THE INFLUENCE OF ALTERATIONS IN PLASMA VOLUME ON PHYSIOLOGICAL RESPONSES TO DYNAMIC EXERCISE

by

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Abstract:

This thesis investigated the hypothesis that the level of resting plasma volume (PV) is an important determinant of the responses that occur in the cardiovascular, metabolic and substrate, thermoregulatory and endocrine systems during prolonged exercise. Two strategies were employed to alter resting PV, namely diuretics, which produced a reduction in resting PV, and Pentispan or Dextran, which produced an increase in PV.

In the plasma volume expansion study (PVX), eight healthy untrained males performed 90 min of cycle ergometry at 62% of peak aerobic power (VO_2peak) twice, prior to (CON) and following acute plasma volume expansion (PVX). Oxygen uptake (VO_2) was similar between the two conditions. The PVX condition resulted in a calculated 15.8% increase in resting PV ($P<0.05$). During PVX, heart rate was lower and stroke volume and cardiac output were higher during the exercise. Mean arterial pressure and total peripheral resistance were not different between the two conditions. The core temperature response to exercise was not affected by PVX. It was also observed that acute PV expansion altered both the fluid regulatory hormones and catecholamine responses to exercise. Exercise induced increases in plasma norepinephrine, renin, aldosterone, and vasopressin were attenuated with PVX. No differences in the glucose rate of appearance/disappearance, glycerol rate of appearance, and rates of substrate oxidation were observed between conditions. Muscle glycogen depletion and muscle metabolite concentrations were also unaltered by PVX during exercise.

In the PV reduction study, ten untrained males performed 60 min of cycle exercise at 61% of VO_2peak while on a diuretic (DIU) and under control (CON) conditions. Participants consumed either a diuretic (Novotriamazide) or a placebo, in random order, for 4 days prior to the exercise. The diuretic resulted in a 14.3% reduction in resting PV. Hypovolemia resulted in a reduction in SV and an increase in HR, both at rest and during exercise. The reduced PV led to greater circulating concentrations of norepinephrine during exercise. The hormonal response to the exercise was also altered, such that greater circulating concentrations of plasma renin, aldosterone, and angiotensin I, and a reduction in atrial natriuretic peptide were observed, during exercise. Furthermore, plasma glucagon concentrations were also increased during DIU. The diuretic induced reductions in PV also led to alterations in the response of glucose kinetics, but not whole body lipolysis, during exercise. Rates of total carbohydrate

and fat oxidation during exercise were not affected by the two conditions. Muscle metabolism was also unaltered by DIU, as indicated by the concentrations of muscle glycogen, ATP, PCr, Cr, Pi and lactate, both at rest and during exercise.

To investigate the effects of induced increases and decreases in plasma volume (PV) on submaximal exercise oxygen consumption (VO_2) and the dependent processes, cardiac output (Qc) and arterio-venous O_2 differences (a-vO_2 diff), and metabolite exchange across the working limb, 8 untrained males performed 2 legged kicking ergometry on 3 occasions at 69 ± 2.7% of peak kicking oxygen uptake (VO_2peak) for 10 min. Compared to a control condition (CON), PV was 20.9% higher in PVX and 15.1% lower in DIU. During exercise, cardiac output varied directly with plasma volume status for PVX, CON and DIU, respectively. Also during exercise, the a-vO_2 diff was similar in CON and PVX, but increased in DIU. Exercise VO_2 (l/min) was similar among conditions. Leg blood flow was higher ($P<0.05$) by 3 min of exercise with PVX as compared to CON and DIU. No differences were observed for net release or uptake of blood metabolites among the three conditions. Circulating concentrations of norepinephrine were increased with DIU both at rest and during exercise, while no differences were observed for epinephrine.

It is concluded that alterations in plasma volume have notable effects on a number of physiological systems during exercise. Alterations in PV directly or indirectly influence the cardiovascular and hormonal systems, thermoregulation, and the responses of blood flow and substrate turnover to dynamic exercise. What appears evident is that muscle metabolism and VO_2 are maintained or protected, despite changes in arterial oxygen content (CaO_2). These observations demonstrate that integration between central (cardiovascular) and peripheral mechanisms is occurring, in order to compensate for the changes in CaO_2. In addition, increases in PV are better tolerated by most physiological systems, whereas reductions in PV induce greater strain on most physiological systems during exercise.

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List of Studies:

This thesis is based on the following studies. They will be referred to throughout by their chapter designation.

Chapter II: The Physiological Influence of an Acute Increase in Plasma Volume on the Physiological Response to Prolonged Dynamic Exercise.

Chapter III: The Physiological Influence of a Diuretic Induced Decrease in Plasma Volume on the Physiological Response to Prolonged Dynamic Exercise.

Chapter IV: Influence of Plasma Volume on Substrate Delivery and Utilization During Dynamic Exercise.

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List of Abbreviations

(with units where appropriate)

α-ANP – atrial natriuretic peptide ($pg \cdot ml^{-1}$)

ALD – aldosterone ($pg \cdot ml^{-1}$)

ANG I – angiotensin I ($ng \cdot ml^{-1}$)

ANG II – angiotensin II ($ng \cdot ml^{-1}$)

ATP – adenosine triphosphate ($mmol \cdot kg^{-1}$ dw)

a-vO_2 diff – arterial venous oxygen difference ($ml \cdot 100\ ml^{-1}$)

AVP – arginine vasopressin ($pg \cdot ml^{-1}$)

BV – blood volume

CaO_2 – arterial oxygen content of blood ($ml \cdot 100\ ml^{-1}$)

CON – control condition

Cr – creatine ($mmol \cdot kg^{-1}$ dw)

CvO_2 – venous oxygen content of blood ($ml \cdot 100\ ml^{-1}$)

CVP – central venous pressure (mmHg)

DIU – diuretic condition

EPI – epinephrine ($pg \cdot ml^{-1}$)

FBF – forearm blood flow ($ml \cdot min^{-1}$)

GCMS – gas chromatography mass spectrometry

Hb – hemoglobin (g%)

Hct – hematocrit (%)

HPLC – high performance liquid chromatography

HR – heart rate (beats·min^{-1})

IMP – idenosine monophosphate (mmol·kg^{-1} dw)

MAP – mean arterial pressure (mmHg)

NE – norepinephrine (pg·ml^{-1})

OSM – osmolality (mOSM·kg^{-1} H_2O)

PCO_2 – partial pressure of carbon dioxide (mmHg)

PCr – phosphocreatine (mmol·kg^{-1} dw)

Pi – inorganic phosphate (mmol·kg^{-1} dw)

PO_2 – partial pressure of oxygen (mmHg)

PRA – plasma renin activity (ng·ml^{-1}·h^{-1})

PV – plasma volume

PVX – plasma volume expanded condition

Q – leg blood flow (ml·min^{-1})

Qc – cardiac output (l·min^{-1})

R_a – rate of appearance (μmol·kg^{-1}·min^{-1})

RAA – renin, angiotensin, aldosterone

RCV – red cell volume

R_d – rate of disappearance (μmol·kg^{-1}·min^{-1})

SNS – sympathetic nervous system

SV – stroke volume (ml·beat^{-1})

TBV – total blood volume

TCr – total creatine ($mmol \cdot kg^{-1}$ dw)

T_{es} – esophageal temperature (°C)

TPR – total peripheral resistance ($mmHg \cdot l^{-1} \cdot min^{-1}$)

T_{re} – rectal temperature (°C)

T_{vent} – ventilatory threshold

VCO_2 – carbon dioxide production ($l \cdot min^{-1}$)

V_E – ventilation ($l \cdot min^{-1}$)

VO_2 – oxygen uptake ($l \cdot min^{-1}$)

VO_2peak – maximal oxygen uptake ($l \cdot min^{-1}$)

CHAPTER I

INTRODUCTION, REVIEW OF THE LITERATURE AND STATEMENT OF THE PROBLEM

INTRODUCTION

Fluid within the human body is distributed within two primary compartments, the intracellular compartment and the extracellular compartment. The intracellular compartment contains approximately 28 L of fluid (average 70 kg male), and represents the fluid within all of the different cells in the human body (Guyton & Hall, 1996). The extracellular compartment, which represents the fluid found outside of the cells, contains approximately 14 L. The extracellular compartment can be further subdivided into the interstitial compartment and the vascular compartment. The interstitial compartment represents approximately 75% of the extracellular compartment, whereas the plasma compartment or volume makes up approximately 25% of the extracellular compartment (Guyton & Hall, 1996). Plasma, the non-cellular part of the blood, interacts continuously with the interstitial compartment. Plasma/blood is considered its own fluid compartment because it is contained within the vascular system.

Plasma volume (PV) and red cell volume (RCV) constitute the total blood volume (TBV). An increase in either PV or RCV can result in an increase in TBV (hypervolemia). A decrease in either PV or RCV results in the opposite, a decrease in TBV (hypovolemia). An average TBV of a normal adult is approximately 5 L, where 60% of the TBV is PV, and the remaining 40% represents primarily RCV.

Homeostasis is critically dependent on being able to maintain fluid level and balance within relatively narrow limits. Alterations in fluid balance can disturb a wide range of processes, including cardiovascular and kidney functions, thermoregulation, metabolism and protein turnover to name a few (Guyton & Hall, 1996).

In humans, PV can be altered by a number of different stimuli. These stimuli include

exercise, heat, a combination of heat and exercise (Nielsen *et al.*, 1993), fluid restriction (Montain *et al.*, 1998; Zappe *et al.*, 1993), altitude exposure (Reeves *et al.*, 1991), and hypogravity (Leach *et al.*, 1996). Acutely, all of these stimuli lead to hypovolemia. Repeated chronic exposure to these stimuli generally results in hypervolemia (Convertino, 1991; Green *et al.*, 1990; Senay, 1972; Senay *et al.*, 1976; Wyndham *et al.*, 1976). As an example, a rapid and pronounced increase in PV occurs with endurance training. Training consisting of cycling for 2 h per day at approximately 60% of maximal oxygen consumption (VO_2max), leads to increases in PV of 15-20% (Convertino, 1991; Green *et al.*, 1987; Green *et al.*, 1990; Helyar *et al.*, 1996). Initial increases occur very rapidly, and can be observed within 24 h after a single exercise session.

Changes in RCV tend to occur in response to more chronic stimuli and do not usually occur over the short term. Both chronic exposure to altitude or endurance training have been associated with increases in RCV (Green *et al.*, 1991b; Reeves *et al.*, 1991), whereas hypogravity has been associated with reductions in RCV (Leach *et al.*, 1996).

These observations invite two questions, namely what are the mechanisms controlling PV levels and what are the physiological implications of alterations in PV during the exercise state. Alterations in PV could potentially influence the responses of a number of physiological systems to exercise. These systems range from the cardiovascular to thermoregulatory, fluid and electrolyte balance, and substrate turnover and metabolism. Alterations within the vascular compartment appear to have important implications for homeostasis, particularly during the exercise state (Parker, 1993).

CARDIOVASCULAR RESPONSES TO ALTERATIONS IN PLASMA VOLUME

Both hypervolemia and hypovolemia appear to have profound effects on cardiovascular function (Candas *et al.*, 1988; Fortney *et al.*, 1981a; Fortney *et al.*, 1983; Gonzalez-Alonso *et al.*, 1995; Grant *et al.*, 1997; Heaps *et al.*, 1994; Helyar *et al.*, 1996; Kanstrup & Ekblom, 1982; Montain & Coyle, 1992b). Decreases in TBV are associated with an increase in heart rate (HR) and a decrease in stroke volume (SV) and cardiac output (Qc) during exercise (Montain & Coyle, 1992b). For example, Nadel et al. (1980) observed that hypovolemia, resulting from 4 days of diuretic administration, resulted in a greater increase in HR during exercise that occurred in conjunction with a reduction in Qc compared to a normovolemic condition (Nadel *et al.*, 1980). Similarly, heat and exercise induced decreases in PV (dehydration) are also associated with a decrease in Qc and SV, and an increase in HR (Montain & Coyle, 1992b).

The changes that are observed in cardiac function during exercise with hypovolemia are also accompanied by an increase in total peripheral resistance (TPR) (Gonzalez-Alonso *et al.*, 1995). It appears that an increase in TPR is an attempt to maintain mean arterial pressure (MAP), central venous pressure (CVP) and venous return. The increase in TPR is likely mediated by an increase in sympathetic nervous system activity, since sympathetic vasoconstriction have been observed to occur in the leg during exercise (Buckwalter *et al.*, 1997; Pawelczyk *et al.*, 1992). In addition, there is some recent in vitro evidence that increases in temperature in combination with sympathetic stimulation leads to vasoconstriction of the vessels isolated from the splanchnic region (Massett *et al.*, 1999). Depending on the degree of hypovolemia, the increase in TPR may not be able to protect MAP during exercise performed in warm or hot conditions. For example, exercise in the heat, resulting in

dehydration and hypovolemia, leads to a decline in MAP, especially late in exercise (Rowell, 1986; Rowell, 1993). These observations suggest that the response of MAP varies depending on the degree of cardiovascular strain induced by the intensity of exercise and environmental conditions.

Two primary strategies appear to be used to minimize the cardiovascular instability that occurs with exercise induced hypovolemia. These involve both short-term and long-term regulation of PV. Short-term regulation occurs during the exercise itself, and is directed at minimizing additional loses of PV. Long-term regulation attempts to adjust the level of PV during recovery and prior to the exercise.

In contrast to hypovolemia, increases in both TBV and PV lead to an increase in SV and Qc, and a decrease in HR, both at rest and during exercise (Fortney *et al.*, 1981a; Fortney *et al.*, 1983; Gillen *et al.*, 1994; Grant *et al.*, 1997; Helyar *et al.*, 1996; Kanstrup & Ekblom, 1982; Kanstrup *et al.*, 1992). For example, during exercise Fortney et al. (1981a) observed increases in both SV and Qc and a decrease in HR when an increase in TBV was induced by an infusion of whole blood. Similarly, Helyar et al. (1996) also observed an increase in SV and Qc and a decrease in HR with an artificial increase in PV that was induced by an infusion of a large molecular weight compound (Dextran) diluted in saline. These changes, observed at rest and early in exercise, persisted throughout a prolonged session of cycling exercise.

The relationship between MAP, Qc and TPR (MAP = Qc x TPR) indicates that an acute increase in Qc, as observed with hypervolemia, should result in an increase in MAP, unless a decrease in TPR occurs. Hypervolemia does not appear to have the same degree of influence on the response of TPR and MAP, as does hypovolemia. Generally, slight reductions are observed in TPR with an acute increase in PV, whereas varying results have

been observed with MAP and hypervolemia (Grant *et al.*, 1997; Helyar *et al.*, 1996; Mier *et al.*, 1996). For example, it has been observed that a decrease in MAP occurs both at rest and during exercise with hypervolemia (Grant *et al.*, 1997), while others have observed no influence of hypervolemia on MAP during exercise (Helyar *et al.*, 1996; Kanstrup *et al.*, 1992). Together, these studies demonstrate that hypervolemia leads to a reduction in TPR. However, depending on the magnitude of the increase in Qc that also occurs with hypervolemia, varying alterations in MAP are observed.

In summary, both increases and decreases in TBV, resulting from alterations in PV, can alter the cardiovascular response to submaximal exercise. An increase in PV promotes a more stable cardiovascular response to prolonged endurance exercise (Helyar *et al.*, 1996), while a decrease in PV increases cardiac instability (Montain & Coyle, 1992b; Nadel *et al.*, 1980). It appears that the cardiovascular system is more tolerant of hypervolemia, where protection of MAP is easily managed through a reduction of TPR, in response to the increase in Qc. Such is not the case for hypovolemia. Although the increase in TPR appears as an important response to defend MAP, in the face of a decrease in Qc, this strategy is not always successful as indicated by the decline in MAP in some conditions.

OXYGEN CARRYING CAPACITY OF BLOOD AND PLASMA VOLUME

Changes in PV can lead to alterations in the carrying capacity and content (CaO_2) of the blood for oxygen. With hypervolemia, as an example, CaO_2 is reduced as a result of the dilutional effects of the increase in PV on hemoglobin concentration (Hb). The change in CaO_2 has potentially important consequences on the availability of O_2 in the working muscle. If O_2 availability is altered within the muscle, this could lead to alterations in oxidative

phosphorylation and substrate utilization (Fortney *et al.*, 1981a; Green *et al.*, 1991b). Some form of compensation must occur to offset the decrease in O_2 carrying content, since no changes in whole body oxygen uptake (VO_2) are observed with exercise following acute PV expansion compared to normovolemia (Helyar *et al.*, 1997). It is possible that with situations where O_2 demand is increased (high intensity exercise), the combined effect of increased O_2 demand and the decrease in CaO_2 with hypervolemia could limit the delivery of oxygen, unless some form of compensation occurs. Two possibilities exist: one possibility is that blood flow is increased to maintain O_2 delivery to the working muscle. A second possibility is that O_2 extraction is maintained, thus translating into the maintenance of arterio-venous oxygen difference (a-vO_2 diff).

Reductions in PV, in contrast to increases in PV, have the opposite effect on Hb and CaO_2. As with hypervolemia, VO_2 is also unaltered during exercise with a reduction in PV (Zappe *et al.*, 1996), suggesting that some form of compensation also occurs with oxygen delivery and/or a-vO_2 diff. The potential alterations in Qc and TPR with hypovolemia could potentially compromise muscle blood flow and metabolism (Fortney *et al.*, 1981a; Green *et al.*, 1989). A reduction in muscle blood flow could jeopardize the delivery of exogenous substrates and result in an increased reliance on intramuscular substrates. In addition, a reduction in blood flow could lead to a decrease in the clearance of metabolic by products such as lactate and ammonia. It is possible that a critical level of hypovolemia must be achieved, resulting in a decline in Qc, before a reduction in muscle blood flow occurs. Reductions in muscle blood flow have been observed late in prolonged exercise when a decline in PV and dehydration are present (Gonzalez-Alonso *et al.*, 1998b). In situations where Qc is reduced during exercise, increases in vasoconstriction have been observed in the working muscle

(Buckwalter *et al.*, 1997; Pawelczyk *et al.*, 1992), suggesting that a reduction in muscle blood flow could occur even though the metabolic demands of the muscle are increased. Despite these observed effects on vasoconstriction during exercise, others have observed no alterations in blood flow during exercise performed in a hot environment (Nielsen *et al.*, 1990; Savard *et al.*, 1988). In these studies, the exercise may not have been of an adequate duration to induce reductions in Qc sufficient to impact on leg blood flow.

Investigations into the effects of varying levels of iso-volemic hemodilution in animal preparations have demonstrated that significant declines in CaO_2 result in an increase in resting blood flow to various tissues, including, the heart (Chapler & Cain, 1986; Krieter *et al.*, 1995), brain (Chapler & Cain, 1986; Hudetz *et al.*, 1999; Krieter *et al.*, 1995), liver (Krieter *et al.*, 1995) and skeletal muscle (Hutter *et al.*, 1999; Krieter *et al.*, 1995). In contrast, no consistent effects of iso-volemic hemoconcentration on blood flow distribution have been detected. Observations obtained using different models have indicated decreases in resting splanchnic (Fan *et al.*, 1980) and muscle blood flow (Kuo & Pittman, 1990), increases in splanchnic blood flow (Ackermann & Veress, 1980), and no alterations in muscle blood flow (Ackermann & Veress, 1980). It is possible that these inconsistencies may relate to inter-species differences due to the variety of different animal models used. Alterations in blood viscosity have been suggested as a possible mechanism for altering muscle blood flow with hemoconcentration, however, other factors, such as metabolic influences, likely contribute to the alterations in regional blood flows (Ackermann & Veress, 1980). Decreases in the mean red blood cell velocity have also been observed with iso-volemic hemoconcentration (Kuo & Pittman, 1990). Despite these potential effects, O_2 delivery and uptake do not appear to be compromised in either splanchnic (Fan *et al.*, 1980) or skeletal muscle tissue (Kuo & Pittman,

1990) at rest. Therefore, hemodilution and hemoconcentration, appear to be well tolerated and alterations in regional blood flow seem to compensate for change in CaO_2, thereby maintaining regional O_2 delivery.

Alterations in CaO_2 have also been implicated in the control of Qc and leg blood flow in the human. It has been observed that blood flow to the working muscle varies with the Hb concentration during exercise (Saltin *et al.*, 1986). Specifically, leg blood flow was observed to be lower in individuals with an elevated Hb, whereas it was increased in individuals with a lower Hb level (Saltin *et al.*, 1986). The importance of Hb concentration with respect to control of leg blood flow during exercise, appears to be related to CaO_2 and the delivery of oxygen to the working muscle. Roach et al. (Roach *et al.*, 1999) observed that regulation of Qc, leg blood flow and arterial O_2 delivery is dependent on CaO_2, not PaO_2. Using a model that allowed for manipulation of both arterial oxygen content (CaO_2) and the partial pressure of oxygen in the artery (PaO_2) at various Hb concentrations, they observed that Qc and leg blood flow were unaltered with alterations in PaO_2, but when CaO_2 was decreased (with a decrease in hemoglobin concentration), there was a compensatory increase in Qc and leg blood flow (Roach *et al.*, 1999). These changes were independent of any changes in MAP, suggesting that any increase in blood flow was likely due to vasodilation. These observations contribute to the growing evidence that alterations in CaO_2, due to alterations in PV, likely lead to alterations in regional blood flow distribution in the human.

HORMONAL RESPONSES TO ALTERATIONS IN PLASMA VOLUME

A number of hormones are involved in the maintenance of cardiovascular integrity with alterations in both TBV and PV. These hormones, collectively referred to as fluid and

electrolyte hormones, serve in the regulation of cardiovascular and kidney function, and fluid and electrolyte balance (Guyton & Hall, 1996). The fluid and electrolyte hormones include, angiotensin I and II (ANG I, ANG II), aldosterone (ALD), renin (PRA), vasopressin (AVP), and α-atrial natriuretic peptide (α-ANP) (Guyton & Hall, 1996).

The SNS has been suggested to be important in the control of some of the fluid and electrolyte hormones (Guyton & Hall, 1996). The two hormones involved in the SNS are norepinephrine (NE) and epinephrine (EPI). The primary source of circulating norepinephrine during exercise is the nerve endings of sympathetic fibers (Savard *et al.*, 1987). Epinephrine is released from the adrenal medulla in response to increases in sympathetic stimulation (Guyton & Hall, 1996; Kaplan, 1992; Kjaer, 1998). Alterations in vascular volumes result in changes in the SNS response to dynamic exercise. Hypervolemia, induced prior to exercise, is associated with a reduced SNS response to exercise (Grant *et al.*, 1996; Hargreaves *et al.*, 1996b; Helyar *et al.*, 1997), whereas, exercise hypovolemia induced a more pronounced increase in the SNS response to exercise (Gonzalez-Alonso *et al.*, 1995).

The renin-angiotensin-aldosterone system (RAA) is recognized as being intimately involved with fluid and electrolyte homeostasis and cardiovascular regulation. Renin is secreted from the juxtaglomerular cells of the kidney in response to alterations in the perfusion pressure. Renin serves to convert the precursor of ANG I, angiotensinogen, into the active form. Angiotensin I is rapidly converted into its active form, ANG II, by angiotensin converting enzyme. Angiotensin II has widespread systemic effects, including vasoconstriction, increased cardiac contractility, stimulation of ALD release from the adrenal cortex, central nervous system regulation, and stimulation of water absorption from the small intestine (Greenstein, 1994). Aldosterone stimulates sodium (Na^+) transport in the epithelial

cells of the distal tubule of the kidney (Greenstein, 1994).

Concentrations of RAA, AVP and α-ANP, are all influenced by alterations in PV. Generally, hypervolemia results in an attenuation of the response of these hormones to exercise (Grant *et al.*, 1996), whereas hypovolemia appears to exaggerate the normal response (Montain *et al.*, 1997). Similarly, the responses of RAA and AVP, during exercise, are attenuated when subjects are hyperhydrated (likely hypervolemic or normovolemic) compared to when the same subjects are dehydrated (hypovolemic) (Brandenberger *et al.*, 1989).

A potentially important function of the fluid and electrolyte hormones with alterations in PV is to contribute the cardiovascular responses, especially during prolonged exercise. Changes in ANG II concentrations have the potential to alter the hemodynamic response to exercise (Stebbins & Symons, 1995). It has been observed that blocking ANG II receptors during dynamic exercise results in an attenuation of the MAP changes, a greater decrease in systemic vascular resistance, and greater blood flow to the splanchnic region (Stebbins & Symons, 1995). Similar results have been observed with other fluid regulatory hormones such as vasopressin (Stebbins & Symons, 1993). More specifically, Stebbins et al. (1993) observed that administration of a vasopressin antagonist led to a reduction in MAP and an increase in Qc during dynamic exercise. Therefore, if an alteration in PV alters the response of vasopressin during exercise, alterations in blood flow distribution could be expected to occur. Taken together, all of the fluid and electrolyte hormonal changes, due to changes in PV, have the potential to influence the regional distribution of blood flow.

Clearly, alterations in PV alter the hormonal response to exercise. These changes are evident with acute PV expansion (Grant *et al.*, 1996; Helyar *et al.*, 1997; Kanstrup *et al.*, 1992), differing levels of hydration (Brandenberger *et al.*, 1989) and acute dehydration with

exercise (Brandenberger *et al.*, 1989; Grant *et al.*, 1996). Moreover, hypovolemia most often results in more pronounced increases in both resting and exercise concentrations of the fluid and electrolyte hormones and catecholamines, suggestive of a compensatory response to the increased strain that occurs with hypovolemia. In contrast, hypervolemia leads to both declines in resting concentrations and blunted responses of these hormones during exercise. It would appear that the changes in these hormones may be related to the alterations in PV directly, however, many other factors have also been implicated in their control (Christensen, 1993; Greenstein, 1994; Guyton & Hall, 1996; Share, 1996). Hormonal regulation could also be related to alterations in the cardiovascular system directly, in an attempt to adjust cardiovascular function to either compensate for the added strain due to hypovolemia, or the decreased strain with hypervolemia.

THERMOREGULATORY RESPONSES TO ALTERATIONS IN PLASMA VOLUME

Prolonged endurance exercise increases heat production, which results in a progressive increases in core temperature (Sawka *et al.*, 1996). Depending on the environmental conditions and the nature of the exercise, the temperature could continue to progressively increase, or a new steady state value could be reached (Sawka *et al.*, 1996). The increase in core temperature is due to the increased rate of heat production, produced primarily by the working muscles. The rate of increase in core temperature is dependent on a number of factors including, intensity and duration of exercise (Sawka *et al.*, 1996), environmental conditions including ambient temperature and humidity (Sawka *et al.*, 1996), hydration state (Candas *et al.*, 1988; Montain *et al.*, 1995), the amount of fluid replacement (Candas *et al.*, 1986; Hargreaves *et al.*, 1996b; Nadel *et al.*, 1980), and the degree of heat acclimation (Harrison,

1996; Mitchell *et al.*, 1976; Nielsen *et al.*, 1993; Senay *et al.*, 1976; Wyndham *et al.*, 1976). Alterations in any or all of these variables can lead to changes in the thermoregulatory response to prolonged exercise.

Typically, the onset of dynamic exercise is accompanied by an early increase in core temperature due to the imbalance between heat production and heat dissipation. The increase in core temperature stimulates a number of heat dissipating physiological responses, including, increases in skin blood flow and increases in the rate of sweating (Gleeson, 1998; Sawka *et al.*, 1998; Sawka *et al.*, 1996). These changes lead to an increase in both convective and evaporative heat loss (Gleeson, 1998; Sawka *et al.*, 1996). As the exercise continues, the thermoregulatory system adapts to balance the rate of heat dissipation with heat production. If the exercise is not too intense and the environmental conditions not too severe, a steady state core temperature is achieved (Gleeson, 1998; Sawka *et al.*, 1996).

As the duration of exercise increases, the heat dissipation mechanisms (primarily evaporation) lead to reductions in PV, due to the continued fluid loss that occurs via sweating (Armstrong & Maresh, 1998; Gleeson, 1998; Sawka *et al.*, 1996). If the decline in PV becomes too severe and MAP is threatened, heat dissipation mechanisms may be compromised due to the instability that occurs in cardiovascular function (Coyle, 1998; Gonzalez-Alonso, 1998a; Sawka *et al.*, 1996), and further increases in core temperature are observed (Coyle, 1998; Gleeson, 1998; Sawka *et al.*, 1996). The decline in PV that occurs during exercise results in increased demands on the cardiovascular system, such that the available Qc is required in large part both for the working muscle and the cutaneous circulation. If the loss becomes such that MAP is threatened, cutaneous circulation is reduced (Mora-Rodriguez *et al.*, 1996). In addition, reductions in Qc, and increases in core temperature, have also been

associated with a reduction in muscle blood flow (Gonzalez-Alonso *et al.*, 1998b). The exercise induced declines in PV have been prevented with exogenous administration of fluids, which improves cardiac stability (Hamilton *et al.*, 1991) and attenuates the increase in core temperature (Hargreaves *et al.*, 1996b). Reductions in PV that occur with prolonged exercise are associated with a number of alterations in various physiological systems, including the cardiovascular (Coyle, 1998; Gonzalez-Alonso *et al.*, 1995), endocrine (Brandenberger *et al.*, 1989; Rocker *et al.*, 1989; Shoemaker *et al.*, 1998), and sympathetic nervous system (Gonzalez-Alonso *et al.*, 1995). These responses resemble those observed with exercise and acute hypovolemia (Fortney *et al.*, 1981a; Nadel *et al.*, 1980; Zappe *et al.*, 1996).

In situations where heat dissipation cannot be matched with heat production, the increase in core temperature continues. Excessive increases in core temperature during dynamic exercise ($\geq$ 40°C) can result in fatigue (Gonzalez-Alonso *et al.*, 1999), defined as the inability to continue at a given work output. As discussed previously, under such conditions exercise can lead to reductions in muscle blood flow (Gonzalez-Alonso *et al.*, 1999). It has been suggested that fatigue, under such circumstances, is a result of feedback from the central nervous system (Gonzalez-Alonso *et al.*, 1999). The inhibitory feedback may provide a mechanism that protects the organism from increases in core temperature into a range where serious injuries, such as heat stroke, could result.

Acute increases in heat stress also have implications for both substrate utilization and metabolism during exercise (Febbraio *et al.*, 1994b; Febbraio *et al.*, 1996; Hargreaves *et al.*, 1996a; Hargreaves *et al.*, 1996b; Parkin *et al.*, 1999). Exercise in the heat results in increases in the endogenous rate of glucose appearance and disappearance (Hargreaves *et al.*, 1996a), muscle glycogenolysis (Febbraio *et al.*, 1994b; Febbraio *et al.*, 1996) muscle lactate

concentrations (Febbraio *et al.*, 1996) and lactate release from the working muscle (Gonzalez-Alonso *et al.*, 1999). Interestingly, these alterations in muscle metabolism during prolonged exercise also occur within a similar time frame as the reductions in PV. When the reduction in PV that occurs during prolonged exercise is prevented with the consumption of water, decreased rates of muscle glycogenolysis and a lower core temperature occur (Hargreaves *et al.*, 1996b). Therefore, it appears that altered thermoregulation alters both substrate utilization and muscle metabolism. It is possible that the alterations that are observed are mediated by the increases in the endocrine and SNS responses that are also observed during exercise and thermal stress.

Several days of repeated bouts of dynamic exercise in a warm or hot environment leads to physiological changes that result in improved exercise performance in the heat. This process of adaptation is termed heat acclimation. Heat acclimation is characterized by declines in HR and core temperature, and an earlier and more rapid increase in sweat rate (Mitchell *et al.*, 1976; Nielsen *et al.*, 1993; Sawka *et al.*, 1996; Senay *et al.*, 1976; Wyndham *et al.*, 1976). Acclimation also results in a rapid increase in PV, which leads to the reduction in HR (Convertino *et al.*, 1980a; Harrison, 1996; Mitchell *et al.*, 1976; Nielsen *et al.*, 1993; Sawka *et al.*, 1996). The increase in PV results from an increase in circulating protein (albumin) (Senay, 1972). The increase in PV appears to be important in the circulatory adjustments that occur with heat acclimation, such as an increase in cutaneous blood flow and evaporative heat loss. In contrast, acute increases in PV, induced by osmotic particles diluted in saline (Dextran), do not appear to alter the thermoregulatory response during exercise (Grant *et al.*, 1997; Sawka *et al.*, 1983). However, acute increases in PV induced by saline infusion have been observed to alter the thermoregulatory response to exercise (Nose *et al.*, 1990). The

addition of saline to the vascular compartment could lead to alterations in other fluid compartments within the body, depending on the tonicity of the saline. For example, a hypo-osmotic saline solution would rapidly diffuse out of the vascular compartment and into the interstitial and intracellular space due to the osmotic gradients (Guyton & Hall, 1996). It is unclear what influence an increase in interstitial or intracellular volume would have on the thermoregulatory response to exercise. Alterations in the interstitial and intracellular space would create difficulty in determining the importance of changes in PV alone. Therefore, based on the current literature changes in PV alone, do not appear to lead to improvements in thermoregulation. However, when the increase in PV occurs in combination with other adaptations, an improved thermoregulatory capacity is observed. It is possible that adaptations in other fluid compartments also occur and that the combined effects of these alterations are essential for heat acclimation processes to occur.

It is evident that thermoregulation during exercise is intimately related to the TBV and maintenance of cardiovascular stability. Abnormal elevations in core temperature and muscle temperature have also been demonstrated to lead to an increased dependency on carbohydrate metabolism. In addition, excessive increases in core temperature can lead to fatigue that appears to involve the central nervous system. Repeated exposure to thermal stress leads to a process termed acclimation, which allows improved control of heat accumulation. Acclimation results in increases in PV, but other simultaneously occurring adaptations also appear to be important, as indicated by the lack of an effect of an acute increases in PV on the thermoregulatory response to prolonged exercise.

BLOOD FLOW AND ALTERATIONS PLASMA VOLUME

Skeletal muscle blood flow is under complex regulation, by a large number of different variables. Blood flow control both during the onset of exercise and during steady state exercise has been extensively reviewed (Clarke *et al.*, 1998; Delp, 1999; Delp & Laughlin, 1998; Green *et al.*, 1996; Hughson & Tschakovsky, 1999; Joyner & Dietz, 1997; Joyner & Proctor, 1999; Laughlin & Schrage, 1999; Rådegran & Saltin, 1998; Saltin, 1988; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). Identifying the potential mechanisms underlying the role of alterations in PV on muscle blood flow regulation is important in the context of the current thesis.

Blood flow through the vasculature can be described using an adaptation of Ohm's law, which relates flow to the change in pressure and the resistance to flow through the vessel. The equation is as follows; $Q = \Delta P / R$, where Q is blood flow through the vessel, ΔP is the pressure gradient between two points in the vascular bed, and R is the resistance of the vasculature, which is also equal to 1/C, the conductance of the vessel (Guyton & Hall, 1996).

With the onset of exercise, a rapid increase in blood flow is observed with the first few muscle contractions (Rådegran & Saltin, 1998; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). The increase in blood flow to the working muscle is accompanied by a slightly delayed corresponding increase in VO_2 across the working limb (Grassi *et al.*, 1996). The initial increase in skeletal muscle blood flow, termed exercise hyperemia, can be separated into a number of specific stages (Delp, 1999; Rådegran & Saltin, 1998; Saltin *et al.*, 1998). The initial increase in blood flow, observed during the first few contractions, appears to be due to mechanical changes such as the muscle pump (Delp, 1999; Rådegran & Saltin, 1998; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). During the subsequent stage, the increase that

occurs appears to be due to dilation, apparently mediated by feedback regulation (Rådegran & Saltin, 1998; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). Numerous metabolic factors have been suggested to have a role in the control of muscle blood flow (Delp, 1999; Green *et al.*, 1996; Joyner & Dietz, 1997; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). Some of these factors include; potassium ions, hyperosmolality, hydrogen ions, adenosine, CO_2, Pi, ammonia, prostaglandins, endothelium-derived nitric oxide, and acetylcholine (Delp, 1999; Green *et al.*, 1996; Joyner & Dietz, 1997; Saltin *et al.*, 1998; Shoemaker & Hughson, 1999). Ultimately, the increase in blood flow appears to be attempt by the system to match oxygen delivery to metabolic demands of the working muscle. Interestingly, the increase in VO_2 with the onset of exercise appears to be explained by an increase in leg blood flow, rather than an increase in O_2 extraction (Grassi *et al.*, 1996). However, soon after the onset of exercise, an increase in O_2 extraction is also observed (Grassi *et al.*, 1996). This suggests, feedback control, with regulation based on levels of O_2 delivery to the working muscle.

Alterations in PV appear to modify muscle blood flow both at rest and during exercise. As discussed earlier, acute increases in PV lead to a decline in CaO_2, while a decrease in PV leads to an increase in CaO_2. In the human, alterations in CaO_2 likely lead to changes in muscle blood flow, since no changes in VO_2 have been observed during exercise with either acute increases (Grant *et al.*, 1997; Green *et al.*, 1987; Watt *et al.*, 1999), or decreases (Caldwell *et al.*, 1984; Zappe *et al.*, 1996) in PV. With acute increases in PV, an increase in blood flow to the working muscle likely occurs to compensate for the decline in CaO_2. Possible mechanisms that could lead to an increase in muscle blood flow with acute PV expansion, are endothelium-derived nitric oxide (NO) and/or other endothelium derived vasoactive agents. Nitric-oxide is released by endothelial cells in response to shear-stress

(Green *et al.*, 1996; Joyner & Dietz, 1997). With an acute increase in PV, Qc is increased (Grant *et al.*, 1997; Helyar *et al.*, 1996; Kanstrup *et al.*, 1992). In addition, hemodilution has also been observed to lead to increases in the velocity of red blood cells (Hudetz *et al.*, 1999). These factors taken together, could lead to an increase in shear stress, resulting in an increase in NO and vasodilation.

Changes in hemoglobin concentration have also been suggested to result in alterations in the level of endothelium-derived NO. Hemoglobin has been demonstrated to be a NO scavenger in an O_2-dependent manner, such that with decreasing O_2 saturation, more NO is available for local vasodilation (Stamler *et al.*, 1997). Oxygen-dependent ATP release from red blood cells has also been proposed as a possible mechanism to alter blood flow with alterations in CaO_2 (Ellsworth *et al.*, 1995). In addition, the RAA system (Stebbins & Symons, 1995) and the SNS also have the potential to impact on the control of blood flow (Buckwalter *et al.*, 1997). There may also be other mechanisms that could impact on blood flow with changes in PV and CaO_2 that have yet to be identified. However, before mechanisms can be examined it is first necessary to accurately describe the effect of alterations in PV on blood flow responses across the working limb.

MUSCLE METABOLISM, SUBSTRATE TURNOVER AND PLASMA VOLUME

There is a possibility that alterations in PV may affect muscle metabolism and substrate selection during exercise. It has been established that both heat acclimation (Kirwan *et al.*, 1987; Nielsen *et al.*, 1993; Young *et al.*, 1985) and endurance training (Green *et al.*, 1991; Green *et al.*, 1989; Green *et al.*, 1990; Green *et al.*, 1991b) result in a significant increase in PV. During exercise, hypervolemia has been associated with muscle glycogen sparing

(Febbraio *et al.*, 1994a; Green *et al.*, 1989), decreased RER (Febbraio *et al.*, 1994a; Kirwan *et al.*, 1987; Young *et al.*, 1985), and a decreased lactate accumulation (Young *et al.*, 1985). The metabolic changes that occur with hypervolemia during exercise are also accompanied by a decreased catecholamine response (Febbraio *et al.*, 1994a; Green *et al.*, 1989). For example, Green et al. (1991) observed that short-term endurance training (2h/day, 10-12 days) resulted in a rapid increase in PV that occurred within the first few days of training. In addition, decreases in plasma lactate, ammonia and uric acid were also observed following a similar amount of training (Green *et al.*, 1991a). Using a similar training protocol, Phillips et al. (1996a) observed less of a decrease in phosphocreatine (PCr) and muscle glycogen, and less of an increase in the accumulation of muscle lactate and inosine monophosphate (IMP) during submaximal exercise with 5 days of training. This form of training is also associated with an attenuation of the catecholamine response to sub-maximal exercise (Febbraio *et al.*, 1994a; Green *et al.*, 1989; Helyar *et al.*, 1997). It is possible that the alterations in muscle metabolism observed early in training could be partially mediated by a decrease in plasma catecholamines as a result of hypervolemia. In addition, the alterations could be related to changes in the oxidative capacity of the muscle, which have also been observed with training (Holloszy & Coyle, 1984). However, the changes in PV, blood catecholamines and muscle metabolism have all been observed to occur prior to changes in muscle oxidative capacity (Green *et al.*, 1992). There is controversy as to the time course of when oxidative capacity is first increased with training (Green *et al.*, 1999; Spina *et al.*, 1996). Specifically, some authors belief that an increase in oxidative capacity occurs early in training, and corresponds with alterations in muscle metabolism that are also observed (Spina *et al.*, 1996). However, recent evidence suggests that some changes in muscle metabolism occur within the first 2 days of training, in

the absence of changes in muscle oxidative potential (Green *et al.*, 1999).

Changes in muscle metabolism, that occur with endurance training, appear to occur before any changes in muscle oxidative potential. Increases in oxidative potential do occur with both endurance training (i.e. 1-2 h per day, 50-80% peak oxygen uptake) (Green *et al.*, 1999; Phillips *et al.*, 1996a; Spina *et al.*, 1996) and sprint training (MacDougall *et al.*, 1998). The training state of the participants has also been hypothesized to influence the rate of mitochondrial adaptations. However, recent work demonstrated that training state, as indicated by VO_2peak, did not influence the initial rate of mitochondrial adaptations (Green *et al.*, 1999). Despite the controversy regarding the time course of adaptations in mitochondrial potential, what is clear is that alterations in muscle metabolism do occur early in training, before any measurable adaptation in muscle oxidative capacity (Green *et al.*, 1999). Of particular concern is whether the alterations in muscle metabolism that occur with short-term training can be explained by other adaptations, such as an increase in PV.

To investigate the possible influence of an increase in PV on muscle metabolism and substrate utilization during dynamic exercise, PV has been acutely expanded prior to the initiation of prolonged, low intensity cycle exercise (46% VO_2peak) (Green *et al.*, 1997). These investigators observed lower muscle lactate and pyruvate concentrations early in exercise, which persisted throughout the remainder of the exercise (Green *et al.*, 1997). However, others using a similar model of PV expansion, but higher intensity exercise (72% VO_2peak) have not observed any effect on either lactate or muscle metabolite concentrations (Watt *et al.*, 1999). In the study by Watt et al., there appeared to be a trend for muscle lactate to be lower at the end of the 40 min of exercise with acute PV expansion compared to control conditions. Finally, the investigators did not observe any attenuation of the catecholamine, or

HR response with PV expansion, that is normally observed with such protocols (Grant *et al.*, 1996; Helyar *et al.*, 1997). It is curious as to why no decline in HR was observed, despite the 12% increase in PV that was induced (Watt *et al.*, 1999). Since HR was similar and PV was increased during the experimental trial, Qc should have been increased. Any increase in Qc, may have been directed to the working muscle in an attempt to maintain O_2 delivery due to the hemodilution that would have occurred as a result of the PV expansion. Since the greater exercise intensity employed by Watt et al. (1999), would have resulted in an increased O_2 demand at the working muscle, the increase in O_2 demand may have overcome any benefit of the increase in PV. Taken together, it appears that an acute increase in PV influences muscle lactate in a manner similar to that observed following training, but with more moderate exercise intensities no such benefit has been observed, possibly due to the increased O_2 demands of the greater workload.

Heat acclimation also results in a hypervolemia similar to that observed with training. This form of hypervolemia is also associated with an attenuation of the catecholamine response to exercise, glycogen sparing, decreased muscle lactate accumulation and an attenuation in the decrease in PCr (Febbraio *et al.*, 1994a; Kirwan *et al.*, 1987; Young *et al.*, 1985). These findings further support the notion that the increase in PV may have an impact on muscle metabolism.

Alterations in thermoregulation, manifested by changes in core and muscle temperature, can directly alter muscle metabolism (Febbraio *et al.*, 1996; Febbraio *et al.*, 1994b; Febbraio *et al.*, 1996; Snow *et al.*, 1993; Yaspelkis *et al.*, 1993) and whole body substrate turnover (Hargreaves *et al.*, 1996a). It has been shown that increases in core and muscle temperature, induced by increases in ambient temperature and direct heating of skeletal

muscle, increase reliance on skeletal muscle carbohydrate metabolism (Febbraio *et al.*, 1996; Febbraio *et al.*, 1994b; Febbraio *et al.*, 1996; Yaspelkis *et al.*, 1993), liver glycogenolysis (Hargreaves *et al.*, 1996a), and decreased reliance on extramuscular substrates (Hargreaves *et al.*, 1996a). Several possible mechanisms have been suggested to explain the temperature effects on muscle metabolism. One hypothesis is based on the direct effect of raised temperatures on metabolic reactions (Q_{10} effect) (Edwards *et al.*, 1972; Kozlowski *et al.*, 1985). However, the Q_{10} for enzyme mediated reactions (2.0-3.0) cannot account for the changes observed in muscle glycogenolysis or muscle lactate accumulation (Febbraio *et al.*, 1994a). A second possibility is that the increase in temperature leads to alterations in the distribution of muscle blood flow, resulting in an decrease in O_2 delivery (Kozlowski *et al.*, 1985). It has been observed that muscle blood flow is decreased late during cycling exercise in the heat (Gonzalez-Alonso *et al.*, 1998b). However, others have observed little influence of acute heat stress on leg blood flow during treadmill exercise (Nielsen *et al.*, 1990). Another possibility is that the increased concentrations of blood EPI observed with acute heat stress, promote an increased dependency on carbohydrate metabolism (Febbraio *et al.*, 1994a). At present, it is unclear what the exact mechanism underlying these alterations is related to. It is possible that changes in PV could alter thermoregulation, and since temperature directly alters metabolism, PV could then alter muscle metabolism and substrate turnover through indirect mechanisms.

A decline in PV normally occurs with prolonged dynamic exercise. If the decline is attenuated or prevented, the metabolic response to the exercise is significantly altered (Hargreaves *et al.*, 1996b). For example, Hargreaves et al. (1996b) observed significant glycogen sparing, and a decreased accumulation of muscle lactate, when the decline in PV is

prevented with water consumption during exercise. The catecholamine response to the exercise bout was also attenuated (Hargreaves *et al.*, 1996b). The alterations in EPI that occur with changes in PV not only could impact on muscle metabolism, but they could also impact on the mobilization of both fatty acids from adipocytes and glucose from the liver (Wasserman & Cherrington, 1997). Changes in plasma EPI could also influence other regulatory hormones, such as insulin and glucagon, both which are also important in the regulation of extramuscular fuel sources (Wasserman & Cherrington, 1997). Since catecholamines are known to influence muscle metabolism (Febbraio *et al.*, 1998) and substrate mobilization (Wasserman & Cherrington, 1997) during prolonged exercise, it is possible that an attenuated catecholamine response, with an increase in PV, could influence both substrate mobilization and muscle metabolism.

Exercise induced dehydration, which results in a reductions in PV, has been observed to alter both muscle metabolism (Gonzalez-Alonso *et al.*, 1999; Hargreaves *et al.*, 1996b) and muscle blood flow (Gonzalez-Alonso *et al.*, 1998b; Gonzalez-Alonso *et al.*, 1999). Cycle ergometry (61% VO_2peak) performed in the heat, without fluid replacement, resulted in declines in PV and muscle blood flow, and increases in carbohydrate oxidation and lactate release across the leg (Gonzalez-Alonso *et al.*, 1998b; Gonzalez-Alonso *et al.*, 1999). These observations suggest that dehydration during prolonged exercise promotes an increased carbohydrate oxidation and lactate production. These alterations could be related directly to the decline in PV that occurs with dehydration.

Alterations in the fluid and electrolyte regulatory hormones (AVP and ANGII) and the catecholamines (EPI and NE) have the potential to alter the hemodynamic response to exercise (Buckwalter *et al.*, 1997; Stebbins & Symons, 1993; Stebbins & Symons, 1995).

Hypervolemia blunts the increased release of many of these hormones (Brandenberger *et al.*, 1989; Francesconi *et al.*, 1985; Melin *et al.*, 1997), possibly preventing some of the shunting of blood away from the splanchnic region during exercise (Stebbins & Symons, 1993; Stebbins & Symons, 1995). Increases in flow to the liver may allow for increase in the clearance of metabolities, increased delivery of gluconeogenic precursors, and improved regulation of glucose homeostasis. In addition, alterations in the release and delivery of other metabolically active hormones could also occur. A decline in hepatic blood flow could have the potential to alter hepatic metabolism due to splanchnic hypoxia (Rowell *et al.*, 1968). It has been suggested that splanchnic hypoxia could lead to an increase in hepatic glucose production via glycogenolysis (Rowell *et al.*, 1968). Increases in hepatic glucose production have been observed during acute heat-stress and exercise (Hargreaves *et al.*, 1996a). Alterations in the mobilization of extramuscular substrates can directly influence metabolism in the working muscle (Howlett *et al.*, 1998; Odland *et al.*, 1998; Wasserman & Cherrington, 1997). Therefore, alterations in splanchnic blood flow, possibly due to alterations in the circulating concentrations of fluid regulatory hormones, could alter substrate mobilization from the liver, which, in turn, could influence metabolism within the working muscle.

In summary, alterations in PV have the potential to impact on both substrate mobilization and substrate delivery during dynamic exercise. These alterations could result from changes in the response of a number of hormones, the sympathetic nervous system, and/or possible alterations in the distribution of blood flow during exercise.

STATEMENT OF THE PROBLEM:

The central objective of the following studies was to determine the effects of alterations in PV, both increases and decreases, on physiological responses to exercise. The physiological responses included cardiovascular, thermoregulatory, endocrine, substrate mobilization and muscle metabolism.

The specific hypotheses are:

1. Oxygen uptake during dynamic exercise are not altered by changes in PV, however, the relative contribution of the variables that contribute to VO_2 would be altered depending on whether an increase or decrease in PV occurred. Specifically, an increase in PV would lead to an increase in Qc and a decrease in a-vO_2 diff, whereas a decrease in PV would lead to an decrease in Qc and an increase in a-vO_2 diff.
2. Hypervolemia would lead to a stabilization of cardiovascular function, while hypovolemia would result in an increase in cardiovascular strain, during prolonged exercise.
3. Thermoregulation would not be influenced by hypervolemia, but hypovolemia would lead to an increase in thermoregulatory strain during prolonged dynamic exercise.
4. The endocrine response to dynamic exercise, including the fluid and electrolyte hormones, and the catecholamines, would be attenuated with hypervolemia (except for α-ANP). In contrast, hypovolemia would lead to a increase in the concentrations of these hormones (except for α-ANP). In the case of α-ANP, hypervolemia would result in an increase in this hormone, both at rest and during exercise. With hypovolemia, α-ANP concentrations would be decreased both at rest and during exercise.
5. An increased blood flow to the working limb will occur during exercise with hypervolemia compared to control conditions. This increase is postulated to compensate for the decrease

in CaO_2 and oxygen delivery, and therefore, exercise VO_2 will be defended. Hypovolemia is expected to result in a decrease in leg blood flow during exercise. The decrease in blood flow will occur in response to the increase in CaO_2. Therefore, O_2 delivery will be maintained despite the decrease in CaO_2.

6. Hypervolemia is postulated to result in an increased dependency on fat oxidation, whereas hypovolemia should lead to an increased dependency on carbohydrate metabolism, both at rest and during exercise.

CHAPTER II

THE INFLUENCE OF AN ACUTE INCREASE IN PLASMA VOLUME ON THE PHYSIOLOGICAL RESPONSES TO PROLONGED DYNAMIC EXERCISE.

ABSTRACT

To investigate the influence of an acute increase in plasma volume on the physiological responses to prolonged dynamic exercise, eight healthy untrained males performed 90 min of cycle ergometry at 62% of peak oxygen uptake (VO_2peak) twice, both prior to (CON) and following acute plasma volume expansion (PVX). Plasma volume expansion resulted in a calculated 15.8% increase in resting PV. During exercise following PVX, heart rate was lower ($P<0.05$) and stroke volume and cardiac output were higher ($P<0.05$). Mean arterial pressure and total peripheral resistance during exercise were not different between the two conditions. The exercise induced increase in core temperature was not affected by PVX. The prolonged exercise resulted in increases ($P<0.05$) in plasma vasopressin (AVP), plasma renin activity (PRA), aldosterone (ALD), atrial natriuretic peptide (α-ANP), glucagon, norepinephrine (NE) and epinephrine (EPI), while a decrease ($P<0.05$) in plasma insulin was observed. Plasma volume expansion blunted the increase ($P<0.05$) in AVP, PRA, ALD, NE and EPI, during the exercise itself. The concentration of α-ANP was increased ($P<0.05$) and the concentration of glucagon decreased ($P<0.05$) during exercise following PVX, an effect that could be attributed to the altered resting level of PV. No differences in osmolality (OSM), or in the serum concentrations of sodium (Na^+) and potassium (K^+) were observed with PVX. Glucose rates of appearance (R_a) and utilization (R_d), and glycerol R_a although progressively increased ($P<0.05$) with exercise, were not different between conditions. Similarly, no differences in substrate oxidation, either fat or carbohydrate, were observed between the two conditions. Muscle metabolism was also unaltered by PVX during exercise. These results indicate that acute PVX alters cardiovascular performance and the fluid and electrolyte regulatory hormone response without affecting the thermoregulatory response to prolonged cycle exercise. Despite

a calculated decrease in the O_2 content mediated by PVX, glucose kinetics, glycerol kinetics, substrate oxidation, and muscle metabolism were unaltered during moderate intensity dynamic exercise.

INTRODUCTION

One of the earliest and most pronounced acute adaptations observed with prolonged exercise training is an increase in plasma volume (PV) that is unaccompanied by changes in osmolality (OSM) (Convertino *et al.*, 1980a; Convertino *et al.*, 1983; Shoemaker *et al.*, 1998). Interestingly, training is also accompanied by changes in the cardiovascular, hormonal and muscle metabolic responses to short-term, submaximal steady-state exercise. Conceptually, increases in PV could result in alterations in all of these variables.

The adaptations in the cardiovascular response have been typically represented as an increase in stroke volume (SV) and a decrease in heart rate (HR) with unchanged cardiac output (Qc) and oxygen consumption (VO_2) at the same submaximal power output (Rowell *et al.*, 1996). Mean arterial blood pressure (MAP) and total peripheral resistance (TPR) also appear unaltered by the training (Convertino, 1991; Rowell *et al.*, 1996).

These training responses are believed to display their important consequences during prolonged submaximal exercise by providing for improved cardiovascular stability and thermoregulation (Convertino, 1991; Harrison, 1996; Nose *et al.*, 1990). According to current theory, the increase in PV elevates central circulatory blood volume and central venous pressure (CVP), allowing for greater cardiac filling and end-diastolic volume (Convertino, 1991; Nose *et al.*, 1990). The elevated CVP, in turn, acts as a reserve during prolonged effort, allowing SV and MAP to remain above the critical level needed to allow cutaneous blood flow and sweating to progressively increase.

The obstacle to this hypothesis is the need to maintain O_2 delivery to the working muscle. Existing evidence indicates that any reduction in arterial O_2 content (CaO_2), which would accompany PV expansion, would result in a compensatory increase in blood flow

(Curtis & Cain, 1992; Saltin *et al.*, 1986). As a result, it is possible that a substantial fraction of the elevated central blood volume may be directed toward the working muscle and not toward the cutaneous vasculature. If such is the case, the increase in PV noted with training may have minimal effect on thermoregulation during sustained effort. Although hypervolemia has been cited to have profound beneficial effects on cardiovascular stability and thermoregulation in hot environments (Nose *et al.*, 1990), the experimental evidence is not compelling.

The most definitive evidence of the role of PV in cardiovascular and thermoregulation comes from experiments in which saline was infused during the exercise. A saline infusion was observed to lower HR and esophageal temperature, and increase forearm blood flow (FBF) late in exercise in the heat, however, the infusion had no effect on exercise performed in a cool environment (Nose *et al.*, 1990). These effects have also been confirmed by Montain and Coyle (1992), who demonstrated that fluid ingestion during exercise in the heat, attenuated the time-dependent reduction in SV and increase in HR (cardiovascular drift) and core temperature increases and resulted in improved FBF. Interestingly, the infusion of Macrodex during exercise, a PV expander, although protecting cardiovascular stability, failed to alter FBF or core temperature (Montain & Coyle, 1992a).

Acute increases in PV of both 14% and 21%, have been observed to result in a persistent increase in Qc and SV, and a lower HR, throughout prolonged exercise conducted in a thermoneutral environment (Grant *et al.*, 1997). The increase in PV was without effect in reducing the increase in core temperature that was observed throughout the exercise. Even though a 2 hour exercise protocol was used, the light intensity, approximately 45% of maximal aerobic power (VO_2peak), may have been insufficient to promote a significant enough threat to

thermoregulation.

Prolonged dynamic exercise not only leads to increases in core temperature, but also results in alterations in the blood concentrations of a large variety of hormones. Among the most conspicuous of these changes are in the hormones involved in fluid and electrolyte balance and substrate mobilization, notably arginine vasopressin (AVP), plasma renin (PRA), serum aldosterone (ALDO), plasma atrial natriuretic peptide (α-ANP) (Fallo, 1993; Wade & Freund, 1990), epinephrine (EPI) and norepinephrine (NE) (Green *et al.*, 1991a; Mendenhall *et al.*, 1994), insulin and glucagon (Green *et al.*, 1987; Shoemaker *et al.*, 1998). The changes that occur in the fluid and electrolyte hormones appear to occur in response to disturbances in both the fluid compartments and electrolyte concentrations. Prolonged exercise leads to a decrease in plasma volume (PV) and an increase in OSM (Sawka & Coyle, 1999). The role of alterations in the fluid regulatory hormones is to minimize the potential imbalance in fluid volumes and OSM that occurs with prolonged exercise (Wade & Freund, 1990). The hormones have a number of different functions, ultimately acting on the cardiovascular system, sweat glands and the kidney. The net result is to alter hemodynamics, sweat rate and electrolyte excretion (Fallo, 1993).

Most of the fluid and electrolyte regulatory hormones also display a dependency on exercise intensity and duration (Wade & Freund, 1990). With increasing exercise intensity and duration the concentration of these circulating hormones is increased (Freund *et al.*, 1991; Freund *et al.*, 1988; Kanstrup *et al.*, 1992; Viru, 1992). The increase is likely a response to the greater disturbance in fluid and electrolyte homeostasis that occurs (Freund *et al.*, 1991; Viru, 1992).

Endurance training also leads to alterations in the hormonal response to exercise. For

example, Shoemaker et al. (1998) has previously observed an attenuation in ALD that was present during each of the three stages of a progressive standardized cycle exercise test. In contrast, AVP and EPI were blunted at the highest exercise intensity (75% VO_2peak). No effect of training was observed for α-ANP or NE (Shoemaker *et al.*, 1998). The 6 day training protocol resulted in higher PV during exercise, which occurred in the absence of changes in OSM. Collectively, the results suggest that the changes in the hormones associated with fluid and electrolyte homeostasis maybe dependent both on the nature of the adaptation that occurs with training, and on the nature of the exercise protocol employed to assess the training adaptations.

Acute PV expansion prior to prolonged exercise (Grant *et al.*, 1996) resulted in similar alterations in the hormonal response to those observed with endurance training (Shoemaker *et al.*, 1998). These included a pronounced reduction in ALD, which was evident early in exercise and persisted throughout. Reductions in EPI were also common in both studies. However, unlike the training study, reductions in NE, AVP and α-ANP occurred. In the case of AVP and α-ANP, the altered concentrations observed during exercise could be explained by the altered resting concentrations (Grant *et al.*, 1996). Since this experiment only resulted in an expansion of PV, without changes in OSM, it is unclear why the differences in hormonal responses between the studies were observed.

The differences in the exercise protocol used to examine the effects of training versus acutely mediated increases in PV may be important in explaining the different hormonal profiles that were observed. Unlike the training study which employed a progressive exercise protocol at 50%, 65% and 75% of VO_2peak, the acute PV expansion study used a protocol which consisted of 2 hours of light to moderate intensity (46% VO_2peak) cycle exercise.

Given the effects of exercise intensity on strain imposed on the fluid and electrolyte balance, hormonal profiles would be expected to change accordingly.

An acute increase in PV not only influences the hormonal response to exercise, but also appears to influence substrate turnover and oxidation. The effects of such an alteration on PV on glucose and free fatty acid (FFA) turnover and oxidation during exercise remains unclear. Conceptually, alterations in substrate turnover could occur either as a direct consequence of alterations in blood flow to the working muscles or as a result of changes in the hormonal response. Increases in blood flow could promote a greater substrate delivery to the working muscle, potentially increasing utilization. This may be particularly important in the case of plasma FFA where increased delivery, at least as mediated via increase blood concentrations, can increase oxidation at the expense of carbohydrates (CHO) (Romijn *et al.*, 1995). Moreover, an independent effect of an alteration in PV could be postulated as the basis of the changes in the hormonal response to exercise, which in turn could lead to changes in substrate turnover and oxidation.

The influence of acute increases in PV on glucose and free fatty acid turnover has been investigated using the stable isotopes [6,6-$^2H_2$] glucose and [$^2H_5$] glycerol (Phillips *et al.*, 1997). Although whole body lipolysis was reduced with PV expansion, as measured from glycerol rate of appearance (R_a), no effect on glucose kinetics, specifically glucose rate of appearance (R_a) and disappearance (R_d), were observed during exercise. The inability to detect a more substantial effect, particularly on fat oxidation, may have been due to the relatively low exercise intensity (approximately 45% VO_2 peak) that was employed (Phillips *et al.*, 1997). During light intensity exercise, plasma FFA is a dominant substrate oxidized (Romijn *et al.*, 1993). As exercise intensity increases, the mobilization and rate of utilization

of plasma FFA declines (Romijn *et al.*, 1993). Increasing plasma FFA availability to the working muscle has been shown to increase utilization at higher exercise intensities (Romijn *et al.*, 1995).

There is evidence to suggest that an acute increase in PV may also influence muscle metabolism, particularly early in prolonged exercise (Green *et al.*, 1997). As an example, a reduction in muscle lactate was observed with acute PV expansion at 3 min of low intensity exercise compared to a control condition. However, others using a similar model of acute PV expansion, but a higher intensity of exercise, have not observed any differences in muscle lactate (Watt *et al.*, 1999).

In the present study, our objective was to investigate the influence of hypervolemia on a number of physiological variables, including, cardiovascular and thermoregulatory behavior, fluid and electrolyte hormone responses, and substrate oxidation and turnover during prolonged exercise at a moderate intensity, namely 60% VO_2peak. We have hypothesized that:

1) The higher exercise intensity used in this study compared to our previous study (46% VO_2peak)(Grant *et al.*, 1997) will induce a greater challenge to cardiac function by reducing the time available for cardiac filling, thus promoting a greater dependency on central blood volume and CVP. Since the increased work load would cause a greater heat production and increased need for cutaneous blood flow, we have also hypothesized that hypervolemia will attenuate the cardiovascular drift and thermal load during prolonged exercise.

2) The higher exercise intensity will result in a more pronounced endocrine response to the exercise, than what is observed during low-intensity exercise following PV expansion (Grant *et al.*, 1996).

3) An increase in plasma FFA oxidation and a reduction in CHO oxidation will occur with plasma volume expansion. In addition, we have postulated that the acute increase in plasma volume will lead to a reduction in muscle lactate due to either the decreased reliance on CHO, or due to an increase in blood flow to the working muscle, to compensate for the hemodilution and decline in CaO_2 with hypervolemia.

METHODS

Participants. Eight untrained but active males were recruited and screened (by questionnaire) to ensure that they were healthy prior to entry into the study. Their age, body mass, peak aerobic power (VO_2 peak), and maximal heart rate (means ± SE) were 21.8 ± 0.5 yr, 80.6 ± 3.6 kg, 3.52 ± 0.12 $l \cdot min^{-1}$, and 192.3 ± 2.5 beats/min, respectively. The experimental procedures, risks and benefits were explained to each subject before written consent was obtained. Written consent was obtained after approval of the study by the Office of Human Research (University of Waterloo, Waterloo, ON).

Design and procedures. Measurements were made during a standardized prolonged cycling test at a moderate intensity of approximately 62% of VO_2peak on two separate occasions: under control conditions with no PV expansion (CON), and after an infusion of a solution of either Macrodex or Pentaspan (PVX). Failure to secure additional supplies of the Macrodex solution necessitated the use of Pentaspan solution in 3 participants. The Macrodex consists of 6% Dextran dissolved in normal saline while the Pentaspan is 10% Pentastarch dissolved in 0.9% sodium chloride. The exercise tests, conducted in ambient temperatures (22-24°C) and humidities (35-45%), were separated by a minimum of one week, and assigned in a randomized, single blind order. The two tests were performed at approximately the same time of day for each subject. No differences in environmental conditions (temperature and humidity) were observed between tests. Subjects consumed a standardized snack 4 to 6 h before each exercise test (Ensure liquid, 1045 kJ: 14.8% protein, 31.5% fat, and 53.7% carbohydrate; Ross Laboratories, Montreal, PQ). Only water (ad libitum) was allowed between consumption of the snack and arrival at the laboratory. All participants were instructed not to engage in any vigorous physical activity, except as required by the

experiment, and to follow a normal balanced diet.

Participants reported to the laboratory approximately 150 min before the exercise test. During this period, body weight was determined, an 18-gauge catheter was inserted into an antecubital vein, blood samples were collected, and the infusion of either the 6% Macrodex solution (Macrodex, Medisan Pharma Inc. Uppsala Sweden) or 10% Pentaspan solution (DuPont Pharma, Mississauga, ON. Canada) was initiated. In addition, a rectal thermometer (Model 401, Yellow Springs Instruments, S1, Yellow Springs, OH. USA) was inserted 20 cm past the anal sphincter for measurement of core temperature (Convertino, Greenleaf and Bernhauer, 1980). It has been previously shown (Montain and Coyle, 1992) that temperature measurements recorded with a rectal probe (T_{re}) produce the same time-dependent effects during exercise as esophageal (T_{es}) measurements. The Macrodex or Pentaspan was pre-warmed to ~35°C, and infused over an ~45 min period into the antecubital vein. The volume of infused solution was calculated on the basis of 6.7 $ml \cdot kg^{-1}$ body mass. This protocol resulted in a calculated 15.8 ± 2.2% expansion of PV, estimated from hematocrit (Hct) determinations (van Beaumont *et al.*, 1972) obtained after the subjects had been in a seated position for at least 15 min. The changes in PV were calculated using the following equation:

$$\%\Delta PV = \frac{100}{100 - HCTa} \times \frac{HCTa - HCTb}{HCTb} \times 100$$

where %ΔPV is the percentage change in plasma volume, HCTa and HCTb are the pre-hematocrit and post-hematocrit values, respectively (van Beaumont *et al.*, 1972). Under the conditions of the experiment, differences in Hct would be expected to provide an approximation of the changes in PV (van Beaumont *et al.*, 1972). For the CON condition, all procedures were identical, except that the participants received a sham infusion. The only

difference between the infusion and sham infusion was that during the sham infusion no fluid was allowed to enter the antecubital vein. During both conditions, the bottle of infusate was hidden from view of the participants. No differences were found between the resting pre-infusion Hct for the two conditions, suggesting that resting PV was comparable between conditions. Fluid consumption was not permitted either during the exercise test or during the preparatory period following arrival at the laboratory.

Following completion of the infusion, the *vastus lateralis* muscles of each leg were prepared for biopsies according to the procedures of Bergström et al. (1971). At this time, a total of four incisions were made in the two muscles and used for a pre-exercise sample, a sample at 3 min, a sample at 45 min, and a sample at the completion of the exercise. The pre-exercise biopsy was collected while the subject was resting in the supine position. Following collection of the resting muscle sample, the participants sat upright on an electronically braked cycle ergometer (Quinton Excalibur, Germany) and the seat height was adjusted to provide slight flexion at the knee. The same seat height was used for each subject for each condition. After approximately 20 min of quiet resting on the cycle ergometer, measurements of Qc, HR, and blood pressure (BP) were performed. In addition, measures were made over a 4-5 min period prior to 15, 30, 60 and 90 min of exercise. Qc measurements were performed as described previously (Green *et al.*, 1990), using the CO_2 rebreath technique (Collier, 1956), as modified in our laboratory (Inman *et al.*, 1985). As with our previous work with PV expansion, Qc values were not corrected for the change in CO_2 dissociation that occurs with the alteration in PV and hemoglobin concentration (Grant *et al.*, 1997). Standard electrocardiography techniques were used to monitor HR, and stroke volume (SV) was calculated from the Qc and HR determinations. Blood pressure was measured by

sphygmomanometer and cuff placed around the upper arm. Total peripheral resistance (TPR) was calculated as the ratio of Qc and the mean arterial blood pressure (MAP). MAP was calculated using the following equation:

$$MAP = \frac{SBP + 2(DBP)}{3}$$

where, SBP represents systolic blood pressure and DBP represents diastolic blood pressure. Whole body arterial-venous oxygen difference (a-vO_2 diff) was also calculated using VO_2 and Qc, according to the Fick equation (VO_2 = Qc x a-vO_2 diff).

VO_2 measurements. Each participant performed a progressive exercise test for measurement of VO_2peak, consisting of 15 W increments in power output each min until fatigue approximately one week before the first prolonged exercise test. Ventilatory and gas exchange measures were determined using an open-circuit system as previously described (Hughson *et al.*, 1980). Measurements of VO_2, using a 4-5 minute collection period, were made prior to (0 min) and intermittently (15, 30, 60 and 90 min) during each test.

Indirect calorimetry. Stoichiometric equations and appropriate caloric equivalents (Frayn, 1983; Wolfe, 1992) were used to calculated carbohydrate (CHO) and fat oxidation rates during exercise. It was assumed that the nitrogen excretion rate was 135 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (Romijn *et al.*, 1993) (Appendix II).

Blood Measurements. Arterialized venous blood samples were collected at standardized intervals during the prolonged exercise tests (0, 15, 30, 45, 60, 75, and 90 min). Samples were collected from a 20-guage catheter inserted into a heated dorsal hand vein of the arm opposite from the infusion catheter. Standard radioimmunoassay methods were used to determine the concentration of arginine-vasopressin (AVP) (Penninsula Laboratories Inc,

Belmont CA.), aldosterone (ALD) (Coat-A-Count, Diagnostic Products, Intermedico, Toronto, ON.), α-atrial natriuretic peptide (α-ANP) (Penninsula Laboratories Inc, Belmont CA.), plasma renin activity (PRA) (NEN, Billerica, MA), and plasma insulin and glucagon (Coat-A-Count, Diagnostic Products, Intermedico, Toronto, ON). For AVP, ANP and ALD, the methods of collection and analysis have been previously described (Grant *et al.*, 1996). Briefly, for ANP and AVP, 3 ml of whole blood was centrifuged (1,600 g) for 15 min in chilled polypropylene tubes containing EDTA, aprotinin (ANP), and heparin (AVP). The plasma was collected and stored at -80°C until analysis. For ALD, serum was collected from whole blood and stored at -80°C until analysis. As in our previous assays (Grant *et al.*, 1996), the intra-assay coefficients of variation ranged between 3% (ANP) and 5% (AVP and ALD). For PRA, 2 ml of whole blood were transferred into a chilled polypropylene tube containing heparin, the tubes were then centrifuged at 1,600 g and 4°C for 15 min, the plasma collected and stored at -80°C for later analysis. For analysis, samples were first thawed at 4°C and then 500 μl of each sample was aliquoted into two separate tubes. To each of these tubes, 10 μl of Dimercaprol solution, 10 μl of 8-hydroxyquinoline solution and 1 ml of maleate buffer were added and the tubes vortexed. Following mixing, 1 ml aliquotes from each tube were transferred to new tubes. The new tubes were then incubated for 1 hr at 37°C, while the remaining tubes were maintained at 4°C. At the end of the incubation, the tubes incubated at 37°C were matched with 4°C tubes and ANGI concentrations determined for all tubes by standard radio-immunoassay procedures. The differences between the concentrations at 37°C and 4°C allowed determination of PRA. The intra-assay coefficient of variation for PRA was 6.2%. All intra-assay coefficients of variation were within the described limits of the RIA

procedures. In addition, all antibodies used in the RIA procedures were 100% specific to the peptides of interest.

Plasma catecholamines, both epinephrine (EPI) and norepinephrine (NE) were determined using high-performance liquid chromatography (HPLC) and electrochemical detection (Waters 712 Wisp) according to the methods described by Weicher et al. (1984) and modified by Green et al. (1991a). For this assay, 3 ml of whole blood was collected in a chilled glass tube containing EDTA and glutathione as antioxidants. The plasma was removed and stored at -80°C after centrifugation (2,000 g) at 4°C for 15 min. In preparation for analysis, 1.5 ml of plasma were mixed with alumina for 10 min. The plasma was then removed and discarded and the alumina rinsed at least 5 times with distilled water. Perchloric acid was then added, the samples vortexed and then centrifuged (5,000 g) for 10 min at 4°C. The resulting supernatant was used for HPLC analysis.

All blood samples for a given assay and a given individual were analyzed in duplicate during the same analytical session. Hematocrit was also determined for each blood sample (in triplicate). Hematocrit was used to calculate changes in PV (van Beaumont *et al.*, 1972) both between conditions at rest, and during exercise within each condition. All Hct values were corrected for trapped plasma (0.96) and venous-to-whole body Hct differences (0.91). Serum osmolality, sodium (Na^+) and potassium (K^+) were determined in duplicate using an automated analyzer (NovaStat Profile Plus 9, Waltham, MA.).

The samples were also used for the determination of blood lactate, glucose, glycerol, and serum FFA. For determination of blood metabolites (lactate, glucose and glycerol), some blood was deproteinized using ice-cold perchloric acid. Following centrifugation for removal of the precipitated proteins, ice-cold $KHCO_3$ was added to neutralize the acid. For analysis of

serum FFA, 1.5 ml of blood was allowed to clot, the sample was then centrifuged and the resulting serum was stored until analysis. Blood samples for the determination of glucose and glycerol enrichment were added to heparinized tubes, the tube centrifuged and the resulting plasma was stored at for later analysis. All samples were stored at -80°C before analysis. Blood concentrations of lactate, glucose, glycerol, and serum FFA were determined using fluorometric methods, as described previously (Green *et al.*, 1991a).

Isotopes. The isotopes used for determination of substrate turnover, [6,6-$^2H_2$]glucose and [$^2H_5$]glycerol (98% enriched; MassTrace Inc., Woburn, MA. USA) were diluted in sterile 0.9% saline under aseptic conditions and were filtered through a 0.2 μm filter (Pall Gelman Sciences, Ann Arbor, MI. USA) before infusion. Both a priming dose of glucose (14 μmmol·kg^{-1}) and glycerol (1.3 μmmol·kg^{-1}) were administered before the initiation of the constant infusion (0.22 ± 0.03 μmmol·kg^{-1}·min^{-1} for [6,6-$^2H_2$]glucose; 0.1 ± 0.03 μmmol·kg^{-1}·min^{-1} for glycerol). Specific infusion rates were determined for each isotope by multiplying the infusate concentration (determined fluorometrically), by the infusion rate. When exercise was initiated, the infusion rate was doubled (compared to rest) for both isotopes. The specific infusion rates and infusates were kept constant for each subject over each condition to avoid biasing the data. (Please see Appendix I for a brief review of stable isotope methodology).

Tracer Enrichment. Glucose and glycerol enrichments were determined by making the pentaacetate derivative of glucose and the trimethylsilyl (TMS) derivative of glycerol. For glucose, 250 μl of plasma was deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The resulting supernatant was then deionized by passing it over a mixed-bed anion-cation exchange chromatographic column (AG-1-X8 and AG 50W-X8; Sigma Chemical). The

eluted fluid from this column was then lyophilized to dryness. To the lyophilized extract, 100 µl of a 2:1 solution of acetic anhydride and pyridine was added. Samples were then incubated at 80°C for 15 min. For glycerol, 1000 µl of plasma was deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The resulting supernatant was then deionized by passing it over a mixed-bed anion-cation exchange chromatographic column (AG-1-X8 and AG 50W-X8; Sigma Chemical). The eluted fluid from this column was then lyophilized to dryness. To the dry extract, 100 µl of 2:1 N,O-bis (trimethylsilyl)trifluroacetamide) (BSTFA) and pyridine. Extracts were then incubated at 80°C for 30 min.

GC-MS. Enrichment of each derivative was measured by injection of 1 µl of extract into a Hewlett-Packard 6890 GC oven (Fullerton, CA. USA). An HP-5 fused silica capillary column (15 m x 0.32 mm, 0.25 µm film thickness) was used in the GC oven (Hewlett-Packard, Fullerton, CA. USA). Mass analysis was performed using a Hewlett-Packard 5973 mass spectrometer, operating in EI mode. Data was processed using HP-Chemstation software (Hewlett-Packard, Fullerton, CA. USA).

Selected ion masses were monitored depending on which derivative was injected. Mass-to-charge ratios (m/z) were determined for 200, 202 and 205, 208 atomic mass units for determination of glucose and glycerol enrichment, respectively. These enrichments gave the expected M + 2/M + 0 or M + 3/M + 0 ratios, and thus indicated that there were no other interfering products or masses. All masses were also corrected according to a linear peak area standard curve for glucose and glycerol.

Muscle Measurements. For analysis of the muscle tissue, the rapidly extracted biopsy samples were immediately frozen in liquid N_2 and subsequently stored at -80°C.

Before analysis, samples were freeze-dried, weighed, and extracted for measurements of ATP, creatine phosphate (PCr), creatine (Cr), inorganic phosphate (Pi), glycogen and lactate using fluorometric techniques according to the procedures described by Harris et al. (1974) and modified by Green et al. (1987). With the exception of glycogen and lactate, the samples for a given individual were corrected to the total creatine content averaged for that individual (Sabina *et al.*, 1984). Lactate was not corrected because this compound exists both in the muscle and interstitial space (Katz *et al.*, 1986). In general, for the muscle data, samples for a given subject were analyzed in triplicate during the same analytical session under conditions as nearly identical as possible.

Calculations. Isotope kinetics, or the rate of appearance (R_a) and rate of disappearance (R_d) of glucose and glycerol were calculated using steady-state tracer dilution equation for rest (Phillips *et al.*, 1995). With exercise, the turnover and concentration of these metabolities change, therefore it was necessary to calculate substrate R_a and R_d according to the Steele equation as modified for stable isotopes, since the amount of tracer infused was no longer negligible (Wolfe, 1992).

The effective volumes of distribution were assumed to be 230 ml/kg for glycerol and 100 ml/kg for glucose. It has been previously observed that such volumes of distributions appear to be adequate under a variety of conditions (Phillips *et al.*, 1997; Phillips *et al.*, 1995; Phillips *et al.*, 1996b). Increasing (+50%) or decreasing (-50%) the volume of distribution had little effect on the results obtained and therefore it appears that these volumes of distribution were adequate.

The total amount of glycerol released during exercise is the sum total of release from peripheral and intramuscular lipolysis. Since the activity of glycerol kinase within skeletal

muscle is negligible (Newsholme & Taylor, 1969; Romijn *et al.*, 1993), and the majority of glycerol reesterification occurs in the liver during exercise (Romijn *et al.*, 1993), we have assumed that glycerol R_a is representative of whole body lipolysis. Therefore, using these assumptions, three times the rate of lipolysis should represent the maximum FFA availability, since there are very few monoglyceride or diglyceride pools with skeletal muscle or adipocytes (Romijn *et al.*, 1993).

We also estimated the minimal rate of muscle glycogen oxidation, as previously described (Phillips *et al.*, 1997). Briefly, whole body carbohydrate (CHO) oxidation was calculated from indirect calorimetry and we assumed that 100% of glucose R_d was oxidized during exercise (Phillips *et al.*, 1995; Romijn *et al.*, 1993). In situations where plasma glucose is converted directly to lactate instead of being completely oxidized, this method will lead to an underestimation of the minimal rate of glycogen oxidation (Romijn *et al.*, 1993).

Statistics. Data were analyzed using a two-way repeated measures ANOVA for experimental condition (CON, PVX) and time (0-90 min). When a significant interaction was found ($P<0.05$), the Newman-Keul post-hoc technique was used to determine pairwise differences. Where only main effects occurred, a summary is provided in the legends of the tables and figures. Body weight changes and whole body lipolysis were analyzed using paired t-tests. To examine the relationship between ΔPV, ΔOSM, ΔNa^+, ΔK^+ and the hormonal measures and the interrelationship between the hormonal changes, Pearson product-moment correlation coefficients (r) were calculated. The relationships between these variables were determined during exercise within each condition (PVX and CON). To examine the independent effects of ΔPV and ΔOSM on the hormonal changes and the interrelationships between the hormones with the potential effects of ΔPV and ΔOSM removed, second-order

partial correlational procedures were used. The relationships are based on all measurements within a condition during exercise, collapsed across time. All data are expressed as means ± standard error of the mean.

RESULTS

PV Changes. The infusion of 10% Pentaspan or 6% Macrodex induced a 15.8 ± 2.2 % increase in resting PV. The prolonged exercise resulted in a decrease in PV for both conditions that occurred within the first 15 min of the exercise (Figure 2.1). No further decreases were observed for the remainder of the exercise. PV was consistently higher in the PVX condition than the CON condition at rest and at each exercise time point (Figure 2.1).

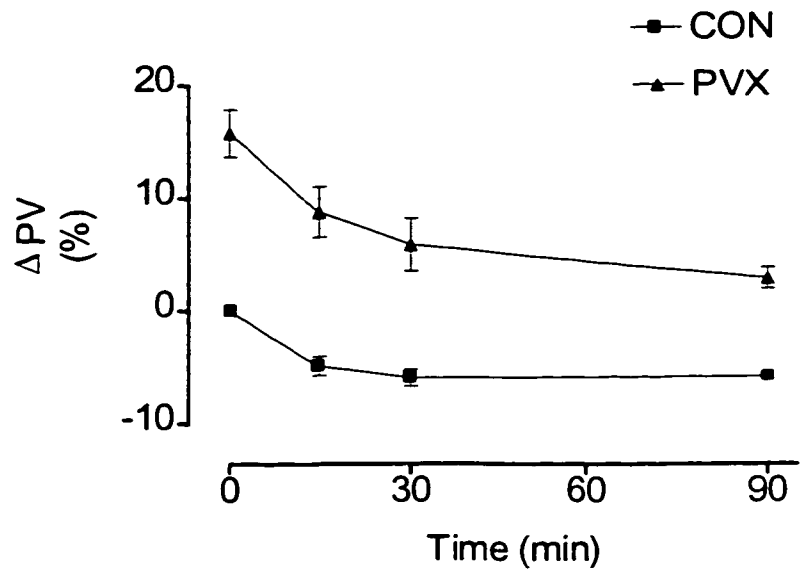

Figure 2.1. *Changes in plasma volume (PV) with exercise during control (CON) and hypervolemia (PVX). Main effects (P<0.05) of both condition and time were found. For condition, PVX>CON. For time, 0>15, 30, 60 min.*

Submaximal VO_2 and Cardiovascular Function. Acute PV expansion had no effect on VO_2, VCO_2, or RER during the prolonged exercise (Table 2.1). However, the prolonged exercise did result in a slight but significant drift in VO_2. When averaged over both conditions, an increase of 7% was observed between 15 and 90 min of exercise (2.07 ± 0.08 vs. 2.22 ± 0.09 $l \cdot min^{-1}$).

Cardiac function was altered both at rest and during exercise in PVX compared to CON (Table 2.1, Figure 2.2). At rest, PVX resulted in a 34% greater increase in Qc. Approximately the same absolute increase in Qc persisted throughout the exercise. The greater increase in Qc was also accompanied by a higher SV during both rest and exercise (Figure 2.2). During exercise, time-dependent changes were not observed for either Qc or SV. In contrast to the increase in SV and Qc, PVX resulted in a decrease in HR at rest and during exercise compared to CON (Figure 2.2). Exercise also resulted in a significant upward drift in HR over time.

The a-vO_2 diff, calculated from the Fick equation, increased from rest to exercise in both conditions (Table 2.1). Although there was a trend for the a-vO_2 to increase with exercise during CON, this was not significant. PVX did not alter the a-vO_2 diff either at rest or during exercise.

MAP was similar between conditions both at rest and during exercise (Table 2.2). The onset of exercise did result in a significant increase in MAP, the magnitude of which did not change with exercise duration. Exercise also resulted in a decrease in TPR as compared to rest for both conditions (Table 2.2), and this decrease persisted throughout the exercise.

Table 2.1. *VO_2, VCO_2, respiratory exchange ratio, cardiac output and arterio-venous oxygen difference during exercise following hypervolemia.*

	Time (min)				
	0	15	30	60	90
VO_2 ($l \cdot min^{-1}$)					
CON	0.34 ± 0.00	2.10 ± 0.10	2.16 ± 0.10	2.12 ± 0.10	2.18 ± 0.10
PVX	0.39 ± 0.00	2.04 ± 0.10	2.14 ± 0.10	2.24 ± 0.10	2.26 ± 0.10
VCO_2, l/min					
CON	0.35 ±0.02	2.07 ± 0.06	2.11 ± 0.06	1.98 ± 0.06	2.06 ± 0.06
PVX	0.34 ± 0.02	2.04 ± 0.10	2.09 ± 0.10	2.00 ± 0.09	2.08 ± 0.07
RER					
CON	0.86 ± 0.02	0.99 ± 0.01	0.98 ± 0.01	0.95 ± 0.01	0.95 ±0.02
PVX	0.88 ± 0.03	1.00 ± 0.02	0.98 ± 0.03	0.92 ± 0.02	0.92 ± 0.02
Qc($l \cdot min^{-1}$)					
CON	5.33 ± 0.81	16.5 ± 0.5	16.0 ± 1.1	15.9 ± 0.6	16.2 ± 0.8
PVX	7.16 ± 0.70	17.4 ± 0.4	17.7 ± 0.8	18.2 ± 0.9	18.6 ± 0.8
a-vO_2 diff ($ml \cdot l^{-1}$)					
CON	69.9 ± 5.9	127.3 ± 3.2	133.6 ± 5.9	130.4 ± 3.3	134.8 ± 3.8
PVX	60.6 ± 7.5	122.1 ± 6.5	131.3 ± 8.3	119.6 ± 4.4	122.1 ± 5.3

Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion. VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; Qc, cardiac output; a-vO_2 diff, arterio-venous oxygen differences. Main effects for time ($P<0.05$) were found for VO_2, VCO_2, RER, Qc, and a-vO_2 diff while main effects for condition ($P<0.05$) were found for Qc and a-vO_2 diff. For time, VO_2, 0<15, 30, 60 and 90 min; 15<90 min; VCO_2, 0<15, 30, 45, 60, and 90 min; RER, 0<15, 30, 45, 60, and 90 min Qc, 0<15, 30, 60, and 90 min; a-vO_2 diff, 0<15, 30, 60, and 90 min. For condition, Qc, PVX >CON; a-vO_2 diff, CON>PVX.

Table 2.2. *Effects of induced hypervolemia on mean arterial pressure and total peripheral resistance during exercise.*

	Time (min)				
	0	15	30	60	90
MAP(mmHg)					
CON	89.7 ± 1.3	93.9 ± 1.4	95.7 ± 0.9	98.7 ± 1.1	100.8 ± 1.1
PVX	87.2 ± 1.4	93.4 ± 1.4	96.8 ± 1.8	98.5 ± 1.2	97.9 ± 1.2
TPR($mmHg \cdot l^{-1} \cdot min^{-1}$)					
CON	17.1 ± 1.2	5.7 ± 0.2	5.8 ± 0.3	6.2 ± 0.3	6.1 ± 0.3
PVX	13.2 ± 1.7	5.5 ± 0.2	6.0 ± 0.4	5.5 ± 0.3	5.2 ± 0.3

Values are means ± SE; n=8. MAP, mean arterial pressure; TPR, total peripheral resistance; CON, Control; PVX, plasma volume expansion. A main effect ($P<0.05$) for time was found for MAP and TPR. For MAP, 0<15=30=60=90 min. For TPR, 0>15,30,60 and 90 min.

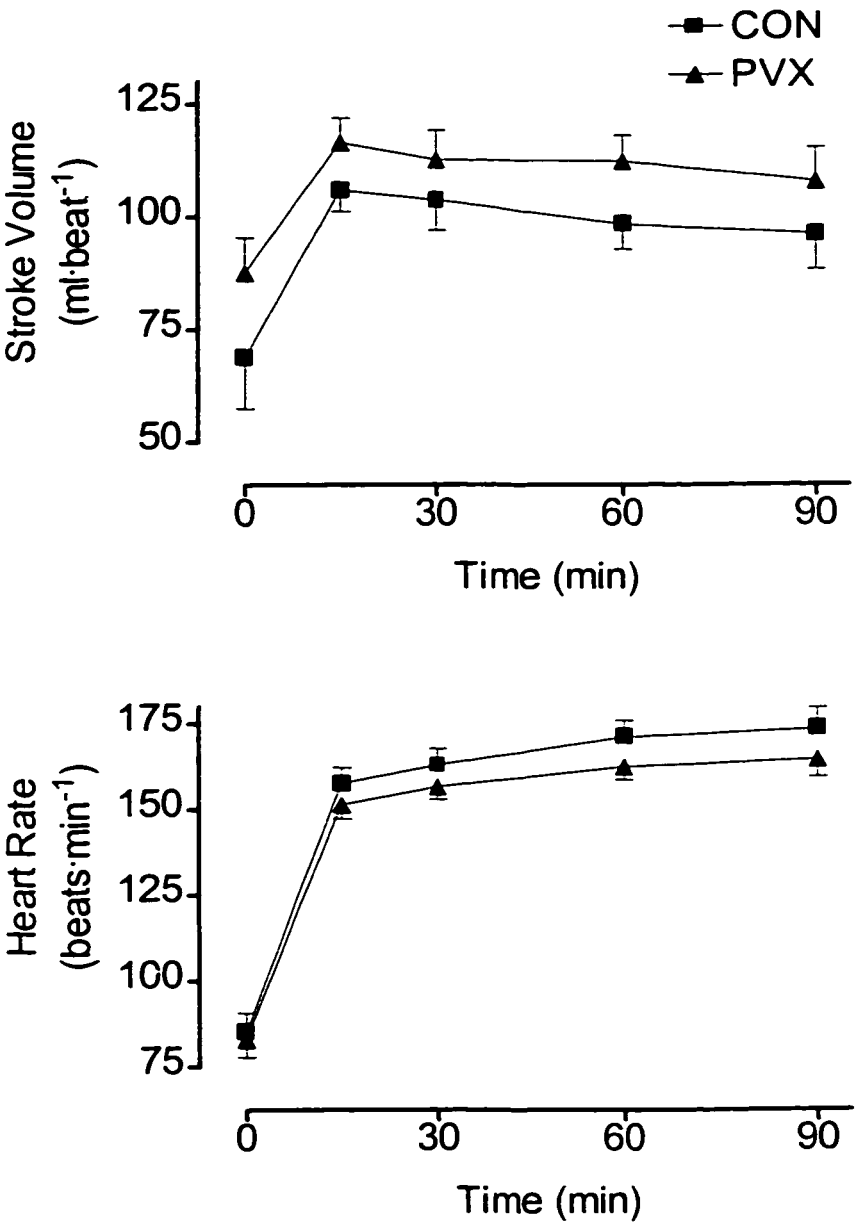

Figure 2.2. *Stroke volume (A) and heart rate (B) with exercise during control (CON) and hypervolemia (PVX). Main effects ($P<0.05$) of both condition and time were found. For condition, SV, CON<PVX; HR, CON>PVX. For time, SV and HB 0<15, 30, 60, 90 min; for HR 15<60, 90 min, and 30, 60<90 min.*

Rectal temperature. In both conditions, the exercise resulted in a steady progressive rise in T_{re}(Figure 2.3). PVX had no effect on the magnitude of the increase in T_{re}. In general, 90 min of exercise resulted in a 1.5 ± 0.02 and 1.5 ± 0.01°C increase in T_{re}, for both CON and PVX, respectively. The greatest increase occurred over the first 15 min of exercise for both conditions (~0.5°C, for both conditions). Rectal temperatures at rest prior to the exercise were 37.3 ± 0.1 and 37.2 ± 0.1°C for the CON and PVX, respectively.

Each condition also resulted in a similar decrease in body mass. The average decline in body weight during exercise was 1.2 ± 0.2 kg and 0.9 ± 0.1 kg for CON and PVX, respectively. These decreases translated into a 1.6 ± 0.2% decline in body mass for the CON condition and a 1.2 ± 0.1% decline for PVX. No fluid replacement was permitted during the exercise.

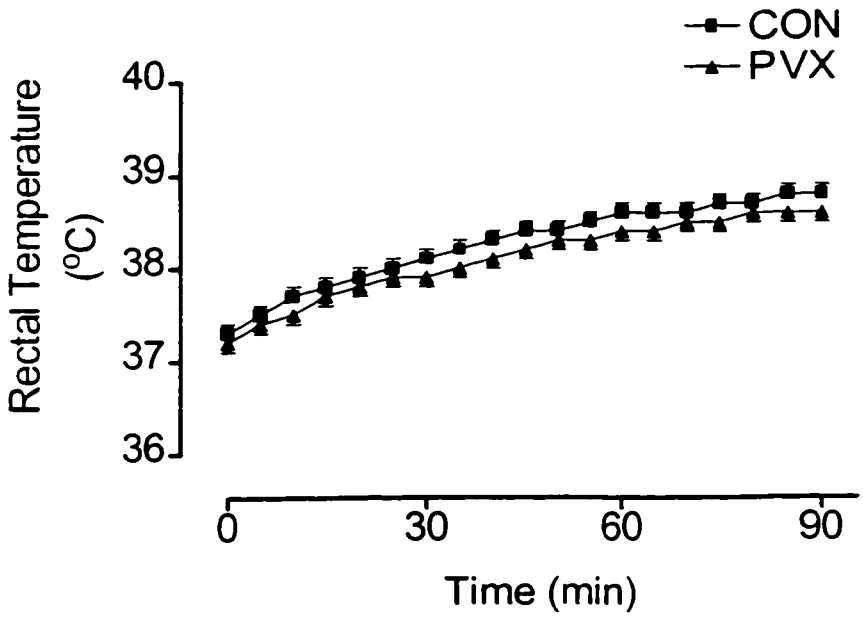

Figure 2.3. Core temperature responses to exercise during control (CON) and hypervolemia (PVX). Only a main effect ($P<0.05$) of time was found. For time, 0<5<10, 15<20<25<30, 35, 40, 45<50, 55<60, 65 ,70<75, 80<85<90 min.

Serum osmolality and electrolytes. Serum osmolality (OSM) was not different between the two experimental conditions (Table 2.3). The exercise led to an increase in OSM from rest to 30 min. regardless of condition. This increase in OSM was maintained for the duration of the exercise. Exercise resulted in an increase in both serum Na^+ and K^+ (Table 2.3). No differences were observed for either Na^+ or K^+ between the two conditions, both at rest and during exercise.

Table 2.3. *Effects of acute plasma volume expansion and exercise on serum osmolality, sodium and potassium.*

	Time, min			
	0	30	60	90
OSM(mOSM·kg^{-1} H_2O)				
CON	287.0 ± 0.8	292.9 ± 2.0	291.1 ± 1.9	290.2 ± 1.7
PVX	284.4 ± 0.8	290.3 ± 0.8	288.9 ± 0.8	289.7 ± 0.9
Na^+ (mmol·l^{-1})				
CON	146.8 ± 0.7	149.0 ± 1.0	149.1 ± 1.7	148.3 ± 0.8
PVX	144.6 ± 0.5	148.1 ± 0.5	147.4 ± 0.6	149.0 ± 0.9
K^+ (mmol·l^{-1})				
CON	4.4 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.7 ± 0.3
PVX	4.3 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.3 ± 0.2

Values are means ± SE; n=8. OSM, osmolality; Na^+, sodium; K^+ potassium; CON, control; PVX, plasma volume expansion. Main effects ($P<0.05$) of time were found for OSM, Na^+ and K^+.

Blood hormones. In general, in the CON condition, progressive increases in NE were observed with the initial increase evident by 15 min of exercise (Figure 2.4). Plasma NE response to the exercise was blunted by the PVX condition as compared to the CON. There was a trend for NE to be lower at all of the exercise time points, however, this trend only became significant at 45 min, and remained significant for the duration of the exercise. The effect of acute PV expansion and exercise on plasma EPI was not as pronounced as observed

with NE (Figure 2.4). The exercise induced increase in EPI was also blunted during the PVX condition, but only very late in the exercise, namely at 90 min. Although initial increases in were observed in EPI by 15 min, no further increases were observed until 75 min of exercise. At 90 min, further increases occurred.

With increasing duration of exercise, there was a trend for an increase in plasma glucagon to occur ($P = 0.06$) (Table 2.4). This was accompanied by a significant decline in plasma insulin (Table 2.4) for both conditions. As expected, the lowest values for glucagon were observed at rest and the greatest values were observed at 90 min. The opposite was observed with insulin, such that the greatest values were observed at rest, with a rapid decline observed by 30 min of exercise and no further changes as the exercise progressed. A main effect of condition was observed for glucagon, with PVX being significantly lower than CON. No effect of experimental condition was observed for insulin.

PRA was altered both by exercise and PV expansion (Figure 2.5). Values were similar between the two conditions at rest, however, as the exercise progressed, a greater increase was observed for CON as compared to PVX. For exercise, an increase in PRA was evident by 30 min in CON, and by 60 min in PVX. PRA was greater for CON at 30, 60 and 90 min of exercise as compared to PVX.

Table 2.4. *Effect of exercise and plasma volume expansion on plasma glucagon and insulin.*

	Time (min)			
	0	30	60	90
Glucagon, $pg \cdot ml^{-1}$				
CON	77.4 ± 3.3	95.3 ± 7.9	115.6 ± 17.5	125.0 ± 32.5
PVX	71.4 ± 4.4	82.1 ± 7.5	96.1 ± 10.5	109.3 ±22.1
Insulin, $\mu IU \cdot ml^{-1}$				
CON	18.9 ± 4.3	9.7 ± 0.7	6.5 ± 0.2	5.7 ± 0.4
PVX	20.2 ± 4.8	10.7 ± 1.7	6.6 ± 0.6	5.9 ± 0.6

Values are means ± SE (n=7 for Glucagon, n=8 for Insulin). CON, control; PVX, plasma volume expansion. A main effect for condition was observed for glucagon (P<0.05); PVX was less than CON (P<0.05). A significant main effect for time was observed for insulin (P<0.01); 0 > all other time points.

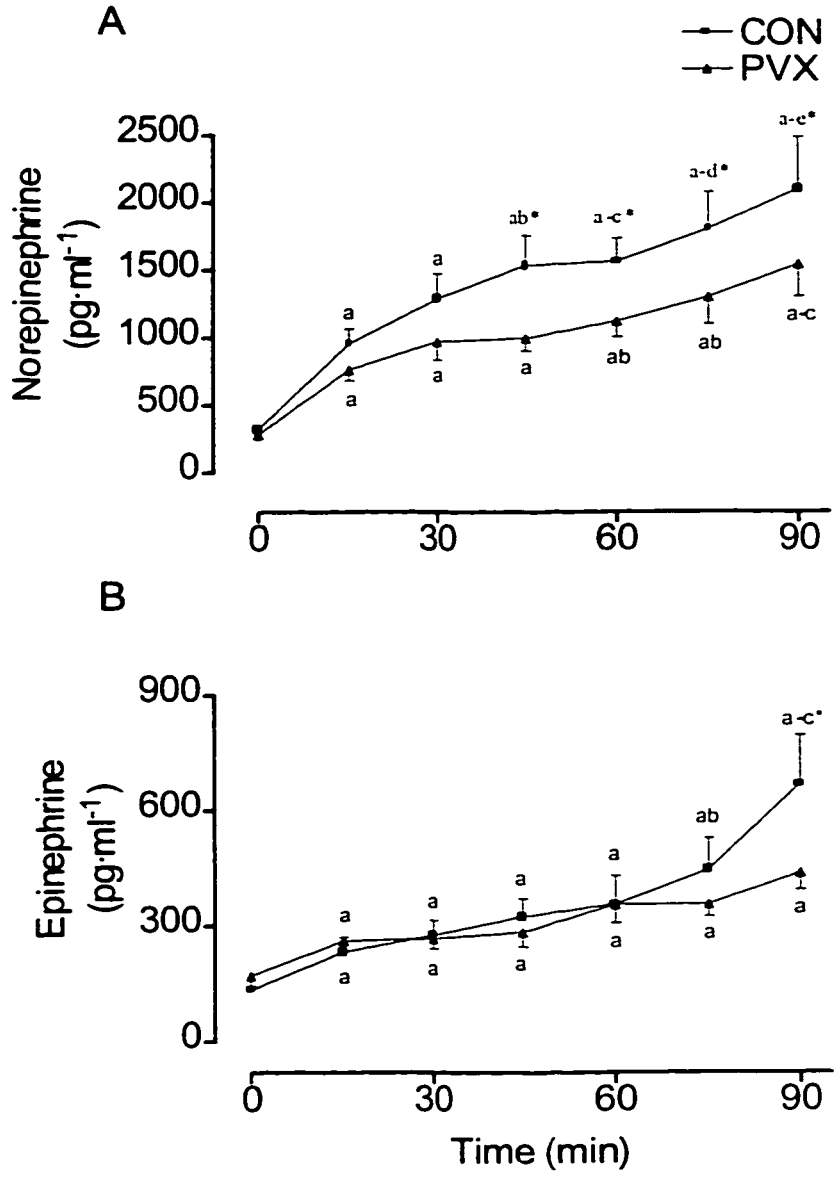

Figure 2.4. *Effects of acute plasma volume expansion on plasma norepinephrine and epinephrine concentrations. Values are means ± SE; n = 8 subjects.. *Significantly different from PVX (P < 0.05). [a]significantly different from 0; [b]significantly different from 15 min; [c]significantly different from 30 min; [d]significantly different from 60 min; [e]significantly different from 75 min, (P<0.05).*

Plasma ALD was also altered by both exercise and PV state (Figure 2.6). In the CON condition, an increase of ALD was found by 30 min of exercise, with progressive increases noted at 30, 60, and 90 min. For the PVX condition, initial increases were not observed until 60 min of exercise. PVX attenuated the ALD response to exercise as compared to CON at all exercise time points.

Plasma AVP was also altered by exercise and hypervolemia (Figure 2.7). With exercise resulting in an increase in AVP that was observed at 60 and 90 min. Plasma AVP was greater for CON at 60 and 90 min of exercise as compared to PVX.

As with plasma ALD and AVP, both exercise and acute PV expansion altered plasma α-ANP concentration (Figure 2.8). In the case of exercise, an increase was observed from 0 to 30 min, and persisted for the remainder of the exercise. PVX resulted in a general increase in circulating α-ANP concentrations. This difference could be explained by increased resting levels which persisted throughout exercise.

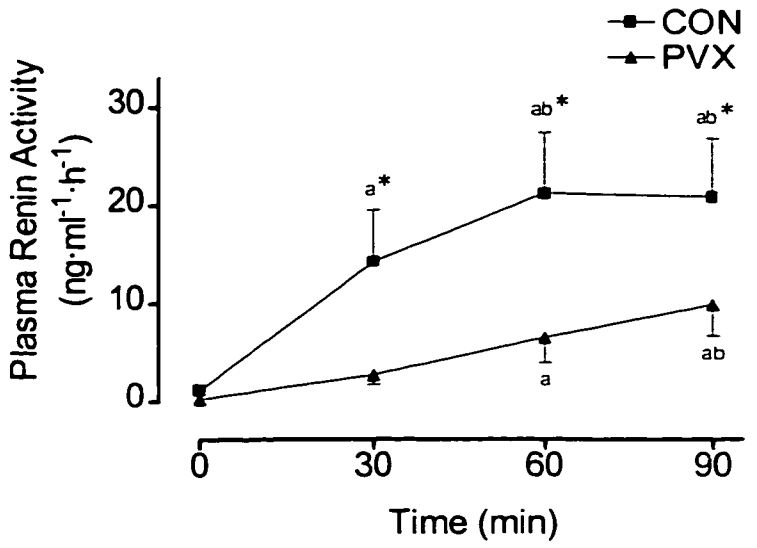

Figure 2.5. *Effects of acute plasma volume expansion on plasma renin activity. Values are means ± SE; n = 7 subjects. *Significantly different from PVX (P < 0.05). [a]Significantly different from 0; [b]Significantly different from 30 min, (P<0.05).*

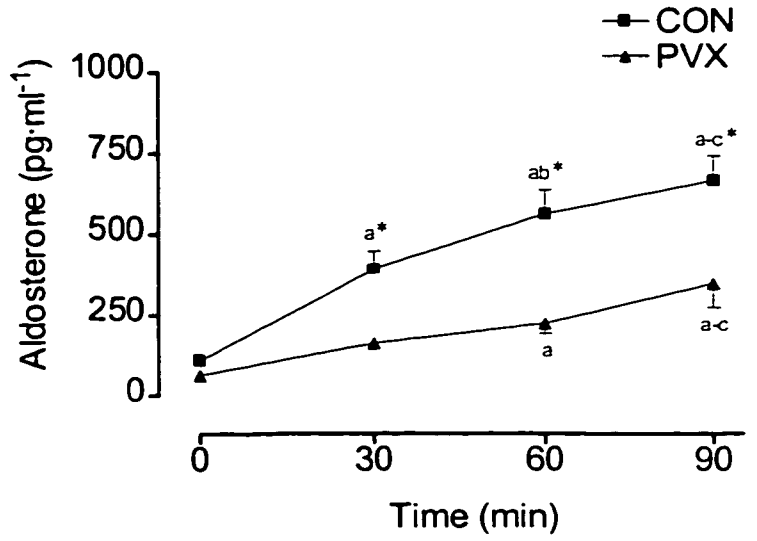

Figure 2.6. *Effect of acute plasma volume expansion on exercised-induced changes in serum aldosterone. Values are means ± SE; n = 8 subjects. *Significantly different from PVX (P<0.05). [a]Significantly different from 0; [b]Significantly different from 30 min; [c]Significantly different from 60 min, (P<0.05).*

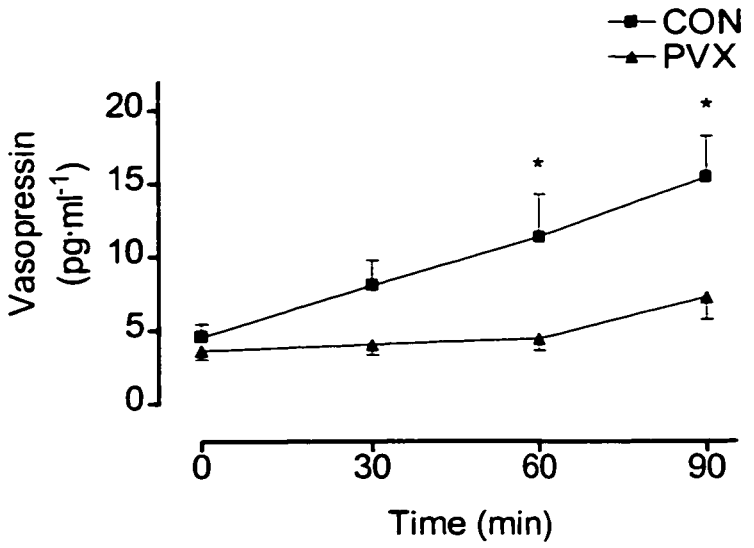

Figure 2.7. *Effect of acute plasma volume expansion on exercise-induced changes in plasma arginine vasopressin. Values are means ± SE; n = 7 subjects. *Significantly different than PVX. Main effects for both time and condition (P < 0.05) were found. For time, O < 60 and 90 min. For condition, CON > PVX.*

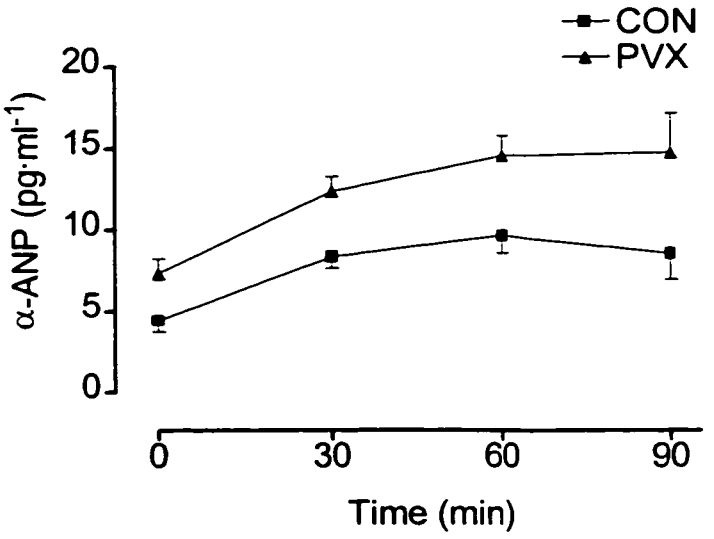

Figure 2.8. *Effects of exercise and acute plasma volume expansion on plasma α-atrial natriuretic peptide (α-ANP). Values are means ± SE; n=7 subjects. Main effects for both time and condition (P < 0.05) were found. For time, 0 < 30, 60 and 90 min. For condition, PVX > CON.*

Since changes in OSM and PV have been implicated in the changes that occur in the hormonal concentrations during exercise, we have examined these relationships, both overall and with the effects of one held constant (Table 2.5, Table 2.6). Second-order (partial) correlations were necessary since the change in OSM and the change in PV during exercise were inversely correlated. For the CON condition, r = -0.60 and for the PVX condition r = -0.83 were found. Overall, inverse relationships were found between the changes in PV and the changes in ALD (CON), α-ANP (PVX) and NE (PVX). When the effects of changes in OSM were held constant, only NE remained correlated with PV. Changes in OSM, during exercise were positively correlated with both changes in α-ANP (CON) and NE (CON and PVX). With the exception of NE (CON), no correlation was found between these two variables when the effects of the changes in PV were held constant.

We have also conducted a similar correlational analyses for the changes in K^+ and Na^+ during exercise and the changes in the hormones examined (Table 2.7, Table 2.8). The changes in K^+ during exercise were directly correlated with changes in Na^+. A significant positive relationship was found for NE and Na^+ (PVX), however, when the effect of K^+ was eliminated, the relationship was not significant. In addition, changes in ALD were found to relate to changes in Na^+ but only when the effect of K^+ was controlled for. In the case of changes in K^+ during exercise, positive relationships were found with the changes in NE both overall and when Na^+ was isolated (PVX).

Interrelationships between changes in the different hormones during exercise were only found for PRA and ALD (CON), PRA and NE (CON), and ALD and NE (CON). In all cases, the relationships were positive. When the effects of changes in both PV and OSM were held constant, the relationships between PRA and ALD (CON), PRA and NE (CON), and ALD and

NE (CON) persisted. These same relationships also persisted when the effects of changes in Na^+ and K^+ were held constant. In addition, changes in AVP were found to be inversely correlated with changes in EPI (CON). Changes in α-ANP (CON) and EPI (CON) were also found to be directly related to the changes in NE when the Na^+ and K^+ effects were isolated.

Table 2.5. *Acute plasma volume expansion and the relationships between the changes in plasma volume, plasma osmolality, and blood hormones.*

	PV		OSM	
	Overall	Partial (OSM)	Overall	Partial (PV)
OSM				
CON	-0.60*			
PVX	-0.83**			
AVP				
CON	-0.10	-0.26	-0.14	-0.24
PVX	-0.22	0.12	0.34	0.29
ALD				
CON	-0.56*	-0.37	0.51	0.26
PVX	-0.21	-0.14	0.15	-0.04
PRA				
CON	-0.35	-0.06	0.51	0.40
PVX	0.27	0.17	-0.21	0.02
α-ANP				
CON	-0.47	-0.18	0.58*	0.42
PVX	-0.54*	-0.27	0.49	0.09
EPI				
CON	-0.33	-0.16	0.34	0.19
PVX	-0.09	0.34	0.32	0.44
NE				
CON	-0.63*	-0.29	0.83**	0.73**
PVX	-0.85**	-0.75**	0.63**	-0.27

*Values represent first-order correlation coefficients (overall) and second-order (partial) correlation coefficients with effects of changes in osmolality (OSM) and plasma volume (PV) held constant. *Significantly different from 0 (P<0.05). **Significantly different from 0 (P<0.01). AVP, arginine vasopressin; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; PVX, plasma volume expansion.*

Table 2.6. *Acute plasma volume expansion and the interrelationships between the changes in fluid and electrolyte hormones with the effects of changes in osmolality and plasma volume held constant.*

	AVP	PRA	ALD	ANP	EPI	NE
AVP						
CON		-0.02	0.05	0.08	-0.60*	-0.26
PVX		-0.01	-0.12	-0.25	-0.41	-0.40
PRA						
CON			0.81**	-0.34	0.06	0.78**
PVX			0.29	-0.51	-0.27	-0.01
ALD						
CON				-0.32	-0.01	0.61*
PVX				-0.21	-0.16	0.50*
α-ANP						
CON					0.30	-0.03
PVX					0.16	-0.13
EPI						
CON						0.38
PVX						0.16
NE						
CON						
PVX						

*Values represent second-order correlation coefficients (r) with effects of changes in osmolality (OSM) and plasma volume (PV) held constant. AVP, arginine vasopressin; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; PVX, plasma volume expansion. *Significantly different from 0 (P<0.05). **Significantly different from 0 (P<0.01).*

Table 2.7. *Acute plasma volume expansion and the relationships between the changes in serum sodium, potassium, and blood hormones.*

	Na^+		K^+	
	Overall	Partial (K^+)	Overall	Partial (Na^+)
K^+				
CON	0.62*			
PVX	0.68**			
AVP				
CON	0.10	0.27	-0.17	-0.30
PVX	0.38	0.43	0.13	-0.21
ALD				
CON	0.45	0.67**	0.12	-0.05
PVX	0.22	-0.02	0.40	0.32
PRA				
CON	0.51	0.29	0.15	-0.12
PVX	-0.41	-0.39	-0.19	0.01
α-ANP				
CON	0.05	-0.11	0.13	0.16
PVX	0.49	0.45	0.25	-0.07
EPI				
CON	-0.08	-0.08	0.14	0.10
PVX	0.33	0.43	-0.03	-0.36
NE				
CON	0.53	0.52	0.48	0.11
PVX	0.63*	0.03	0.86**	0.76**

*Values represent first-order correlation coefficients (overall) and second-order (partial) correlation coefficients with effects of changes in sodium (Na^+) and potassium (K^+) held constant. AVP, arginine vasopressin; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; PVX, plasma volume expansion. *Significantly different from 0 ($P<0.05$). **Significantly different from 0 ($P<0.01$).*

Table 2.8. *Acute plasma volume expansion and the interrelationships between the changes in fluid and electrolyte hormones with the effects of changes in serum sodium and potassium held constant.*

	AVP	PRA	ALD	α-ANP	EPI	NE
AVP						
CON		-0.22	-0.05	0.07	-0.50	-0.22
PVX		-0.15	-0.12	-0.34	-0.47	-0.40
PRA						
CON			0.78**	-0.09	0.40	0.82**
PVX			0.33	-0.44	-0.09	-0.01
ALD						
CON				0.16	0.35	0.75**
PVX				-0.17	-0.12	0.20
α-ANP						
CON					0.44	0.54*
PVX					0.00	0.34
EPI						
CON						0.62*
PVX						0.16
NE						
CON						
PVX						

*Values represent second-order correlation coefficients (r) with effects of changes in serum sodium (Na^+) and potassium (K^+) held constant. AVP, arginine vasopressin; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; PVX, plasma volume expansion. *Significantly different from 0 (P<0.05). **Significantly different from 0 (P<0.01).*

Muscle metabolites. In general, PVX had little effect on any of the muscle metabolite measures, during exercise (Table 2.9). No differences were observed with ATP during rest or exercise for both conditions. However, exercise did lead to a reduction in PCr and an increase in Cr and Pi (P<0.05), these changes were similar between the two conditions. Similar alterations in muscle lactate were observed between the two conditions. Due to technical difficulties rest values could not be obtained.

Blood metabolite concentration and turnover. Arterialized venous blood lactate concentrations peaked between 15 and 30 min of exercise and then declined during the remaining 60 min of exercise (Figure 2.9). No differences in lactate concentration were found between the conditions. Serum FFA concentration (Figure 2.10) decreased with the onset of exercise. After the initial decline, serum FFA progressively increased with the time of exercise. By 75 min of exercise, serum FFA levels returned to resting values. As with plasma lactate, PVX had no effect on the serum FFA response to exercise as compared to CON.

Prolonged moderate, intensity exercise led to a progressive decline in plasma glucose (Figure 2.11A). The PV expansion had no effect on the response of plasma glucose to the exercise. Glucose R_a increased in the rest-to-exercise transition and continued to increase with increasing exercise duration (Figure 2.11B). As with plasma glucose, glucose R_a was not affected by PVX.

Exercise led to an increase in plasma glycerol concentration (Figure 2.12A). PV expansion had no effect on the changes that occurred in plasma glycerol concentration during exercise. Glycerol R_a increased at the onset of exercise, and then remained stable as the exercise continued (Figure 2.12B). As with plasma glycerol concentration, PVX had no effect on glycerol R_a, either at rest or during exercise. The integrated lipolytic response during exercise, expressed as the area under the glycerol R_a vs. time curve from rest to 90 min, was not different between the two conditions (Figure 2.13).

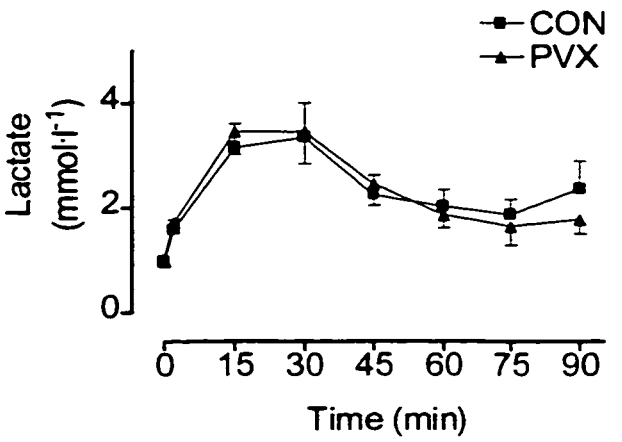

Figure 2.9. *Blood lactate concentration with exercise and acute plasma volume expansion. Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion. A main effect of time was found (P <0.01); 0 min < all exercise times, 0, 2 < 15, and 30 min, and 15, 30 > 60, 75 and 90 min.*

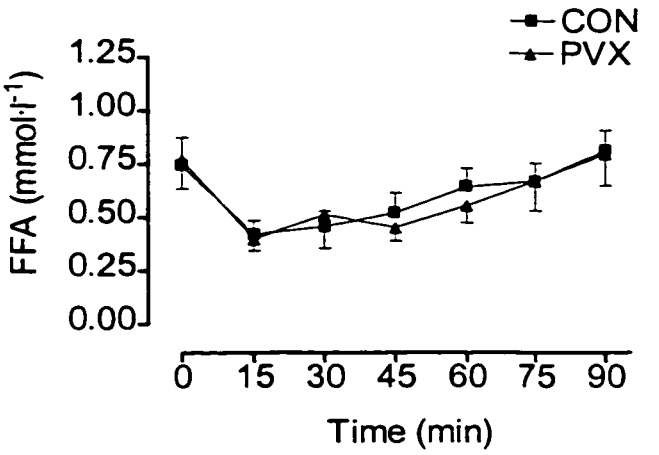

Figure 2.10. *Serum free fatty acid (FFA) concentration with exercise and plasma volume expansion. Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion. A main effect for time was found (P <0.01): 0 = 75 = 90 > 15, 30, 45, and 60 min.*

Substrate oxidation. Acute PV expansion had no effect on calculated whole body fat and carbohydrate oxidation (Table 2.10). Calculated whole body CHO oxidation did not change over time, however, whole body fat oxidation increased with exercise time during both conditions. We assumed that 100% of glucose R_d measured by tracer turnover was oxidized. Glucose oxidation increased over the duration of the exercise, with no differences being observed between the two conditions. Calculated muscle glycogen oxidation declined with increasing exercise time to a similar extent during both conditions. Glycogen oxidation was greatest during the first 30 min of exercise, and was significantly lower at 90 min of exercise. Acute PV expansion had no effect on muscle glycogen oxidation.

Table 2.9. *Effect of acute plasma volume expansion on muscle metabolites during prolonged exercise.*

	Time (min)			
	0	3	45	90
ATP				
CON		20.1 ± 2.7	21.5 ± 0.4	21.2 ± 1.3
PVX		21.4 ± 0.9	20.7 ± 1.2	21.9 ± 1.2
PCr				
CON		43.5 ± 6.8	40.9 ± 7.4	41.9 ± 6.4
PVX		55.3 ± 6.5	45.1 ± 7.0	38.0 ± 6.3
Cr				
CON		76.5 ± 9.8	90.8 ± 8.5	87.5 ± 7.3
PVX		71.3 ± 5.3	81.2 ± 4.4	88.5 ± 4.3
Pi				
CON		52.0 ± 6.7	78.3 ± 5.5	70.6 ± 6.3
PVX		48.8 ± 2.7	65.6 ± 7.1	80.1 ± 8.2
TCr				
CON		120.1 ± 8.8	125.9 ± 3.6	129.4 ± 3.5
PVX		126.5 ± 6.0	126.3 ± 8.6	126.5 ± 6.0
Lactate				
CON		34.5 ± 5.9	29.5 ± 4.5	18.9 ± 3.3
PVX		25.5 ± 2.3	19.0 ± 4.6	17.2 ± 7.9
Glycogen				
CON	301.8 ± 16.7	279.6 ± 24.3	208.6 ± 43.8	112.1 ± 16.9
PVX	300.8 ± 16.9	273.9 ± 38.6	206.2 ± 39.0	115.0 ± 38.2

Values are means ± SE in mmol·kg^{-1} dry wt; n=7. CON, control; PVX, hypervolemia. All measures were determined by fluorometric techniques. ATP, adenosine triphosphate; PCr, creatine phosphate; Cr, creatine; Pi, inorganic phosphate; TCr, total creatine. Main effects for time (P<0.05) were found for Cr, Pi, and glycogen. For Cr and Pi, 3 < 45, and 90 min. For glycogen, 0, 3, > 45 > 90 min.

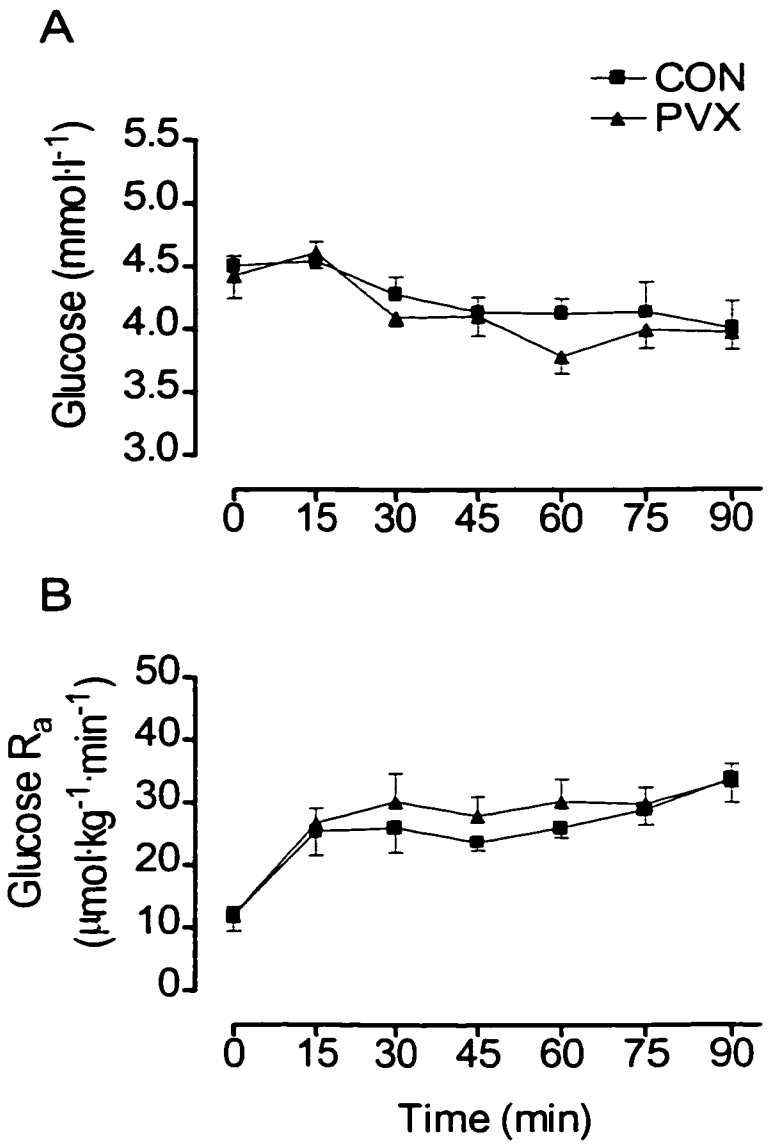

Figure 2.11. *Blood glucose concentration (A) and glucose rate of appearance (R_a) (B) with exercise and plasma volume expansion. Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion. A significant main effect for time was found for plasma glucose ($P < 0.01$): 0 > 60 min, and 15 > 45, 60, 75 and 90 min. A significant main effect for time was also observed for glucose R_a ($P < 0.01$): 90 > 75 > 60 = 45, 30, and 15 > 0 min.*

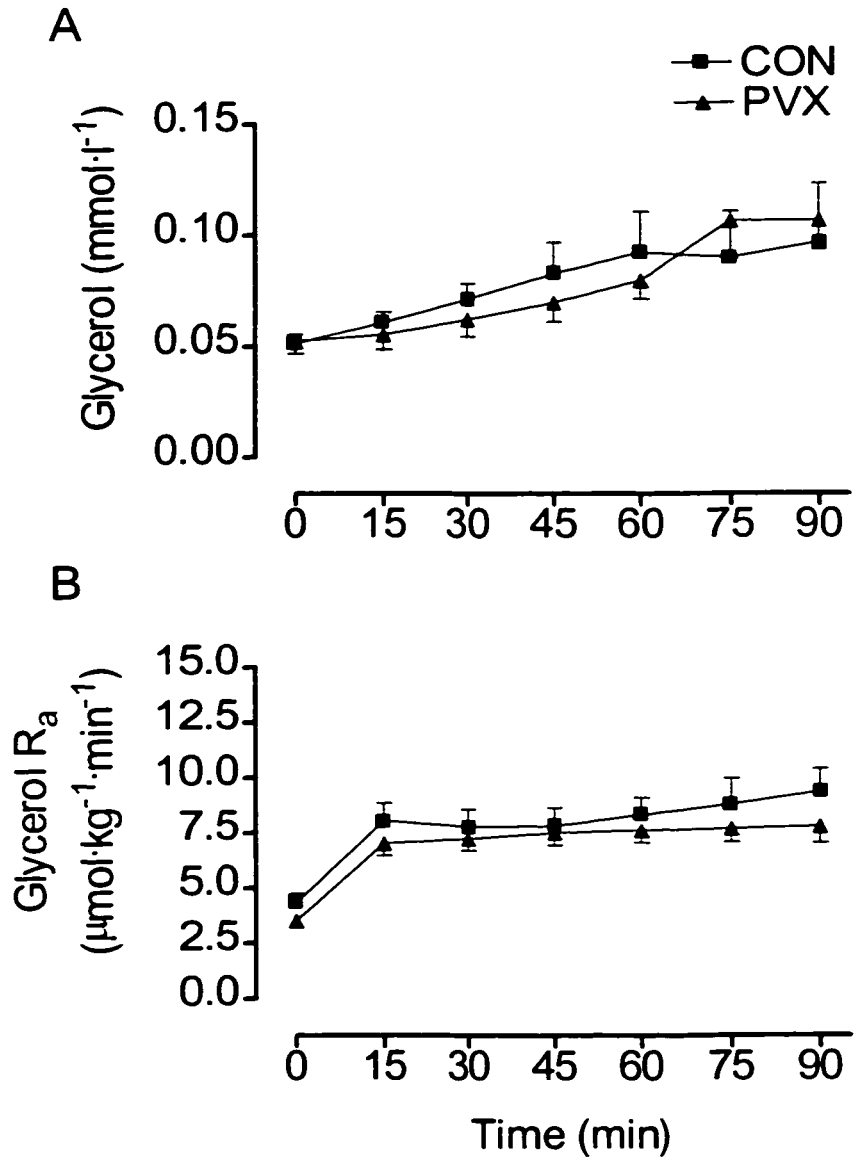

Figure 2.12. *Blood glycerol concentration (A) and glycerol rate of appearance (R_a) (B) with exercise and plasma volume expansion. Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion. A main effect for time was found for plasma glycerol and glycerol R_a ($P < 0.01$). For glycerol; 0, 15, 30, 45 < 75 and 90 min. For glycerol R_a; 0 < all exercise times.*

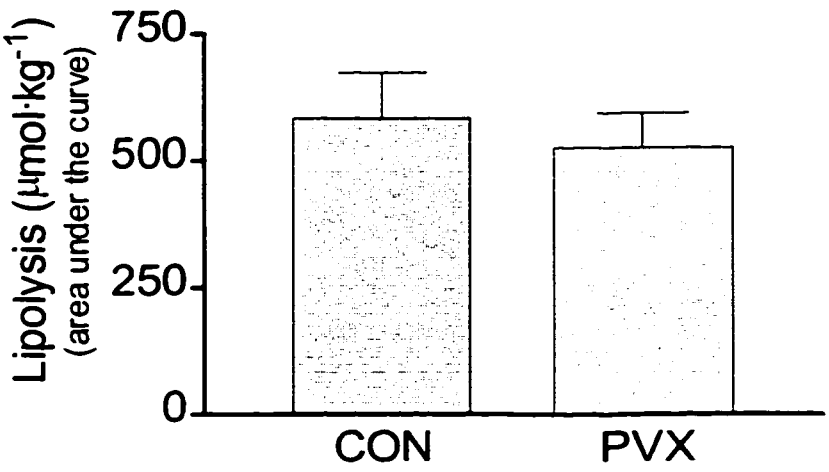

Figure 2.13. *Integrated lipolytic response during exercise with acute plasma volume expansion, expressed as area under glycerol R_a curve (μmol/kg). Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion.*

Table 2.10. *Effects of exercise and acute plasma volume expansion on calculated substrate oxidation.*

	Time, min		
	30	60	90
Carbohydrate Oxidation			
CON	177 ± 8	170 ± 9	168 ± 13
PVX	167 ± 12	150 ± 8	158 ± 10
Fat oxidation			
CON	1.5 ± 0.3	2.2 ± 0.5	3.3 ± 0.8
PVX	2.1 ± 0.8	4.4 ± 0.9	4.4 ± 1.0
Glucose oxidation			
CON	25.9 ± 3.9	25.9 ± 1.6	33.8 ± 3.8
PVX	30.1 ± 4.4	30.1 ± 3.6	33.5 ± 2.6
Glycogen oxidation			
CON	151 ± 9	145 ± 9	134 ± 11
PVX	137 ± 13	123 ± 7	125 ± 9

Values are means ± SE in μmol·kg^{-1}·min^{-1}; n= 8. A main effect for time was found for fat oxidation, glucose oxidation and glycogen oxidation (P<0.01). Fat oxidation: 90 > 60 = 30; glucose oxidation: 30 < 60 < 90; glycogen oxidation: 30 = 60 > 90. See METHODS for explanation of how substrate oxidation was derived.

DISCUSSION

Two major findings of the current study were that acute PV expansion altered the cardiovascular response to prolonged moderate exercise, while the thermoregulatory response was unaltered. In addition, the responses of the fluid and electrolyte regulatory hormones (PRA, ALD, AVP, and α-ANP), catecholamines (NE and EPI) and glucagon were different between CON and PVX during prolonged, moderate intensity exercise. The results also indicate that PVX had no effect in altering whole body lipolysis, glucose R_a and R_d, glycogen oxidation, or the proportion of energy derived from fat or CHO during the exercise. The level of PV expansion used in the current study (15.8 %) was approximately that which is observed with short periods of training (Green *et al.*, 1991; Green *et al.*, 1987). This increase in PV is similar to what has been previously observed with low intensity, prolonged dynamic exercise (Grant *et al.*, 1996; Grant *et al.*, 1997; Phillips *et al.*, 1997).

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The cardiovascular response that was observed during prolonged exercise following PVX is consistent with what has been found previously in our laboratory with lower intensity exercise (Grant *et al.*, 1997; Helyar *et al.*, 1996), and by others (Sawka *et al.*, 1983). These changes are also similar to those observed with short-term endurance training (Green *et al.*, 1987; Green *et al.*, 1990; Green *et al.*, 1987). The current data clearly demonstrates no significant interaction for HR, SV or Qc with exercise time between the two conditions, PVX and CON. This response is different from what might be expected if an increase in cutaneous blood flow was occurring (Nose *et al.*, 1990). However, there are differences between the current study and what is observed with long-term endurance training. With long-term endurance training, there is an attenuation of the cardiovascular drift during exercise as

indicated by time-dependent reductions in the slope for changes in HR and SV (Convertino, 1991; Rowell, 1993). The cardiovascular drift has typically been defined as the downward drift in CVP and SV and rise in HR that occurs with prolonged exercise (Rowell, 1986).

The increase in SV and Qc with PVX would be expected to result primarily from an increase in left ventricular end diastolic volume, consequent to an increase CVP (Kanstrup *et al.*, 1992). However, the increase in Qc with PVX is not a consistent finding, since two other studies (Hopper *et al.*, 1988; Mier *et al.*, 1996) have found no effect of PVX on Qc and HR during short-term submaximal exercise.

One of the primary interests of the current study was to determine if a moderate exercise intensity (~60% VO_2max) would alter the cardiovascular response (i.e. cardiovascular drift) as compared to what has been observed with lower intensity exercise (Grant *et al.*, 1997). The higher intensity exercise would have resulted in a greater metabolic demand at the active muscle mass, resulting in greater heat production. With the greater intensity there should have been a greater competition between the need to deliver blood to the active muscle mass and the need to dissipate heat by increasing cutaneous circulation. It has been previously observed that acute elevations in total blood volume result in an increase in cutaneous circulation with exercise (Fortney *et al.*, 1981a). However, since it appears that blood flow to the active muscle mass is maintained during exercise in the heat (Savard *et al.*, 1988), increased cutaneous blood flow must occur via a redistribution of blood flow from areas other than active muscle or from the central circulation. Since blood flow to vascular beds other than muscle is relatively low during exercise, redistribution of blood from the central circulation to the cutaneous vessels would appear of primary importance (Grassi *et al.*, 1996; Rowell, 1986). Under such conditions, cardiovascular instability occurs, cutaneous blood flow is

compromised, and thermoregulatory strain increases (Rowell, 1986).

Theoretically, increasing blood volume or PV should allow for a decrease in thermoregulatory load (Convertino, 1991; Mack *et al.*, 1994; Senay *et al.*, 1976). The findings of the current study do not support this hypothesis, since PV expansion had no effect on the increase in T_{re} during exercise. Although, there does appear to be a trend for T_{re} in the PVX condition to be lower than the CON condition at all measured points, this trend was not significant and likely resulted from the infusion of the Dextran or Pentaspan. The Dextran or Pentaspan was pre-warmed prior to the infusion; however, during the infusion the infusate sat at room temperature. It is apparent, however, that not keeping the infusate warmed throughout the infusion had minimal effects, since resting T_{re} was not different between conditions.

There are a number of possibilities as to why there was no effect of PVX on the T_{re} response to the exercise. It is possible the exercise intensity employed in this study did not result in a thermal load that adequately stressed the thermoregulatory system. There may have been adequate reserve in the system, before PVX, to deal with the increase in thermal load associated with the exercise. The majority of studies looking into the role of PV on thermoregulation utilize an external heat load as well as exercise (Fortney *et al.*, 1981a; Fortney *et al.*, 1983; Mack *et al.*, 1994; Savard *et al.*, 1988). All measurements taken during the current study were done at an ambient temperature of ~23°C, well below the experimental temperatures used in other studies (Fortney *et al.*, 1981a; Fortney *et al.*, 1983; Mack *et al.*, 1994; Savard *et al.*, 1988; Savard *et al.*, 1988; Fortney *et al.*, 1983). However, the change in T_{re} induced by our 90 min increase protocol was well in excess of that observed by shorter exercise protocols performed in the heat (Nose *et al.*, 1990). Others have previously reported a major attenuation in cardiovascular drift and thermal stress with fluid replacement using

similar environmental conditions (Hamilton *et al.*, 1991). Interestingly, Dextran-induced PVX during exercise did not affect T_{re} even at high temperatures (Montain & Coyle, 1992a). This is in contrast to the very substantial effect that occurs with saline infusion (Nose *et al.*, 1990).

Another possibility as to why we did not observe a change in thermoregulation with PVX may be due to the fact that the increase in cardiac output that occurred with PVX was directed to the working muscles. Associated with acute PV expansion is a decrease in the oxygen carrying capacity (CaO_2) of the blood (Chapler & Cain, 1986; Curtis & Cain, 1992; Kanstrup & Ekblom, 1982; Roach *et al.*, 1999). To overcome the decrease in O_2 content, a fundamental strategy appears to involve an increase in blood flow to the working muscles (Roach *et al.*, 1999; Saltin *et al.*, 1986). It is likely that the increase in Qc, observed in the present study, enhanced blood flow to the active muscle to compensate for the decrease in CaO_2. Whole body VO_2 was similar between the two conditions. Increases in Qc have been previously observed with acute PV expansion (Grant *et al.*, 1997; Helyar *et al.*, 1996; Kanstrup & Ekblom, 1982). However, it is unclear to what extent the increased Qc is directed to the working muscles in prolonged cycling exercise. Since there was no time-dependent change in cardiac function with PVX compared to CON, and no effect on T_{re}, the extra PV would appear not to be routed to the cutaneous vasculature.

The third possibility as to why no difference was observed in thermoregulatory response between the two conditions is that PV expansion per se does not improve thermoregulation. This is in contradiction to a popular hypothesis (Convertino, 1991; Harrison, 1996; Mack *et al.*, 1994; Nose *et al.*, 1990; Senay, 1972; Senay *et al.*, 1976). However, based on this study and others (Montain & Coyle, 1992a; Sawka *et al.*, 1983), it is clear that the expansion of PV prior to exercise, by itself, does not alter T_{re}. Unlike saline infusion, which

has a distinct influence in altering cutaneous blood flow and thermoregulation (Hamilton *et al.*, 1991; Montain & Coyle, 1992a; Nose *et al.*, 1990), the acute expansion of PV using albumin or a synthetic compound does not produce the same result. One major difference between the two methods of PV expansion relates to the fluid lost from the vascular to extravascular space. With saline infusion, the loss is pronounced (Haskell *et al.*, 1997), whereas with large molecular weight compounds are retained within the vascular space. Interestingly, training causes a PV expansion via albumin not unlike the current procedures (Convertino, 1991; Nagashima *et al.*, 1999). The mechanisms, if any, underlying the role of fluid shifts in cutaneous blood flow and thermoregulation remain to be identified.

Fluid and Electrolyte Hormones:

Acute iso-osmotic PV expansion appears to influence most of the hormones in a similar manner. For PRA, ALD, AVP, NE and EPI, the effect is manifested during exercise itself. For all of these hormones, PVX resulted in a blunting of the exercise response. Differences were noted between the hormones in the exercise time needed for PVX to have an effect. In contrast, the resting concentration of α-ANP was elevated, while glucagon was decreased, with these difference being manifested throughout exercise. Although our results are generally similar to what has been reported previously, using a similar expansion of PV but low intensity exercise (Grant *et al.*, 1996), some differences do exist. The differences in AVP observed between conditions, occurred at rest and became greater during the exercise, whereas the increases in α-ANP was observed at rest and persisted throughout the exercise. This was unlike an earlier study where the increases observed for both AVP and α-ANP occurred at rest and persisted throughout exercise (Grant *et al.*, 1996).

Although there are a large number of variables that have been implicated in the

regulation of the fluid regulatory hormones described above, the principal factors appear to be PV and osmolality (Convertino *et al.*, 1980a; Convertino *et al.*, 1983). Many other factors have also been directly or indirectly implicated in the modification of the hormonal response to exercise, such as; systemic blood pressure, cardiac output and stroke volume, thermal load, and the metaboreflex (O'leary *et al.*, 1993; Stebbins *et al.*, 1994; Viru, 1992; Wade & Freund, 1990). In the current study, no differences were observed between PVX and CON for mean arterial pressure or T_{re}, however, differences in cardiac function were observed, as previously discussed.

As expected, the exercise led to similar decreases in PV for both conditions, despite the larger PV in the PVX condition (Grant *et al.*, 1996; Harrison, 1996). The decreases in PV due to exercise in the current study (62% VO_2peak) were greater than what has been previously observed using a low-intensity exercise protocol (46% VO_2peak) (Grant *et al.*, 1996). This was expected, since it is well established that with increasing exercise intensity there is a greater exercise induced decline in PV (Wade & Freund, 1990). Serum OSM was similar both at rest and during exercise for PVX and CON, which has been observed previously (Grant *et al.*, 1996).

The exercise as performed in CON resulted in an increase in AVP. This increase was expected, based on a number of previous studies (Convertino *et al.*, 1983; Freund *et al.*, 1991; Grant *et al.*, 1996). The increase in the current study was greater than had been previously observed with lower intensity exercise (Grant *et al.*, 1996). These observations support the concept that AVP release is intensity and duration dependent (Convertino *et al.*, 1983; Freund *et al.*, 1991). PV expansion clearly blunted the exercise induced increase, in contrast to our earlier work that indicated that the blunting of AVP during exercise, following PVX, was due

to the depression observed at rest.

Since both OSM and PV were significantly altered during exercise with the present study, it is possible that both could be implicated in AVP release (Convertino *et al.*, 1983). However, no differences existed in OSM between the two conditions. This suggests that the lower AVP observed with PVX in the present study is dependent on changes in one or more unidentified factors occurring during exercise itself. Interestingly, within a condition, we could find no significant relationships between changes in AVP during exercise and the changes in either PV, OSM, Na^+ and K^+.

The AVP results from the current study closely resemble those obtained using a model of hypohydration and hyperhydration, induced by diuretic consumption and excess fluid consumption, respectively (Brandenberger *et al.*, 1989). These authors found that both PV and OSM influence AVP secretion. The results from the current study do not support the influential role of PV and OSM on AVP secretion, especially late in exercise. The NE, EPI, angiotensin II (ANG II), pain, and alterations in PaO_2 and $PaCO_2$ have also been implicated in AVP release (Toto, 1994). However, in this study, the only relationship (inverse) that was observed was between increases in AVP and the increases in EPI for the CON condition. Since this relationship occurred with the effects of the changes in OSM and PV held constant, it would appear that increases in EPI in itself relates to a lower increase in AVP.

At present, although it is unclear how the increase in PV attenuates the exercise-induced increase in AVP, feedback signals mediated by the increased intravascular volume would appear to be involved. The secretion of AVP could be reduced by alterations in baroreceptor stimulation, which could be related to the degree of left atrial stretch or wall tension (Share, 1996; Toto, 1994). Since depression in the other fluid and regulatory

hormones also occurred with PVX compared to CON, it is possible that the depression in AVP could occur as a direct result of changes in one or more of these hormones, secondary to changes in intravascular volume (Toto, 1994; Toto, 1994).

Acute PV expansion had the greatest effect on plasma PRA and serum ALD. It was observed that PVX suppressed the exercise induced increase in both these hormones. Previously, an attenuation of the serum ALD response to low intensity prolonged dynamic exercise with acute PV expansion has also been observed (Grant *et al.*, 1996). Despite the higher exercise intensity used in the current study, the response of ALD was similar to that observed previously. Increasing exercise intensity has been shown to result in increasing levels of PRA and ALD (Freund *et al.*, 1991). The reductions in ALD are also consistent with what we have observed with short-term training which induced an approximate 14% increase in PV (Shoemaker *et al.*, 1998).

To gain insight into the mechanisms governing the blunting of ALD during exercise following PVX, we examined a number of factors associated with the increase observed during CON. There was a highly significant, positive correlation between the increases in PRA and the increases in ALD during exercise. This relationship would suggest that exercise-induced reductions in renal blood flow resulted in an increase in PRA which, in time, increased angiotensin I (ANG I) and ANG II and ultimately ALD (Fallo, 1993; Melin *et al.*, 1997; Wade & Freund, 1990). The sympathetic system could also be directly involved in the stimulatory cascade or indirectly involved by modulating renal blood flow and renal perfusion (Fallo, 1993; Melin *et al.*, 1997; Share, 1996; Wade & Freund, 1990). This could explain the high correlations that we have also observed between the changes in PRA and NE and the changes in ALD and NE during exercise in CON.

Additional factors appear to be involved in suppressing ALD during exercise in the PVX condition since the correlations between ALD and PRA and between ALD, PRA and NE were all insignificant. It is not clear what these additional factors might be. However, since the correlation between the changes in PRA and ALD were insignificant, altered control may be elicited at the level of ANG I and ANG II (Guyton & Hall, 1996).

Angiotensin II, as an example, has been implicated in the hemodynamic response to exercise (Stebbins & Symons, 1995). It has been observed that ANG II contributes to the redistribution of Qc that occurs with exercise, specifically, it was observed that ANG II receptor (AT_1 receptor) blockade lead to an increase in splanchnic blood flow during exercise in swine (Stebbins & Symons, 1995). It is possible that ANG II levels were higher during CON as compared to PVX in this study, as a result of an altered distribution of Qc and a reduction in splanchnic blood flow during exercise that would be expected.

Since no differences were found between conditions in plasma OSM, Na^+ and K^+ concentrations, the modulatory effects would appear to associate with the changes in intravascular volume. As with AVP, other fluid and regulatory hormones could be directly involved (Dostal & Baker, 1999; Montain *et al.*, 1997; Wade & Freund, 1990). However, at least within a condition, either CON or PVX, the correlations were generally low and insignificant. Clearly, many different factors may be involved with the regulation of ALD, with control exerted at a number of different levels (Dostal & Baker, 1999) and with the different intermediates exerting their own independent effect (Reid, 1996).

Plasma α-ANP was altered by both exercise and acute PV expansion in the current study. These findings are similar to what has been observed previously (Grant *et al.*, 1996). Both an acute elevation in PV (Grant *et al.*, 1996; Kanstrup *et al.*, 1992) and dynamic exercise

can increase circulating levels of α-ANP (Grant *et al.*, 1996; Kanstrup *et al.*, 1992; Shoemaker *et al.*, 1998). In the current study, exercise led to an early increase in α-ANP during both PVX and CON. Beyond 30 min of exercise, no further changes in α-ANP occurred. This is a typical response pattern and is likely due to changes in CVP and atrial distension that occur in a time-dependent fashion during prolonged exercise. Initially, α-ANP levels rise at the onset of exercise due to the increase in CVP and atrial distension, but as exercise progresses, there is a decrease in both of these factors, and thus a decrease in the stimulus of α-ANP release (Freund *et al.*, 1988). Cardiovascular data from the current study support this interpretation. Plasma α-ANP levels during exercise in the current study were greater than those observed during lower intensity exercise (Grant *et al.*, 1996). This suggests that α-ANP release is also intensity dependent which has also been observed by others (Freund *et al.*, 1988). The increase in α-ANP that we observed during exercise with PVX could clearly be explained by the increased resting levels. The elevated resting level would appear to result primarily from the increased atrial distension, secondary to increases in central blood volume and atrial filling (Kanstrup *et al.*, 1992; Wade & Freund, 1990). Other factors also appear to be involved, since the changes in α-ANP were correlated with the changes in NE during CON, when the effects of Na^+ and K^+ were held constant. In addition, an overall correlation was found between the changes in α-ANP and OSM. These factors have previously been implicated in α-ANP secretion (Christensen, 1993; Hossack *et al.*, 1990; Mannix *et al.*, 1990).

Substrate and Muscle Metabolism:

The current results suggest that the increase in fat oxidation and the decrease in CHO oxidation, that are observed with short periods of training (Bergman *et al.*, 1999b; Friedlander

et al., 1998; Martin *et al.*, 1993; Phillips *et al.*, 1996b), cannot be explained by PV increases. Moreover, it also appears that, in general, whether the exercise is of light or moderate intensity, has little effect on the pattern of substrate utilization following PV expansion.

The failure to find an effect of acute PV expansion, particularly during moderate intensity exercise, was unexpected since the conditions appeared favourable for an increase in fat oxidation to occur. The similar concentrations of FFA, and the likely increase in leg blood flow with PVX (based on finding from Chapter IV), we assumed an increase in delivery would lead to an increase in blood FFA oxidation based on previous work (Romijn *et al.*, 1995). Moreover, the changes in the hormonal milieu with PVX would suggest a shift towards increased fat and decreased CHO oxidation. The hormonal alterations with PVX included an attenuation of the adrenergic response and a reduction in plasma glucagon both at rest and during exercise, while insulin was unaffected.

The hormonal response that we observed during exercise with PVX is generally similar to that observed with training (Green *et al.*, 1991a; Mendenhall *et al.*, 1994; Shoemaker *et al.*, 1998). With training, there is a reduction in adrenergic drive, as indicated by reduced NE spillover into the blood from nerve endings and by the reduction in EPI, which is released from the adrenal medulla (Kjaer, 1998; Ray & Hume, 1998). The increase in plasma glucagon that normally occurs with exercise was blunted (Green *et al.*, 1991a; Mendenhall *et al.*, 1994), while for insulin the reduction that typically occurs during exercise was not as pronounced (Green *et al.*, 1991a; Mendenhall *et al.*, 1994). These effects appeared to occur only late in prolonged exercise.

Although, it is generally accepted that, in males, training results in an increase in fat oxidation and a decrease in CHO oxidation (Mendenhall *et al.*, 1994; Phillips *et al.*, 1996b),

there continues to be disagreement as to the pool of substrate that is effected. It has been reported, based on stable isotope tracers, that fat oxidation increases progressively with training duration, with intramuscular substrate stores representing the primary substrate particularly late in exercise (Phillips *et al.*, 1996b). Oxidation of plasma FFA is increased with training as well, but only early in exercise (Phillips *et al.*, 1996b). The reduction in whole body CHO oxidation occurs both as a result of decreases in blood glucose (Mendenhall *et al.*, 1994; Phillips *et al.*, 1995) and muscle glycogen utilization (Phillips *et al.*, 1995), with the latter being most important during exercise of moderate intensity.

Our failure to find a reduction in CHO oxidation and in glucose R_a and R_d with PVX, in spite of an environment generally favourable to changes in substrate turnover, suggests that other factors are important in its regulation. One factor may be the blood glucose concentration, deviations of which could have profound effects on CHO and fat oxidation (Hargreaves, 1995). However, in this study not only did the dilutional effects of PVX fail to lower blood glucose concentrations, the exercise response was also similar between the two conditions. Blood FFA concentrations, both at rest and exercise, were also unaffected by PVX. Since blood flow to the leg was likely increased with PVX (based on findings in Chapter IV), the delivery of both substrates would have been enhanced. However, since the R_d's were unchanged, increases in oxidation of both glucose and FFA did not occur. Since increases in the utilization of these blood substrates do occur, however, with increased delivery, it would appear that increases in the arterial blood concentration of both glucose (Howlett *et al.*, 1998) and FFA (Romijn *et al.*, 1995) is a necessary pre-requisite.

In studies using stable isotopes to access substrate turnover and oxidation, several assumptions were necessary. In the present study we assumed that 100% of glucose R_d was

oxidized, and represented blood glucose oxidation. This assumption allowed the calculation of whole body glycogen oxidation by subtracting glucose R_d from total CHO oxidation as determined from indirect calorimetry (Table 2.10) (Phillips *et al.*, 1996b; Romijn *et al.*, 1993). As expected, total CHO, glucose and glycogen oxidation where greater in the present study as compared to what has been observed with acute PV expansion and low intensity exercise (Phillips *et al.*, 1997). In addition, total fat oxidation was also lower in the current study as compared to previous work (Phillips *et al.*, 1997). Based on the influence of exercise intensity on substrate turnover and oxidation (Romijn *et al.*, 1993), these findings would be expected.

In the current study, we also assumed that glycerol R_a was representative of whole body lipolysis. There has been some controversy as to the validity of glycerol R_a being representative of whole body lipolysis (Elia *et al.*, 1993; Jensen, 1999). For glycerol R_a to be used for whole body lipolysis, it is essential that no other processes other than lipolysis leads to glycerol formation, and the only site of significant glycerol use is the liver. The first assumption appears to be valid, however, it is the second assumption that has been recently challenged. Significant uptake of glycerol has been observed both across the forearm and leg (Coppack *et al.*, 1999; Elia *et al.*, 1993; Jensen, 1999). However, in one of these investigations, the amount of glycerol uptake across the limb was approximately one tenth of splanchnic glycerol uptake and only occurred after meal ingestion at rest (Jensen, 1999). Despite the observation of glycerol uptake across the limb, there has still not been any evidence of significant in-vivo activity of glycerol kinase in skeletal muscle. In studies that have observed uptake across the limb, it was assumed that skeletal muscle accumulated the glycerol (Coppack *et al.*, 1999; Elia *et al.*, 1993; Jensen, 1999), but such a model cannot

differentiate between the tissues within the limb. At present, the evidence suggesting that skeletal muscle is a site of glycerol disposal is indirect.

There is impressive evidence to suggest that glycerol R_a is representative of whole body lipolysis. First, in the present study the maximal availability of FFA (3 times glycerol R_a) exceeds the rate of FFA oxidation at all times. This is a consistent finding when glycerol R_a is used to estimate whole body lipolysis during exercise (Phillips *et al.*, 1997; Romijn *et al.*, 1993). Secondly, when both FFA R_a and glycerol R_a are assessed simultaneously at rest, the ratio of these two variables is approximately 3:1 (Friedlander *et al.*, 1999; Phillips *et al.*, 1996b). Based on these findings we, and others (Friedlander *et al.*, 1999; Klein *et al.*, 1996; Romijn *et al.*, 1998) believe that glycerol R_a is a reliable indicator of whole body lipolysis during prolonged endurance exercise.

Summary:

In summary, we observed that PVX increased cardiac output and stroke volume and decreased heart rate both at rest and during moderate intensity prolonged exercise, in comparison to CON. In addition, PVX had no effect on the T_{re} response to the exercise or on the cardiovascular drift. It appears that some other adaptation consequent to an elevation in PV must occur with training to explain the improved thermoregulation that results. In addition, we also observed that acute PV expansion alters the endocrine response to moderate intensity dynamic exercise, in a manner generally similar to that observed following short-term training induced alterations in PV. The specific mechanisms underlying these alterations remain unclear, but appear to be related directly or indirectly to the increase in PV.

In addition, the results of the present study show that acute PV expansion had no effects on whole body lipolysis, glucose metabolism, substrate oxidation, and muscle

metabolism during moderate intensity prolonged exercise. Also, no differences in glucose R_a and R_d where observed, despite alterations in the glucoregulatory hormone response to exercise with PV expansion that would appear favourable to altering extramuscular substrate mobilization and utilization.

The results also suggest that increased delivery of substrate, both glucose and FFA, mediated by a possible increase in blood flow that occurs to working muscle with PV expansion, is also without consequence in altering utilization. Our results with PV expansion also suggest that the hormonal adaptations typically observed following training and believed to be intimately associated with substrate turnover, may be more related to protecting fluid and electrolyte balance.

CHAPTER III

THE INFLUENCE OF A DIURETIC INDUCED DECREASE IN PLASMA VOLUME ON THE PHYSIOLOGICAL RESPONSES TO PROLONGED DYNAMIC EXERCISE.

ABSTRACT

To examine the role of a reduction in plasma volume (PV) on the physiological responses to submaximal exercise, ten untrained males performed 60 min of cycle exercise at ~61% of peak aerobic power (VO_2peak) while on a diuretic (DIU) and under control (CON) conditions. Participants consumed either Novotriamazide, (a diuretic) or a placebo, in random order, for 4 days prior to the exercise. The diuretic resulted in a 14.6% reduction in resting PV. During DIU, heart rate was higher ($P<0.05$) and stroke volume was lower ($P<0.05$) at rest and during exercise, while cardiac output was only reduced ($P<0.05$) during exercise. Exercise VO_2 was similar between conditions. Whole body a-vO_2 difference was greater ($P<0.01$) for DIU both at rest and during exercise compared to CON. Rectal temperature (T_{re}) was higher ($P<0.05$) during DIU from 10 min to the end of exercise. Plasma concentrations of norepinephrine were higher ($P<0.05$) during DIU as compared to CON at 15 min of exercise and beyond. Exercise resulted in increases ($P<0.05$) in plasma renin (PRA), angiotensin I (ANG I), aldosterone (ALD), vasopressin (AVP), and atrial naturetic peptide (α-ANP). DIU led to a more pronounced increase ($P<0.05$) in PRA, ANG I, and ALD, and blunted the increase in α-ANP at all time points, as compared to CON. α-ANP was lower ($P<0.05$) at all measured time points with DIU compared to CON. Plasma glucose, glucagon and glycerol, were increased ($P<0.05$) at all time points during DIU compared to CON. The DIU condition led to lower rates of glucose appearance (R_a) and disappearance (R_d) at rest, 15, and 30 min of exercise, but by 60 min rates were increased compared to CON. DIU had no effect on rates of glycerol appearance and whole body lipolysis. Also, no differences in total carbohydrate and fat oxidation were observed between the two conditions. No differences were observed for muscle phosphocreatine (PCr), creatine (Cr), total creatine (TCr), inorganic phosphate (Pi),

lactate, and glycogen between the two conditions, both at rest and during exercise. Therefore, it appears that the diuretic induced reduction in PV led to greater circulating concentrations of norepinephrine which likely result from increased cardiac and thermoregulatory stresses. The DIU condition also resulted in an alteration in the fluid regulatory hormone response to exercise, which suggests that the level of PV is important in the regulation of the exercise response of these hormones. Finally, the results also indicate that diuretic induced reductions in PV alters glucose kinetics during moderate intensity dynamic exercise in the absence of changes in total carbohydrate and fat oxidation, muscle metabolites and muscle glycogen. The specific mechanisms underlying the alterations in glucose kinetics remain unclear, but could be related to either the alterations in the hormonal response or possibly alterations in blood flow distribution.

INTRODUCTION

Prolonged dynamic exercise generally leads to a steady yet progressive increase in heart rate (HR), and a decline in stoke volume (SV) (Rowell, 1993). This phenomena is termed cardiovascular drift. Generally, drift results from a progressive redistribution of blood flow to the cutaneous circulation (Rowell, 1993). These alterations results in a decrease in central venous pressure (CVP), thus resulting a decrease in ventricular filling and end-diastolic volume. This redistribution of blood serves to increase cutaneous blood flow and sweat rate, in order to eliminate the heat generated by the active muscles. As the exercise continues, CVP and cardiac filling may be further compromised due to fluid loss, resulting in a progressive increase in cardiovascular strain as indicated by the continued drift in HR and SV (Sawka & Coyle, 1999). Accompanying these changes may be an increased thermal strain as a result of inadequate adjustments in cutaneous blood flow and sweat rate (Sawka & Coyle, 1999).

To investigate the influence of blood volume and PV on the physiological responses to prolonged dynamic exercise, including cardiovascular and thermal strain, different models of hypohydration/dehydration have been used (Candas *et al.*, 1988; Gonzalez-Alonso *et al.*, 1995; McConell *et al.*, 1997; Montain & Coyle, 1992b; Nadel *et al.*, 1980; Sawka *et al.*, 1996). It appears that hypohydration leads to an increase in cardiovascular instability, as indicated by a greater increase in HR and decrease in SV (Gonzalez-Alonso *et al.*, 1995; Montain & Coyle, 1992b; Nadel *et al.*, 1980; Sawka & Coyle, 1999; Sawka *et al.*, 1996). If the reduction in total blood volume is too severe, Qc could be compromised resulting in an increased peripheral vasoconstriction in an attempt to defend blood pressure (Sawka & Coyle, 1999). Increased peripheral vasoconstriction could conceivably compromise arterial oxygen delivery to the working muscle, resulting in alterations in substrate utilization and metabolism.

This sequence of events are well documented effects of hypohydration induced by either heat exposure or exercise-induced dehydration prior to another bout of exercise (for review see Sawka and Coyle, 1999). However, what remains unclear is whether a similar scenario accompanies diuretic-induced dehydration. Diuretic-induced hypohydration, unlike exercise and heat hypohydration, results in an iso-osmotic hypovolemia. With iso-osmotic hypovolemia, a greater fluid loss from the extracellular and a smaller fluid loss from the intracellular spaces, occurs (Sawka & Coyle, 1999). Moreover, the effects of acute exposure to heat or exercise, designed to induce the hypohydration, could complicate the effects of hypohydration per se, on the response to prolonged exercise.

The defense of cardiovascular integrity and PV appears to be regulated by a number of hormones associated with fluid and electrolyte function. The principal hormones have been identified as arginine vasopressin (AVP), renin-angiotensin-aldosterone (RAS), and atrial natriuretic peptide (α-ANP) (Fallo, 1993; Montain *et al.*, 1997; Wade & Freund, 1990). Although norepinephrine (NE) and epinephrine (EPI), two hormones under the control of the sympathetic nervous system, are not normally recognized as part of the fluid and electrolyte hormonal complex, their importance is well established (Wade & Freund, 1990). These hormones not only influence other fluid regulatory hormones, but also have direct effects on the cardiovascular system, sweat glands and the kidney (Dostal & Baker, 1999; Reid, 1996; Share, 1996; Wade & Freund, 1990). If PV loss becomes too extreme, cardiovascular function, sweat rate and cutaneous blood flow can all be compromised (Sawka & Coyle, 1999).

Prolonged exercise training, which is known to result in an early and pronounced increase in PV (Convertino, 1991; Gillen *et al.*, 1991; Green *et al.*, 1987), is also accompanied

by a blunting of the fluid and electrolyte hormones (ALD, AVP) and catecholamines (NE and EPI) (Convertino *et al.*, 1983; Shoemaker *et al.*, 1998). When PV is increased artificially, at rest by an infusion of high molecular weight solutions, similar to that observed with training, essentially the same effect is observed, namely less of an increase in ALD and AVP, and a greater increase in α-ANP response to prolonged exercise (Grant *et al.*, 1996). Since the high molecular weight solutions (Dextran/Pentispan) produce and iso-osmotic hypervolemia, it would appear that hormonal secretion is directly influenced by the increase in PV.

Decreases in resting PV would be expected to have the opposite effect during prolonged exercise, namely higher ALD, AVP, NE and EPI responses, and a lower α-ANP response. However, it is not clear if this is what occurs, since few studies have induced a reduction in PV prior to exercise, independent of changes in osmolality (OSM). Changes in OSM have been implicated in the secretion of a number of fluid and electrolyte hormones, and in particular, AVP (Share, 1996; Stebbins *et al.*, 1994). The models that have been used to lower PV, namely heat dehydration (Convertino *et al.*, 1980b), fluid restriction (Zappe *et al.*, 1993), and exercise and heat (Harrison, 1996; Montain *et al.*, 1997; Senay *et al.*, 1976), could potentially alter a variety of factors, in addition to altering OSM, all of which could alter the hormonal response.

The compensatory responses to hypohydration, both cardiovascular and hormonal, suggest that a shift in substrate turnover and oxidation may occur. The increase in catecholamines, as an example, would be expected to promote an increased mobilization and utilization of carbohydrates (CHO). In addition, if blood flow is compromised, O_2 availability to the mitochondria may be threatened. Under such conditions, CHO oxidation becomes more emphasized, ostensibly because it provides the highest ATP yield per mole of O_2 (Astrand &

Rodahl, 1986). Increased blood glucose utilization is a classic response to submaximal exercise in hypoxia, even though oxidative phosphorylation is maintained (Brooks *et al.*, 1991).

Prolonged exercise leading to dehydration results in alterations in muscle metabolism. The alterations in muscle metabolism may be related to a decrease in muscle blood flow (Gonzalez-Alonso *et al.*, 1998b) and an increased reliance on carbohydrate metabolism and lactate production (Gonzalez-Alonso *et al.*, 1999). The results of experiments investigating the influence of fluid consumption during prolonged exercise provides additional support for the effect of dehydration on muscle metabolism. Hargreaves et al. (1996) observed that fluid intake during prolonged exercise reduced muscle glycogen use and core temperature as compared to controls (Hargreaves *et al.*, 1996b). Together, these findings suggest that dehydration leads to an increased reliance on muscle glycogenolysis and that this may be related to an increase in thermal load.

The purpose of this study was to investigate the influence of iso-osmotic hypohydration, induced by 4 days of diuretic administration, on the cardiovascular, thermoregulatory, endocrine, substrate turnover and metabolic responses to prolonged exercise in a thermal neutral environment. The advantage of such a model is that no other mitigating factors such as changes in osmolality, previous exercise, and prolonged heat exposure are necessary to induce the hypovolemic state. We have hypothesized that hypohydration, as compared to euhydration, would result in an exaggeration of cardiovascular and thermal strain, as indicated by an increase in cardiovascular drift and core temperature. It was further hypothesized that the hypohydration would result in a greater concentration in blood AVP, RAA, and a lower concentration of α-ANP during prolonged exercise and that these changes

would be accompanied by greater increases in both EPI and NE. It was also hypothesized that hypohydration would alter glucose kinetics, resulting in increases in both glucose release from the liver and glucose oxidation by the working muscle. These changes would occur in association with a decline in both the mobilization and utilization of blood free fatty acids (FFA). An increased rate of muscle glycogen depletion, an increase in muscle lactate, and a greater reduction in muscle PCr would represent the muscle metabolic response to hypohydration as compared to euhydration.

METHODS

Participants. Ten young males were recruited and screened to ensure that they were healthy and untrained. Their age, weight, peak aerobic power (VO_{2peak}), and maximal heart rate (mean ± S.E.) were 20.3 ± 0.4 yr, 78.1 ± 3.0 kg, 3.96 ± 0.14 $l \cdot min^{-1}$, and 199.0 ± 2.5 bpm, respectively. All experimental procedures, including the risks involved, were explained to each subject before written consent was obtained. All protocols and procedures were approved by the Office of Human Research (University of Waterloo, Waterloo, ON), prior to obtaining written consent.

Design and procedures. Measurements were made during a standardized prolonged, moderate, submaximal cycling test on two separate occasions: under control conditions with no diuretic (CON), and after diuretic administration (DIU). During the four days prior to each submaximal test, subjects consumed either the diuretic (Novotriamazide; 100mg triamterene and 50mg hydrochlorothiazide) or a placebo. The experimental design and procedures were virtually identical to those described in Chapter II, except that muscle biopsies were collected at rest, 3 min, 45 min and on completion of exercise. The exercise test, performed at approximately 61% VO_2peak, was planned for a 90 min duration. However, since some subjects were unable to complete 90 min of exercise, and since blood sampling was a problem in some subjects, we report only on the first 60 min of exercise.

O_2 consumption measurements (VO_2). Measurements of respiratory gas exchange were made using the equipment and procedures described in Chapter II. These measures were made prior to (0 min) and intermittently (15, 30 and 60 min) during each test. Gas collection measurements, which ranged between 3 and 5 min, were made in concert with cardiac output (Qc) and heart rate (HR). After approximately 20 min of quiet resting on the cycle ergometer,

the initial measurements were performed. Cardiac output measurements were performed as described previously (Chapter II), using the CO_2 rebreath technique. Standard electrocardiography techniques were used to monitor HR. Stroke volume (SV) was calculated from the Qc and HR determinations. Whole body a-vO_2 difference (a-vO_2 diff) was also calculated using VO_2 and Qc, according to the Fick equation (VO_2 = Qc x a-vO_2 diff).

Indirect calorimetry. Stoichiometric equations and appropriate caloric equivalents (Frayn, 1983; Wolfe, 1992) were used to calculated carbohydrate (CHO) and fat oxidation rates during exercise as previously described (Chapter II) (Appendix II).

Blood Measurements. Blood was collected both prior to (0 min) and intermittently (15, 30 and 60 min) during the exercise bouts. Standard radioimmunoassay methods (as described in Chapter II) were used to determine the concentration of AVP (Penninsula Laboratories Inc, Belmont CA.), ALD (Coat-A-Count, Diagnostic Products, Intermedico, Toronto, ON.), α-ANP (Penninsula Laboratories Inc, Belmont CA.), PRA (NEN, Billerica, MA.), and plasma insulin and glucagon (Coat-A-Count, Diagnostic Products, Intermedico, Toronto, ON.). Plasma EPI and NE, were determined using high-performance liquid chromatography and electrochemical detection as described previously (Chapter II). Hematocrit (Hct) was also determined for each blood sample (in triplicate) and used to calculate changes in PV (van Beaumont *et al.*, 1972), as previously described in Chapter II. Blood samples were also used for the determination of blood lactate, glucose, glycerol, and serum FFA (as described in Chapter II).

Isotopes. The isotopes used for determination of substrate turnover, [6,6-$^2H_2$]glucose and [$^2H_5$]glycerol (98% enriched; MassTrace Inc., Woburn, MA. USA) were prepared as described in Chapter II. Both a priming dose of glucose (14 $\mu mmol \cdot kg^{-1}$) and glycerol (1.3

μmmol·kg^{-1}) were administered before the initiation of the constant infusion (0.22 ± 0.03 μmmol·kg^{-1}·min^{-1} for [6,6-$^{2}H_2$]glucose; 0.1 ± 0.03 μmmol·kg^{-1}·min^{-1} for [$^{2}H_5$]glycerol). Specific infusion rates were determined as described in Chapter II. When exercise was initiated, the infusion rate was doubled (as compared to rest) for both isotopes. The specific infusion rates and infusates were kept constant for each subject over each condition to avoid biasing the data.

Tracer Enrichment and GC-MS. Glucose and glycerol enrichments were determined by making the pentaacetate derivative of glucose and the trimethylsilyl (TMS) derivative of glycerol, respectively. Enrichment of each derivative was measured by the methods described in Chapter II.

Muscle Measurements. The muscle samples were analyzed for PCr, Cr, Pi, lactate and glycogen using the procedures outlined previously (Chapter II). However, only data obtained at 0, 3, and 45 min of exercise were used to compare conditions. In situations were participants could not complete the 90 min, a biopsy was collected at the point of exhaustion. The data collected for the final or 90 min biopsies was not included in the analysis.

Calculations. Isotope kinetics, or the rate of appearance (R_a) and rate of disappearance (R_d) of glucose and glycerol were calculated according to the methods previously described (Chapter II).

The effective volumes of distribution used in the calculation of substrate kinetics were similar to those used in Chapter II. These were assumed to be 230 ml/kg for glycerol and 100 ml/kg for glucose. Glycerol R_a has also been assumed to represent whole body (assumptions stated in Chapter II). In addition, the minimum rate of muscle glycogen oxidation was also

calculated based on the difference between whole body carbohydrate oxidation and blood glucose oxidation (see METHODS section of Chapter II).

Statistics. Data were analyzed using a two way repeated measures analysis of variance (ANOVA) for experimental condition (CON, DIU) and time (0-60 min). When significance was found ($P<0.05$), the Newman-Keul post-hoc technique was used to determine pairwise differences. Average rate of T_{re} increase and changes in body mass were compared using paired studentized t-tests. To examine the relationship between ΔPV, ΔOSM, and the hormonal measures and the interrelationship between the hormonal changes, Pearson product-moment correlation coefficients (r) were calculated. The relationships between these variables were determined during exercise within each condition (DIU and CON). To examine the independent effects of ΔPV and ΔOSM on the hormonal changes and the interrelationships between the hormones with the potential effects of ΔPV and ΔOSM removed, second-order partial correlational procedures were used. The relationships are based on all measurements within a condition during exercise, collapsed across time. All data are expressed as means $\pm$ standard error of the mean.

RESULTS

PV Changes. Administration of the diuretic for 4 days induced a 14.6 ± 3.3 % decrease in resting plasma volume. The prolonged exercise resulted in a decrease in PV, regardless of condition, that occurred during the first 15 min and persisted throughout the remainder of the exercise (Figure 3.1). Plasma volume was consistently lower for the DIU condition than the CON condition at rest and throughout the exercise (Figure 3.1).

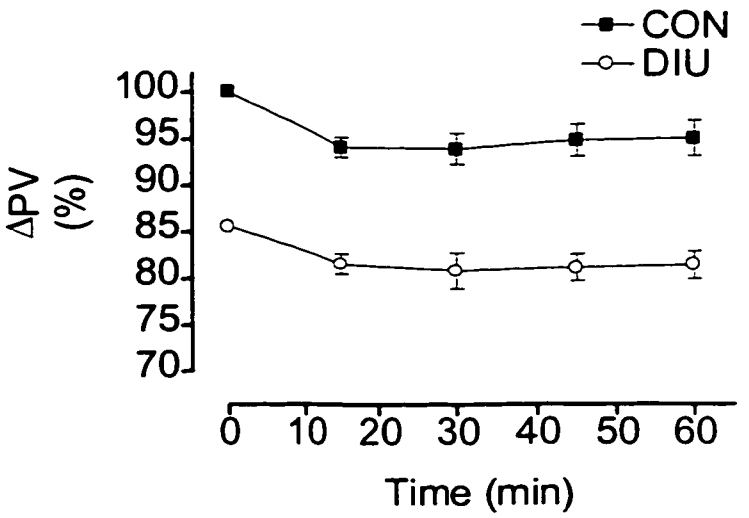

Figure 3.1. *Changes in plasma volume (PV) with exercise. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. Main effects ($p<0.05$) for both condition and time were found. For condition, CON>DIU. For time, 0 > 15, 30 and 60 min.*

Submaximal VO_2 and Cardiovascular Function. Diuretic administration had no effect on VO_2 during exercise (Table 3.1). Exercise resulted in a drift in VO_2 such that the value recorded at 60 min was greater than that at 15 min. Cardiac function was altered both at rest and during exercise in the DIU condition compared to the CON condition (Table 3.1, Figure 3.2). Heart rate was persistently higher in the DIU condition compared the CON condition, both at rest and during exercise. A progressive upward drift in HR was also

observed over time. Stroke volume was lower both at rest and during exercise for the DIU condition as compared to CON (Figure 3.2A). Exercise resulted in an early increase in SV. Beyond 15 min of exercise, namely at 30 and 60 min, SV declined. As with SV, exercise Qc was lower with DIU compared to CON (Table 3.1). Exercise resulted in an increase in Qc at 15 min. No further changes in Qc were observed as the exercise progressed.

Exercise resulted in an increase in the whole body a-vO_2 diff, as compared to rest, regardless of condition (Table 3.1). Compared to CON, a-vO_2 diff for DIU was greater during both rest and exercise.

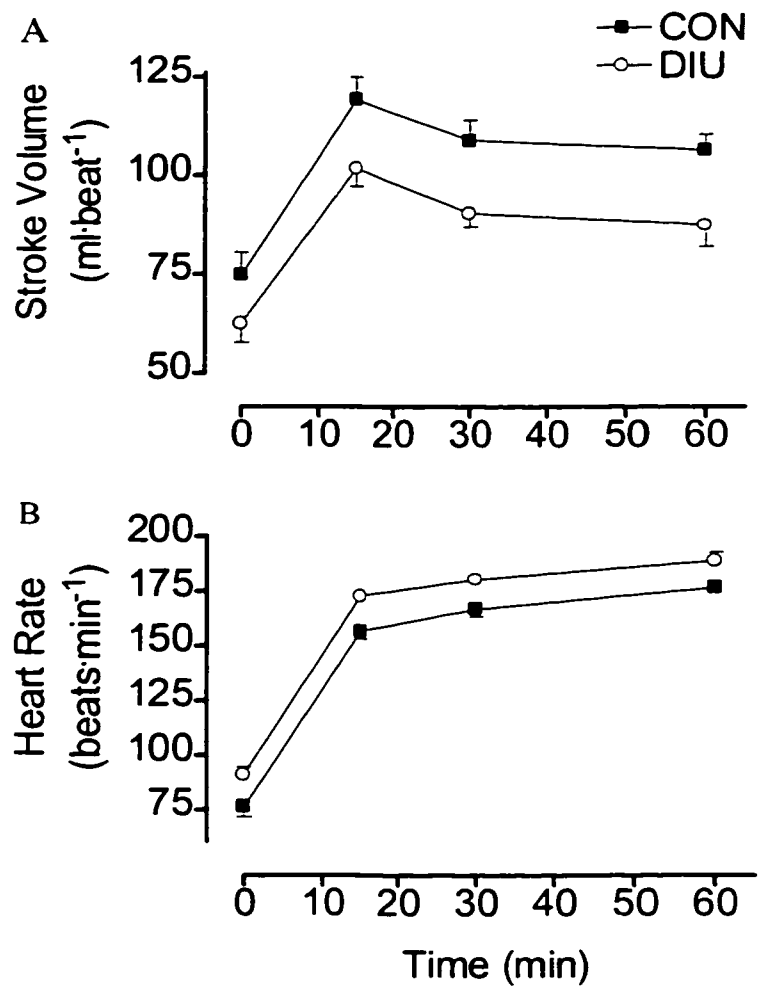

Figure 3.2. *Changes in stroke volume (A) and heart rate (B) with exercise. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. Main effect ($p<0.05$) for time was found for both SV and HR. Main effect ($p<0.05$) for condition was also found for SV and HR. For time, SV 0 < 15, 30, and 60 min, and 15 > 30, and 60 min; and HR 0 < 15 < 30 < 60 min. For condition, SV, CON > DIU; and for HR, CON < DIU.*

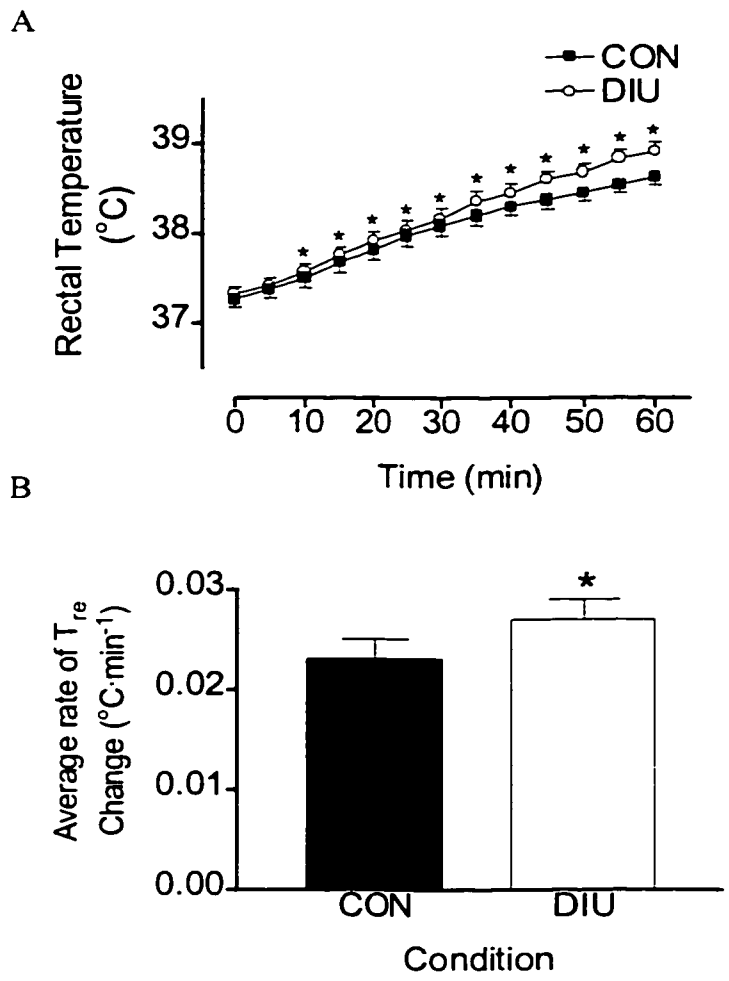

Figure 3.3. *Rectal temperature responses (A) and mean rate of rectal temperature change (B) during exercise. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON (p<0.05). Main effects (p<0.05) for time and condition were also found. For time, 0, 5 min < all times greater; 10 min < all times greater; 15 min < all times greater; 20 min < all times greater; 25 min < all times greater; 30 min < all times greater; 35 < 45-60 min; 40 < 50-60 min; 45 < 55, and 60 min; 50 < 55, and 60 min.*

Rectal temperature. For both conditions, exercise resulted in a progressive rise in T_{re} (Figure 3.3A). However, DIU resulted in a greater increase in T_{re} that was first observed at 15 min of exercise. This difference became greater as the exercise continued. In general, the 60 min of exercise resulted in a 1.3 ± 0.1°C and 1.6 ± 0.1°C increase in T_{re}, for CON and DIU respectively. The average rate of T_{re} increase was greater for DIU as compared to CON

(Figure 3.3B).

Each condition also resulted in similar declines in body weight. The average decrease in body weight was 1.3 ± 0.1 kg and 1.0 ± 0.1 kg for CON and DIU, respectively. These reductions translated into a 1.7 ± 0.1% decline in body weight for the CON condition and a 1.3 ± 0.2% decline for DIU.

Serum Osmolality. The prolonged exercise led to similar increases in OSM in both conditions (Table 3.2). This increase was evident by 30 min, and no further increases in OSM were observed as the exercise progressed.

Table 3.1. *VO_2, VCO_2, respiratory exchange ratio, cardiac output and arterio-venous oxygen difference during exercise following diuretic administration.*

	Time (min)			
	0	15	30	60
VO_2 ($l \cdot min^{-1}$)				
CON	0.47 ± 0.03	2.38 ± 0.07	2.39 ± 0.09	2.56 ± 0.08
DIU	0.51 ± 0.05	2.44 ± 0.08	2.43 ± 0.08	2.53 ± 0.12
VCO_2 ($l \cdot min^{-1}$)				
CON	0.46 ± 0.04	2.30 ± 0.08	2.26 ± 0.08	2.35 ± 0.08
DIU	0.45 ± 0.05	2.37 ± 0.06	2.35 ± 0.07	2.40 ± 0.08
RER				
CON	0.97 ± 0.06	0.97 ± 0.02	0.94 ± 0.02	0.92 ± 0.01
DIU	0.91 ± 0.05	0.98 ± 0.01	0.97 ± 0.01	0.96 ± 0.02
Qc ($l \cdot min^{-1}$)				
CON	5.6 ± 0.5	18.5 ± 0.5	17.9 ± 0.5	18.6 ± 0.4
DIU	5.7 ± 0.5	17.2 ± 0.6*	16.2 ± 0.4*	16.5 ± 0.7*
a-vO_2 diff ($ml \cdot l^{-1}$)				
CON	82.4 ± 5.1	129.7 ± 5.1	134.4 ± 5.9	138.6 ± 5.9
DIU	90.4 ± 6.7	142.3 ± 3.6	150.3 ± 5.2	158.8 ± 7.8

*Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. VO_2, oxygen consumption; VCO_2, carbon dioxide production; RER, respiratory exchange ratio; Qc, cardiac output; a-vO_2 diff, arterio-venous oxygen differences. *Significantly different from CON. Main effects for time ($p<0.05$) where found for VO_2, VCO_2, Qc, and a-vO_2 diff. Main effect for condition ($p<0.05$) was found for a-vO_2 diff. For time, VO_2 and VCO_2, 0<15, 30, and 60 min, and 15<60 min; Qc 0<15, 30, and 60 min; a-vO_2 diff, 0<15, 30, and 60 min.*

Table 3.2. *Effects of diuretic administration and exercise on serum osmolality.*

	Time (min)		
	0	30	60
CON	293.9 ± 2.7	302.9 ± 2.2	303.1 ± 1.9
DIU	295.7 ± 3.4	301.4 ± 1.5	303.8 ± 2.7

Values are means ± SE in mOSM/kgH_2O; n=9 subjects. CON, placebo; DIU, after 4 days of diuretic administration. A main effect for time was found; 0 < 30 and 60 min (P<0.01).

Blood Hormones. As expected, exercise led to a rise in the concentrations of both plasma EPI (Figure 3.4A) and NE (Figure 3.4B), the magnitude of which was dependent on the time of exercise. Despite the exercise induced changes, no difference in response was observed for plasma EPI between CON and DIU. In contrast, plasma NE concentrations were higher at 15 min of exercise during DIU compared to CON. No differences were observed between conditions for resting NE.

In both conditions, exercise resulted in an increase and decrease for plasma glucagon and insulin, respectively (Table 3.3). For plasma glucagon, the concentration was greater by the end of exercise (60 min) compared to rest. In contrast, exercise led to a rapid decline in plasma insulin that was evident by 30 min of exercise. No further alterations in plasma insulin were observed as the exercise continued. The diuretic condition had no effect on plasma insulin concentrations. However, for plasma glucagon, the concentration with DIU was greater than CON.

Exercise led to a progressive increase in PRA during DIU (Figure 3.5). This increase in PRA was much more pronounced with DIU, such that by 30 min, PRA was 5 fold greater compared to CON. This difference was maintained through the remainder of the exercise. A difference in resting PRA was also observed between the two conditions.

Plasma ANG I followed a similar pattern to that which was observed for PRA (Figure 3.6). ANG I was altered by exercise but only in the experimental condition. Values were similar at rest between the two conditions. With DIU, exercise led to an increase at 30 min followed by a further increase at 60 min. However, by 30 min, DIU was greater than CON and this difference was maintained through the completion of the exercise.

With DIU, plasma ALD also altered to a greater extent at rest and during exercise as compared to CON (Figure 3.7). For DIU, the resting concentrations of ALD were approximately 8 fold greater than CON. At both 30 and 60 min of exercise the difference was approximately 6 fold (Figure 3.7).

Unlike ALD, PRA and ANG I, no differences were observed between conditions for AVP either at rest or during exercise (Figure 3.8). However, exercise led to a progressive increase in AVP concentrations for both conditions.

Exercise altered plasma α-ANP concentration, regardless of condition (Figure 3.9). With exercise, an increase was evident for both conditions by 30 min, which was followed by a further increase at 60 min of exercise. DIU blunted plasma α-ANP concentrations at all measured time points, both at rest and during exercise.

Table 3.3. *Effects of diuretic administration and exercise on plasma insulin and glucagon.*

	Time (min)		
	0	30	60
Insulin ($\mu IU \cdot ml^{-1}$)			
CON	14.1 ± 1.1	7.8 ± 1.1	6.4 ± 0.9
DIU	15.8 ± 1.9	8.3 ± 0.9	7.5 ± 1.1
Glucagon ($pg \cdot ml^{-1}$)			
CON	104.5 ± 6.1	114.0 ± 4.8	119.8 ± 3.1
DIU	125 ± 6.8	128.2 ± 6.6	134.5 ± 6.3

Values are means ± SE; n=10. CON, control; DIU, diuretic. A main effect for condition was observed for glucagon (P<0.05); DIU > CON. A main effect for time was observed for insulin and glucagon (P<0.01). For insulin: 0 > 30 and 60 min. For glucagon: 0 and 30 < 60 min.

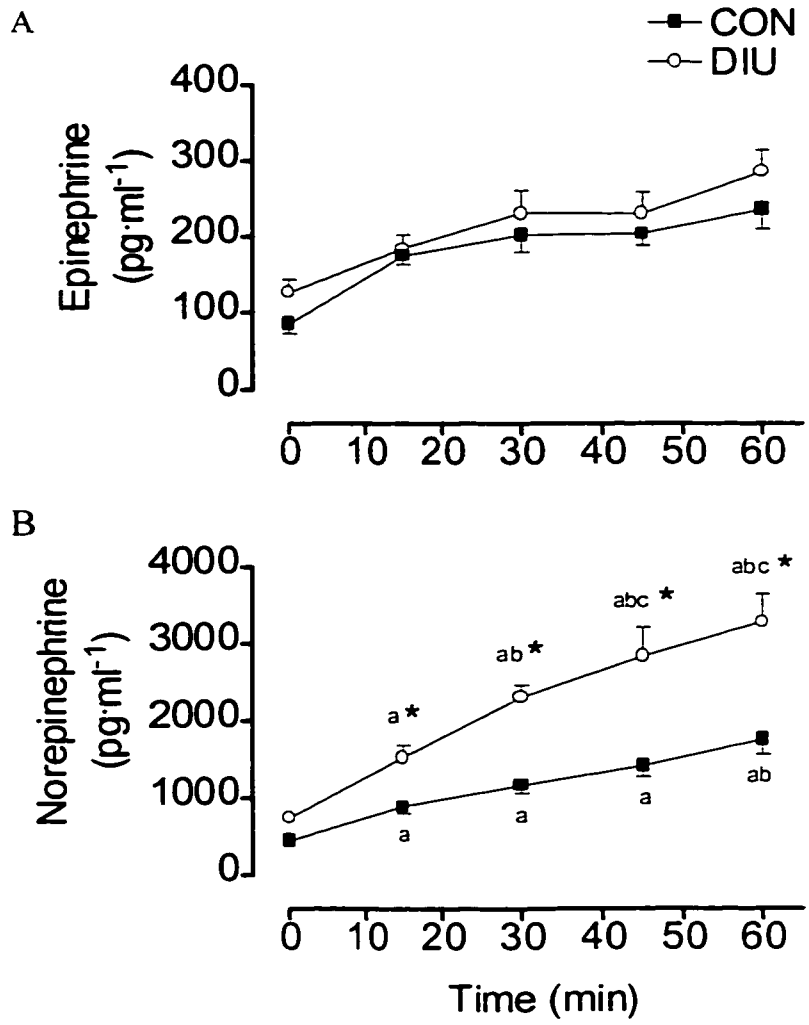

Figure 3.4. *Epinephrine (A) and norepinephrine (B) responses to exercise. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON ($p<0.05$). [a]Significantly different from 0, [b]significantly different from 15 min, [c]significantly different from 30 min.*

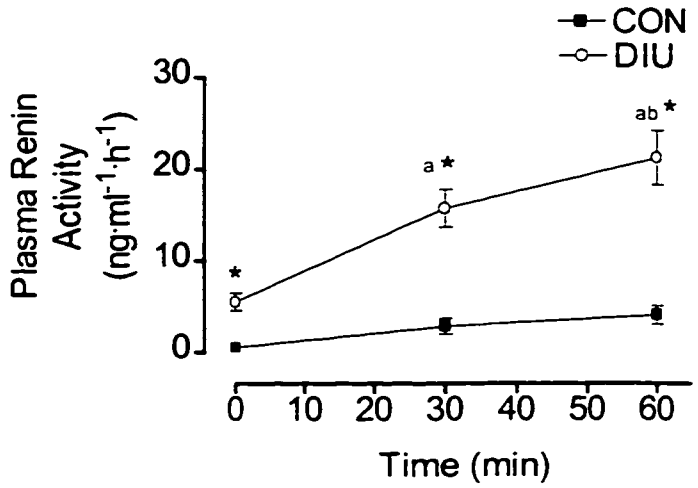

Figure 3.5. *Effects of acute diuretic administration and exercise on plasma renin activity. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON, P < 0.01. [a]Significantly different from 0, [b]significantly different from 30 min. A main effect for condition was observed (P<0.01); CON < DIU.*

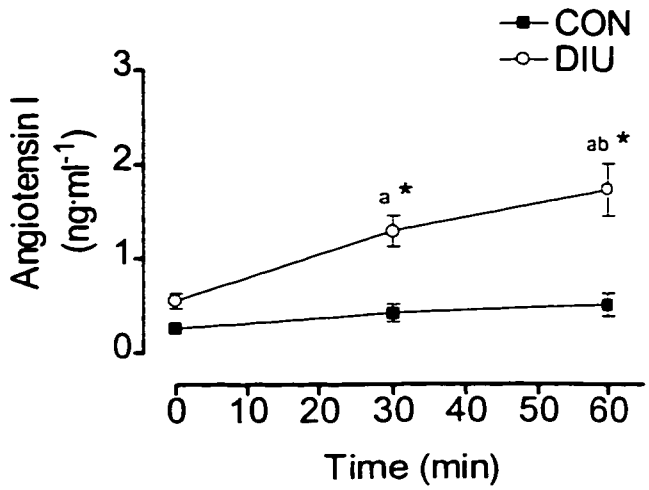

Figure 3.6. *Effects of acute diuretic administration and exercise on angiotensin I concentration. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON, (P<0.01). [a]Significantly different from 0, [b]significantly different from 30 min. A main effect for condition was observed (P<0.01); CON < DIU.*

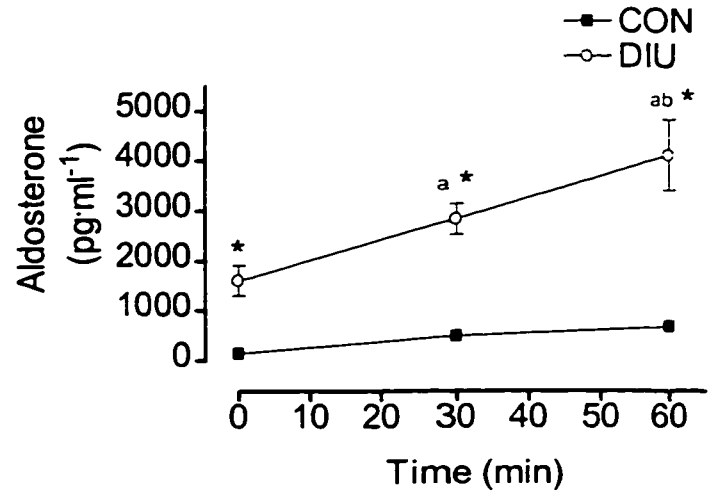

Figure 3.7. *Effects of acute diuretic administration and exercise on serum aldosterone. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON, (P<0.01). [a]Significantly different from 0, [b]significantly different from 30 min. A main effect for condition was observed (P<0.01); CON < DIU.*

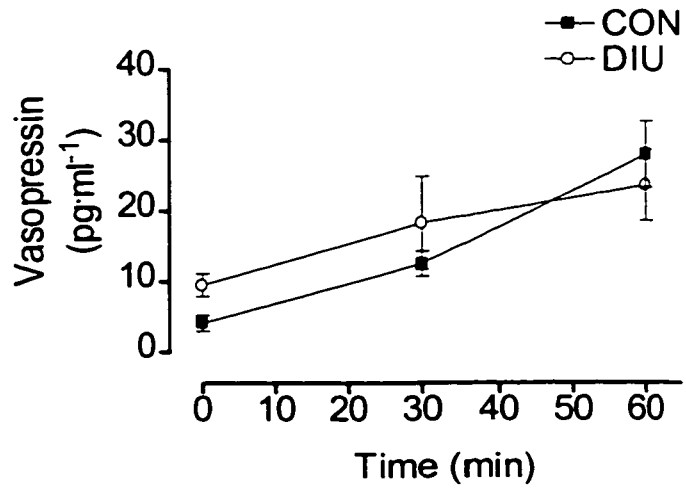

Figure 3.8. *Effects of acute diuretic administration and exercise on plasma arginine vasopressin. Values are means ± SE; n=7 subjects. CON, control; DIU, diuretic. A main effect for time was found (P<0.01); 0 < 30 < 60 min.*

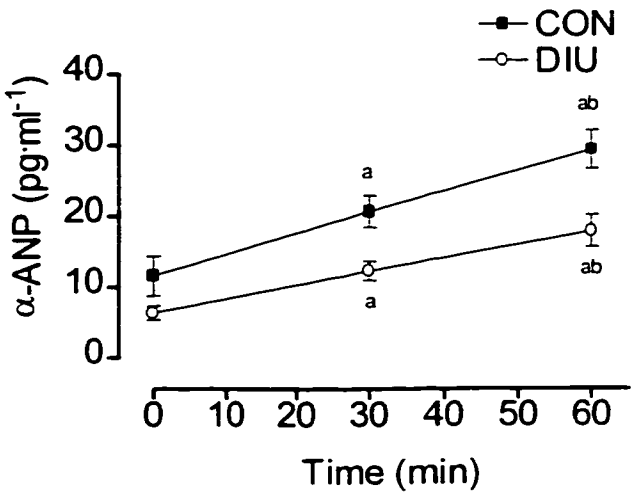

Figure 3.9. *Effects of acute diuretic administration and exercise on plasma α-atrial natriuretic peptide (α-ANP). Values are means ± SE; n=7 subjects. CON, control; DIU, diuretic. [a]Significantly different from 0, [b]significantly different from 30 min. A main effect for condition was observed (P<0.05); CON > DIU.*

No significant relationship was found between the exercise-induced changes in PV (ΔPV), and OSM (ΔOSM) for either the CON or DIU condition (Table 3.4). Correlation coefficients (r) calculated between ΔPV and the hormonal changes were all insignificant, both with and without the effect of ΔOSM. In general, a similar result was found for ΔOSM and the hormonal changes. The only exceptions were for ΔOSM and ΔAVP where a positive relationship (r=0.46) was found for the DIU condition when the effect of ΔPV was held constant and for ΔOSM and ΔANG I for DIU overall (r=0.51) and independent of PV (r=0.52).

The interrelationships between the exercise-induced changes in the different hormones revealed few significant relationships regardless of condition (Table 3.5). Exceptions were noted for ΔPRA and ΔALD for CON (r=0.51), ΔALD and ΔNE for CON (r=0.58), ΔPRA and ΔANG I for CON (r=0.92), and ΔAVP and Δα-ANP for DIU (r=-0.51). All of these

relationships were unaffected when ΔPV and ΔOSM were held constant (Table 3.6).

Table 3.4. *Diuretic administration and the relationships between the changes in plasma volume, serum osmolality, and blood hormones.*

	PV		OSM	
	Overall	Partial (OSM)	Overall	Partial (PV)
OSM				
CON	-0.37			
DIU	-0.20			
AVP				
CON	0.32	0.16	-0.13	-0.11
DIU	-0.01	0.05	0.41	0.46*
ANG I				
CON	-0.08	-0.13	-0.06	-0.12
DIU	-0.04	0.10	0.51*	0.52*
ALD				
CON	-0.25	-0.06	0.03	0.09
DIU	-0.12	-0.11	0.07	0.05
PRA				
CON	0.04	0.08	-0.03	0.02
DIU	-0.13	-0.10	0.31	0.32
α-ANP				
CON	-0.06	-0.09	-0.06	-0.09
DIU	0.19	0.11	-0.30	-0.24
EPI				
CON	-0.34	-0.16	0.15	0.15
DIU	-0.20	-0.22	0.28	0.32
NE				
CON	-0.30	-0.37	-0.12	-0.25
DIU	0.13	0.11	-0.16	-0.13

*Values represent first-order correlation coefficients (overall) and second-order (partial) correlation coefficients with effects of changes in osmolality (OSM) and plasma volume (PV) held constant. *Significantly different from 0 ($P<0.05$). AVP, arginine vasopressin; ANG I, angiotensin I; ALD, aldosterone; PRA, plasma renin activity; α-α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; DIU, after 4 days of diuretic administration.*

Table 3.5. *Diuretic administration and the interrelationships between the changes in the fluid and electrolyte hormones.*

	AVP	ANG I	PRA	ALD	α-ANP	EPI	NE
AVP							
CON		-0.29	-0.23	-0.29	-0.31	-0.11	-0.42
DIU		0.06	0.19	0.30	-0.52*	0.27	0.21
ANG I							
CON			0.92*	0.36	0.13	0.03	0.18
DIU			0.10	-0.15	-0.08	-0.02	0.04
PRA							
CON				0.51*	-0.03	-0.01	0.14
DIU				0.04	0.10	0.13	0.15
ALD							
CON					0.00	0.01	0.58*
DIU					-0.02	-0.06	0.00
α-ANP							
CON						0.04	-0.01
DIU						-0.08	0.37
EPI							
CON							0.44
DIU							0.33
NE							
CON							
DIU							

*Values represent first-order correlation coefficients (r). AVP, arginine vasopressin; ANG I, angiotensin I; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; DIU, after 4 days of diuretic consumption. *Significantly different from 0 ($P<0.05$).*

Table 3.6. *Diuretic administration and the interrelationships between the changes in fluid and electrolyte hormones with the effects of changes in osmolality and plasma volume held constant.*

	AVP	ANG I	PRA	ALD	α-ANP	EPI	NE
AVP							
CON		-0.30	-0.25	-0.23	-0.31	-0.02	-0.26
DIU		-0.20	0.08	0.31	-0.47*	0.19	-0.40
ANG I							
CON			0.92*	0.35	0.13	-0.04	0.16
DIU			-0.06	-0.21	0.08	-0.18	0.15
PRA							
CON				0.53*	-0.03	-0.05	0.15
DIU				0.01	0.23	0.03	0.22
ALD							
CON					-0.02	-0.11	0.55*
DIU					0.02	-0.10	0.02
α-ANP							
CON						0.02	-0.05
DIU						0.02	0.33
EPI							
CON							0.40
DIU							0.42
NE							
CON							
DIU							

*Values represent second-order correlation coefficients (r) with effects of changes in osmolality (OSM) and plasma volume (PV) held constant. AVP, arginine vasopressin; ANG I, angiotensin I; ALD, aldosterone; PRA, plasma renin activity; α-ANP, atrial natriuretic peptide; EPI, epinephrine; NE, norepinephrine; CON, control; DIU, after 4 days of diuretic administration. *Significantly different from 0 (P<0.05).*

Muscle Metabolites. No differences in muscle metabolites were observed between the two experimental conditions, both at rest and during exercise (Table 3.7). The onset of dynamic exercise led to a decline in PCr and a parallel increase in Cr and Pi during both conditions. An early increase in muscle lactate was also observed, which declined by 45 min, but remained greater than resting values. The prolonged exercise also led to a progressive decline in muscle glycogen for both conditions, which was evident by 3 min of exercise, with

further declines observed at 45 min of exercise.

Table 3.7. *Effect of diuretic administration on muscle metabolites during prolonged exercise.*

	Time (min)		
	0	3	45
ATP			
CON	21.3 ± 0.8	21.0 ± 0.5	19.6 ± 0.8
DIU	21.4 ± 1.0	20.0 ± 0.9	20.5 ± 0.3
PCr			
CON	64.8 ± 3.1	34.2 ± 3.0	32.7 ± 4.1
DIU	62.3 ± 2.9	35.3 ± 2.2	36.4 ± 3.0
Cr			
CON	52.3 ± 3.3	81.2 ± 3.6	90.2 ± 6.0
DIU	51.9 ± 3.6	83.1 ± 4.5	81.5 ± 5.2
Pi			
CON	30.4 ± 1.8	49.5 ± 3.5	54.4 ± 5.5
DIU	29.2 ± 2.0	43.7 ± 3.5	51.3 ± 4.8
TCr			
CON	117.1 ± 2.6	115.3 ± 4.4	122.9 ± 5.2
DIU	114.2 ± 3.4	118.4 ± 3.6	118.0 ± 3.6
Lactate			
CON	4.9 ± 0.8	29.1 ± 5.1	16.1 ± 4.0
DIU	4.1 ± 0.8	26.8 ± 4.5	18.7 ± 4.5
Glycogen			
CON	501.5 ± 18.9	420.4 ± 22.5	260.9 ± 29.6
DIU	465.7 ± 16.9	396.1 ± 20.4	243.1 ± 20.4

Values are means ± SE in mmol/kg dry wt; n=10. CON, control; DIU, diuretic administration. ATP, adenosine triphosphate; PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate; TCr, total creatine. Main effects for time (P<0.05) were found for CP, Cr, Pi, lactate and glycogen. For CP, 0>3, and 45 min. For Cr and Pi, 0<3, 45 min. For lactate, 0<3>45 min, and 0<45 min. For glycogen, 0>3>45 min.

Blood metabolite concentration and turnover. Arterialized venous blood lactate concentrations were increased at 15 min of exercise (Figure 3.10). No further changes were

observed as the exercise progressed. In addition, no differences were observed for DIU as compared to CON. Serum FFA decreased during the early phase of exercise, but returned to resting levels by 60 min of exercise (Figure 3.11). As with plasma lactate, DIU had no effect on serum FFA response to exercise as compared to CON.

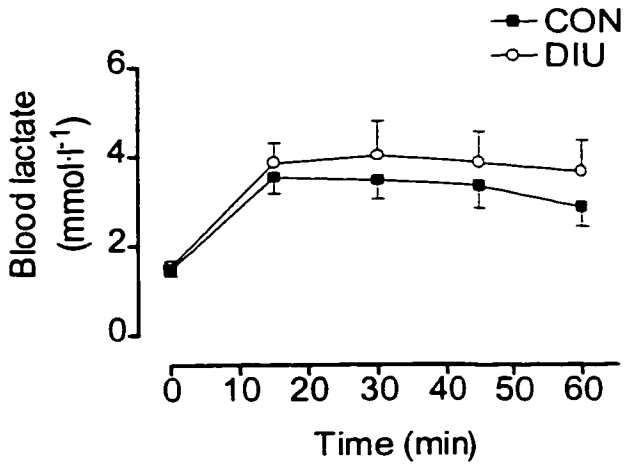

Figure 3.10. *Blood lactate concentrations with exercise and diuretic administration. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. A main effect for time was found (P<0.01): 0 min < all exercise time points.*

Plasma glucose concentrations remained constant throughout the 60 min of exercise for both conditions (Figure 3.12A). Blood glucose was generally higher during both rest and exercise with DIU. Exercise resulted in a progressive rise in glucose R_a (Figure 3.12B). Glucose R_a was lower at rest, 15 and 30 min of exercise, and elevated at 60 min of exercise for DIU as compared to CON. The metabolic clearance rate of glucose was not different between the experimental conditions. The onset of exercise led to an increase in glucose MCR. No further increases were observed until 60 min of exercise (Table 3.8).

Exercise resulted in an increase in plasma glycerol by 30 min of exercise, with further increases observed at 45 and 60 min of exercise (Figure 3.13A). In general, plasma glycerol

concentrations were greater with DIU as compared to CON. Glycerol R_a was unaffected by DIU (Figure 3.13B). Exercise resulted in a general increase in glycerol R_a regardless of condition. The increase that was first evident at 15 min of exercise, was progressive with time. Similarly, no differences were observed between the two conditions for whole body lipolysis, as estimated from the area under the glycerol R_a curve (Figure 3.14).

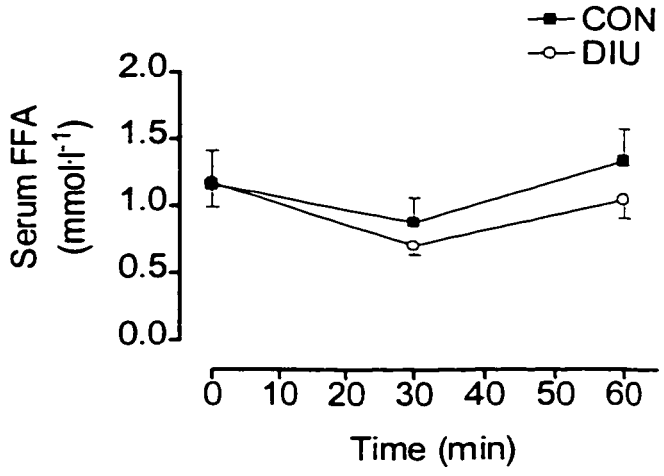

Figure 3.11. *Serum free fatty acid concentration with exercise and diuretic administration. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. A main effect for time was found (P<0.05): 30 min < 0 and 60 min.*

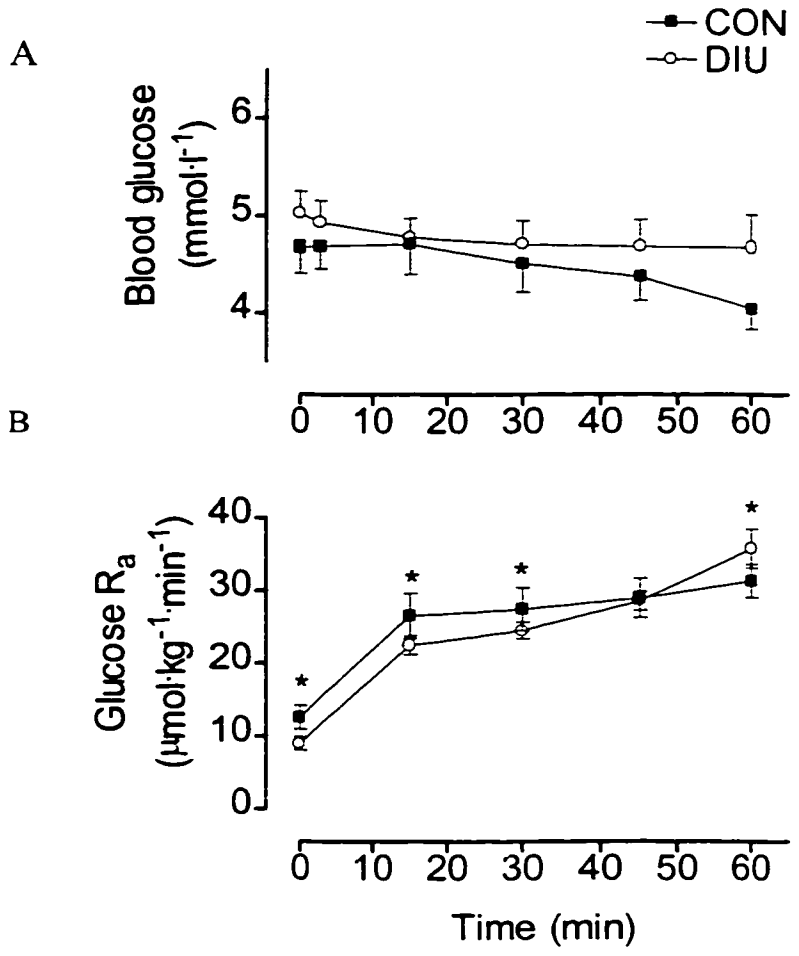

Figure 3.12. *Blood glucose concentrations (A) and glucose rate of appearance (R_a) (B) with exercise and diuretic administration. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. *Significantly different from CON (P<0.05). A main effect for condition was found for blood glucose (P<0.05): DIU > CON. A main effect for time was found for glucose R_a (P<0.01): 0 < 15, 30 < 45 < 60 min.*

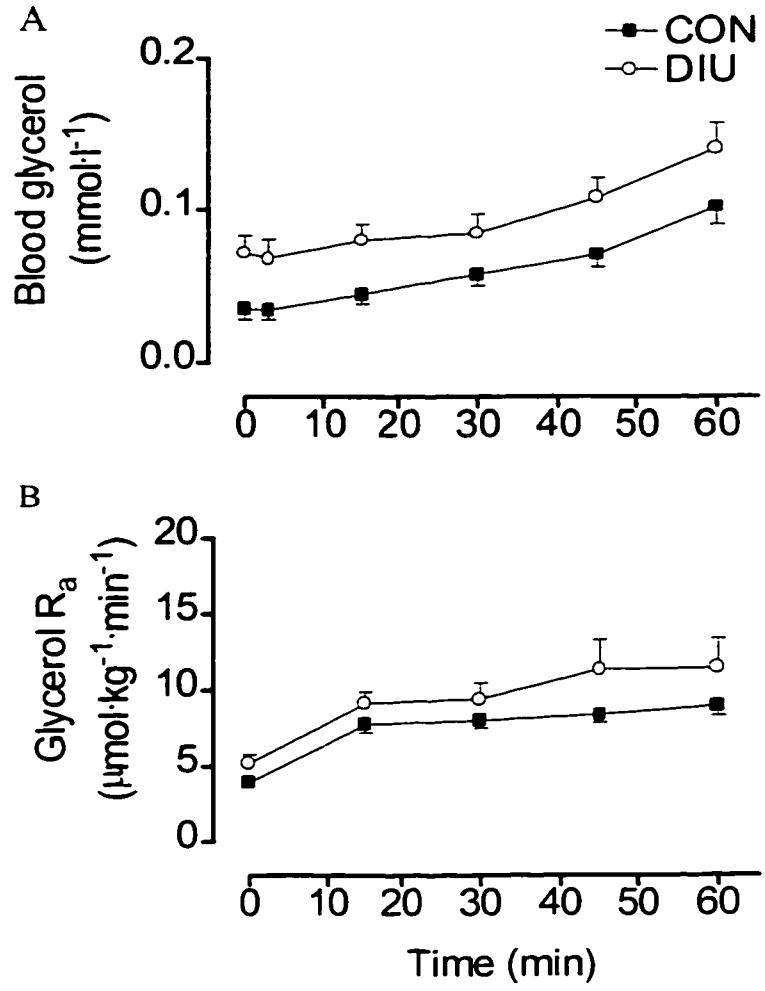

Figure 3.13. *Blood glycerol concentration (A) and glycerol rate of appearance (R_a) (B) with exercise and diuretic administration. Values are means ± SE; n=10 subjects. CON, control; DIU, diuretic. A main effect for condition was found for blood glycerol (P<0.05): DIU > CON. A main effect for time was found for blood glycerol and glycerol R_a (P<0.05). For glycerol; 0, 15 < 30 < 45 < 60 min. For glycerol R_a; 0 < 15, 30, 45 and 60 min, 0, 15, 30 < 60 min.*

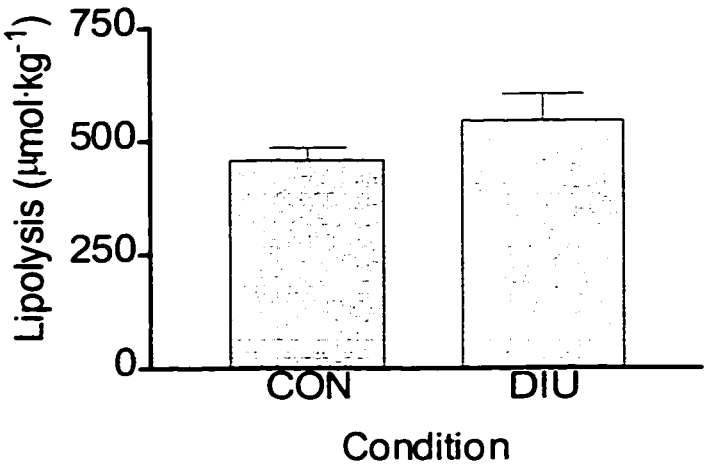

Figure 3.14. *Integrated lipolytic response during exercise with diuretic administration, expressed as area under the glycerol R_a curve (µmol·kg^{-1}). Values are means ± SE; n = 10 subjects. CON, control; DIU, diuretic.*

Substrate Oxidation. The hypohydration had no effect on whole body CHO and fat oxidation during exercise (Table 3.9). With the onset of exercise, whole body CHO oxidation increased. Whole body fat oxidation also increased during exercise, but only at 60 min. Blood glucose oxidation was different between the two experimental conditions (Table 3.9). DIU resulted in lower blood glucose oxidation at 0, 15 and 30 min of exercise, but by 60 min of exercise, blood glucose oxidation was not different between DIU as compared to CON. Exercise also led to a progressive increase in blood glucose oxidation in both conditions. Calculated muscle glycogen oxidation was not different between the two conditions (Table 3.9). In general, exercise led to a decline in muscle glycogen oxidation at 60 min.

Table 3.8. *Effect of exercise and diuretic administration on glucose metabolic clearance rate.*

	Time (min)				
	0	15	30	45	60
CON	2.89 ± 0.37	6.10 ± 0.72	6.41 ± 0.70	6.97 ± 0.71	7.86 ± 0.69
DIU	2.03 ± 0.28	4.91 ± 0.40	5.44 ± 0.53	6.28 ± 0.62	7.92 ± 1.03

Values are means ± SE in $\mu mol \cdot kg^{-1} \cdot min^{-1}$*; n=10. CON, control; DIU diuretic. A main effect for time was found; 0<15, 30, 45<60 min.*

Table 3.9. *Effects of exercise and diuretic administration on calculated substrate oxidation.*

	Time (min)			
	0	15	30	60
Carbohydrate oxidation				
CON	41 ± 10	199 ± 12	186 ± 12	178 ± 11
DIU	32 ± 9	210 ± 8	207 ± 8	195 ± 8
Fat oxidation				
CON	1.3 ± 0.3	3.1 ± 0.7	3.6 ± 0.9	5.1 ± 1.1
DIU	2.1 ± 0.5	2.6 ± 0.8	2.4 ± 0.7	4.7 ± 1.1
Glucose oxidation				
CON	13 ± 1	28 ± 3[a]	28 ± 3[a]	32 ± 2[ab]
DIU	10 ± 1*	24 ± 1*[a]	25 ± 1*[a]	36 ± 3[ab]
Glycogen oxidation				
CON	32 ± 11	172 ± 11	157 ± 11	146 ± 11
DIU	26 ± 9	187 ± 9	182 ± 8	159 ± 7

Values are means ± SE in $\mu mol \cdot kg^{-1} \cdot min^{-1}$*; n=10. CON, control; DIU, diuretic. *Significantly different from CON. [a]Significantly different from 0 (P<0.05); [b]Significantly different from 15 and 30 min (P<0.05). Significant main effect for time was found for carbohydrate oxidation, muscle glycogen oxidation, glucose oxidation and fat oxidation (P<0.05). For carbohydrate oxidation, 0<15, 30, 60 min. For glycogen oxidation, 0< 15, 30 > 60 min. For fat oxidation, 0, 15, 30 < 60 min.*

DISCUSSION

The major findings of the current study were that the 14.6% decrease in PV, induced by 4 days of diuretic administration, resulted in progressively greater heat storage during submaximal exercise than observed during a euhydrated condition. The increased storage of heat could not be explained by differences in core temperature prior to the start of exercise. The hypohydration also resulted in a greater cardiovascular strain during exercise, as indicated by the higher HR and lower SV, as predicted. Unlike core temperature, these differences were manifested at rest and persisted during the exercise state. Prolonged exercise in itself did not exaggerate the cardiovascular strain. Thus, the greater thermoregulatory imbalance that occurs is not accompanied by a greater cardiovascular drift.

Another major finding of the current study was that lower pre-exercise PV altered the exercise blood concentration of most, but not all of the fluid and electrolyte hormones that were examined. During prolonged exercise, higher PRA, ANG I, and ALD, and lower levels for α-ANP were observed with DIU compared to CON. The concentration of AVP was not effected by PV. For the catecholamines, EPI and NE, the hypovolemia only altered the NE response, resulting in higher exercise concentrations.

The current study also demonstrated that the diuretic induced reductions in PV altered the glucoregulatory response to prolonged, moderate intensity exercise. Both glucose R_a and R_d, and the rate of glucose oxidation were depressed early in exercise as predicted. However, by 60 min of exercise, glucose R_a, R_d and glucose oxidation were all increased with DIU compared to CON, which was unexpected. Interestingly, the alterations in blood glucose regulation and metabolism were not accompanied by changes in fat oxidation, whole body lipolysis and total CHO oxidation. In addition, no differences were observed for plasma

insulin concentrations between the two conditions, while plasma glucagon was elevated following the reduction in PV.

Cardiovascular Function and Thermoregulation:

The current results regarding the effects of hypohydration on thermoregulatory behaviour appears at odds with previous work (Nadel *et al.*, 1980) in which the effects of a 21% diuretic-induced decrease in PV was examined during 30 min of exercise in the heat (35°C). Although, the authors concluded that heat storage during hypohydration greatly exceeded the euhydrated condition, the results of Nadel et al. (1980) do not support such a conclusion. The change in temperature from 2 to 30 min of exercise, between the two conditions, does not appear significantly different. A similar study by this group also failed to demonstrate a greater time-dependent effect of DIU on core temperature during exercise (Fortney *et al.*, 1981a). However, others (Candas *et al.*, 1986) have found a greater disturbance in thermoregulation during exercise and hypohydration. It is possible that the severity of the exercise protocol and the environmental conditions in which the exercise is performed are important in allowing thermoregulatory mechanisms to be sufficiently challenged for differences to be observed.

Thermoregulatory behaviour during exercise may well depend on the technique used to induce hypohydration and whether the hypohydration is induced prior to exercise or during the exercise itself. When hypohydration is induced prior to the exercise, body heat storage appears to be disproportionately increased as compared to euhydrated conditions. The disproportionate increase in core temperature appears to be due to both an abnormal adjustment in skin blood flow and sweating rate (Fortney *et al.*, 1981b; Fortney *et al.*, 1983). Hyperosmolality can further exaggerate the effect (Sawka & Coyle, 1999). Hypohydration

induced by heat, exercise or a combination of both, induces a hyperosmotic hypohydration. Diuretic-induced PV loss results in an iso-osmotic hypohydration (Fortney *et al.*, 1981b). The current experimental protocol resulted in iso-osmotic hypohydration.

The most dramatic effects on thermal strain appear to result when dehydration occurs during exercise itself. When prolonged exercise is preformed in the heat, fluid replacement results in a substantial decrease in the rate at which body temperature increases (Gonzalez-Alonso *et al.*, 1995). The reduction in thermal strain appears to be closely related to the increase in skin blood flow and sweating rate which occurs (Gonzalez-Alonso *et al.*, 1995). In the current study, sweat-induced dehydration would also be expected to contribute to the exercise-induced increases in body temperature since no fluid replacement was provided and body weight significantly decreased. However, the loss of body weight during the exercise was similar between the two conditions, and by implication sweat rates would not be substantially different.

It has been previously indicated that diuretic-induced hypohydration results in an exaggerated cardiovascular drift during exercise as indicated by progressive differences in HR and SV (Fortney *et al.*, 1981a; Nadel *et al.*, 1980). However, the greater cardiovascular strain mediated by the lower PV that occurred with DIU appeared to be almost fully manifested during rest or early in the exercise. There is little evidence in these studies to suggest that a greater cardiovascular instability results. What is clear from these studies, is that forearm blood flow is reduced with hypohydration, an effect that is manifested early in exercise and which has been attributed to a greater venomotor tone (Fortney *et al.*, 1983).

The most compelling mechanism to explain the change in cardiac performance with hypohydration would appear to involve the decline in central blood volume and central venous

pressure (CVP). The lower central blood volume would compromise cardiac filling, resulting in a lower end-diastolic volume and stroke volume. During submaximal exercise, a reserve exists for HR, this reserve allows increases in HR to occur in order to compensate for the decrease in SV, preventing a reduction in Qc. This does not appear to happen, since Qc is reduced during exercise. This appears to be a consistent finding as reported both in this study and others (Fortney *et al.*, 1983; Nadel *et al.*, 1980).

At present, it is unclear why HR does not compensate to maintain a constant Qc. Rather, a different strategy appears to have been selected to protect cardiovascular function. Although, blood pressure was not monitored in this study, it has been measured in earlier work, using essentially the same experimental protocol (Zappe *et al.*, 1996). In the earlier study, no differences in mean blood pressure were observed between euhydrated and hypohydrated conditions, either at rest or during prolonged exercise. Since Qc was depressed, a higher total peripheral resistance (TPR) would be expected. Based on the fact that most of the Qc is directed to the working muscles (Grassi *et al.*, 1996), an increase in leg vasoconstriction would probably result during hypohydration as compared with the euhydrated condition. This is essentially what has been found when cardioselective (β1) andrenergic blockade is used to reduce Qc (Pawelczyk *et al.*, 1992). The larger increase in plasma NE during exercise with hypohydration is indicative of an increased sympathetic drive, perhaps needed to protect blood pressure, given the lower PV that resulted (Rowell, 1986). The increase in sympathetic drive may well be implicated in the lower cutaneous blood flow observed during exercise with diuretic-induced PV reduction (Rowell, 1986).

It is of interest that aerobic metabolism during exercise was unaltered in the hypovolemic condition, in spite of the lower Qc and ostensibly the lower blood flow to the

working muscles. Under these conditions, it is possible that arterial O_2 delivery could be protected as postulated (Saltin *et al.*, 1986), given the increase in hemoglobin concentration that occurred with the loss of PV and the increase in CaO_2 that would be expected. Alternatively, VO_2 could have been defended by increasing a-vO_2 difference, as a result of lowering venous O_2. Unfortunately, our results are not definitive since muscle blood flows were not obtained.

Fluid and Electrolyte Hormones:

For the RAA system, higher levels of ANG I occurred during the exercise itself while for ALD, the differences observed at rest became more pronounced during the exercise. In contrast, the differences in α-ANP that were observed between conditions could be explained by differences in the resting concentration. For NE, the lower PV exaggerated the time-dependent increase in that occurred during CON. These observations are consistent with the notion that lower PV levels, although important in altering exercise hormonal levels, do so in different ways and by different stimuli.

The changes that occurred in the hormones of the RAA system following DIU were the most dramatic. The changes in these hormones became so large that they masked the changes that occurred during exercise in CON. Based on the two way ANOVA, the changes in ANG I, PRA, and ALD were all non-significant. However, changes were clearly indicated as would be expected (Fallo, 1993). During the 60 min exercise period, ANG I, PRA and ALD increased 2.5 and 6 fold, respectively. When these changes were examined using a one-way ANOVA, the increases that were observed in all hormones during exercise were all highly significant.

As indicated, prolonged exercise with normal PV resulted in a progressive increase in

ALD which was accompanied by increases in both PRA and ANG I. This response pattern is consistent with what has been reported earlier (Fallo, 1993; Wade & Freund, 1990) and suggests that under normal conditions, the secretion of ALD is primarily controlled by a cascade of events involving increases in PRA, ANG I and ANG II (Fallo, 1993; Wade & Freund, 1990). Although PRA release may be affected by a number of factors, a decrease in intrarenal perfusion pressure, mediated by a reduction in renal blood flow, is probably of fundamental importance (Fallo, 1993). Reductions in renal blood flow are progressive with time during submaximal exercise and appear to occur as a consequence of sympathetic mediated vasoconstriction of the golumerular afferent arteriole (Rowell, 1993). In addition, it is possible that increases in circulating catecholamines and particularly NE, may directly increase renin secretion (Fallo, 1993).

To investigate the role of different factors in the changes that were observed in the hormones of the RAA system during exercise, we examined the relationship with changes in a number of variables, such as PV and OSM, previously shown to be implicated in RAA regulation (Fallo, 1993; Wade & Freund, 1990). No significant relationships were found between ΔPV and either ΔPRA, ΔANG I, and ΔALD, with and without the changes in OSM held constant. Similarly, no relationships could be found between ΔOSM and the changes in these hormones. This was not surprising since the reduction in PV and the increase in OSM that occurred early in exercise, probably as a result of the loss of PV from the vascular space (Harrison, 1996). All of the hormones of the RAA system increased progressively throughout exercise. As expected, the ΔNE was positively correlated with ΔALD. However, no relationship was found between ΔNE and ΔPRA or between ΔNE and ΔANG I. This finding would suggest that at least for the exercise challenge that was used in the current study, the

sympathetic system, and in particular NE, may be exerting control on ALD secretion more upstream and specifically at the level of ANG II as proposed (Fallo, 1993; Wade & Freund, 1990). It should be emphasized that ΔPRA was highly correlated with ΔANG I in the CON condition.

The loss of PV induced by the diuretic resulted in dramatic changes in the hormones of the RAA system, ANG I, PRA, and ALD. To some extent, the differences that were observed during exercise, could be attributed to higher resting levels. However, for all hormones, the rate of increase in concentration was much more pronounced with DIU than with CON. Since OSM was not different between conditions, the reduction in PV would appear to be directly responsible for the elevated activation of the RAA system. As noted for exercise in the CON condition, the elevated resting and exercise levels of ALD are probably mediated by classical control, namely an increase in PRA which initiates a sequence of events, ultimately culminating with increases in ALD.

The correlational analysis failed to provide any insight into the factors regulating the increases in ALD during exercise following PV loss. Although ANG I and PRA both increased in conjunction with ALD, no significant interrelationships were found between ΔANG I, ΔPRA, and ΔALD. Similarly, changes in NE, observed to correlate with changes in ALD during exercise in the CON, were unrelated in the DIU condition. Collectively, the observations indicate that other factors, different from ones investigated, must regulate RAA changes during diuretic-induced PV loss.

Of the few studies which have examined changes in ALD during exercise following DIU (Zappe *et al.*, 1996), all have been consistent with the results shown here, namely a higher resting level compared to normovolemia and a more exaggerated exercise response.

Several studies have been published, however, examining the hormonal response following PV loss induced by passive thermal dehydration (Melin *et al.*, 1997) or dehydration induced by exercise and heat (Francesconi *et al.*, 1985; Melin *et al.*, 1997) and PV loss induced by fluid deprivation (Brandenberger *et al.*, 1989). For all of these models, essentially the same results are found as with DIU, namely an increase in resting PRA (Brandenberger *et al.*, 1989; Melin *et al.*, 1997) and ALD (Brandenberger *et al.*, 1989; Melin *et al.*, 1997). However, with DIU, the increase in resting PRA and ALD, even at similar reductions in PV, appears to be much more pronounced. Differences also appear to exist in the exercise response. In the current study, we found a greater increase in PRA and ALD with DIU as compared to CON, whereas other studies have attributed the elevated exercise response during PV loss to a higher resting level (Francesconi *et al.*, 1985; Melin *et al.*, 1997). One difference between the models used to decrease PV is in OSM. In contrast to other models which produce an increase in OSM compared to CON, diuretic induced PV loss results in an iso-osmotic hypovolemia. Changes in OSM, and in particular Na^+, have been implicated in the control of RAA during exercise (Fallo, 1993; Melin *et al.*, 1997).

Since plasma OSM has been suggested as the major factor controlling AVP secretion (Melin *et al.*, 1997; Share, 1996), and since diuretic administration resulted in an iso-osmotic hypovolemia, it was not surprising to observe that PV loss did not influence AVP concentration at rest or during exercise. Increases in the resting concentration of AVP are commonly observed when PV loss is induced by passive heating (Melin *et al.*, 1997), fluid restriction (Brandenberger *et al.*, 1989), and heat plus exercise (Francesconi *et al.*, 1985). However, all of these models also resulted in an increase in OSM. Increases in resting OSM also appears to be either totally responsible (Francesconi *et al.*, 1985; Melin *et al.*, 1997), or

partially responsible (Brandenberger *et al.*, 1989; Francesconi *et al.*, 1985) for the increases in AVP concentration observed during exercise. The exercise protocol employed appears to be an important factor in the changes that occur in AVP following hypohydration (Francesconi *et al.*, 1985).

The lower α-ANP concentrations observed during exercise in the current study with PV loss can clearly be explained by the lower resting concentrations. This is much like the response observed following acute plasma volume expansion, induced prior to exercise, where the elevation which occurs at rest persists during exercise (Grant *et al.*, 1996). Plasma volume loss, produced by thermal dehydration, however, appears to be without effect either at rest or during exercise (Melin *et al.*, 1997). The most plausible mechanism to explain the apparent reduction in α-ANP secretion observed in this study following PV loss is a reduction in atrial distension, secondary to a reduction in central blood volume and atrial filling (Share, 1996; Toto, 1994). As discussed earlier, SV increased with exercise following PV loss, however, the magnitude is blunted. The increase in atrial distension that accompanies the exercise is probably important in the secretion of α-ANP (Share, 1996; Toto, 1994). No significant relationships were observed between the changes in α-ANP during exercise, regardless of condition, and the changes in either PV or OSM. In addition, the changes in α-ANP were also unrelated to the changes in EPI and NE.

Prolonged exercise resulted in a progressive increase in both EPI and NE. When the exercise was proceeded by a decrease in PV, the sympathetic nervous system was activated to a greater extent, particularly late in exercise, as indicated by the NE spillover measured in the blood. Plasma EPI, secreted from the adrenal medulla, was not affected by PV. The increased

sympathetic response was likely driven by the challenge to blood pressure, which occurs both as a result of the lower PV and the cardiovascular drift that occurs (Rowell, 1993; Sawka & Coyle, 1999). Increased sympathetic activation has also been observed during exercise when PV is reduced using hypohydration (Melin *et al.*, 1997).

Substrate and Muscle Metabolism:

Diuretic administration altered the hormonal response to exercise in a manner opposite to what is normally observed with endurance training. With training there is a reduction in the sympatho-adrenal drive, as indicated by the reduction in NE, which is most dramatic late in exercise (Coggan & Williams, 1995). Unlike the DIU model, there is also a large reduction in the blood EPI response (Coggan & Williams, 1995). In addition, plasma glucagon was elevated and not decreased as observed during exercise following training (Coggan & Williams, 1995). Despite these differences both at rest and during exercise, glucose R_a was significantly lower at rest, 15 and 30 min of exercise. This suggests that the elevated levels of glucagon did not influence glucose R_a, despite suggestions that glucagon is one of the major regulators of hepatic glucose production (Kjaer, 1995; Wasserman & Cherrington, 1997). It appears that the elevated plasma glucose concentration occurred as a result of a decline in the uptake of plasma glucose by the various tissues in the body. Support for this possibly is found with the glucose R_d data, which demonstrates that glucose R_d was attenuated both at rest and early in exercise (15 and 30 min) during DIU, as compared to CON. It is possible that the elevation in blood glucose was also involved in inhibiting glucose output as has been previously observed when blood glucose is elevated (Hargreaves, 1995). Similarly, Gonzalez-Alonso et al. (1999) observed that exercise induced dehydration led to similar alterations in blood glucose concentrations late during prolonged exercise (Gonzalez-Alonso *et al.*, 1999).

They also observed greater increases in the concentration of plasma glucagon with dehydration. Therefore, it appears that both diuretic and exercise dehydration induced hypovolemia influences blood glucose regulation. However, it should be noted that in the current study blood metabolites and hormones were taken from blood sampled from a peripheral vein and may not reflect concentrations in the portal vein where control of blood glucose concentration is initiated. Finally, others factors have also been implicated in the control of endogenous glucose production (Wasserman & Cherrington, 1997), and further work is required to determine the mechanisms which are involved in the observed changes.

Generally, the onset of exercise leads to a very rapid and large increase in glucose R_a, which then continues to increase at a much more moderate and linear rate of increase as the exercise progresses (Coggan, 1996; Kjaer, 1995). In the present study, a linear increase in glucose R_a was observed with CON as the dynamic exercise progressed, whereas DIU led to a curvilinear increase in glucose R_a during the exercise. The uptake of glucose, as estimated by glucose R_d, followed a similar pattern to what was observed with endogenous glucose production. It appears that early in exercise DIU led to a decreased reliance on circulating blood glucose, however, as the exercise continued there was an exponential increase in both endogenous glucose production (R_a) and glucose uptake (R_d). These changes appeared to be independent of alterations in plasma glucose, insulin and glucagon concentrations. Plasma glucagon and glucose concentrations were increased at rest with DIU, however, these differences at rest persisted through out the exercise. Therefore, if the alterations in glucose regulation would have been due to changes in either plasma glucose or glucagon, different responses in glucose R_a and R_d would likely have been observed.

The alterations in glucose R_a and R_d in the current study may have been related to the

differences in thermoregulation that were also observed. It has been previously observed that heat stress significantly alters endogenous glucose production during exercise (Hargreaves *et al.*, 1996a). Specifically, exercise in a higher ambient temperature led to increased glucose R_a and RER, suggesting an increased rate of carbohydrate oxidation (Hargreaves *et al.*, 1996a). The increase in glucose R_a and RER was associated with an increase in plasma glucagon that was also observed late in exercise with the added heat stress (Hargreaves *et al.*, 1996a). An increased rate of muscle glycogenolysis and an increased RER, has also been observed with a combination of heat stress and exercise (Febbraio *et al.*, 1994b), which supports the notion of a shift towards carbohydrate utilization.

In the current study, we observed a trend for total CHO oxidation and RER to be greater during exercise with DIU as compared to CON. There was also a trend for glycogen oxidation to be greater with DIU at all time points. In contrast, glucose oxidation, particularly early in exercise, was decreased. Since glucose oxidation represents less that 15% of the total CHO oxidation, and since the reduction is glucose oxidation was so small with DIU, the impact on total CHO oxidation is minimal.

Abnormal elevations in muscle temperature, which would be expected to occur with DIU, given the increase in core temperature that was observed, have been demonstrated to alter muscle metabolism and substrate oxidation (Febbraio *et al.*, 1996; Febbraio *et al.*, 1994b; Febbraio *et al.*, 1996; Hargreaves *et al.*, 1996a). Work in the heat, which resulted in elevations in muscle temperature, led to increased rates of muscle glycogenolysis and a decreased reliance on fat oxidation (Febbraio *et al.*, 1994b; Hargreaves *et al.*, 1996a; Hargreaves *et al.*, 1996b). Our results appear to be generally consistent with these findings. Interestingly, the fact that we did not find any differences in blood EPI concentrations during exercise between

the two conditions would suggest that some other factors are involved in mediating the increase in glycogenolysis. Increased EPI levels have been shown previously to enhance muscle glycogenolysis during exercise (Febbraio *et al.*, 1998).

The reduction in PV induced by DIU did not alter glycerol R_a or whole body lipolysis during exercise, but did lead to consistently elevated concentrations of plasma glycerol, both at rest and during exercise. The trend towards an increase in whole body lipolysis with DIU was not significant. It is noteworthy that resting values for both blood glycerol and lipolysis appear to account for changes observed during exercise. Since NE was not elevated during this period following PV loss, the elevated glucagon levels would appear to be involved. However, plasma NE levels were greatly exaggerated late in exercise. Despite these differences in NE, no differences in lipolysis or utilization were observed at this time. These observations would appear to challenge the role of NE in fat turnover (Martin, 1996). Collectively, it appears that the reduction in PV may influence lipolysis, possibly through an alteration in the endocrine response to exercise. The increase in blood glycerol concentration with DIU was unexpected, since no changes were observed in glycerol R_a. With limitations in Qc (as discussed earlier), it is possible that splanchnic blood flow could have been reduced. Such a reduction in flow could result in a decline in glycerol clearance, since the liver is the primary site of glycerol uptake (Coppack *et al.*, 1994; Larsen, 1963).

Summary:

In summary, we have observed that diuretic-induced hypohydration induced prior to exercise, resulted in a greater thermal imbalance when prolonged, moderate exercise is performed in temperate conditions. The increase in thermal strain during exercise was not accompanied by a greater cardiovascular instability. Rather, the higher heart rates and lower

Qc and stroke volumes that were observed with the exercise protocol employed, are attributed to the changes that occur either at rest or early in exercise.

The current study also demonstrated that a reduction in PV, induced by acute diuretic administration, altered the endocrine response to moderate intensity dynamic exercise. Specifically, it lead to an increase in the circulating concentrations of PRA, ANG I, ALD, NE, and a reduction in α-ANP. This suggests that the level of PV is important in the regulation of the endocrine response to exercise. The specific mechanisms underlying these alterations remain unclear, but appear to be related directly to the decline in PV.

It was also observed that reductions in PV, mediated by a diuretic administration, altered glucose kinetics during the exercise. The specific effect depended on the time of exercise. Early in exercise, glucose mobilization and utilization were decreased, while late in exercise, these effects were reversed. Although not significant, a strong trend was shown for both glycogen and total CHO oxidation. These changes occurred in the absence of changes in whole body lipolysis and fat oxidation. These results strongly suggest that the greater thermoregulatory strain which accompanies exercise induced dehydration is involved in the altered substrate response. Hypohydration and core temperature would appear to be important factors in understanding the changes in control of substrate turnover and oxidation that occur with exercise and training.

CHAPTER IV

INFLUENCE OF PLASMA VOLUME ON SUBSTRATE DELIVERY AND UTILIZATION DURING DYNAMIC EXERCISE

ABSTRACT

To investigate the effects of artificially induced increases and decreases in plasma volume (PV) on submaximal exercise oxygen consumption (VO_2), the dependent processes, cardiac output (Qc) and arterio-venous O_2 differences (a-vO_2 diff), and metabolite exchange across the working limb, 8 untrained males performed 2 legged kicking ergometry on 3 occasions at 69 ± 3% of peak kicking oxygen uptake (VO_2peak) for 10 min. In one condition (PVX), subjects were infused with 10% Pentispan, in a second condition (DIU), subjects consumed a diuretic (Novotriamzide) for 4 days, while a third condition served as a control (CON). Compared to CON, PV was 20.9 ± 1.2% higher ($P<0.05$) in PVX and 15.1 ± 2.0% lower ($P<0.05$) in DIU. During exercise, VO_2 (l/min) was similar between conditions, whereas Qc varied directly with PV status, such that PVX > CON > DIU ($P<0.05$). Whole body a-vO_2 diff also varied directly with treatment during exercise, such that DIU > CON > PVX ($P<0.05$). With respect to measurements across the working limb, VO_2 was not different between the conditions, whereas leg blood flow (Q_L) was increased ($P<0.05$) during exercise (3, 5, and 9 min) with PVX as compared to CON and DIU. Similar to whole body a-vO_2 diff, leg a-vO_2 diff varied directly with treatment, such that DIU > CON > PVX ($P<0.05$). With respect to blood metabolites, arterial concentrations of lactate and glycerol were increased ($P<0.05$), and FFA reduced ($P<0.05$) with DIU during rest and exercise. No differences were observed for net flux of these metabolites among the three conditions. Circulating concentrations of norepinephrine were increased ($P<0.05$) with DIU during exercise, while no differences were observed for epinephrine. It was concluded that the contribution of Qc and a-vO_2 diff in maintaining whole body VO_2 during exercise depends on PV, with hypervolemia promoting a greater dependence on Qc and hypovolemia a reduced dependence. Despite the alterations in

Qc, Q_L, and whole body and leg a-vO_2 difference, no differences in metabolite exchange were observed across the working limb. This suggests that adequate compensation in Q_L and oxygen uptake occurs with varying levels of PV to prevent any alterations in oxidative metabolism during short term, moderate intensity kicking ergometry. Moreover, the compensation mechanisms used to protect VO_2 did not appear to alter metabolite exchange across the working limbs.

INTRODUCTION

Acute alterations in plasma volume (PV), both increases and decreases, appear to alter the basic responses to exercise in a wide range of physiological systems. One system that is notably affected is the cardiovascular system. Plasma volume expansion, as an example, can result in a higher cardiac output (Qc) and stroke volume (SV), and a lower heart rate (HR), than normally observed (Gillen *et al.*, 1994; Grant *et al.*, 1997; Kanstrup & Ekblom, 1982). In contrast, acute reductions in PV lead to a decline in SV and an increase in HR, and if the reduction in PV is sufficiently severe, a reduction in Qc (Fortney *et al.*, 1983; Zappe *et al.*, 1996). Changes in PV also have potential consequences to peripheral conductance, blood flow and substrate exchange across the working muscle. Acute alterations in PV alter O_2 carrying content of the blood (CaO_2), as a result of changes in hemoglobin (Hb) concentration. Decreases in PV result in an increase in Hb concentration and CaO_2, while increases in PV result in a decrease in Hb concentration and CaO_2. Despite the alterations in CaO_2 which occur with PV manipulations, no changes in steady-state oxygen consumption (VO_2) (Grant *et al.*, 1997; Helyar *et al.*, 1996), or maximal O_2 uptake (VO_2max) (Kanstrup & Ekblom, 1982) are observed when the changes are modest. Some form of accommodation must be occurring to compensate for these changes.

To maintain VO_2 across the working limb, two primary strategies are available, namely an alteration in blood flow to the working muscle or a change in O_2 extraction (a-vO_2 difference). Alterations in blood flow, either an increase or decrease, depending on PV, could attempt to correct for the changes in CaO_2 by maintaining arterial O_2 delivery to the working muscles. With this strategy, a-vO_2 difference is maintained, even though venous O_2 would be altered given the altered CaO_2. The second potential strategy involves maintaining a-vO_2

extraction, in the absence of changes in arterial blood flow. This would mean that adjustments are made at the level of the muscle tissue and not in the vascular regulation of blood flow.

A number of studies have been published examining cardiovascular regulation with altered Hb concentrations (Chapler & Cain, 1986; Ferretti *et al.*, 1992; Hutter *et al.*, 1999; Saltin *et al.*, 1986). Under these conditions, blood flow adjustments and maintenance of arterial O_2 delivery appear to be of fundamental importance. Arterial CaO_2 has also been manipulated by altering the partial pressure of O_2 (PO_2) in the inspired air (Roach *et al.*, 1999). The measurements obtained using this model indicated that regardless of whether CaO_2 is decreased by hypoxia or increased by hyperoxic gas mixtures, the strategy is the same, namely altering blood flow to maintain arterial O_2 delivery to the working muscles. Collectively, these studies suggest that over a range of submaximal work intensities, oxidative phosphorylation appears to remain unchanged as a result of the compensation that occurs.

If alterations in PV influenced oxidative phosphorylation, changes in substrate utilization and muscle metabolism would be expected. Alterations in PV (Green *et al.*, 1997) have been observed to alter muscle metabolism during prolonged exercise. Although, the high energy phosphate potential, as indicated by muscle adenosine triphosphate (ATP) and phosphocreatine (PCr), was unaltered, acute PV expansion was associated with a reduction in muscle lactate concentration, especially early in exercise (Green *et al.*, 1997). An increase in muscle blood flow could be a mechanism by which the clearance of lactate from skeletal muscle is increased, resulting in a lower concentration of lactate within the muscle. Similar considerations would apply to other substrates such as glucose (gluc) and free fatty acids (FFA). Increases in PV could lower arterial concentrations and delivery of substrates, as a dilutional effect, unless an increase in muscle blood flow occurs. In such circumstances, if the

changes in PV are not compensated for by changes in substrate secretion or elimination, the arterial concentration will change as in the case of O_2. The question is whether alterations in blood flow, demonstrated to maintain O_2 delivery, maintain arterial substrate delivery.

The purpose of this study was to determine the influence of pre-exercise alterations in PV, both an increase and decrease, on the mechanisms used to maintain VO_2 given the alterations in arterial O_2 content. We hypothesized, based on previous studies, that VO_2 during submaximal exercise would not be altered by PV status. In addition, we have also hypothesized that muscle blood flow would be adjusted to maintain O_2 delivery, regardless of condition. It is postulated that the adjustments in muscle blood flow would alter muscle metabolite exchange, and in particular, lactate. Increases in blood flow would lead to an increase in lactate clearance, whereas a decrease in blood flow would lead to a decrease in metabolite clearance.

METHODS

Participants. Eight healthy males were recruited and screened to ensure that they were healthy and inactive or only active on an occasional basis. Their mean (± SE) age, weight, peak aerobic power (VO_2peak), and maximal heart rate were 21.9 ± 0.8 yr, 75.5 ± 4.0 kg, 3.43 ± 0.11 $l \cdot min^{-1}$, and 200.0 ± 1.0 bpm, respectively. Prior to obtaining written consent, all of the experimental procedures, risks and benefits were explained to each subject. All protocols and procedures were approved by the Office of Human Research (University of Waterloo, Waterloo, ON), prior to recruitment of the participants.

General research design. The basic experimental design consisted of performing a standardized kicking ergometry task on three separate occasions: under control conditions with a placebo consumed during the 4 days prior to the exercise protocol (CON); after 4 days of diuretic administration (DIU) (Novotriamazide; 100mg triamterene and 50mg hydrochlorothiazide), and after an infusion of a Pentaspan solution (PVX). The three exercise tests were separated by a minimum of one week, and performed at ambient temperatures (22-24°C) and humidities (35-45%) in a randomized, single blind order. All studies were conducted at approximately the same time of day for each subject. To control for nutritional intake on each experimental day, subjects consumed a standardized snack approximately 3-4 h before each exercise test (Ensure liquid, 1045 kJ: 14.8% protein, 31.5% fat, and 53.7% carbohydrate; Ross Laboratories, Montreal, PQ). Only water (ad libitum) was allowed between consumption of the snack and the beginning of the exercise test. All participants were instructed not to engage in any vigorous physical activity for at least 24 hr prior to each exercise test, not to initiate any new exercise programs and to follow a normal balanced diet for the duration of the study.

Testing and Sampling Protocols. Participants reported to the laboratory ~1 h before the exercise test. After application of a local anesthetic (2% Xylocaine with epinephrine), a 16-guage central venous catheter (16 cm, single port; Arrow Medical Products, Mississauga, ON) was inserted retrograde into the femoral vein, 1-2 cm distal to the inguinal ligament. The catheter was advanced ~10 cm and then secured with a single suture. A second catheter (22-guage; Angiocath, Becton Dickinson, Mississauga, ON.) was then inserted into the distal radial artery of the wrist in a anterograde manner, under a local anesthetic (2% xylocaine with epinephrine). After placement of the catheters and after a period of quiet resting in the supine position, a resting blood sample was collected from the femoral vein for determination of resting hematocrit (Hct) and hemoglobin (Hb) concentrations. Catheters were kept patent with a flush of saline (0.9% NaCl; ~15 ml/h) after collection of each blood sample. During PVX, ~500 ml of a 6% Pentispan solution was then infused through the catheter into the femoral vein over a 30 min period. The Pentispan solution was pre-warmed to 37°C to avoid any alterations in body temperature. Following the infusion, participants were then moved to the kicking ergometer in preparation for the standardized exercise test. In the other two conditions, CON and DIU, the procedures were identical except that no infusion of Pentispan solution was performed.

Dynamic two-legged knee extension/flexion exercise was performed with participants seated on an electronically-braked custom made kicking ergometer. Three pre-test sessions on the ergometer were completed to acclimatize participants to the exercise mode. The final session involved an incremental test to fatigue in order to determine each participant's peak work rate and peak aerobic power (VO_2peak). Fatigue was defined as an inability to maintain a kicking frequency of 25-30 rpm, or when excessive trunk rotation and/or shoulder movement

were observed. Gas exchange data collected during this test were used to calculate the ventilatory threshold (Tvent) and the standardised exercise test work rate. The ventilatory threshold was determined using a computer program that utilised the V slope method (First Breath, St. Agatha, ON). With this method, VCO_2 and VO_2 are plotted against time and the point of deflection is detected (Beaver *et al.*, 1986).

The standardized test involved 10 min of exercise at a workload that elicited ~85% Tvent (61 ± 4 W). Following 2 min of resting data collection, the flywheel of the ergometer was manually cranked so that the participants would not be required to overcome inertia when starting the exercise. This basic protocol was used during all 3 conditions.

Breath-by-breath gas exchange and ventilation were measured with a computer software system (First Breath, St. Agatha, ON) (Hughson *et al.*, 1991). This system determines fractional concentrations of O_2, CO_2 and N_2 with a mass spectrometer (MGA-1100, Marquette Electronics Inc., Milwaukee, WI), and inspired and expired volumes with a volume turbine (VMM-110, Alpha Technologies, Laguna Beach, CA). The mass spectrometer was calibrated using two tanks of known gas mixtures. Calibration of the volume turbine was done by manual pumping of a 3000 ml syringe at flow rates similar to those achieved during the exercise tests.

A pneumatic finger cuff (Ohmeda 2300, Finapres, Lakewood, CO), placed on the middle finger of the hand, was used to measure mean arterial pressure (MAP). Cardiac output (Qc), by CO_2 rebreathing, was determined at rest and during the last minute of exercise (Jones & Campbell, 1982).

Blood flow was calculated from the product of mean blood velocity (MBV) and femoral artery diameter. Beat-by-beat MBV was determined using 4 MHz pulsed Doppler

probe (Model 500V Multigon Industries, Mt. Vernon, NY) held on the skin overlying the femoral artery (2-3 cm distal to the inguinal ligament). Constant intensity insonation (insonation angle 45°) across the entire vessel was ensured by setting the ultrasound gate at full width. Auditory and visual feedback were used to ensure optimal ultrasound beam alignment. An internal signal representing a Doppler shift frequency elicited by blood flowing at 1m/s at a 0° insonation angle was used to calibrate the signal. B-mode echo Doppler (Model SSH1140A, Toshiba Inc., Tochigi-Ken, Japan) using a 7.5 MHz probe was used to collect an image of the femoral artery at rest, 2 min and 9.5 min of exercise. Images were stored on VHS videotape for later analysis.

Heart rate (HR), MAP, and femoral artery blood velocity were recorded continuously throughout the tests at a frequency of 200 Hz with an analog-to-digital converter and computer.

Simultaneous blood samples were drawn from both the radial artery and femoral vein catheters at rest, 20 and 40 sec, and 1, 3, 5, and 9 min of exercise. Immediately prior to each time point, ~3 ml of blood was drawn from each catheter to ensure the catheter was clear and patent. Two samples were drawn from each catheter at each time point, one into a 1 ml heparinized syringe and the other into a 5 ml syringe. The 1 ml heparinized syringes were gently mixed and placed on ice. Within one hour of collection, the 1 ml heparinized samples were analyzed for PO_2, PCO_2, Na^+, K^+, and Hct by selective electrodes in a blood gas-electrolyte analyzer (NovaStat Profile Plus 9, Waltham, MA). The analyzer was calibrated at regular intervals during analyses. Oxygen saturation and content were obtained from the analyzer after automated application of standard equations (Stat Profile Plus 9 Analyzer Reference Manual, Waltham, MA). The standard equations for oxygen saturation and content

are as follows:

$$\text{oxygen saturation (\%)} = \frac{(pO_2^{\,1})^3 + 150(pO_2^{\,1})}{(pO_2^{\,1})^3 + 150(pO_2^{\,1}) + 23400} \times 100$$

$$\text{where } pO_2^{\,1} = pO_2 \times e[2.3026 \times (0.48(pH - 7.4) - 0.0013\,([HCO_3^{-}] - 25))]$$

$$O_2 \text{ content } (ml \cdot 100\,ml^{-1}) = 1.39\,[Hb] \times \frac{O_2 \text{ saturation}}{100}$$

where pO_2 is the partial pressure of oxygen, HCO_3^- is the bicarbonate concentration, and Hb is the hemoglobin concnetration.

The other blood sample (5 ml) was used for the determination of blood lactate, glucose, glycerol, serum FFA, and catecholamine concentrations. For determination of blood metabolites (lactate, glucose and glycerol), a sample of blood was deproteinized using ice-cold perchloric acid. Following centrifugation for removal of the precipitated proteins, ice-cold $KHCO_3$ was added to neutralize the acid. For analysis of serum FFA, 1.5 ml of blood was allowed to clot, the sample was then centrifuged and the resulting serum was extracted and stored. All samples were stored at -80°C before analysis. Blood concentrations of lactate, glucose, glycerol, and serum FFA were determined using fluorometric methods, as previously described (Green *et al.*, 1991a). Plasma catecholamines, epinephrine (EPI) and norepinephrine (NE), were determined using high-performance liquid chromatography and electrochemical detection according to the methods described by Weicher et al. (1984) and modified by Green et al. (1991a).

Hematocrit was also measured on the initial resting blood sample that was extracted before any infusion and immediately before exercise (in triplicate). Hematocrit and Hb were used to calculate changes in PV both between conditions at rest, and during exercise within

each condition. The equations of van Beaumont et al. (van Beaumont *et al.*, 1972) were used to calculate the changes in PV. Similar values were obtained when Hct and Hb where used to calculate changes in PV compared to when Hct was used alone. Thus, only values calculated from Hct are reported. All Hct values were also corrected for trapped plasma (0.96) and venous-to-whole body Hct differences (0.91).

Data analysis and calculations. A Doppler signal processor determined the instantaneous mean velocity from the Doppler shift spectra. The instantaneous mean velocity was integrated between consecutive R waves of the electrocardiogram to yield a mean blood velocity value for each heart beat. The Doppler equation was then applied to the area under the instantaneous blood velocity curve. The Doppler principle calculates velocity from the magnitude of the Doppler frequency shift using the equation:

$$V = \frac{f_D \cdot c}{2 \cdot f_t \cos(\alpha)}$$

where V = velocity of the red blood cells in cm/sec, f_D = Doppler shift frequency, c = velocity of sound in the tissues, f_t = transmitted frequency and α = angle of insonation (Burns & Jaffe, 1985).

Femoral artery diameter was determined as the average of 3 separate frozen screen images, using 3 pairs of electronic callipers placed on the arterial walls of each screen, at rest, 2 min and 9.5 min of exercise. Blood flow was then calculated at the times corresponding to the blood samples taken at rest, 20 and 40 sec, and 1, 3, 5, and 9 min of exercise.

Rest and exercise cardiac outputs were calculated by the indirect Fick procedure (Jones & Campbell, 1982) using the mixed venous PCO_2 ($PvCO_2$) determined by CO_2 rebreathing and $PaCO_2$ estimated from end-tidal PCO_2 immediately before rebreathing. Stroke volume

was calculated using cardiac output and heart rate values for a given time point. Vascular resistance across the leg was calculated based on the following equation;

$$LVR = \left(\frac{MAP}{Q_L}\right) \times 1000$$

where LVR is leg vascular resistance, MAP is mean arterial pressure at the level of the femoral artery, and Q_L is leg blood flow (Shoemaker *et al.*, 1996).

Net metabolite exchange differences were calculated using the equations described by Bergman et al. (Bergman *et al.*, 1999a; Bergman *et al.*, 1999b). Briefly, net exchange differences were calculated by the product of leg blood flow and a-v differences where arterial and venous Hct values were used to correct for changes in PV.

Net glucose uptake ($mmol \cdot min^{-1}$)

$$= 2\ (Q_L)([glucose]_a - ((Hct_a/Hct_v)[glucose]_v)$$

Net lactate release ($mmol \cdot min^{-1}$)

$$= 2\ (Q_L)(((Hct_a/Hct_v)[lactate]_v) - [lactate]_a)$$

Net glycerol release ($mmol \cdot min^{-1}$)

$$= 2\ (Q_L)(((Hct_a/Hct_v)[glycerol]_v) - [glycerol]_a)$$

Net FFA uptake ($mmol \cdot min^{-1}$)

$$= 2\ (Q_L)([FFA]_a - ((Hct_a/Hct_v)[FFA]_v)$$

where 2 corrects for both legs, Hct is hematocrit, Q_L is single leg blood flow, and a and v represent arterial and venous, respectively.

Whole body oxygen delivery was calculated by multiplying Qc by arterial oxygen content. Whereas, leg oxygen delivery was calculated using the following formula;

$$= 2\ (Q_L)(CaO_2)$$

where CaO_2 is the arterial oxygen content of the blood and Q_L is single leg blood flow.

Whole body a-vO_2 difference was calculated using the following rearranged Fick equation;

$$a - vO_2\,diff = \frac{VO_2}{Qc}$$

where a-vO_2 diff is whole body arterial-venous oxygen difference, VO_2 is whole body oxygen uptake as measured at the mouth, and Qc is cardiac output as estimated using the CO_2 rebreath procedure.

Oxygen uptake across the leg was calculated using the following equation;

$$Leg\ VO_2 = \frac{2(Q_L)(CaO_2 - CvO_2)}{100}$$

where leg VO_2 represents oxygen uptake across the leg, Q_L is single leg blood flow, CaO_2 is arterial oxygen content in the radial artery and CvO_2 content is femoral vein oxygen content.

Statistical analysis. Data were analyzed using a two way repeated measures ANOVA for experimental condition (CON, PVX, DIU) and time. When a significant difference was found, Newman-Keul post-hoc technique was used to determine pairwise differences. Also, when appropriate, significant differences were also identified using paired t-tests. The level of significance was set at $P < 0.05$. Data are presented as means ± S.E., unless otherwise stated.

RESULTS

PV changes. At rest and during exercise, PV was different among the three conditions ($P < 0.05$) (Figure 4.1). At rest, PV with DIU was reduced by 15.1 ± 2.0 % compared to CON, while with PVX, it was increased by 18.2 ± 1.3 %. Exercise had similar effects on all conditions. A rapid decline in PV was observed with the onset of exercise. As the exercise continued there was further, albeit small, decline ($P < 0.05$).

Respiratory Gas Exchange. Exercise lead to rapid increases in VO_2, which plateaued by 4 min of exercise in all conditions. PVX and DIU had no effect on VO_2 during exercise (Figure 4.2A). Carbon dioxide production (VCO_2) followed a similar pattern to that observed with VO_2 (Figure 4.2B). Ventilation (V_E) (BTPS) also followed a similar pattern, with a rapid progressive increase observed over the initial 3 min (Figure 4.2C). However, for V_E, a further upward drift occurred during the remaining period of exercise. Respiratory exchange ratio (RER) was different between the three conditions (Figure 4.2D). A main effect for condition was observed for RER, such that DIU was greater than both CON and PVX. In addition, exercise lead to an increase in RER for all experimental conditions that was initially observed by 2 min of exercise.

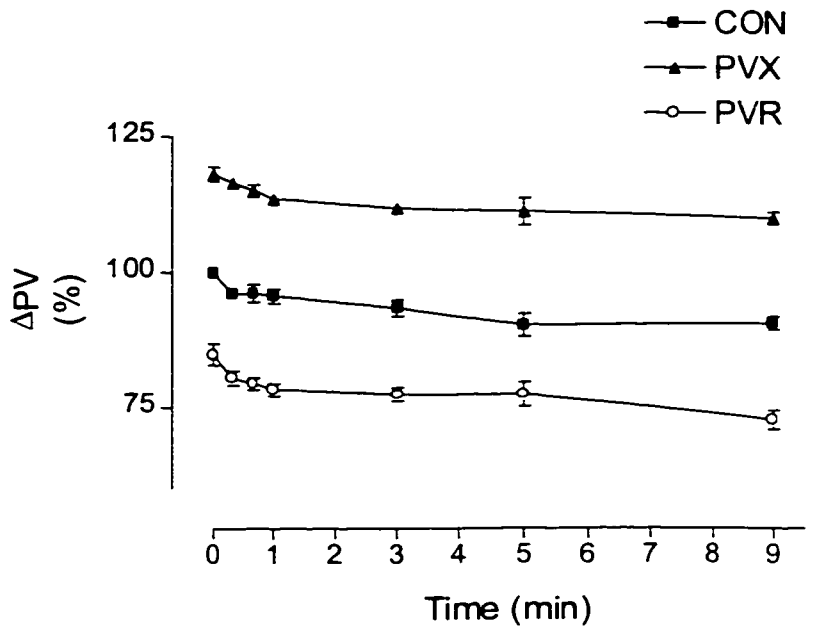

Figure 4.1. *Changes in plasma volume (PV) with exercise. Values are means ± SE; n=7 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. Main effects (p<0.01) for both condition and time were found. For condition, PVX > CON > DIU. For time, 0 > all other time points; 20 sec > 3, 5, and 9 min; 40 sec > 3, 5 and 9 min; and 3 > 9 min.*

Cardiovascular responses. Cardiac output was altered both by experimental condition and exercise (Table 4.1). At rest, Qc was similar between DIU and CON. However, PVX led to an increase Qc at rest compared to CON. Exercise resulted in an increase in Qc during all of the conditions compared to resting values. During exercise, Qc was higher and lower for PVX and DIU, respectively, compared to CON.

A main effect for condition was observed for heart rate, such that DIU was greater than both CON and PVX (Figure 4.3A, Table 4.1). Exercise also led to increases in HR for all conditions. Heart rate was different among the three conditions at various time points both at rest and during exercise. Plasma volume expansion led to higher heart rate values at 1, 3, and 9 min of exercise, compared to CON. Heart rate during DIU was greater at every measured

time point compared to CON. In addition, DIU led to greater heart rate values at 0, 20 sec, 40 sec, 5 min, and 9 min, compared to PVX.

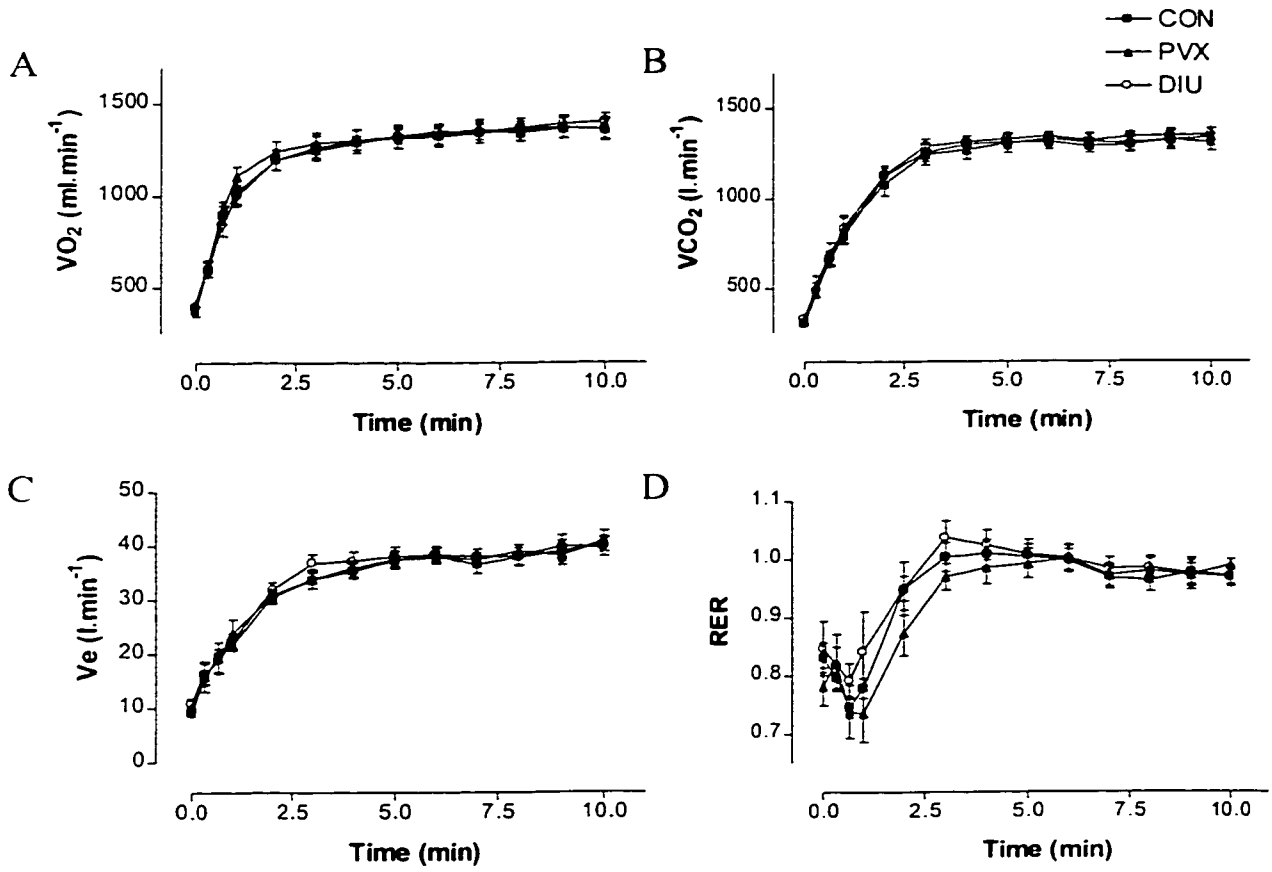

Figure 4.2. *Whole body VO_2 (A), VCO_2 (B), V_E (C), and RER (D) with exercise. Values are means ± SE; n=7 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. For VO_2 and VCO_2, a main effect for time (P<0.01) was observed. For time, 0 < 20 sec < 40 sec < 1 min < 2 min < 3 min < 4, 5, 6, 7, 8, 9, and 10 min. For V_E, a main effect for time (P<0.01) was observed. For time, 0 < 20 sec < 40 sec < 1 min < 2 min < 3, 4, 5, 6, 7, 8 min < 9, and 10 min. For RER, main effects for both condition (P<0.05) and time (P<0.01) were found. For condition, DIU > CON, PVX. For time, 0, 20, 40 sec, 1 min < 2, 3, 4, 5, 6, 7, 8, 9, and 10 min.*

A main effect for condition was also observed for stroke volume (Table 4.1). Stroke volume during DIU was lower than CON and PVX, whereas CON was lower than PVX. During all three conditions, stroke volume remained constant both at rest and during exercise.

At rest, whole body a-vO_2 diff was decreased ($P<0.05$) with PVX, compared to CON and DIU, whereas during exercise, whole body a-vO_2 diff varied directly with PV, such that DIU > CON > PVX (Table 4.1).

Table 4.1. *Influence of acute alterations in PV on cardiovascular variables at rest and during exercise.*

	Time	
	Rest	10 min Exercise
Cardiac output ($l \cdot min^{-1}$)		
CON	6.3 ± 0.6	13.8 ± 0.6
PVX	8.2 ± 0.6*	16.0 ± 0.9*
DIU	6.0 ± 0.3	12.2 ± 0.5*
Stroke volume ($ml \cdot beat^{-1}$)		
CON	93.0 ± 9.8	103.5 ± 6.3
PVX	115.3 ± 8.9	118.3 ± 7.5
DIU	74.1 ± 6.3	85.3 ± 6.9
Heart rate ($b \cdot min^{-1}$)		
CON	72 ± 4	133 ± 6
PVX	72 ± 3	134 ± 5
DIU	90 ± 6*	143 ± 8*
Whole body a-vO_2 diff ($ml \cdot 100ml^{-1}$)		
CON	6.02 ± 0.49	9.96 ± 0.35
PVX	5.04 ± 0.34*	8.86 ± 0.37*
DIU	6.51 ± 0.37	11.50 ± 0.27*

*Values are means ± SE; n=8. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.05). For cardiac output, main effects for time and condition (P<0.01) were observed. For time; Exercise > Rest. For condition; DIU < CON < PVX. For stroke volume, a main effect for condition (P<0.01) was observed. For condition; DIU < CON < PVX.*

The experimental conditions had no influence, either at rest or during exercise, on mean arterial pressure (MAP) (Figure 4.3B). In all 3 conditions, exercise led to a progressive increase in MAP, that was evident by 40 sec of exercise and continued to increase until 3 min, with no further changes observed after 3 min. Exercise led to a decline in vascular resistance in all of the experimental conditions (Figure 4.3C). Despite the increase in vascular resistance at rest with DIU compared to CON and PVX, no differences were observed among the three conditions during exercise.

Whole body oxygen (O_2) delivery was increased both at rest and during exercise with PVX compared to CON and DIU (Figure 4.4). A similar increase in whole body O_2 delivery was observed during exercise for all three experimental conditions.

Oxygen uptake across the leg. Oxygen uptake across the leg (legVO_2) was similar among the three conditions, both at rest and during exercise (Figure 4.5A). Exercise led to a progressive increase in legVO_2, evident by 20 sec, with further increases observed at 40 sec, and 3 min, with no further increases thereafter.

Leg blood flow. Exercise led to an increase in leg blood flow for all conditions (Figure 4.5B). With the onset of exercise, a rapid increase occurred that was evident by 20 sec. Blood flow continued to increase until 3 min, before plateauing. At rest and during the first few min of exercise, blood flow was similar among all conditions. However, by 3 min of exercise, blood flow increased more in PVX compared to both CON and DIU. Blood flow was similar between DIU and CON throughout exercise.

Exercise led to similar increases ($P<0.05$) in leg a-vO_2 diff for all three conditions (Figure 4.6A). A rapid increase was observed by 20 and 40 sec, with no further increases until 9 min. A main effect ($P<0.05$) for condition was also observed, such that DIU > CON > PVX.

Despite the difference in leg blood flow, no differences were observed for leg O_2 delivery between the experimental conditions (Figure 4.6B). However, exercise did lead to a rapid and progressive increase in leg O_2 delivery, during the initial 3 min of exercise, in all conditions.

The DIU and PVX conditions induced alterations in arterial oxygen content (CaO_2), both at rest and during exercise (Figure 4.6C). The DIU condition lead to an increase in CaO_2, while PVX lead to a decrease in CaO_2, compared to CON. In contrast, no differences were observed in CvO_2 for the three conditions either at rest or during exercise (Figure 4.6D). With exercise, CaO_2 increased rapidly during the first 40 sec in all conditions, with no further changes occurring for the remainder of the exercise. With CvO_2, exercise also resulted in a rapid decline that was first evident at 20 sec, then was followed by a further decline at 40 sec. No further declines were observed beyond this time point. Despite the differences in CaO_2, no differences in CvO_2 were observed between the experimental conditions.

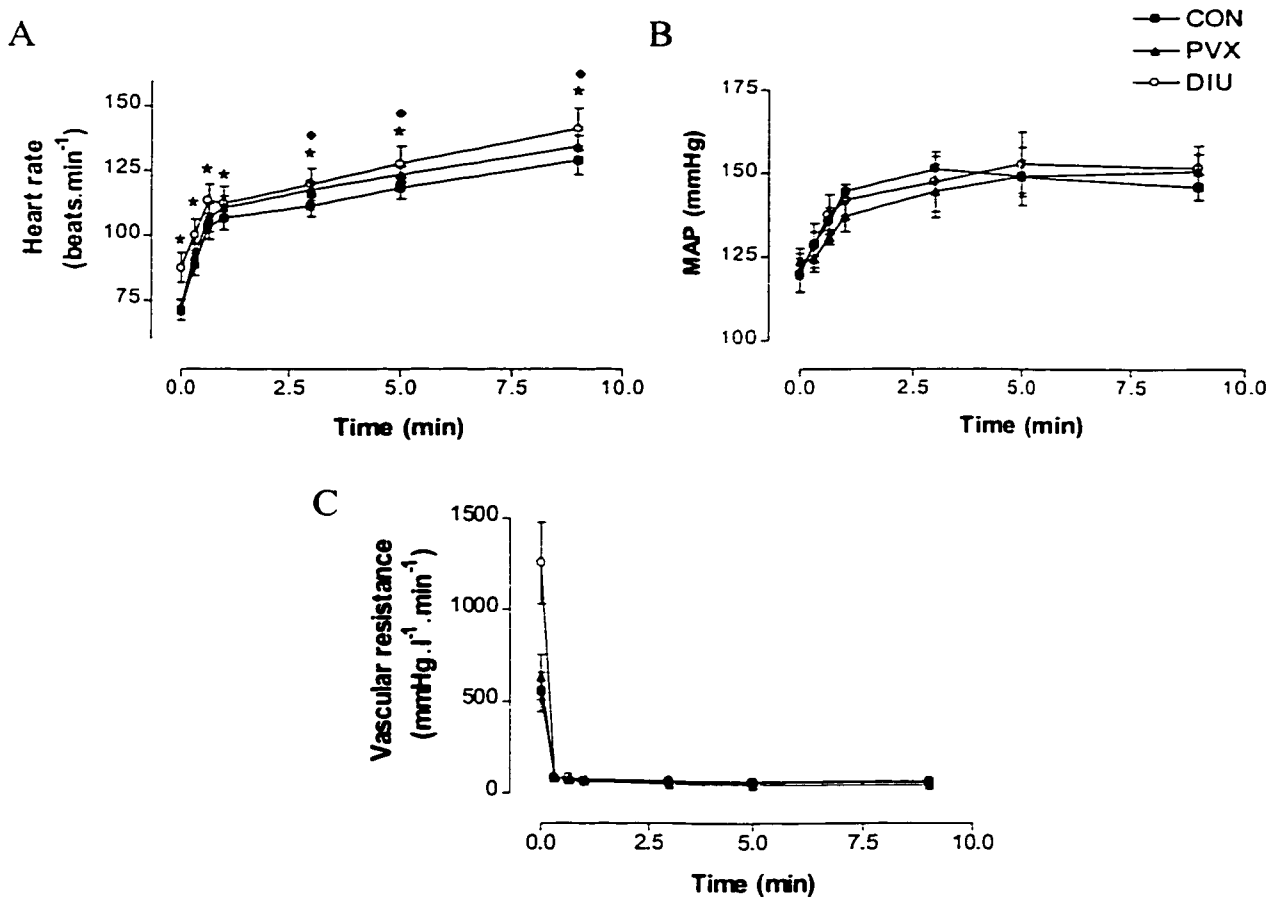

Figure 4.3. *Heart rate (A), mean arterial pressure (B), and vascular resistance (C) with exercise. Values are means ± SE; n=7 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.01). •Significantly different from PVX (P<0.01). For heart rate, main effects (P<0.01) for both condition and time were found. For condition, DIU > CON, PVX. For time, 0 < 20 sec < 40 sec, 1 min < 3 < 5 < 9 min. For mean arterial pressure, a main effect for time was observed (P<0.01), such that; 0, 20 sec < 40 sec, 1 min < 3, 5 , 9 min. For vascular resistance, a main effect for time was observed (P<0.01), such that 0 > all other time points.*

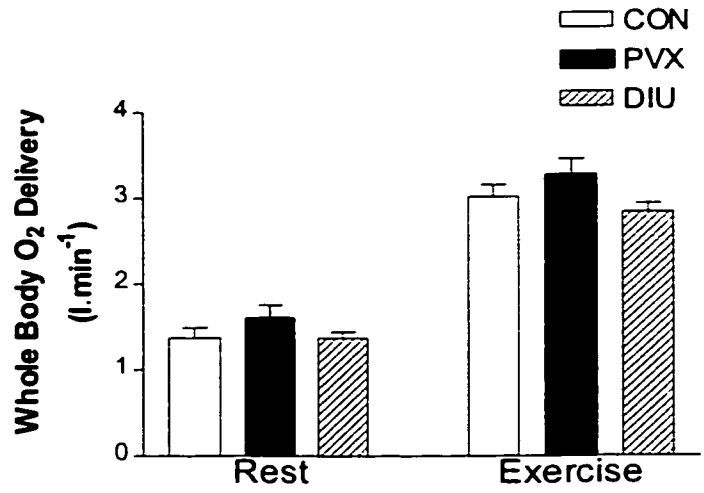

Figure 4.4. *Whole body oxygen delivery with exercise. Values are means ± SE; n=7 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. Main effects for condition and time were observed (P<0.05). For condition, PVX > CON, DIU. For time, Exercise > Rest.*

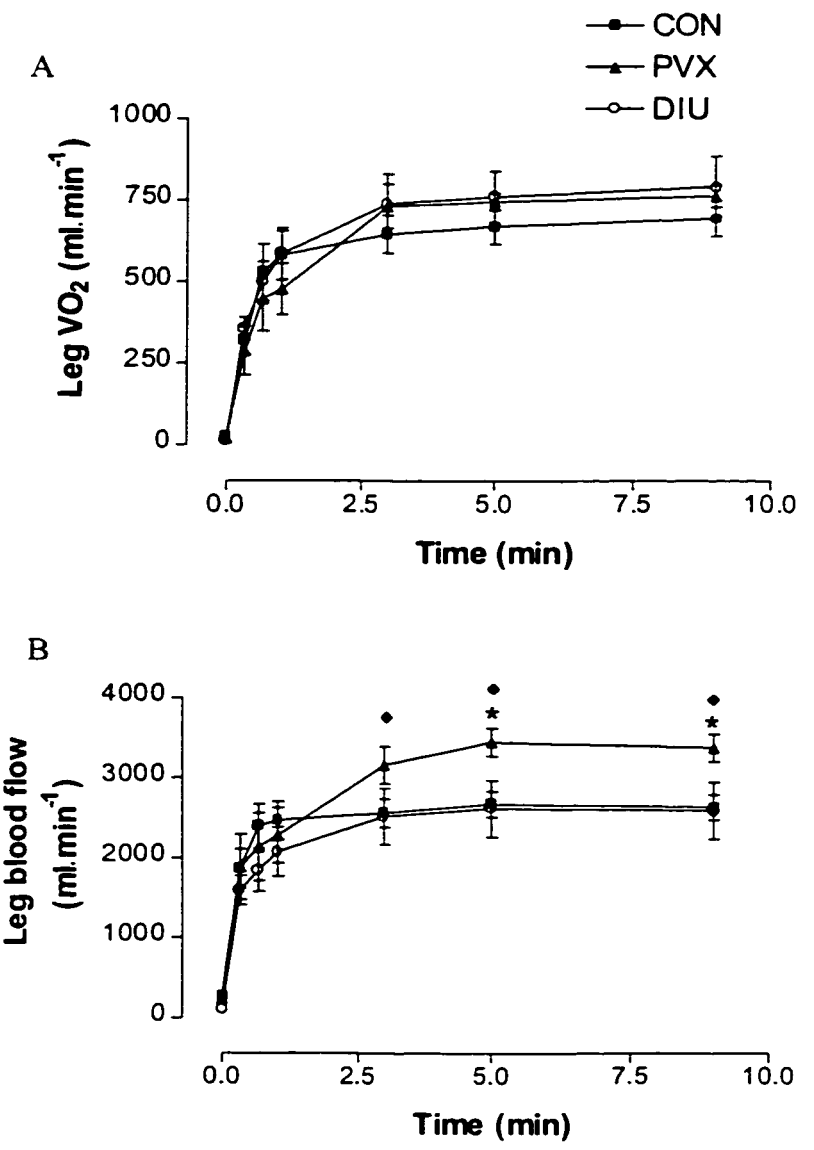

Figure 4.5. *Oxygen uptake across the leg (A) and leg blood flow (B) with exercise. Values are means ± SE; n=6 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.05). •Significantly different from DIU (P<0.01). For leg VO_2, a main effect for time was observed (P<0.01), such that 0 < 20 sec < 40 sec, 1 min < 3, 5, and 9 min.*

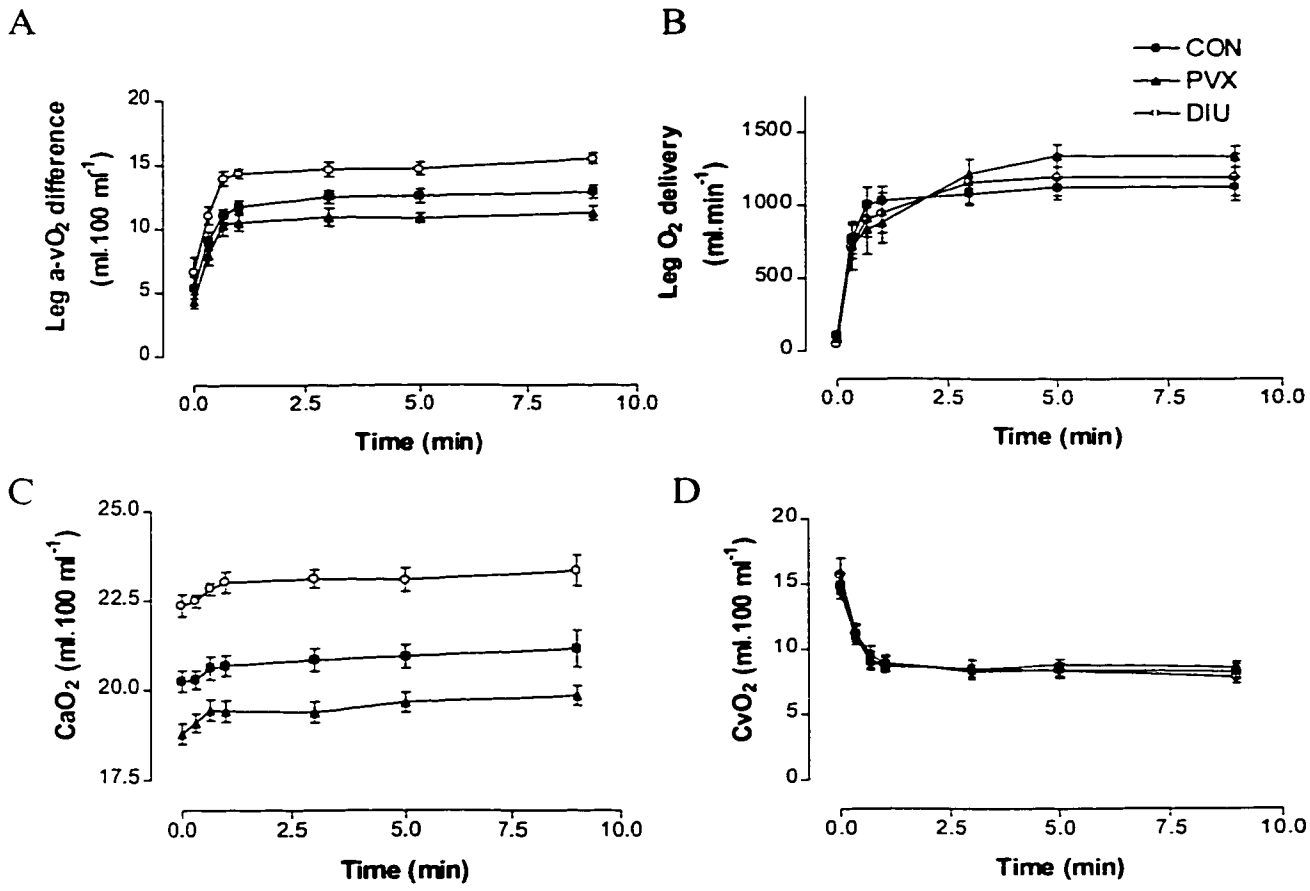

Figure 4.6. *Changes in leg a-vO_2 difference (A), leg O_2 delivery (B), CaO_2 (C), and CvO_2 (D) with exercise. Values are means ± SE; n=7 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.01). For a-vO_2 difference, main effects (P<0.05) for condition and time were observed. For condition, DIU > CON > PVX. For time, 0 < 20 sec < 40 sec < 9 min. For leg O_2 delivery, a main effect for time was observed (P<0.01), such that, 0 < 20 sec < 40 sec < 1 min < 3, 5 and 9 min. For CaO_2, main effects for both condition and time were observed (P<0.01). For condition, DIU > CON > PVX. For time, 0, 20 sec < all other time points. For CvO_2, main effect for time was observed (P<0.01), such that, 0 > 20 sec > all other time points.*

Blood metabolite concentrations and exchange. During DIU, arterial concentrations of lactate were greater compared to CON and PVX, during both rest and exercise (Figure 4.7A), while no differences were observed between CON and PVX. Exercise did lead to a rapid increase in arterial lactate for all conditions. The increase was progressive until 5 min before plateauing. A progressive rise in venous lactate was also observed for all three conditions (Figure 4.7B). Venous lactate was increased ($P<0.01$) at all times points during DIU compared to CON and PVX, except for 40 sec. Exercise also resulted in a progressive increase in net lactate release during the initial 3 min of exercise (Figure 4.7C). No differences in lactate release were observed among the three experimental conditions.

No differences were observed among the experimental conditions for arterial or venous glucose concentrations (Figure 4.8A and B). A main effect for time was observed, such that a decrease in arterial glucose was observed by 9 min of exercise. A main effect for time was also observed for venous glucose, such that an increase was observed by 40 sec, but by 9 min values were similar to resting. Glucose uptake was also influenced by exercise (Figure 4.8C). With the onset of exercise, a rapid increase in glucose uptake occurred during the initial 20 sec. This was followed by a decline back to resting levels by 40-60 sec. However, by 3 min of exercise, glucose uptake was again increased, compared to rest. No further changes were observed after 3 min of exercise. As with net lactate release, no differences were observed for net glucose uptake between the three conditions.

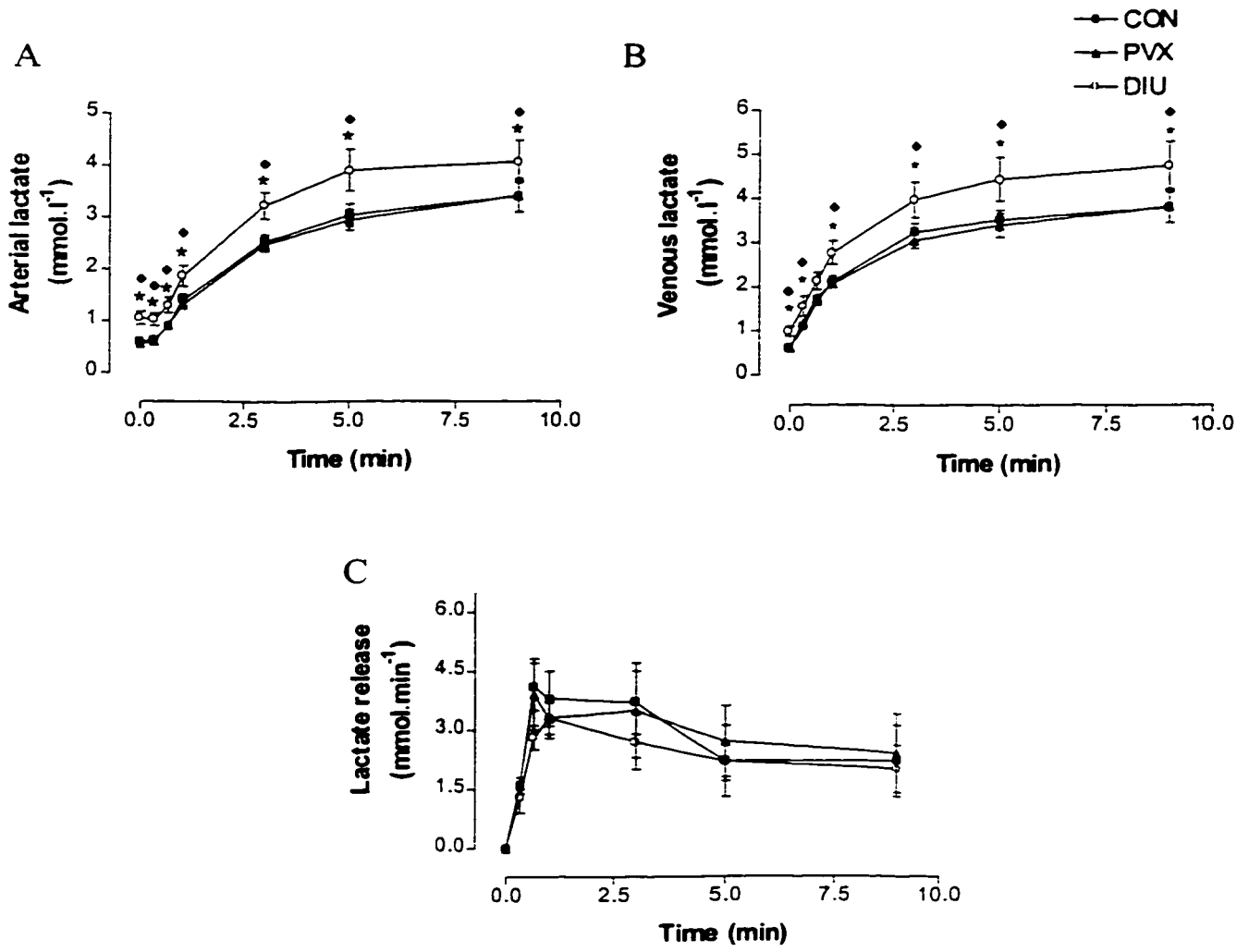

Figure 4.7. *Arterial (A) and venous (B) lactate concentrations and net lactate release (B) with exercise. Values are means ± SE; n=6 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.01). •Significantly different from PVX (P<0.01). For arterial and venous lactates, main effects for both condition and time were observed (P<0.01). For condition, DIU > CON, PVX. Arterial lactate for time, 0 < 1, 3, 5, and 9 min; 1 < 3, 5, and 9 min; 3 < 5, and 9 min. For net lactate release, a main effect for time was observed (P<0.01), 0 < all other time points; 20 sec < 40 sec, 1 min and 3 min.*

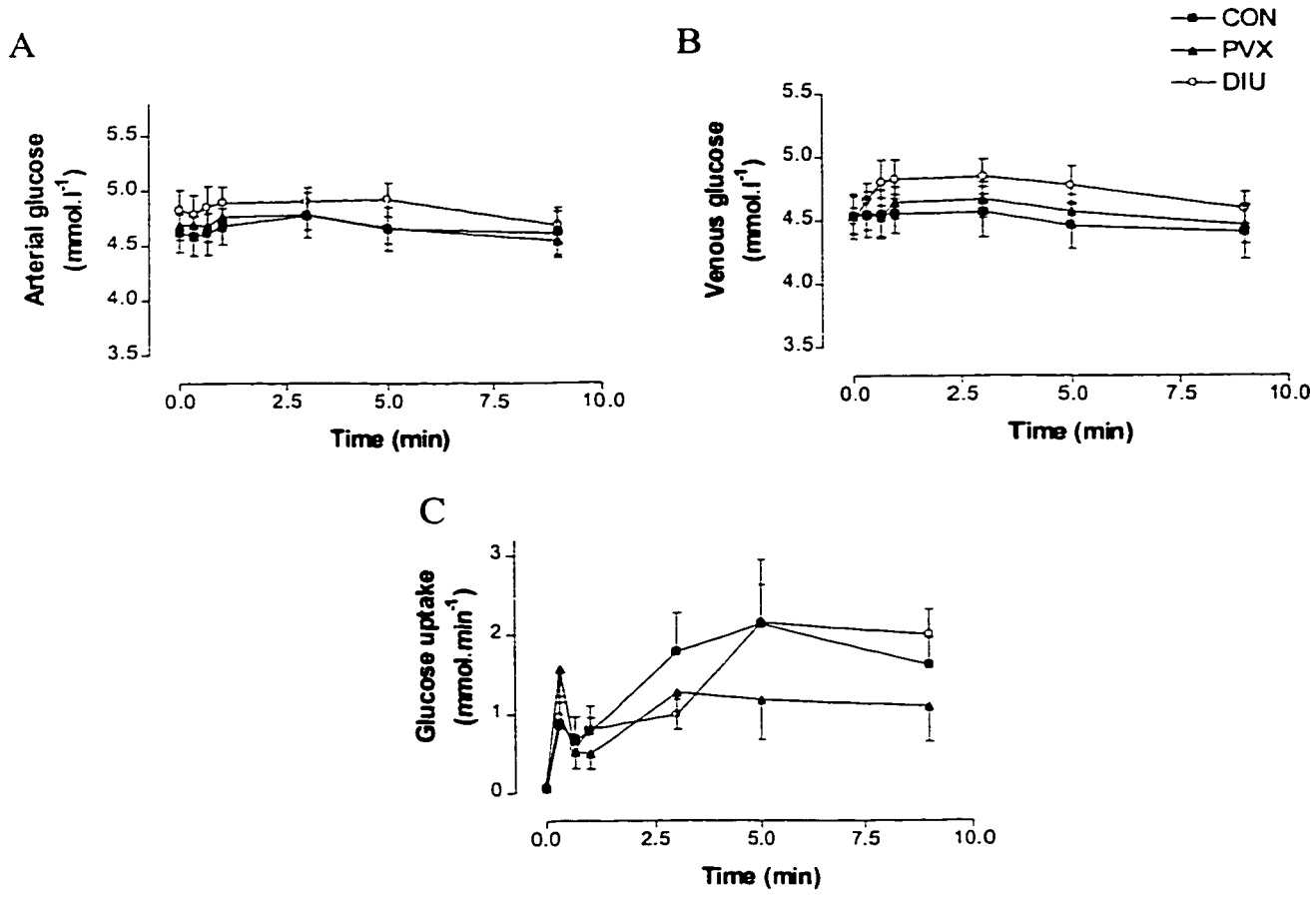

Figure 4.8. *Arterial (A) and venous (B) glucose concentrations and net glucose uptake (B) with exercise. Values are means ± SE; n=6 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. For arterial and venous glucose, main effects for time were observed (P<0.01). For arterial glucose, 9 min < all other time points. For venous glucose, 0 < 40 sec, 1, 3, and 5 min, and 9 < 20 sec, 40 sec, 1, 3, and 5 min. For net glucose uptake, a main effect for time was observed (P<0.01). For time, 0 < 20 sec; 0 = 40 sec = 1 min; 0 < 3, 5, and 9 min.*

Exercise led to a small, but significant increase in arterial glycerol that was only observed by 9 min of exercise (Figure 4.9A). An initial decrease in venous glycerol was observed at 20 sec, which persisted throughout the remainder of the exercise (Figure 4.9B). In

addition, a main effect for condition was also observed for both arterial and venous glycerol concentrations, such that DIU was greater than both CON and PVX. No significant interactions were observed between time and experimental condition for arterial and venous glycerol concentrations. In addition, no differences in glycerol release were observed among the three experimental conditions, both at rest and during exercise (Figure 4.9C).

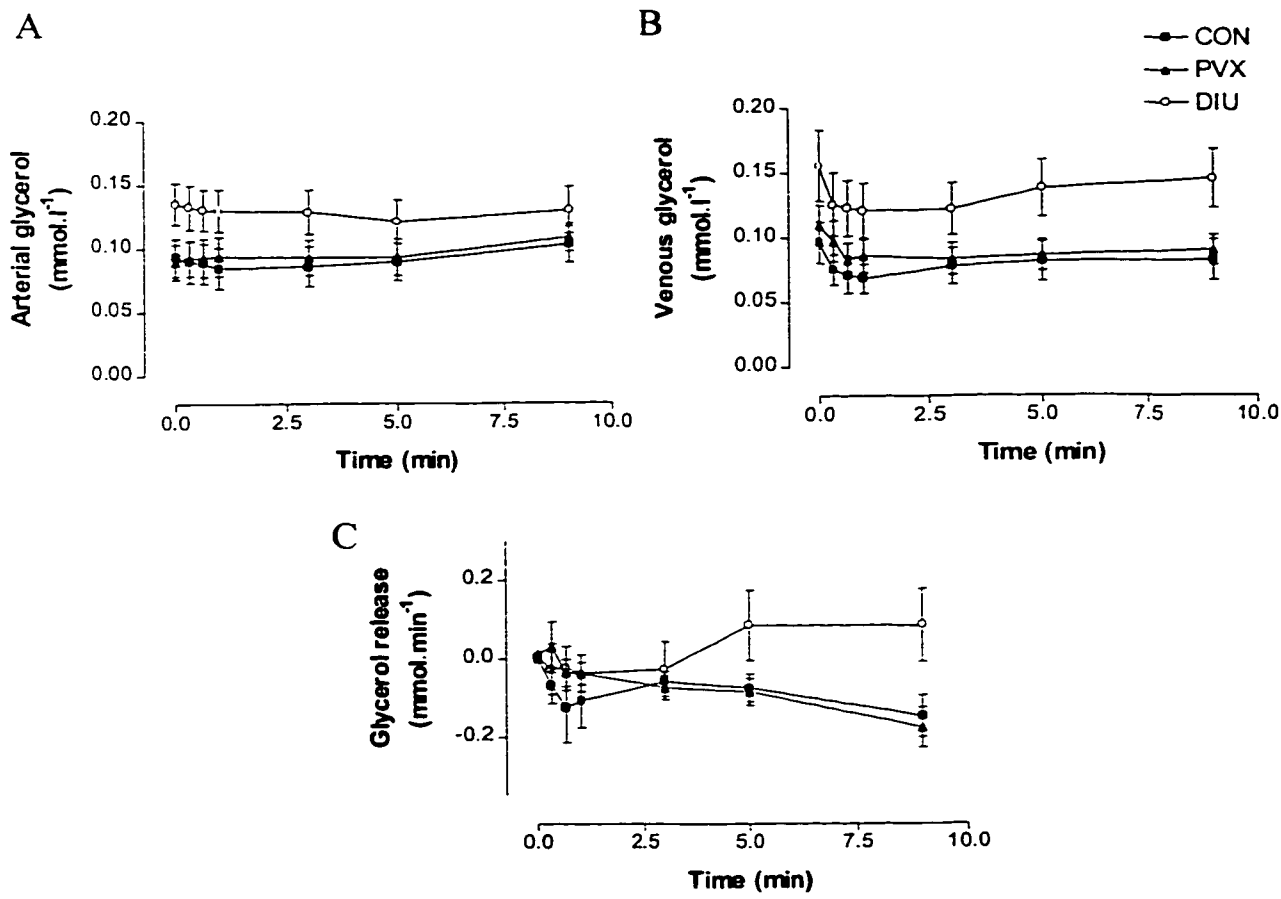

Figure 4.9. *Arterial (A) and venous (B) glycerol concentrations and net glycerol release (C) with exercise. Values are means ± SE; n=6 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. For arterial and venous glycerol concentrations, main effects for condition (P<0.01) and time (P<0.05) were observed. For condition, arterial and venous glycerol, DIU > CON, PVX. For time, arterial; 9 > all other time points, and venous; 0 > all other time points; and 40 sec, 1 min < 9 min.*

Exercise led to a decline in the arterial concentrations of FFA (Figure 4.10A). The decline, which was similar for all three conditions, was evident by 20 sec of exercise and continued until 5 min of exercise. Resting arterial FFA concentrations were lower for both DIU and PVX, compared to CON. The lower arterial FFA persisted throughout exercise during DIU resulting in FFA concentrations that were lower compared to CON. With PVX, arterial FFA concentrations were suppressed at 20 sec and 3 min of exercise, compared to CON. Exercise also lead to a progressive decline in venous FFA concentrations (Figure 4.10B). No differences in venous FFA concentrations were observed among the three conditions. Despite the differences in arterial FFA concentrations, no differences were observed for net FFA uptake with either exercise or experimental condition (Figure 4.10C).

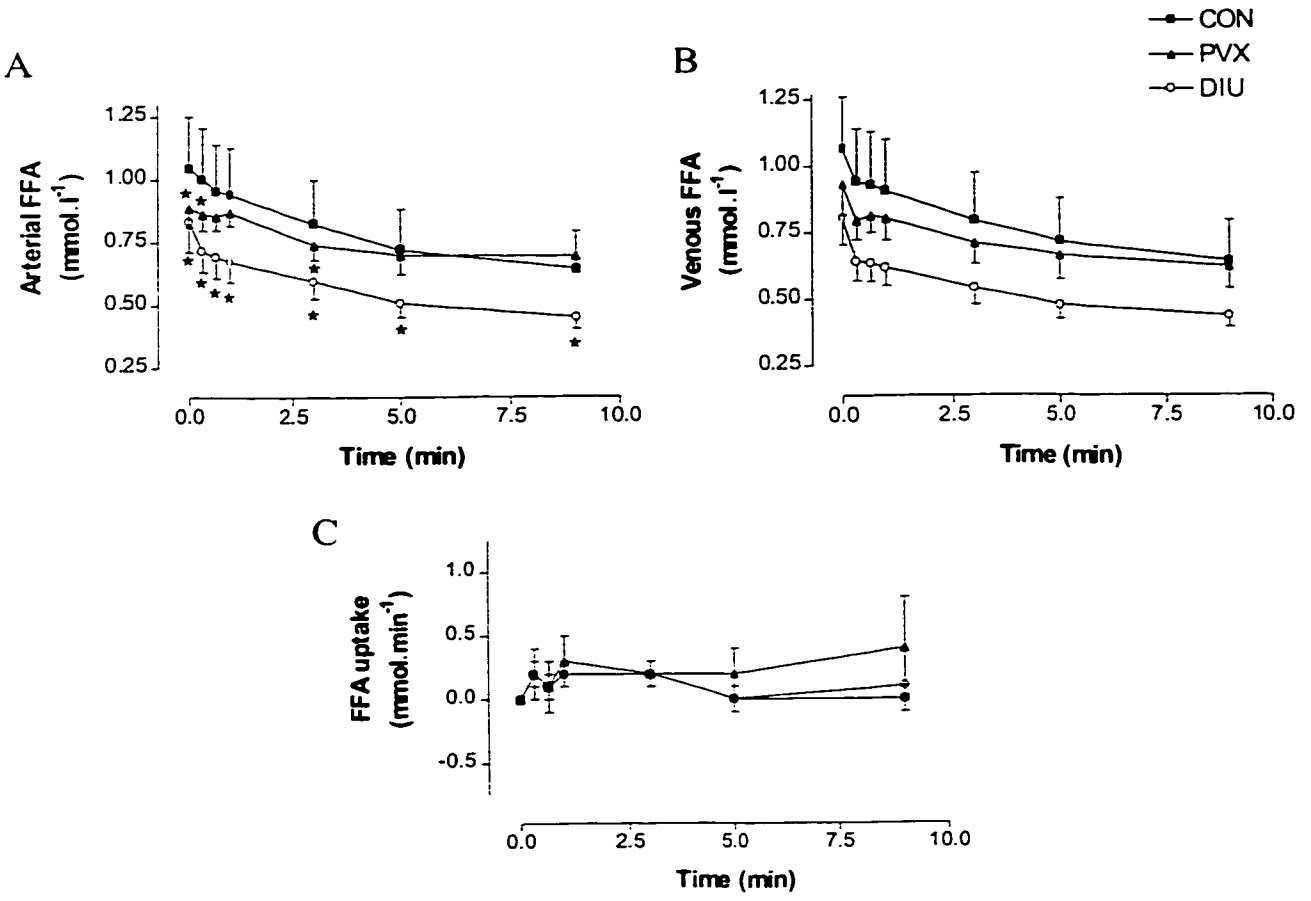

Figure 4.10. *Arterial (A) and venous (B) FFA concentrations and net FFA uptake (C) with exercise. Values are means ± SE; n=6 subjects. CON, control; PVX, plasma volume expansion; DIU, diuretic. *Significantly different from CON (P<0.05). For arterial and venous FFA concentrations, main effects for time (P<0.05) were observed. For arterial FFA, 0 > 20 sec, 40 sec, 1 min > 3 min > 5, and 9 min. For venous FFA, 0 > 20 sec, 40 sec, 1 min > 3 min > 5 min > 9 min.*

Catecholamines. No differences were observed for arterial concentrations of EPI, both at rest and during exercise (Figure 4.11A). Increases in EPI were initially observed at 3 min of exercise. No further changes were observed beyond this time point. For NE, no

differences were observed at rest between the conditions. However, with exercise, a greater increase with DIU was observed compared to both CON and PVX (Figure 4.11B).

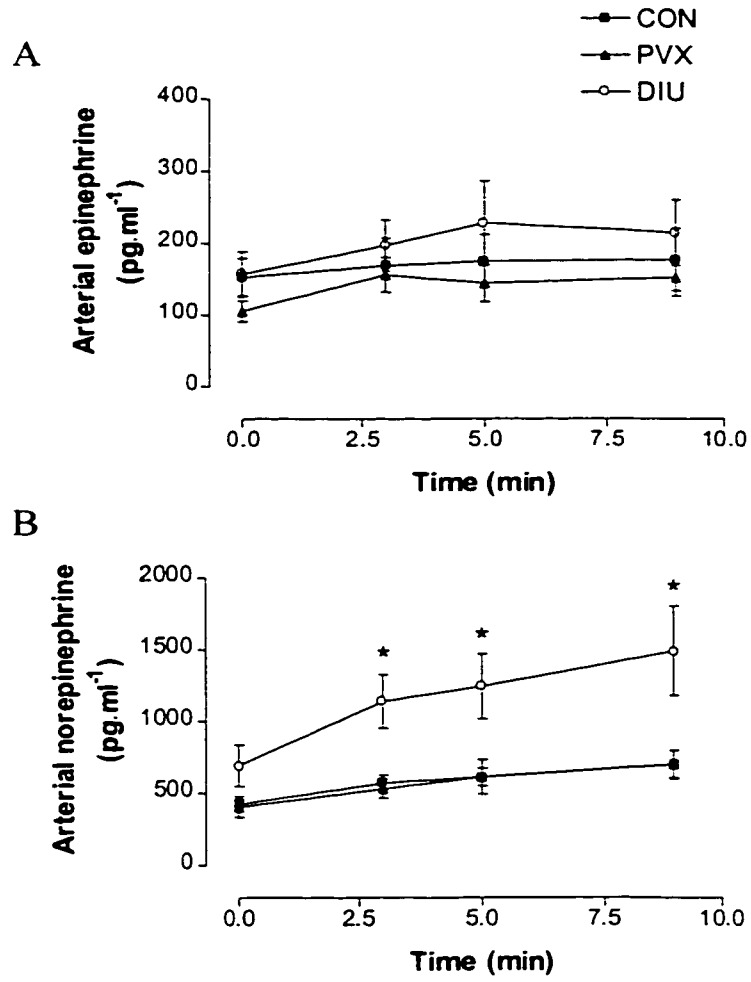

Figure 4.11. *Arterial concentrations of epinephrine (A) and norepinephrine (B) with exercise. Values are means ± SE; n = 6 subjects. CON, control; PVX, plasma volume expansion; DIU, after 4 days of diuretic administration. *Significantly different from CON and PVX (P<0.01). For epinephrine, a main effect for time was observed (P<0.01), such that, 0 < all other time points.*

DISCUSSION

The primary findings of this study were that despite increases or decreases in CaO_2 with hypervolemia and hypovolemia, respectively, VO_2 was maintained at the whole body level and across the working limb, both at rest and during exercise. It was observed that an iso-osmotic increase in PV led to an increase in Qc and SV during exercise, and that these increases contributed to the maintenance whole body O_2 delivery and VO_2, despite the decline in CaO_2, which resulted from the dilutional effect of the increase in PV. In addition, a reduction in whole body a-vO_2 diff was also observed with the increase in PV. In contrast, an iso-osmotic reduction in PV led to a decrease in Qc and SV, an increase in HR, and an increase in whole body a-vO_2 diff. Despite the differences in whole body O_2 delivery and a-vO_2 difference, whole body VO_2 was constant between the conditions.

Across the working limb, hypervolemia led to a larger increase in leg blood flow during exercise. The larger increase in blood flow served to maintain arterial O_2 delivery and VO_2, despite the decrease in CaO_2. The increase in PV also led to a decrease in leg a-vO_2 diff. Whereas, a reduction in PV had no effect on leg blood flow, despite the increase in CaO_2. The reduction in PV also resulted in an increase in leg a-vO_2 diff both at rest and during exercise.

In the current study, a 15.1 ± 2.0 % decline in PV and a 18.2 ± 1.3 % increase in PV occurred with the experimental manipulations. The main advantage of using a diuretic to induce a reduction in PV, is that there was no need for participants to be involved with exercise prior to the last condition to induce dehydration and a reduction in PV. This prevented any possible interaction of prior exercise with the test. Also, the diuretic results in an iso-osmotic reduction in PV, whereas exercise and dehydration led to a hyper-osmotic reduction in PV (Sawka & Pandolf, 1990). The decline in PV with DIU that we observed was

similar to that observed with prolonged exercise in the heat (Coyle & Hamilton, 1990). Declines of 1%, 9%, and 12% in PV have been observed with exercise, sauna and diuretic induced dehydration, respectively (Caldwell *et al.*, 1984). Others have also observed similar decreases in PV using 5 days of diuretic administration (Zappe *et al.*, 1996).

The PV expansion protocol used in this study was similar to what has been used previously to investigate the physiological significance of acute increases in PV (Grant *et al.*, 1996; Helyar *et al.*, 1997; Helyar *et al.*, 1996; Kanstrup & Ekblom, 1982; Phillips *et al.*, 1997). This model of hypervolemia results in an increase in PV, similar in magnitude to what is observed with prolonged endurance training (Convertino, 1991; Green *et al.*, 1987; Senay *et al.*, 1976). Pentispan is a colloid that holds the infused saline within the vascular compartment, thereby not disrupting other body fluid compartments. The infusion of Pentispan is an appropriate model for isolating the independent effects of increases in PV, on the physiological response to exercise.

In the current study, the experimental conditions also led to alterations in a number of cardiovascular variables, both at rest and during exercise. It appears that the alterations in the cardiovascular system served to maintain whole body VO_2, in spite of the alterations of CaO_2. Based on the Fick equation, two possible strategies exist to maintain VO_2 in the face of changes in CaO_2; 1) alter Qc through changes in HR and/or SV, and 2) maintain O_2 extraction despite reductions in CaO_2. An acute increase in PV results in a decrease in CaO_2, which would lead to a reduction in O_2 delivery if uncompensated, whereas a reduction in PV results in an increase in O_2 delivery if uncompensated.

The current study demonstrated that PVX lead to an increase in Qc, resulting from an increase in SV, both at rest and during exercise. Interestingly, during PVX, HR was not

different from CON at rest or exercise. Generally, increases in PV have been observed to results in a systematic reduction in HR, both at rest and during exercise (Grant *et al.*, 1997; Helyar *et al.*, 1996). However, others have observed hypervolemia to result in a reduction in HR at rest, but have no effect during exercise (Fortney *et al.*, 1983). The similar increase in HR during exercise with PVX and CON, was unexpected, and may suggest that the dilutional effect of PVX on CaO_2 could be influential on the increase in Qc that occurs with this protocol. Also, the large increase in PV may have maximized venous return and SV. If SV is near maximal at rest, increases in HR that occur during exercise would result in increases in Qc.

Increases in HR and SV have also been observed in dogs following acute PV expansion using protocols similar to that in the current study (Hutter *et al.*, 1999; Krieter *et al.*, 1995). In the animal models, it appears that an increase in Qc occurred to compensate for the decline in O_2 delivery, since O_2 delivery remained constant (Krieter *et al.*, 1995). In the current study, the relative distribution of Qc to the working muscle (% Qc directed to the legs) remained constant among the three conditions, despite the alterations in Qc. The exact mechanism controlling Qc with alterations in PV and CaO_2 remains unclear, but it has been suggested that feedback from the aortic chemoreceptors maybe important in this increase in Qc (Chapler & Cain, 1986). Therefore, when PV expansion leads to a decrease CaO_2, and a subsequent decline in O_2 delivery, increases in Qc, mediated by increases in SV and HR occur, returning oxygen delivery back to control levels.

The reductions in PV, as a result of DIU, resulted in hemoconcentration and a increase in CaO_2. The increase in CaO_2 was not accompanied by a change in resting Qc, despite a decrease in SV. The decline in SV was compensated for by an increase in HR, thereby

maintaining resting Qc. However, during exercise, Qc was reduced compared to CON. The reduction in Qc occurred despite an increase in HR. In spite of the effects of the reduction in PV on exercise Qc, whole body VO_2 was similar to that observed in the other experimental conditions. During exercise, the maintenance of VO_2 was mediated by an increase in O_2 extraction, as indicated by an increase in whole body a-vO_2 diff.

Even though differences in Qc, HR and SV were observed among the experimental conditions, no differences in MAP and vascular resistance were detected. This is consistent with previous findings during dynamic exercise with both increases in PV (Grant *et al.*, 1997) and decreases in PV (Zappe *et al.*, 1996). In situations were additional strain is placed on the cardiovascular system, in addition to a reduction in PV, such as heat stress, reductions in MAP are observed (Gonzalez-Alonso *et al.*, 1995). The reduction in MAP, under such circumstances, is associated with an increase in vascular resistance (Gonzalez-Alonso *et al.*, 1995). It appears that the reduction in PV, observed in the current study, did not induce enough strain on the cardiovascular system to induce a decrease in MAP and a compensatory increase in TPR.

Based on the changes in Qc that occurred with the experimental conditions during exercise, alterations in blood flow to various tissues must have occurred. In the case of PV expansion, data from the current study demonstrated an increase in leg blood flow occurred during exercise, which served to maintain O_2 delivery to the leg. Others have also observed an increase in leg blood flow in humans during submaximal dynamic exercise, following iso-volemic hemodilution (Roach *et al.*, 1999). Similar results have also been reported in an animal models (Hutter *et al.*, 1999; Krieter *et al.*, 1995). The current study is the first to investigate the influence of a colloid induced increase in PV in humans on the determinants of

VO_2, both at rest and during exercise.

Alterations in CaO_2 have been proposed to have a role in controlling Qc and blood flow (Ferretti *et al.*, 1992; Roach *et al.*, 1999). Three possible mechanisms have been suggested as to how alterations in CaO_2 mediated by altered PV or Hb content could impact on blood flow (Roach *et al.*, 1999). These mechanisms also proposed to alter vascular conductance include, Hb scavenging of nitric oxide (NO), red blood cell ATP release and arachidonic acid metabolite released in response to O_2 levels (Roach *et al.*, 1999). It appears that Hb can act as a NO scavenger in a O_2-dependent manner such that, the less saturated Hb is with O_2, the greater the amount of NO available for vasodilation (Stamler *et al.*, 1997). However, in the current study, no differences in O_2 saturation were observed, suggesting that this was not the mechanism of the increase in blood flow observed with PVX during exercise. Despite the possible mechanisms, the extent to which CaO_2 can control blood flow needs to be further investigated.

We had expected that hypovolemia would lead to a reduction in leg blood flow. Since leg VO_2 was constant between the conditions, and an increase in a-vO_2 diff was observed, according to the Fick equation, a decrease in leg blood flow should have been observed. There are a number of possible explanations as to why no differences in blood flow were observed. First, the technique used to monitor leg blood flow may not have been sensitive enough. This is unlikely, since the combination of pulse and echo Doppler techniques have been widely used and accepted as a reliable, sensitive and valid measure of blood flow at different sites in the body (Shoemaker & Hughson, 1999). Another possible explanation is that we observed a type II error (failure to reject the null hypothesis), due to the small sample size for leg blood flow (n=6). In addition, a large standard deviation was observed during the DIU condition for

the leg blood flow measurements. Repeated trials of each condition would have been beneficial in the blood flow determinations, however, constraints with the study design prevented additional measurements. Repeated trials for measurement of blood flow are commonly used with blood flow determinations made by Doppler ultrasound (MacDonald *et al.*, 1998; Shoemaker *et al.*, 1996). It is possible that an alternative method of blood flow measurement, such as indocyanide green or thermal dilution, would have been more appropriate for the current study. Interestingly, PVX did lead to an increase in blood flow. At present, it remains unclear why we were unable to detect an alteration in leg blood flow, despite the constant VO_2, in the hypovolemic condition.

Hypovolemia, compared to control conditions, resulted in increased concentrations of NE during exercise. The increased concentrations of NE did not appear to influence leg blood flow. Others have reported that when Qc is reduced, an increase in circulating NE is observed, which is associated with a decrease in leg blood flow and an increase in leg vasoconstriction (Pawelczyk *et al.*, 1992). In addition, prolonged exercise resulting in dehydration and a reduction in PV, has also been observed to result in an increase concentration of NE and a reduction in leg blood flow and PV (Gonzalez-Alonso *et al.*, 1998b; Gonzalez-Alonso *et al.*, 1999).

There has been considerable research on the mechanisms that control muscle blood flow both at the onset of and during exercise (Delp, 1999; Delp & Laughlin, 1998; Joyner & Proctor, 1999; Saltin *et al.*, 1998). As discussed earlier, CaO_2 and O_2 delivery maybe more important than capillary PO_2 in the regulation of vasodilation and exercise hyperemia. Leg blood flow at the onset of exercise is characterized by distinct stages (Hughson & Tschakovsky, 1999; Rådegran & Saltin, 1998; Shoemaker & Hughson, 1999). From the

current data, the earlier stages (rest – 1 min) do not appear to be affected by alterations in PV, the differences in blood flow appear in the latter stage of exercise (3 – 9 min). It has been suggested that the later phase of the blood flow response is regulated by feedback mechanisms (Shoemaker & Hughson, 1999). Potential mechanisms include; adenosine, prostaglandins, NO and muscle metabolities (Shoemaker & Hughson, 1999). However, in situations were Qc is limited, neurogenic vasoconstriction of active skeletal muscle has been suggested to be an important controlling factor, capable of overcoming the effects of metabolite vasodilation (Pawelczyk *et al.*, 1992). In the current study, hypervolemia resulted in the increase in Q_L, despite similar concentrations of measured blood metabolites, suggesting that the measured blood metabolites were not involved in the control of blood flow. However, the other possible mechanisms maybe involved.

One of the more interesting finding of the current study was that CvO_2 was constant during exercise across the three experimental conditions. Others have also observed consistent levels of CvO_2 with alterations in hemoglobin concentrations during steady state exercise (Ferretti *et al.*, 1992). The investigators observed that with increasing concentrations of hemoglobin due to training and autologous blood transfusions, Qc appeared to be inversely related to arterial O_2 delivery, while CvO_2 remained unaltered. It is possible that CvO_2 may play a key role in the regulation of the cardiovascular changes and leg blood flow response to exercise. In addition, the data suggests there maybe an O_2 sensor of some type on the venous side of the cardiovascular system, possibly in the right heart or on the arterial side of the pulmonary circulation (Ferretti *et al.*, 1992).

Despite the difference in blood flow and cardiac responses, no differences in the net exchange of any blood metabolites occurred across the working leg. However, DIU did lead

to increased arterial concentrations of lactate and glycerol, and decreased FFA. The elevation of both arterial and venous concentrations of lactate and glycerol do not appear to be the result of a hemoconcentration effect, since FFA concentrations were lower during DIU. The DIU condition resulted in a ~15% decrease in PV, whereas the increases in lactate and glycerol were well in excess of 15%, upwards of ~25% and ~60% for lactate and glycerol, respectively.

Arterial and venous concentrations of metabolities are a function of both release and clearance of the metabolites. Since metabolite exchange across the working limb was unaltered in the current study, it appears that clearance or release of the metabolites by a tissue other than the working limbs was affected by the alterations in PV. The reduction in PV likely lead to a redistribution of blood flow away from regions rich in α-adrenergic receptors, like the splanchnic region, towards the working muscle (McAllister, 1998). Furthermore, the increased concentration of NE observed during DIU could have resulted in an increased vasoconstriction of the splanchnic region, given the abundance of α-adrenergic receptors in the vascular beds of this region (Guyton & Hall, 1996). Norepinephrine has been implicated as a possible factor that could mediate vasoconstriction in the splanchnic region during exercise (McAllister, 1998). A reduction in splanchnic and liver blood flow could lead to a decrease in the clearance of glycerol and lactate, since the liver is a primary site of glycerol and lactate clearance (Kjaer, 1995). Others have also observed increased concentrations of lactate with declines in PV and dehydration during prolonged exercise (Gonzalez-Alonso *et al.*, 1999). The increased concentrations of lactate in the study by Gonzalez et al. (1999) appeared to be due to both an increase in lactate release from the working muscle and a decrease in lactate clearance (Gonzalez-Alonso *et al.*, 1999). Our results for the changes in lactate concentration and release in the current study are difficult to compare to other studies due to differences in

the mode of exercise, duration of exercise, and subject population. Gozalez et al. (1999) used cycle ergometry at ~61% of VO_2 peak, and a prolonged exercise protocol with the first blood measurements performed at 20 min. In addition, the sample population consisted of highly trained cyclists (Gonzalez-Alonso *et al.*, 1999). In the current study, untrained participants performed dynamic knee flexion/extension exercise for only 10 min. The current study appears to be the first to look at alterations in PV and the metabolite balance across the working limb during the onset of exercise. Previously, a decline in muscle lactate was observed with acute PV expansion early in dynamic exercise, which was hypothesized to be related to possible alterations in blood flow (Green *et al.*, 1997). However, the present study does not support this hypothesis, since no differences in net lactate release were observed with PVX, at least during the early phase of dynamic knee flexion/extension exercise.

There have been reports of improved blood glucose regulation with dehydration and prolonged exercise (Gonzalez-Alonso *et al.*, 1999). It has been suggested that the increase and maintenance of blood glucose concentration with dehydration and declines in PV are due to the greater circulating concentrations of catecholamines that are also observed (Gonzalez-Alonso *et al.*, 1999). In the current study, we also observed greater circulating concentrations of norepinephrine, but despite this increase, no differences in blood glucose were observed.

Glucose uptake is mediated by the activity and number of glucose transporters in the sarcolemma (Bernard & Youngren, 1992; Rodnick *et al.*, 1992). These transporters include GLUT1 and GLUT4 (Bernard & Youngren, 1992; Rodnick *et al.*, 1992). Glucose uptake is also dependent on the activity of hexokinase within skeletal muscle (Shulman *et al.*, 1995). Many factors have been suggest to influence the uptake of glucose by skeletal muscle (Hargreaves, 1995). One such factor is NO (Baron, 1996; Bradley *et al.*, 1999). With the

possible alterations in NO and blood flow, as discussed earlier, this could have had a potential influence on glucose uptake in the current study. Despite this possible mechanism, no differences in net glucose uptake were observed.

Circulating concentrations of FFA were also influenced by the level of PV, both at rest and during exercise. DIU resulted in a reduction in the circulating concentration of FFA. This reduction may have been related to a reduction in blood flow to adipose tissue and other sites of FFA mobilization. Clearly, the alterations in blood flow, discussed earlier, likely led to a reduction in splanchnic and abdominal blood flow with DIU. Such a reduction may have led to a reduction in FFA mobilization from the abdominal region (Wasserman & Cherrington, 1997). In addition, the elevated concentrations of lactate could have had an inhibitory effect on FFA concentrations (Wasserman & Cherrington, 1997). It has been observed that increased concentrations of lactate lead to inhibition of lipase activation, and possibly increasing reesterification rates (Wasserman & Cherrington, 1997). Despite the differences in circulating concentrations in FFA, no differences in net FFA uptake were observed. The lack of an effect may have been related to the exercise intensity selected in the current study. FFA uptake and oxidation are related to both the intensity and duration of exercise (Friedlander *et al.*, 1998; Romijn *et al.*, 1993; Wasserman & Cherrington, 1997). The intensity selected for the current study represented ~85% of ventilatory threshold, which represented 60.5 ± 2.2% of the participants knee flexion/extension VO_2peak. In addition, the duration of exercise in the current study was 10 min. Therefore it is not surprising that net FFA uptake was not influenced by the exercise or experimental conditions, since the nature of the exercise itself favoured carbohydrate metabolism and the duration of exercise was not long enough to induce large increases in the circulating concentrations of FFA and increase the rate of fat oxidation.

With more prolonged exercise, it is possible that more pronounced effects of PV alterations and FFA turnover would be evident.

In conclusion, despite the influence of acute alterations in PV on CaO_2, both whole body VO_2 and VO_2 across the working limb were maintained, both at rest and during dynamic exercise. For whole body VO_2, hypervolemia resulted in an increased reliance on Qc, mediated by an increase in SV, to maintain VO_2, despite a decrease in CaO_2. Hypovolemia resulted in an increased reliance on a-vO_2 diff, especially during exercise, since a reduction in Qc was observed. For VO_2 across the working limb, hypervolemia led to an increased reliance on leg blood flow, whereas hypovolemia resulted in an increased reliance on a-vO_2 diff. In addition, CvO_2 in the working limb was constant in the three conditions, suggesting that CvO_2 maybe important in the control of cardiac Qc and leg blood flow. Acute reductions in PV also led to an increase in the circulating concentrations of lactate, glycerol and a decline in FFA.

CHAPTER V

SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

SUMMARY

The focus of this series of studies was to determine the isolated effects of alterations in PV on physiological responses to exercise. The physiological systems of interest included the cardiovascular, thermoregulatory, and endocrine. In addition, leg blood flow, substrate mobilization, and muscle metabolism were also investigated. Two strategies were employed: a reduction in PV, induced by 4 days of diuretic administration; and an increase in PV, induced by an infusion of Dextran or Pentaspan. In addition, two different types of exercise were used. The first two studies involved prolonged dynamic cycling exercise, while a model of dynamic knee flexion/extension exercise was used in the third study.

The acute plasma volume expansion protocol, using Dextran or Pentispan appeared to provide an appropriate model for the investigation of the independent effects of an acute increase in PV. The diuretic protocol did provide a model of an iso-osmotic reduction in PV, however, it is unclear if any other changes, other than the decrease in PV, occurred during the 4 day period. Despite this limitation, the diuretic protocol provided model that allowed the investigation of a reduction in PV independent of other confounding factors.

During the prolonged dynamic cycling exercise no differences in VO_2 were observed with alterations in PV, despite the hemodilution and hemoconcentration that occurred with PVX and DIU, respectively. This suggests that some form of adjustment occurred to counteract the alterations in CaO_2 that occurred with alterations in PV. Based on the Fick equation (VO_2 = Qc x a-vO_2 diff), two possibilities exist to defend VO_2 during submaximal exercise following alterations in CaO_2. In the face of an acute increase in PV (hemodilution, $\downarrow$ CaO_2), which leads to an increase in Qc, a reduction in whole body a-vO_2 diff must occur, if VO_2 is to remain constant. With a decrease in PV (hemoconcentration, $\uparrow CaO_2$), which leads

to a decrease in Qc, an increase in a-vO_2 diff appears to be the primary adjustment. What remains unclear are the mechanisms by which control is exerted at the level of Qc and a-vO_2 diff (O_2 extraction), such that VO_2 is maintained. Using a model of dynamic leg flexion/extension exercise at a similar relative intensity, VO_2 was constant across the working limb during PVX, DIU and CON. Alterations in Q_L and a-vO_2 diff across the working limb were similar to those observed at the whole body level for Qc and whole body a-vO_2 diff, when PV was acutely increased (Chapter IV). However, despite a reduction in Qc with hypovolemia, no decreases in leg blood flow were observed. However, an increase in a-vO_2 diff was observed. Based on metabolite exchange data across the working limb, it appears that at both the whole body level and across the working limb, oxygen delivery and extraction are regulated in such a way the oxidative phosphorylation can be maintained in the face of changes in blood flow and CaO_2.

Alterations in PV were demonstrated to have direct effects on the cardiovascular response to dynamic exercise. As expected, an increase in PV resulted in an increase in Qc and SV, and a decrease in HR (Chapter II), which likely resulted from an increase in CVP and ventricular filling. A reduction in PV, had the opposite effects namely a increase in HR, and decrease in Qc and SV during exercise (Chapter 3), likely resulting from a decline in CVP and ventricular filling. Based on these observations, increases in PV appear to result in an increase in cardiovascular reserve, whereas a reduction in PV leads to an decrease in cardiovascular reserve.

One of the most interesting findings based on the blood flow study was that the venous oxygen content of blood from the working muscle was similar in the three experimental conditions. Venous oxygen content was similar despite the differences in CaO_2, Qc, and Q_L

with the different levels of PV. This finding suggests that some sort of O_2 sensor might exist on the venous side of the vascular system. Such a sensor could contribute to the regulation of the cardiovascular responses to alterations in PV during exercise.

Another issue was whether alterations in PV altered the thermoregulatory response to dynamic exercise. Thermoregulation does not appear to be influenced by acute increases in PV, but is impaired when PV is reduced. Theoretically, an increase in blood volume should lead to an improvement in the thermoregulatory control, allowing for an increase in blood flow to the cutaneous circulation, because of the resulting increase in Qc. This increase in Qc does not appear to be directed to the cutaneous circulation, since no changes in T_{re} were observed with hypervolemia (Chapter II). Alternatively, a decrease in PV induces an increase in the thermoregulatory strain. This may result from the decrease in Qc that occurs with a reduction in PV (Chapters III and IV), which would lead to a reduction in the blood flow available to perfuse the cutaneous circulation. Such a reduction could theoretically contribute to an increase in thermoregulatory strain. Collectively, it appears that blood flow to the working limb is maintained early in exercise, and thermoregulatory capacity is reduced with a decrease in PV, possibly due to a reduction in cutaneous blood flow.

Our results also indicate that the responses of several endocrine hormones to exercise are significantly influenced by the level of PV. Specifically, the response of the catecholamines, glucagon, AVP, and RAA during prolonged exercise, was attenuated with acute increases in PV, whereas with decreases in PV, the hormonal response was more pronounced. Questions still remain as to the significance of these changes. Alterations in the catecholamines could have wide spread implications. The catecholamines have been implicated in blood flow distribution and cardiovascular regulation, endocrine control, muscle

metabolism, and substrate mobilization. The fluid and electrolyte hormones have also been observed to have wide spread physiological influences. As mentioned earlier, ANG II has been implicated in the control of blood flow distribution and vasoconstriction within the splanchnic region (Stebbins & Symons, 1995), as has AVP (Stebbins & Symons, 1993). Aldosterone and AVP are also important in regulation of blood and plasma volume. It was not the focus of this thesis to directly investigate the hormonal influences on the physiological responses during exercise. However, it can be speculated that endocrine induced alterations in blood flow distribution could lead to alterations in the mobilization of extramuscular substrates, such as blood glucose from the liver and FFA from the adipose tissue. Therefore, alterations in the endocrine response to exercise, due to alterations in PV, could have both direct and indirect effects on many different physiological systems.

Alterations in the hormonal response to exercise appear to directly impact on the mobilization of extramuscular substrates (Kjaer, 1995; Wasserman & Cherrington, 1997). Despite the differences in the hormonal response to prolonged dynamic exercise observed in the current studies, the mobilization of extramuscular substrates (hepatic glucose production and whole body lipolysis) with an acute increase in PV were similar to control conditions (Chapter II). In contrast, a much more complex interaction was observed with the diuretic induced decrease in PV (Chapter III). The alterations in hepatic glucose production with hypovolemia, could have resulted from alterations in blood flow to the liver and changes in sympathetic activity. The alterations in hepatic glucose production were not related to the observed changes in glucagon. With DIU, plasma concentrations of glucagon were increased both at rest and throughout exercise, while glucose R_a was reduced at rest and early in exercise. Taken together, the data from these studies suggests that the control of hepatic

glucose output is complex and is likely influenced by a number of redundant systems, possibly including alterations in PV. What remains unclear, is if different levels of PV result in changes in splanchnic blood flow, and if so, what impact would such differences have on hepatic fuel mobilization and metabolite clearance, especially during exercise.

Despite earlier findings that acute PV expansion leads to a reduction in muscle lactate (Green *et al.*, 1997), the current investigations did not observe any mechanisms that may have contributed to this earlier finding. Increases in PV do lead to increases in leg blood flow, but no differences in net lactate release were observed with short-term exercise (Chapter IV). In addition, both an increase and decrease in PV did not lead to any differences in muscle metabolism during moderate intensity dynamic exercise (Chapter II and III). Also, no differences were observed in net metabolite exchange across the working limb during the onset of dynamic exercise (Chapter IV). Others have also observed no differences in muscle lactate with hypervolemia during dynamic exercise (Watt *et al.*, 1999). Therefore, acute alterations in PV do not appear to affect muscle metabolism during moderate intensity dynamic exercise, especially early in exercise. The mechanism leading to the reduction in muscle lactate observed previously with an acute increase in PV does not appear to be related to lactate clearance from the working limb. In addition, no differences were observed for glucose and FFA uptake, and glycerol release across the working limb during the 10 min of leg flexion/extension exercise. It is unclear what effect alterations in PV would have on metabolite exchange across the working muscle with more prolonged exercise, when alterations in the fluid and electrolyte hormones are also observed.

What also remains unclear, are the possible changes in muscle blood flow that could occur during prolonged exercise with alterations in PV. We observed an increase in leg blood

flow with an acute increase in PV during 10 min of dynamic exercise (Chapter IV), but it is unclear if this increase would be maintained if the duration of exercise was increased. There is evidence in the literature which suggests that prolonged exercise, resulting in a reduction in PV, leads to a decrease in muscle blood flow (Gonzalez-Alonso *et al.*, 1998b; Gonzalez-Alonso *et al.*, 1999). Based on these observations, we could speculate that with hypovolemia, leg blood flow may have been reduced during prolonged exercise in the current study. Therefore, further work is warranted to determine the influence of alterations in PV on muscle blood flow, especially an increase in PV, during prolonged dynamic exercise.

CONCLUSIONS

It is evident from the current series of investigations that acute alterations in plasma volume have significant effects on a number of physiological systems during exercise. The exact mechanisms for these wide ranging effects remains to be determined. Alterations in PV, directly or indirectly, influence the cardiovascular, thermoregulatory, and hormonal systems, and the responses of blood flow and substrate turnover to dynamic exercise. What appears evident is that muscle metabolism and O_2 uptake during submaximal moderate intensity exercise is maintained or protected, despite changes in CaO_2. This demonstrates that integration between central (cardiovascular) and peripheral mechanisms occurs, in order to compensate for changes in CaO_2. Clearly, an increase in PV can lead to a reduction in strain for some systems, whereas a decline in PV induces a generally greater strain on a variety of physiological systems during exercise. It is clear that increases in PV represent an adaptation that has wide spread effects in numerous physiological systems, both directly and indirectly during exercise, ultimately leading to a reduction of strain in the affected systems, thereby maintaining homeostasis.

FUTURE DIRECTIONS

The influence of alterations in PV on the determinants of VO_2 requires further investigation. Future work should focus on resolving the exact mechanisms as to how the determinants of VO_2 interact, such that in the face of alterations in CaO_2, VO_2 is maintained. Furthermore, the interaction of the determinants of VO_2 should also be investigated over a wide range of exercise intensities, which challenge VO_2, and the related processes both during steady-state and long-term exercise. In addition, the influence of various levels of hyper and hypovolemia should also be investigated.

Future work should also investigate the coordination of CaO_2, O_2 delivery, O_2 uptake, and CvO_2. The observations from the current study appear to suggest that some form of O_2 sensor may exist on the venous side of the system, since no differences in O_2 uptake were observed in any of the studies, despite clear changes in Qc, O_2 delivery, CaO_2 and a-vO_2 diff. One of the more interesting findings was the same CvO_2 that was observed from the working limb, despite the level of PV. Therefore, future work should explore the interaction of these variables and determine specific points of control, and the possible interaction of these variables in the control of limb blood flow during exercise.

The role of alterations in PV and distribution of blood flow to various vascular beds warrants further investigation. Evidence from the current studies appears to suggest that acute alterations in PV may lead to alterations in blood flow to the liver. Currently there are no feasible and safe methods to measure liver blood flow during whole body dynamic exercise in the human. However, resting blood flow to the liver could be assessed at rest with different levels of PV. When better methods are developed, future work should attempt to investigate the changes in liver blood flow that may occur with alterations in PV. In addition, the

mechanisms which regulate liver blood flow during prolonged dynamic exercise and with varying exercise intensities should also be investigated.

The effects of chronic increases and decreases in PV remains unclear. It is possible that a prolonged increase in PV could be a potential stimulus for angiogenesis. It is also possible that the increase in PV could lead to alterations in protein regulation and gene expression. It is evident that alterations in cell volume can alter gene expression and influence cellular metabolism (Haussinger *et al.*, 1994; Lang *et al.*, 1998). Therefore, alterations in the fluid volumes of other compartments could also impact on both gene expression and cellular metabolism.

One factor not investigated in the current study was the influence of alterations in PV on protein metabolism during exercise. Contribution of protein metabolism to overall substrate turnover is relatively small compared to carbohydrate and fat metabolism, however, there is some suggestion that alterations in osmotic balance within the vascular compartment can influence whole body protein metabolism during exercise (Berneis *et al.*, 1999). Therefore, alterations in PV may also influence whole body protein metabolism, especially during prolonged exercise.

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APPENDIX I

STABLE ISOTOPE METHODOLOGY

Stable isotopes have been used to study substrate turnover for the last 50 years. A stable isotope is an isotope of a given element having the same atomic number (protons), but differing in atomic mass (different number of neutrons). Stable isotopes have been used with greater frequency with the technological advances in gas chromatography/mass spectrometry (GC/MS) and the availability of ^{18}O, ^{2}H, ^{13}C, and ^{15}N. The most commonly used stable isotopes to label amino acids are ^{13}C, ^{15}N, and ^{2}H and their natural abundance's are 1.11%, 0.37% and 0.015%, respectively (Wolfe, 1992). Infusion of an isotope tracer into the venous circulation will increase both the plasma pool and body pool enrichment of the chosen label above the natural background enrichment. The increases in enrichment are usually expressed in atom percent excess (APE). For example, a primed constant infusion (~90 min) of [6,6-$^{2}H_2$] glucose results in APE values of ~2 APE (Phillips *et al.*, 1996b). This indicates that 2 out of every 100 glucose molecules studied is labeled with the tracer above the natural abundance. Several kinetic terms in relation to stable isotope tracers should be discussed; rate of appearance of a labeled tracee in the plasma pool (R_a) and the rate of disappearance of the labeled tracee out of the plasma pool (R_d) into tissues for oxidation or clearance. R_a is considered to represent the total rate of appearance of a tracee into the plasma pool (Wolfe, 1992). R_a is a function of both endogenous production (ie. liver glycogenolysis) and exogenous infusion of the tracee of interest. R_d is the rate of loss of a tracee from a specific body pool (Wolfe, 1992). R_d is the sum of irreversible tissue uptake and loss by other routes. Both R_a and R_d are expressed as mass of substrate per kg body mass per unit of time (i.e. $\mu mol \cdot kg^{-1} \cdot h^{-1}$).

The use of stable isotopes to measure whole body substrate turnover is based on a number of assumptions. These assumptions include: (1) the labeled tracer is biologically

indistinguishable from the naturally occurring tracee, (2) the administration of the tracer dose will not disturb the kinetics of the system, (3) the tracer is rapidly and evenly distributed through all body pools, and (4) during a continuous infusion protocol measurements of kinetics are only made after an isotopic steady-state has been achieved (Wolfe, 1992). These assumptions have been tested and evidence to support them has been presented in a number of other papers (Wolfe, 1992).

Dynamic exercise leads to many alterations in various physiological systems, including those which influence both glucose regulation and whole body lipolysis. Therefore, exercise does not represent steady-state as defined in the assumptions of tracer methodology. An equation to allow calculation of non-steady state kinetics was first described by Steele (Steele, 1959). The Steele equation was developed to allow non-steady state calculation of tracer kinetics using a single pool model. The Steele equation assumes that the pool of interest has a well mixed volume of distribution and there is uniform instantaneous mixing of the infused tracer within the unlabeled tracee. The working form of the Steele equation for use determination of R_a using stable isotope tracers is as follows;

$$R_a = \frac{F - pV[(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2}$$

where F is the infusion of the tracer, pV is the volume of distribution, C_2 and C_1 are the concentrations at times 2 and 1, E_2 and E_1 are tracer enrichments at time 2 and 1, and t_2 and t_1 are times 2 and 1, respectively (Wolfe, 1992). For calculation of Rd, the equation is slightly modified to;

$$R_d = R_a - pV(C_2 - C_1)/(t_2 - t_1)$$

where R_d is the rate of disappearance, R_a is the rate of appearance, pV is the volume of

distribution, C_2 and C_1 are the concentrations at times 2 and 1, and t_2 and t_1 are times 2 and 1, respectively (Wolfe, 1992).

The use of stable isotopes for determination of glucose kinetics has been widely used by a number of different laboratories (Bergman *et al.*, 1999b; Berneis *et al.*, 1999; Coggan *et al.*, 1992; Hargreaves *et al.*, 1996a; Phillips *et al.*, 1996b). Tracer determination of glucose kinetics using a single-pool model meets most of the assumptions of tracer methodology (Wolfe, 1992). However, there is the possibility for certain glucose tracers to be recycled, depending on the positioning of the label. For estimation of hepatic glucose production, a glucose tracer labeled on the 6 carbon will allow determination total new hepatic production of glucose from both glycogen and gluconeogenesis (Wolfe, 1992).

Glycerol kinetics have also been calculated using a single pool model by a number of different laboratories (Friedlander *et al.*, 1999; Klein *et al.*, 1994; Klein *et al.*, 1995; Phillips *et al.*, 1997; Romijn *et al.*, 1993). Glycerol does appear to meet most of the assumptions for stable isotope methodology, however, recently the validity of glycerol Ra representing whole body lipolysis has been questioned (Jensen, 1999). Traditionally, the total amount of glycerol released during exercise was thought to represent the sum total of release from peripheral and intramuscular lipolysis. This was assumed, since the activity of glycerol kinase within skeletal muscle has been difficult to detect (Newsholme & Taylor, 1969; Romijn *et al.*, 1993), and the majority of glycerol reesterification occurs in the liver during exercise (Romijn *et al.*, 1993). Therefore, we and others have assumed that glycerol R_a is representative of whole body lipolysis. The primary argument against the assumptions for glycerol being representative of whole body lipolysis is that significant uptake of glycerol has been observed both across the forearm and leg (Coppack *et al.*, 1999; Elia *et al.*, 1993; Jensen, 1999). However, in one of

these investigations the amount of glycerol uptake across the limb was approximately one tenth of splanchnic glycerol uptake and occurred only after meal ingestion at rest (Jensen, 1999). Despite these observations of glycerol uptake across the limb, as previously mentioned, no measures of glycerol kinase have been made in skeletal muscle. In the few studies that have observed glycerol uptake across the limb, it was assumed that skeletal muscle was taking up the glycerol (Coppack *et al.*, 1999; Elia *et al.*, 1993; Jensen, 1999). Therefore, it is only indirect evidence suggesting that skeletal muscle is a site of glycerol disposal. Whereas, there is consistent evidence to suggest that glycerol R_a does represent whole body lipolysis. First, in most studies using glycerol R_a to estimate lipolysis, the availability of FFA (3 times glycerol R_a) exceeds the rate of FFA oxidation at all times (Phillips *et al.*, 1997; Phillips *et al.*, 1996b; Romijn *et al.*, 1993). Secondly, when both FFA R_a and glycerol R_a are assessed simultaneously at rest, the ratio of these two variables is approximately 3:1 (Friedlander *et al.*, 1999; Phillips *et al.*, 1996b). Based on these findings, it appears that glycerol R_a is a reliable indicator of whole body lipolysis during prolonged endurance exercise.

In summary, stable isotope tracer methodology provides a useful means to investigate the influence of various interventions on the response of liver glycogenolysis and whole body lipolysis. With the large number of assumptions involved, the data provides insight as to the directional changes in these factors.

APPENDIX II

CALCULATIONS OF SUBSTRATE OXIDATION

This appendix is to serve as a brief overview of the stoichiometric calculations used for the estimation of substrate oxidation, based on indirect calorimetry. For a more extensive review of these equations please see Frayn, (1983) and Wolfe (1992) (Frayn, 1983; Wolfe, 1992). The calculation of substrate oxidation from measurements of O_2 consumption (VO_2) and CO_2 production involves a number of assumptions. The primary basis for these calculations is the summarized chemical reactions for the oxidation of glucose and a typical fat. The complete oxidation of glucose leads to the following summary reaction;

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 .$$

Therefore, for each mol of glucose that is oxidized, 6 mol of O_2 are consumed and 6 mol of CO_2 are produced. Calculation of the respiratory exchange ratio (RER = VCO_2/VO_2) in this situation would give a value of 1. Whereas, the oxidation of a typical fat (triglyceride) leads to the following summary reaction;

$$C_{55}H_{104}O_6 + 78O_2 \rightarrow 55CO_2 + 52H_2O .$$

Therefore, for each mol of triglyceride oxidized, 78 mol of O_2 are consumed and 55 mol of CO_2 are produced. Calculation of the RER in this situation gives a value of 0.7. The contribution of protein oxidation must also be considered. Empirical observations demonstrate that the oxidation of 1 g of protein (average protein) leads to the consumption of 0.966 l of O_2 and the production of 0.782 l of CO_2, which yields and RER value of 0.81 (Wolfe, 1992).

Knowing that one mol of a gas occupies 22.4 l, allows the conversion of the above relationships into values which represent liters of CO_2 produced or O_2 consumed per g or mmol of substrate oxidized. Based on these values, formula can be derived which allow the calculation of the separate oxidation rates of carbohydrate and fat, based on values of VO_2 and VCO_2. However, values of protein oxidation must also be incorporated into these calculations.

Protein oxidation rates can be estimated by a number of methods including; stable isotope methods and urine urea nitrogen excretion. However, more often empirical values are used, since the relative contribution of protein oxidation, especially during submaximal dynamic exercise is relatively small in comparison to carbohydrate and fat oxidation. In the current series of studies nitrogen excretion rate was assumed to be 135 $\mu g \cdot kg^{-1} \cdot min^{-1}$(Romijn *et al.*, 1993). The calculation for determination of carbohydrate oxidation is as follows;

$$CHO\,(mmol \cdot min^{-1}) = 25.196(VCO_2) - 17.749(VO_2) - 0.2139(n)$$

where CHO is the oxidation rate in $mmol \cdot min^{-1}$, VCO_2 is the rate of CO_2 excretion in $l \cdot min^{-1}$, VO_2 is the rate of O_2 uptake in $l \cdot min^{-1}$, and n is the nitrogen excretion rate in $mmol \cdot min^{-1}$.

The calculation for determination of fat oxidation is as follows;

$$Fat\,(mmol \cdot min^{-1}) = 1.9357(VO_2) - 1.9357(VCO_2) - 0.031978(n)$$

where Fat is the oxidation rate of triglyceride in $mmol \cdot min^{-1}$, VCO_2 is the rate of CO_2 excretion in $l \cdot min^{-1}$, VO_2 is the rate of O_2 uptake in $l \cdot min^{-1}$, and n is the nitrogen excretion rate in $mmol \cdot min^{-1}$.