe on a scuba mouthpiece for about 20 minutes while wearing a nose clip. Through the mouthpiece, you will be breathing mostly oxygen with a small amount of carbon monoxide added to it. Carbon monoxide is a colorless, odorless gas and is used to measure the amount of blood in your body.

8. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin. You will then be sent home.

You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the study.

How long will I be in the study?

You will be in the study for two days (initial visit and one study visit). The initial visit will last three hours. The study visit will last five hours. You will need to refrain from eating for three hours prior to each visit.

What are the risks of the study?

1. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a heart attack, the laboratory is equipped with an Automatic Electronic Defibrillator that is located in the same room where the study is taking place. Specifically, this is located in the cupboard above the telephone in the laboratory of room 166 in Esslinger Hall. Dr. Halliwill has up to date Advanced Cardiac Life Support (ACLS) training. In the event of an emergency, the Department of Public Safety (6-6666) will be called in order to activate the emergency medical system (i.e., 911).

2. Body Composition Scan: This research study involves exposure to radiation from a DEXA PRODIGY whole body scan. This radiation exposure is not necessary for your medical care and is for research purposes only. The total amount of radiation that you will receive in this study is equivalent to a uniform whole body exposure of 1 day of exposure to natural background radiation. The scan that you will receive is one twentieth of the radiation that you will receive from a chest X-

ray or one twenty-fifth of the radiation you would receive from a Dental X-ray. This use involves minimal risk and is necessary to obtain the research information desired. However, women who are currently pregnant cannot participate.

3. Intravenous catheters: There may be some discomfort during the insertion of the small flexible needle into your vein. Once the needle is in place, the pain should subside. Blood sampling through the needle should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needle is sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein are a small bruise and pain at the site of the needle location which may last several days after removal of the catheter.

4. Blood withdrawal: In total, 50 ml will be withdrawn during the study visit. Typically if you were to donate blood for Lane Memorial Blood Bank, 450 to 500 ml of blood is drawn, therefore this is one ninth or one tenth of the amount when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. You will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.

5. Blood Volume Measurement: This research involves exposure to a small amount of carbon monoxide. Carbon monoxide is a colorless, odorless gas. When humans are exposed to large amounts of carbon monoxide, carbon monoxide can cause symptoms that include headache, fatigue, shortness of breath, nausea, cherry-red colored lips, dizziness, and death. The amount of carbon monoxide you will be exposed to is less than the amount that normally causes symptoms. During the test we will measure your blood levels of carbon monoxide to make sure your body's carbon monoxide level is below the amount that normally causes symptoms. If your carbon monoxide level is too high or if you have any of the symptoms associated with high carbon monoxide levels, we will treat you with oxygen until the levels return to normal and the symptoms go away. The amount of carbon monoxide you will be exposed to will affect blood levels similarly to being in a tobacco, smoke-filled room, driving in a tunnel or parking structure, or the pollution in a big city such as Los Angeles.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Subjects will be asked to take a total of two pregnancy tests, this will occur on both the initial screening and also on the study visit. Women who can still become

pregnant must have a negative pregnancy test no more than 24 hours before taking part in the protocol orientation trial on the initial visit. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better. Blood and/or urine samples are not being collected for diagnostic purposes. The DEXA scan, electrocardiogram and blood testing are not being conducted for diagnostic purposes. The results will not be reviewed by a physician. However, if results fall outside of the normal range, you will be informed that you should consult your primary care physician for additional medical evaluation.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not pay for any tests or procedures that are done just for this research study. You will get $80 for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated at a rate of $10 per hour of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The University of Oregon is not able to offer financial compensation or absorb the costs of medical treatment should you be injured as a result of participation in this research. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment that will be provided at the usual charge.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped. You will be told of important new findings or any changes in the study or procedures that may happen.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel
Office of the President
University of Oregon
Eugene, OR 97403

Office of Human Subjects Compliance
University of Oregon
Eugene, OR 97403
(541) 346-2510 or (541) 346-3082

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be $100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive $500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a "subject identification number". The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill's office. The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood samples will be destroyed when study results are published or 24 months after your participation, whichever comes first. Data will be kept in our files indefinitely.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Human Subjects Compliance, 5219 University of Oregon, Eugene, OR 97403, 541/346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

_____ _____

(Date) (Signature of Participant)

_____ _____

(Date) (Signature of Individual Obtaining Consent)

APPENDIX C

INFORMED CONSENT FORM C

TITLE: "Cardiac Function during Postexercise Hypotension"
INVESTIGATOR: Dr. J. R. Halliwill and Colleagues
APPROVED BY INSTITUTIONAL REVIEW BOARD: January 16, 2008

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

When humans exercise, there is an increase in blood flow to the exercising muscle. This increase in blood flow lasts for several hours after exercise and is associated with a lowering of blood pressure. The lowering of blood pressure is called "post-exercise hypotension" and is thought to occur in all individuals regardless of fitness training levels. We do not understand all the mechanisms that cause this increase in blood flow or lowering of blood pressure. Historically, the postexercise hypotension in endurance exercise-trained men seems to be different compared to untrained individuals and is thought to be caused by higher sweat rates and alteration in heart function. The exact reason for these changes is in endurance-trained men is unknown and thus, the purpose of this study is to provide more information on the effects of dehydration on postexercise changes in blood pressure. This study will examine what happens in a group of endurance-trained men to determine what role loss of fluids play in blood pressure regulation. We anticipate that 20 men will participate in this research study.

What will happen in the study?

Initial visit

1. You will arrive at Dr. Halliwill's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately one hour. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will be given a physical activity questionnaire, which will allow investigators to determine your activity level. The questionnaire will take

approximately 15 minutes to complete. You will need to wear a t-shirt and refrain from eating for two hours prior to arrival. In addition, you will need to refrain from consuming caffeine (for example, coffee, tea, energy drinks like Red Bull, or soda like Coke, which contains caffeine) or medications for 12 hours prior to the study and abstain from alcohol or exercise for 24 hours prior to the study.

2. Graded Exercise Test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor). After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes on your chest, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study visits. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study visits.

You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, chest pain, chest tightness and nausea.

This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study day. At this time you will be reminded not to donate blood or to participate in other research studies where they will be drawing blood for 8 weeks. Before leaving Dr. Halliwill's laboratory on the screening day, we will discuss with you how and when to take a temperature sensing pill. This pill enables the accurate measurement of core body temperature during the study days. This core temperature pill will be safely passed by your GI tract and does not need to be recovered. You will also be instructed on the amount of water to consume 12 hours prior to the study days and ask to record a diet log in the 12 hours prior to each study visit in order for us to monitor your fluid and food intake.

Study Visit

1. You will then return to Dr. Halliwill's laboratory to participate in the study visit, which will be between 7 and 10 days after the initial visit. Each study visit will be separated from the prior visit by 3 to 7 days. This testing session will take

approximately four hours each. You will need to wear a t-shirt, shorts, and refrain from eating at least two hours prior to arrival.

2. When you arrive, you will be asked to collect a sample of urine in the restroom near the physiology lab so the researchers can verify that you are well-hydrated. If you are not well-hydrated, they will have you drink 500 ml of water.
3. Next, you will be asked to undress to your underwear behind a privacy screen and so that your weight can be measured.
4. During the study visits, your heart rate will be monitored by electrocardiogram electrodes placed on your skin. Your blood pressure will be measured at periodic intervals by the inflation of a blood pressure cuff around your arm. Periodically, you will breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people. This is used to study how much work the heart is performing. A small probe (laser-Doppler probe) will be placed over an area of skin on your forearm and another on your thigh. The laser-Doppler probe uses light to measure skin blood flow in these areas, and is taped in place. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your elbow and an artery at your groin-hip intersection. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in these arteries. Your core temperature will be measured with a core temperature pill that you swallowed the night before the study. Core Temp pill is the size of a multivitamin and this pill will harmlessly pass through your system within 2 days. These measurements will all be taken 45 minutes before exercise and for 2 hours after exercise. During the times when measurements are not being taken you will rest on a bed.
5. Exercise Session: During the study visit, you will pedal on a stationary bike at a moderate rate for 60 minutes.
6. Hot environment: On one of the three study days there will be a 60 minute heat exposure which will consist of a 60 minute exercise session and will take place in a purpose built environmental chamber that will allow us to keep the room at 86°F (30°C). The relative humidity will be kept at 30%, which is considered to be a dry environment similar to a hot summer day in Eugene.
7. Fluid intake: On one of the three study days approximately one liter or 32 ounces of water will be provide for you to drink during the exercise period. During exercise on two of the study days no fluid will be given and this will cause a loss in body fluids that is known as dehydration.
8. At the end of the 60 minutes of exercise, you will then step on a scale so your body weight can be obtained.
9. Venous Catheter: You will have one small flexible needle ("intravenous catheter") placed into a vein near your elbow. The skin will be sterilized before this procedure. This catheter will remain in your skin throughout the four hour

study. The catheter will be attached to a pressure transducer in order to measure the pressure of blood in the veins in your arm. At times, you will be asked to lie on your side with your arm hanging down to assist with this measurement.

10. Blood Sampling: Several blood samples will be collected from the catheter during the four hour study. In total, 6 ml will be withdrawn. This is a little more than 1% of the amount withdrawn when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin. You will then be sent home.
11. Local Heating: Towards the end of the study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 103°F for a period of 40 minutes. This will feel warm but should not cause discomfort.

You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit or the study visit. Some examples of discomfort include shortness of breath, light-headedness, chest pain, chest tightness and nausea.

How long will I be in the study?

You will be in the study for four days (initial visit and three study visits). The initial visit will last two hours. The study visits will last four hours. The total time commitment is fourteen hours. You will need to refrain from eating for two hours prior to each visit.

What are the risks of the study?

1. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a heart attack, the laboratory is equipped with an Automatic Electronic Defibrillator that is located in the same room where the study is taking place. Specifically, this is located in the cupboard above the telephone in the laboratory of room 166 in Esslinger Hall. Dr. Halliwill has up to date Advanced Cardiac Life Support (ACLS) training. In the event of an emergency, the Department of Public Safety (6-6666) will be called in order to activate the emergency medical system (i.e., 911).

2. Intravenous catheters: There may be some discomfort during the insertion of the small flexible needle into your vein. Once the needle is in place, the pain should subside. Blood sampling through the needle should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needle is sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein are a small bruise and pain at the site of the needle location which may last several days after removal of the catheter.

3. Blood withdrawal: In total, 6 ml will be withdrawn during the study visit. Typically if you were to donate blood for Lane Memorial Blood Bank, 450 to 500 ml of blood is drawn, therefore this is a little more than 1% of the amount when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. You will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.

4. Heat Exposure: This study requires a 60 minute heat exposure at 86°F (30°C) while exercising on one of the three study days. You will be continuously monitored throughout the entire exercise protocol. The heat exposure can be physically demanding and can cause light-headedness, low blood pressure, fatigue, nausea and cramps. Therefore, you should keep the investigator informed of your feelings and you should not attempt to prolong the heat exposure if you do not feel well. You are free to stop at any time and the room can be immediately cooled down and/or you can be removed from the room. This environmental condition is similar to a exercising on a hot summer day in Eugene.

5. Dehydration: Fluid levels will be maintained on one of the three study days. During two of the study days no fluids will be given. This is meant to cause a minimal loss in body fluids and a decrease in overall body weight of 1 to 3%. This is considered to be mild to moderate dehydration and body weight losses up to 7% are considered safe. You will be weighed and monitored throughout the study as weights and urine specimens will be taken at the beginning, before exercise, after the exercise bout and at the end of the study. If your body weight loss is greater than 4% or if you feel any adverse symptoms such as dizzy, nausea, fatigue or faint, the exercise session will be stopped. Typically, moderate exercise for approximately one hour cause a loss in body weight of about 2% due to sweating. Upon leaving the lab at the end of the study day you will be instructed to drink fluids to replace what was lost during the study.

Are there benefits to taking part in this study?

This study will not make your health better. Blood and/or urine samples are not being collected for diagnostic purposes. The electrocardiogram and blood testing are not being conducted for diagnostic purposes. The results will not be reviewed by a physician. However, if results fall outside of the normal range, you will be informed that you should consult your primary care physician for additional medical evaluation.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not pay for any tests or procedures that are done just for this research study. You will get $140 for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated at a rate of $10 per hour of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel
Office of the President
University of Oregon
Eugene, OR 97403
(541) 346-3082

Office of Human Subjects Compliance
University of Oregon
Eugene, OR 97403
(541) 346-2510

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be $100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive $500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a "subject identification number". The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill's office. The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood samples will be destroyed when study results are published or 24 months after your participation, whichever comes first. Data will be kept in our files indefinitely.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Office for Protection of Human Subjects, 5237 University of Oregon, Eugene, OR 97403, (541) 346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

_____ _____

(Date) (Signature of Participant)

_____ _____

(Date) (Signature of Individual Obtaining Consent)

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