Short-Term Training-Induced Hypervolemia And Diuresis: Effects On Oxygen Uptake And Left Ventricular Diastolic Function In Older Men

by

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Abstract

Eleven older males (68 yrs) were randomly assigned to: 1) 5 days of 1hr/day high intensity stationary cycling (EXER); 2) 100mg/day of spironolactone (DIUR); and 3) Exercise and diuretic (EXDI) to investigate the effect of changes in plasma volume (ΔPV) on left ventricular diastolic filling (LVDF) and oxygen uptake ($\dot{V}O_2$) response. Doppler echocardiographic indices of LVDF, %ΔPV, $\dot{V}O_2$ kinetics and $\dot{V}O_{2max}$ were determined 48 hours after each condition.

Plasma volume appeared to increase with EXER (6.2%, $p = 0.069$) and decreased with DIUR (-11.5%, $p < 0.001$) and EXDI (-6.8%, $p = 0.038$). Peak early trans-mitral flow velocity / peak late trans-mitral flow velocity (E:A), $\dot{V}O_{2max}$, and $\dot{V}O_2$ kinetics were unchanged. However, E (76.9 cm/s, $p = 0.043$) was higher with EXER than BASE (67.9 cm/s) and E:A approached being correlated ® = 0.934, $p = 0.066$) with %ΔPV across all 4 conditions. The absence of change in both $\dot{V}O_2$ and LVDF responses in this moderately fit group of older men suggests that pump function may not be limited by alterations in PV.

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List Of Abbreviations

$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2max}$	maximal oxygen uptake
τ	"tau" represents 63 percent of the time to achieve steady state $\dot{V}O_2$ in response to the square-wave onset of a workload
HR	heart rate
SV	stroke volume
Q	cardiac output (Q = HR x SV)
$a\text{-}\dot{v}O_{2diff}$	arterial - venous oxygen difference
T_{vent}	ventilatory threshold
Hb	hemoglobin
Hct	hematocrit
PV	plasma volume
LVDF	left ventricular diastolic filling
E	peak early left ventricular diastolic filling
A	peak late left ventricular diastolic filling (atrial systole)
E:A	ratio of early to late LVDF, an index of cardiac filling
DT	deceleration time
IVRT	isovolumic relaxation time
TPVR	total peripheral vascular resistance
RPE	rating of perceived exertion on a scale of 1 to 10

Chapter 1

Introduction

There is an age-related decline in human maximal oxygen uptake and exercise performance of approximately 10 percent per decade (Ogawa et al., 1992; Hagberg et al., 1998). Proposed mechanisms of this decline have stimulated research questions and hypotheses in the hope of identifying ways of delaying the ageing process.

The Fick Equation [$\dot{V}O_2$ = (HR x SV) x ($a\text{-}\bar{v}O_{2diff}$)] is a model of oxygen uptake, transport, and utilization and provides a means by which to investigate the age-related decline in oxygen uptake. Two theories have been proposed to account for the decline observed in aerobic performance with ageing. The transport limitation theory proposes that aerobic performance is limited by central factors such as pulmonary diffusion, blood O_2 carrying capacity, heart rate (HR), stroke volume (SV), peripheral blood flow, or diffusion from the capillaries into the muscle cells. The transport limitation theory proposes that either the heart's ability to supply oxygenated blood or ability to distribute peripheral blood flow limits exercise performance. The utilization limitation theory proposes that aerobic performance is limited by the skeletal muscles' ability to extract and use the oxygen supplied by the blood and is reflected by the magnitude of arterial-venous O_2 difference ($a\text{-}\bar{v}O_{2diff}$). Debate continues as to the limiting factor(s) in sub-maximal and maximal exercise performance with the central question being: Is exercise performance transport or utilization limited (Hughson, 1990; Tschakovsky & Hughson, 1999)? The point that should be taken from this debate is that all of these factors should be considered in the interpretation of exercise performance as

any limitations are specific to individual age and specific fitness. More important, in terms of the age-related decline in exercise performance, is the need to investigate the effect of ageing upon these possible limiting factors.

Short-term training with younger males has resulted in improvements in maximal oxygen uptake ($\dot{V}O_{2max}$) (Convertino, 1983; Nadel, 1985; Green et al., 1991a). Associated with this improvement in $\dot{V}O_{2max}$ has been an increase in plasma volume (PV) (Convertino, 1983; Nadel, 1985). Until recently, most of the research in this regard has been on younger men utilizing exercise training of a moderate intensity (65-70%$\dot{V}O_{2max}$)(Convertino, 1983; Nadel, 1985). Short-term training with older males has resulted in improvements in $\dot{V}O_{2max}$ and associated with increased PV and improved left ventricular diastolic filling (LVDF)(Petrella et al., 1997). Older men have also shown a decline in $\dot{V}O_2$ kinetics in response to the onset of a sub-maximal square wave workload and their $\dot{V}O_2$ kinetics are improved with training (Babcock et al., 1994a; Babcock et al., 1994b). Increases seen in PV response to short term training in younger males have been associated with improved $\dot{V}O_{2max}$ however there remains some disagreement on this issue (Green et al., 1987b; Green et al., 1992).

Increased PV dominates exercise-induced hypervolemia in the first two to four weeks and then the hypervolemic response is shared equally between increases in both plasma and erythrocytes (Convertino, 1991). The mechanism(s) responsible for the PV increase have been associated with blood proteins, electrolytes, and fluid regulating hormones. Although total plasma albumin, sodium, renin, and aldosterone have been shown to decrease with ageing (Tsunoda et al., 1986), and also to increase in response to short-term training in

younger males (Convertino et al., 1980), these variables have not been studied in older males in response to short-term training.

In 1996, Petrella at al. reported in a cross-sectional study of 10 older active, 10 older sedentary, and 10 younger males that older more active subjects had LVDF measures more similar to those of the younger men than the older sedentary men did (Petrella et al., 1996). It was proposed that the since the study did not train the older more active subjects, the observed LVDF measures, which appeared to be those of younger men, could not be attributed to training (Petrella et al., 1996). This led to a subsequent study of short-term training-induced PV increase, $\dot{V}O_{2max}$, and LVDF (Petrella et al., 1997) in which $\dot{V}O_{2max}$, and LVDF were shown to improve in association with PV increase.

There is an age-associated decline in LVDF and an impaired myocardial relaxation (Gerstenblith et al., 1977; Appleton & Hatle, 1992; Klein et al., 1994). Wei (1992) suggested this is of great importance due to the diminished cardiac function of the aged and the associated risks of increased diastolic dysfunction. The aged myocardium requires more time to relax (IVRT) due to decreased compliance of the tissue. This decreased compliance and increased relaxation time has been attributed to an age-related increase in human heart collagen matrix, a decrease in the capacity and rate of calcium resequestration, and lipid deposition (Gerstenblith et al., 1976; Zwolinski et al., 1976; Wei, 1992). Unfortunately, and counter-intuitively, relaxation of the myocardium is an active process and requires substantial energy and oxygen (Wei et al., 1989). The increased isovolumic relaxation time (IVRT) observed with left ventricular diastolic dysfunction and ageing makes older less fit people susceptible to hypoxia and ischemia (Wei et al., 1989; Wei, 1992). Blood oxygen content

also declines 4 mm Hg per decade from 30 to 80 years of age. The combination of lower blood oxygen content and longer IVRT under conditions of hemodynamic stress may result in hypoxia and ischemia in older myocardium.

Both left atrial contraction, to a lesser degree, and left ventricular relaxation to a greater degree, contribute to improved left ventricular diastolic filling (Petrella et al., 1997). Enhanced relaxation of the myocardium enables the left ventricle to fill to a greater degree and eject a larger volume with a greater force resulting in a larger stroke volume. The larger stroke volume allows a lower heart rate at the same $\dot{Q}$. Thus the myocardium does less work and requires less oxygen. The increased stroke volume in trained older persons (a central adaptation) approaches younger values (Hagberg et al., 1985; Ogawa et al., 1992).

The previously-mentioned debate as to whether short-term training-induced hypervolemia improves $\dot{V}O_{2max}$ may be clarified by determining the change in O_2 carrying capacity of the blood. The O_2 carrying capacity of blood can be lowered when plasma volume increases with no change in the number of the red blood cells (Coyle et al., 1990). This is termed hemodilution. Short-term training studies of five days or less in younger men have not shown the increase in $\dot{V}O_{2max}$ that has been seen in longer studies of five to eight days or more (Convertino, 1991). It has been hypothesized that this lack of increase could be due to hemodilution and is overcome once the hemoglobin (Hb) count increases in response to continued training (Convertino, 1991).

Ageing has been reported to slow $\dot{V}O_2$ kinetics (Babcock et al., 1994b). Older male O_2 uptake kinetics are also known to be accelerated and approach values similar to those of younger males following long-term exercise or with an already existing higher level of fitness

(Babcock et al., 1994a; Babcock et al., 1994b; Chilibeck et al., 1996). Oxygen uptake in terms of maximum exercise performance and short-term training-induced PV increase in older males has been described(Petrella et al., 1997). However, $\dot{V}O_2$ kinetics in terms of sub-maximal exercise performance and short-term training-induced PV increase in older males has not been described and could assist in resolving the transport vs utilization limitation argument in older males (Hughson, 1990; Tschakovsky & Hughson, 1999). Petrella et al. (1999) have found cardiac filling and $\dot{V}O_2$ kinetics in older active males to approach the values of younger men in contrast to their older sedentary peers.

Previous research into the short-term training response has concentrated predominantly upon younger persons, and when older persons have been investigated, those investigations have focused upon the contributing factors to the training response as separate research analyses. No previous research has used short-term training-induced PV increase and/or diuretic-induced PV decrease in a systematic investigation of their influence upon left ventricular diastolic function, $\dot{V}O_{2max}$, and $\dot{V}O_2$ kinetics. This has made it difficult to define the nature and the interaction of the hypervolemic response, associated diastolic pump function, and their separate and joint influences upon sub-maximal and maximal $\dot{V}O_2$ in older people. Thus, the general purpose of this study was to further investigate the effect of short-term training and/or a diuretic-induced ΔPV on cardiac diastolic pump function and $\dot{V}O_2$. The following objectives and hypotheses are proposed:

OBJECTIVES:

1. To determine the effect of alterations in blood plasma volume on $\dot{V}O_2$ ($\dot{V}O_{2max}$ and $\dot{V}O_2$ kinetics) following short-term exercise training and diuretic intervention.
2. To determine the effect of alterations in blood plasma volume on Doppler echocardiographic indices of cardiac filling following short-term exercise training and diuretic intervention.

NULL HYPOTHESES:

1. Alterations in blood plasma volume will have no effect on $\dot{V}O_2$ ($\dot{V}O_{2max}$ and $\dot{V}O_2$ kinetics).
2. Alterations in blood plasma volume will have no effect on Doppler echocardiographic indices of cardiac filling.

ALTERNATE HYPOTHESES:

1. Alterations in blood plasma volume will effect $\dot{V}O_2$ ($\dot{V}O_{2max}$ and $\dot{V}O_2$ kinetics).
2. Alterations in blood plasma volume will effect Doppler echocardiographic indices of cardiac filling.

Chapter 2

Literature Review

The present study investigated ΔPV, cardiac filling, and the decline in aerobic performance observed in ageing. Firstly, a review of maximal and sub-maximal investigations of cardiorespiratory function is presented. The manipulation and contrast of these two exercise intensities reveal much about cardiorespiratory control with respect to health, fitness, and age. This is followed by a review of short-term training and its acute influences on blood plasma volume, cardiac function, and oxygen uptake ($\dot{V}O_2$). Short-term training and/or a diuretic intervention are used in this study because they offer methods by which to manipulate the cardiovascular control system and interpret responses in measured variables. Lastly, short-term training research has predominantly used younger subjects. The present study provides additional information by characterizing observations related to performance decline with ageing. This review concludes with the description of age-related changes in cardiac function and mechanisms which may be responsible for this.

2.1 Age Related Decline in Aerobic Performance

2.1.1 Maximal Oxygen Uptake

Age-related decline in aerobic work capacity has been well established in the literature as approximating a 10% per decade decline after 30 years of age (Ogawa et al., 1992; Cunningham et al., 1997; Paterson et al., 1999; Bell et al., 1999). Any ambiguity has been attributed to differences in the population studied. Cross-sectional comparisons of younger and older aerobic performance parameters need to be tightly controlled for disease states,

height, weight, fitness or activity levels, smoking, drinking, medications, and lean mass vs fat mass body composition. The age-associated cardiorespiratory adaptations are quite different for hypertensive persons (Lakatta, 1993) for example, and necessitate screening to even comparisons. Hypertension in older persons has been associated with higher PVR, increased LV hypertrophy, diminished LVDF, and a lower $\dot{V}O_{2max}$ than that observed in a normotensive population (Lakatta, 1993; Missault et al., 1993). Longitudinal studies avoid some of the pitfalls of cross-sectional studies such as the subject selection criteria of the selected population. However, longitudinal studies have their own difficulties with a much greater time and resource commitment, and in tracking and follow-up of subjects before any conclusions can be made. Studies that have enacted tighter controls in their cross-sectional comparisons have still detected a decline in aerobic performance parameters. The variables considered responsible for this decline include HR, SV, and a-$\bar{v}O_{2diff}$.

A maximal heart rate decline is considered the predominant contributing factor in the age-related decline seen in aerobic performance. It is not yet clear what causes the HR_{max} decline but studies have suggested that the cause may be desensitization to β-adrenergic stimulus (Lakatta et al., 1975; Hughson, 1984; Lakatta, 1993).

Although some disagreement exists as to the effect of ageing upon SV (Higginbotham et al., 1986), most studies suggest that SV and age are wholly dependent upon fitness and disease state; that SV does decline with ageing in a healthy population (Gerstenblith et al., 1987; Paterson, 1992; Lakatta, 1993; Thomas et al., 1993).

Stroke volume is affected by three variables: 1) preload; 2) contractility; and 3) afterload; all of which have been shown to be affected by ageing. Preload is influenced by the

magnitude of the venous return and the relaxation and compliance of the ventricle. Total blood volume (TBV) has been shown to decline with ageing (Davy & Seals, 1994). Preload is the volume of blood that fills and stretches the ventricle at end-diastole. The relation between preload and myocardial contraction force is described by the Frank-Starling effect; where the force of contraction of the myocardium increases with an increased end-diastolic volume (EDV) and a greater end-diastolic myocardial fibre length. The mechanisms responsible for the age-related decline in TBV have yet to be determined but it has been hypothesized that it may be an adaptive strategy of the fluid regulating system to maintain blood pressure in the face of an ever-increasing total peripheral vascular resistance (TPVR) (Davy & Seals, 1994). Another factor in the determination of preload is the compliance of the heart tissue in accepting venous return. The young heart has faster relaxation, is more compliant, and fills to a greater degree while the aged heart has slower relaxation, becomes stiff, resistive, and less compliant, thus impairing its ability to fill as well as the young heart. There is a limited period of time per heart cycle for the ventricle to fill (i.e. 500 ms at rest) and if the aged ventricle is less compliant to filling, there is the potential for incomplete filling, particularly during exercise-induced tachycardia.

Contractility is the ability of the myocardium to generate a contractile force and this has not been shown to diminish at rest or during application of a load in senescent rat myocardium (Lakatta & Yin, 1982). The muscle preparations were isolated from neurohumoral influence and subjected to differing Ca^{++} ion concentration. These results are in contrast to the decreased contractility observed in non-isolated studies which suggest biochemical, biophysical and molecular mechanisms of excitation-contraction coupling decline

(Lakatta, 1993). Increased invasion of the myocardium by collagen and lipids compromise the mechanical efficiency and rate of force production of the tissue by increasing the load of the structure (Lakatta, 1993). As the myocardium ages there is a decrease in the number of myocardial cells and a hypertrophy of existing cell size in an attempt to maintain force production. The myocardium has also been shown to be less sensitive to β-adrenergic stimulation. Lakatta et al. (1975) examined the inotropic response of aged rat myocardium to catecholamines, isoproterenol, and Ca^{+} in 6, 12, and 25 month-old heart muscle during measures of active tension, maximal rate of tension, and contraction duration. They observed diminished inotropic response to catecholamines in aged myocardium and concluded that since neither age differences in catecholamine uptake nor contractile response to calcium concentrations were seen, a decreased ability of catecholamines to effect a change in calcium concentration may be responsible.

Afterload is the resistance to cardiac output of the heart. With increased TPVR due to decreased compliance and increased tone of the vasculature, the heart has to do more work to supply oxygenated blood to the working tissues (Wei, 1992, Lakatta, 1993). Increased afterload decreases stroke volume.

Muscle mass has been shown to decrease with age (Fleg et al., 1988) and may account for a large percentage of the decline in aerobic work performance seen in older persons (Fleg et al., 1988). The absolute $\dot{V}O_{2max}$ ($ml \cdot min^{-1}$) will decline if the amount of lean mass to utilize O_2 supply declines (Fleg et al., 1988). Further, if fat mass and total mass increases coincident with a decline in lean mass, the relative $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) will decline as well. This is in addition to observed age-related changes in HR, SV, and $a\text{-}\bar{v}O_{2diff}$ (Ogawa et

al., 1992). Hence, there are many variables that play a part in the age-related decline in maximal $\dot{V}O_2$.

2.1.2 Oxygen Uptake Kinetics

Through the advent of breath-by-breath gas exchange analysis, the nature of the $\dot{V}O_2$ response to the onset of a sub-maximal workload has been reported to be exponential (Beaver et al., 1973; Beaver et al., 1981; Hughson, 1990). Furthermore, the $\dot{V}O_2$ response has been found to be comprised of three distinct components or phases. The time course and nature of these three components provide insight into the physiological control mechanisms responsible for $\dot{V}O_2$, transport, and utilization (Hughson, 1990; Whipp & Ward, 1990; Wasserman, 1994a). The study of the observed exponential $\dot{V}O_2$ response has been termed $\dot{V}O_2$ kinetics and of interest in the present study was the measure of the time for $\dot{V}O_2$ to achieve a steady state in response to the onset of the sub-ventilatory threshold workload (Tschakovsky & Hughson, 1999). A sub-ventilatory threshold workload was chosen for our study to avoid the interference of lactate accumulation in the interpretation of the $\dot{V}O_2$ kinetics (Whipp & Ward, 1990; Wasserman, 1994a).

The three components of the $\dot{V}O_2$ response have been observed in sub- and supra-ventilatory threshold studies (Figure 1). Component 1 is from zero load (G0) until G1 or the end of the cardiodynamic component and consists of an immediate and rapid rise in $\dot{V}O_2$ in the first 10-15 seconds representative of increased pulmonary blood flow and respiratory gas exchange. Component 2 begins at the end of the first component (G1) and continues for approximately 2 to 3 minutes until steady-state (G2). The second component (G2) is

representative of oxygen consumption at the exercising muscle. Component 3 (G3) is only seen in supra-threshold workloads and represents an increased $\dot{V}O_2$ demand commensurate with a significant increase in blood lactate producing a $\dot{V}O_2$ slow-component from G2 until G3. In the present study, oxygen uptake kinetics were fit from the end of the cardiodynamic phase (20 sec) to steady state (6 min) and in this instance tau or "τ" represents 63 percent of the time to achieve steady state $\dot{V}O_2$ in response to the square-wave onset of a workload. Tau has been found to be slower in older or less trained persons in comparison to younger or more fit persons (Babcock et al., 1994b; Chilibeck et al., 1996). Two theories have been put forward to account for this response: 1) $\dot{V}O_2$ is transport limited through HR, SV, or blood flow; 2) $\dot{V}O_2$ is limited by a utilization or metabolic limitation, represented by the a-$\bar{v}O_{2diff}$ (Hughson, 1990; Grassi et al., 1996).

In the present study, repeated testing was used to minimize the biological variability inherent in this type of testing and increase the signal-to-noise ratio for the response. The lactic acidemia associated with supra-threshold work rates accelerates kinetics of subsequent bouts of exercise and therefore the use of supra-threshold work rates were avoided to make it possible to average kinetics from repeated bouts (Gerbino et al., 1996).

As early as 1951, Henry et al. hypothesized that the rate of O_2 consumption was controlled by metabolic demand and oxidative substrates and was independent of supply. Phosphocreatine concentration has been implicated as a possible metabolic control mechanism and possible limitation in $\dot{V}O_2$ (Whipp & Mahler, 1980; Connet, 1988; Meyer, 1988). Grassi et al. (1996) determined the kinetics of alveolar $\dot{V}O_2$ and a-$\bar{v}O_{2diff}$ across the leg to be similar in response and concluded that this implied metabolic control was the limitation of $\dot{V}O_2$

(Grassi et al., 1996). Studies of two-stage workloads have found the kinetics of the first step to be faster than the subsequent step (Hughson & Morrissey, 1983). It was hypothesized that first step kinetics were faster because there was a greater parasympathetic nervous system (PNS) withdrawal for the first step than the second and PNS activity is faster than sympathetic nervous system activation required for the second step (Maciel et al., 1986).

Hypoxia has been shown to slow $\dot{V}O_2$ kinetics in younger men (Linnarson, 1974a; Linnarson et al., 1974b; Hughson, 1990). This has implicated O_2 transport as a limitation of $\dot{V}O_2$ (Linnarson, 1974a; Linnarson et al., 1974b; Hughson, 1990). Oxygen transport is not only affected by O_2 concentration in the blood but also by $\dot{Q}$ and peripheral vascular distribution to the muscle site. Blood flow and $\dot{V}O_2$ have been investigated using body position during single knee extension ergometry and although blood flow velocity was shown to adapt faster than $\dot{V}O_2$, it was noted that the $\dot{V}O_2$ response was slower in the supine condition (Hughson et al., 1997; MacDonald et al., 1998). Femoral artery diameter was smaller and blood flow velocity faster in the supine position with the reverse in the upright position (MacDonald et al., 1998). This illustrates the importance in considering blood flow when considering $\dot{V}O_2$ kinetics (MacDonald et al., 1998) and also begs the question of whether or not the postural change in diameter in the femoral artery was simply a mechanical effect or due to the increased sympathetic drive of the upright position and increased HR accompanied by a vasodilation of the femoral artery.

2.2 Short-Term Training Adaptation

2.2.1 Plasma Volume Expansion

An increase in plasma volume is a well-recognized physiological adaptation to endurance training (Green et al., 1987a; Green et al., 1987; Gillen et al., 1991; Hagberg et al., 1998). Several groups have investigated short-term exercise-induced hypervolemia using brief training protocols ranging from 1-12 days of high intensity exercise (Convertino et al., 1983; Green et al., 1984; Gillen et al., 1991; Petrella et al., 1997; Nagashima et al., 1999; Starrit et al.,1999). These protocols have been conducted only in young men.

Petrella et al. (1997) recently studied the response to a short term exercise program (5 days) in older men and found similar increases in plasma volume and $\dot{V}O_{2max}$ as seen in the studies of younger subjects. Longer duration (six month) training studies of older men (Carroll et al., 1995) have also found increases in plasma volume and $\dot{V}O_{2max}$ similar to those seen in the studies of younger subjects. Studies of active and sedentary older men have shown improved cardiovascular performance and greater blood volume in the active group (Stevenson et al., 1994; Hagberg et al., 1998). Duration, intensity, frequency, of exercise and thermal regulation have all been seen to play a part in total blood volume expansion in response to training and contraction with detraining (Coyle et al., 1986; Shoemaker et al., 1998) respectively. All of these variables should be considered when investigating short-term training cardiovascular outcomes.

It has been observed that training-induced hypervolemia is accompanied by an increase in left ventricular diastolic filling; important since cardiac filling has been shown to decline with age (Arrighi et al., 1994; Petrella et al., 1997) and this decline has been linked to

cardiovascular morbidity (Wei, 1992). Older persons represent a group who are sensitive to changes in blood volume (Hyams, 1986); some have shown lower total blood volume in older compared to younger subjects (Davy & Seals, 1994). Hence, understanding the interaction between ageing, plasma volume, and cardiovascular exercise responses would be important in determining limiting factors in exercise performance in this population.

The mechanisms responsible for the training-induced hypervolemia observed are not entirely understood but may include changes in either plasma albumin content (Gillen et al., 1991; Nagashima et al., 1999) or the renin-aldosterone-angiotensin system (RAAS) (Carroll et al., 1995; Zappe et al., 1996). Plasma albumin has been found to be elevated after short-term training and has been hypothesized to be responsible for the majority of the plasma volume expansion, however there is no firm evidence of the mechanism of this increase in plasma albumin (Convertino et al., 1980; Convertino et al., 1983). During exercise, blood volume decreases and the elevated intravascular hydrostatic pressures and low molecular weight of albumin may allow a fluid and protein shift into the interstitium which then passes into the lymphatic system and is eventually returned to the blood after exercise (Harrison, 1985). Using differential hematocrit and hemoglobin measures in the determination of PV changes should be associated with caution. Diet, environment, and posture all influence plasma volume of the intervascular compartment. A diurnal variation in intervascular plasma volume exists with the changing seasons, so analyses should be performed in a controlled environment (Harrison, 1985). Plasma albumin has been implicated as a carrier molecule with a predominant influence in fluid volume regulation through osmotic control (Rothschild et al., 1988). Plasma albumin is expanded with an increase in TBV seen in fit or younger

persons and may be a reflex response to exercise-induced hypovolemia for the improved maintenance of blood volume in subsequent exercise.

The observed increases in serum aldosterone concentrations during exercise, in response to hypovolemia, and the decreases in aldosterone after exercise, in response to hypervolemia, may be a similar response, as previously proposed for plasma albumin concentrations. Decreased activity of the RAAS has been observed in older persons (Weidmann et al., 1975; Tsunoda et al., 1986; Carroll et al., 1995). Hypertension may be influenced by this age-related decline in RAAS although the decline has also been observed in normotensive older adults. The response of the RAAS to training is also inconclusive (Leutkemeier et al., 1994; Carroll et al., 1995; Grant et al., 1996). Baroreceptor resetting may be an adaptive response to the age-related decline in RAAS activity as well as training-induced blood volume increase but the evidence is inconclusive. If plasma volume is increased or decreased with training or diuretic, the interaction between fluid regulation, cardiovascular circulatory reflexes and exercise responses could be determined (Leutkemeier et al., 1994; Zappe et al., 1996).

Posture during exercise and during testing are further considerations in interpreting study results. Nagashima et al. showed a posture-specific plasma volume increase in response to short-term training implicating central venous pressure in the plasma albumin content and plasma volume expansion (Nagashima et al., 1999). After a single intense exercise bout and 22 hours after exercise, PV increased from the upright exercise condition and decreased from the supine exercise condition (Nagashima et al., 1999). Even with no change in oxygen carrying capacity, increased plasma volume increases stroke volume, deceases heart rate, and

thus decreases myocardial oxygen demand. This may leave more oxygen for skeletal muscle tissue extraction.

Previous work by Petrella et al. (1997) measured the effect of a similar short-term exercise protocol, in seven older men on left ventricular diastolic filling and $\dot{V}O_{2max}$. Studies have been completed which have investigated the mechanism of short-term training induced plasma volume expansion and have used diuretic intervention to interpret aldosterone response and contribution (Leutkemeier et al., 1994; Zappe et al., 1996). None, however have used the combined influences of exercise, diuretic, and their combination in the investigation of RAAS, albumin, electrolytes, $\dot{V}O_2$, and cardiodynamic adaptation to determine both the mechanism of acute plasma volume change and resultant changes in exercise performance.

2.2.2 Oxygen Uptake

Plasma volume expansion in response to endurance training is well documented and is the major contributor to exercise-induced hypervolemia up until two to four weeks training (Convertino, 1991) after which time further hypervolemia is equally accounted for by plasma volume and hemoglobin count expansion (Stevenson et al., 1994). Green et al. (1987) addressed concerns that the hypovolemia observed during exercise and hypervolemia after exercise should be considered in terms of total blood volume and hemoglobin concentration. Performance should be considered in terms of one's actual oxygen carrying capacity, exercise specificity (i.e. treadmill vs cycle ergometer test for a trained cyclist), and environmental conditions (i.e. level of thermoregulatory adaptation). This may explain why some studies

have seen significant improvements in $\dot{V}O_{2max}$ with plasma volume increases (Petrella et al., 1997) and others have not (Green et al., 1987b; Green et al., 1990). Subjects in shorter duration short-term training studies may have been hemodiluted due to a plasma volume increase without any appreciable change in erythrocyte count while subjects in longer duration short-term training studies may not have been hemodiluted because the adaptive increase in erythrocyte count caught up with the plasma volume increase.

Total potential O_2 carrying capacity of the blood is described by the following equation (McArdle et al., 1991):

O_2 carrying capacity of the blood* = (hemoglobin) x (O_2 carrying capacity of hemoglobin)

19.7 ml O_2 = (15 g per 100 ml blood) x (1.31 ml O_2 per gram Hb)

* Oxygen is not entirely soluble in fluids and therefore 100 ml of blood plasma will only carry 0.3 ml of O_2 at an alveolar PO_2 of 100 mm Hg. Hb is the predominant mechanism of O_2 transport in the blood. At a PO_2 of 100 mm Hg, Hb is 98% saturated.

Assuming an average total blood volume of five litres and a hematocrit (Hct) of 0.45, plasma thus represents 55% of the total blood volume. Five litres of blood contains approximately 750 g of Hb or 985 ml O_2. The plasma in five litres of blood equals 2750 ml and carries 8.25 ml O_2. After a typical short-term training-induced plasma volume increase of 6%, average

plasma volume would now equal 2915 ml and Hb concentration would drop to a Hct of 0.417. This represents an O_2 carrying capacity of 19.7 ml per 100 ml of blood before hemodilution and 18.3 ml after. That is a 7.1% drop in total O_2 content. Thus blood plasma serves an important purpose, not as a mechanism of O_2 transport, but in terms of its role in acute hemodilution during early short-term training response and its ability to improve left ventricular diastolic function.

Maximal oxygen uptake represents the maximal ability to uptake (pulmonary diffusion), transport (blood [O_2], HR, and SV), and utilize (a-$\dot{v}O_{2diff}$) oxygen. Maximal oxygen uptake is typically determined by providing the subject with a progressive workload which is performed until volitional fatigue. Traditionally, to confirm that $\dot{V}O_{2max}$ has been achieved, response number one and any one of the other four responses must be observed: 1) a plateau in $\dot{V}O_2$ response defined as no appreciable change in $\dot{V}O_2$ with concomitant increases in the workload; 2) a respiratory exchange ratio (RER) of > 1.1; 3) attainment of age-predicted maximum heart rate (220 - age); 4) a blood [La^-] of > 8.0 mmol; 5) a rate of perceived exertion (RPE) of > 8 on a 10 point scale. However, more recently, Wasserman et al. (1994) have suggested that "maximal" oxygen uptake ($\dot{V}O_{2max}$) is achieved when a plateau is observed and anything less than the plateau but satisfying the other indicators is to be considered a peak or "maximum" oxygen uptake ($\dot{V}O_{2peak}$) (Wasserman et al., 1994b). Others have questioned the requirement of a plateau to achieve $\dot{V}O_{2max}$ (Cumming & Borysyk, 1972; Myers et al., 1990; Noakes, 1998). Many variables must be considered when interpreting the results of a $\dot{V}O_{2max}$ test such as gas exchange sampling interval, age, specific fitness, type of ergometer, and the protocol used (Froelicher Jr et al., 1974; Buchfuhrer et al.,

1983; Myers et al., 1990; Rivera-Brown et al., 1995; Taylor et al., 1955). As in the consideration of sub-maximal $\dot{V}O_2$ kinetics, $\dot{V}O_{2max}$ is considered to be either transport or utilization limited as well. In the present study, the investigation of altered plasma volume, cardiac pump function, and $\dot{V}O_{2max}$ may address transport limitations as a determinant.

2.3 Cardiac Filling and Ageing

2.3.1 Left Ventricular Diastolic Dysfunction and Exercise

Left ventricular diastolic filling is known to decline with ageing (Gerstenblith et al., 1977; Appleton & Hatle, 1992; Cacciapuotti et al., 1992; Wei, 1992; Klein et al., 1994). Specifically, LVDF is compromised by a) reduced rate of LV relaxation; and b) reduced LV compliance (Appleton & Hatle, 1992).

The study of diastolic function has been termed diastology. Diastole consists of four distinct and consecutive phases: 1) isovolumic relaxation time, 2) early filling, 3) diastasis, and 4) atrial contraction or late filling. Isovolumic relaxation time (IVRT) is the time between aortic valve closure, left ventricular pressure decline below left atrial pressure, and mitral valve opening. Isovolumic relaxation, is energy-dependent, requiring ATP for the re-uptake of calcium ions by the sarcoplasmic reticulum. Early filling (E) is a passive phase where blood flows from an area of higher pressure (left atrium) to an area of lower pressure (left ventricle) and is responsible for approximately 80% of left ventricular filling at rest. However, E may still be somewhat energy dependent as the left ventricle's continued relaxation "sucks" blood into the ventricle. The deceleration time (DT) is the time it takes E to drop to baseline or diastasis (See Methods Figure 2) and is prolonged by conditions

including mitral stenosis (narrowing of the mitral valve due to calcification) and reduced LV compliance. Diastasis is the period of time in which left atrial and ventricular pressures have nearly equilibrated, the flow into the left ventricle is predominantly from the pulmonary vein, is not energy dependent, and is responsible for approximately 5% of left ventricular filling at rest. Atrial contraction or systole is energy dependent and responsible for approximately 15% of left ventricular filling at rest. At the end of left atrial contraction the atrial pressure drops below that of the left ventricle and the mitral valve closes marking the end of left diastole.

Pulsed wave Doppler echocardiographic indices of LVDF have been well correlated with invasive techniques including radionucleide angiography (Bonow, 1991; Thomas & Weyman, 1991b) in which the rate of early LV filling is determined by the atrial/ventricular pressure gradient and rate of LV relaxation. A faster LV relaxation rate associated with younger or more fit subjects accelerates the LV pressure drop which in turn draws the mitral valve open more forcefully and increases the early trans-mitral flow and velocity (E) (Petrella, 1996). The opposite is true for a slower rate of LV relaxation. This in turn determines the amount of work left for the late trans-mitral flow velocity (A) of LV atrial systole. The more blood left in the left atrium (LA) after LV relaxation, the more blood the LA will need to contract and expel into the LV.

2.3.2 Left Ventricular Diastolic Filling And Ageing And Exercise

With ageing and/or loss of fitness, E declines, while A increases in order to compensate, and IVRT increases due to decrease in both LV rate of relaxation and compliance. It is important to note that there is a progression of left ventricular filling

abnormalities from normal function through impaired relaxation and then decreased compliance (Appleton & Hatle, 1992). As one ages, early filling decreases and atrial systolic filling increases. This is predominantly due to an ever-increasing impairment of LV relaxation resulting in E:A dropping below 1.0. With continued ageing and progression of cardiac disease, loss of LV compliance plays an increasing role in impaired LV diastolic function. With increased loss of LV compliance, left atrial pressure and volume increase, IVRT decreases, E increases, A decreases, and E:A returns toward normal. This occurrence of a high E:A is often referred to as "pseudonormalization" and without the benefit of other indices, could be falsely interpreted as normal LV diastolic function The term pseudonormalization is used to indicate that although LV diastolic filling appears normal, LV diastolic function is not. Short deceleration times and large pulmonary venous flow reversals are indicators that the apparently normal LV diastolic filling may be due to decreased LV compliance and impaired LV diastolic function. Pseudonormalization can be further diagnosed through the presence of exertional dyspnea and abnormally high LV pressure during atrial contraction (Appleton & Hatle, 1992).

Compliance of the LV refers to the passive mechanical ability of the LV to respond to changes in pressure and the more compliant LV of a younger person fills much more rapidly than the LV in an older person. Afterload, due to increased vascular resistance seen in hypertension or pericardial constraint seen in cardiac pericarditis, will be reflected in Doppler echocardiographic indices as reductions in LV compliance. These changes contribute to the lower SV seen with ageing. Stroke volume decline can be attenuated with improved fitness and in the face of an irreversible age-related decline in maximum heart rate,

the age-related decline in maximum cardiac output, and thus aerobic performance, can be delayed as well (Ehsani et al., 1991; Petrella et al., 1996).

The ratio of early/late peak filling velocity (E:A) is considered to be an acceptable index of LVDF (Table 3)(Petrella et al., 1996). Previous investigations of LVDF in older males who were either already fit, or were unfit and then trained, have shown improved LVDF (Forman et al., 1992; Levy et al., 1993).

2.3.3 Mechanisms of Systolic - Diastolic Dysfunction

As the myocardium ages, myocytes typically decrease in number and increase in size. The increased size of the myocytes have been implicated in the thickening of the left-ventricular walls. This thickening due to myocyte hypertrophy occurs in response to the increased TPVR seen in older persons and is more pronounced in hypertension.

Factors that regulate SV are preload, contractility, and afterload. The slower HR observed in older people allows a longer ventricular diastole which allows a larger EDV which in turn increases SV and helps maintain $\dot{Q}$. Afterload is the pressure the left ventricle must overcome to open the semilunar valve and eject its blood. Hypertension increases afterload and has been shown to be associated with hypertrophy of the left ventricle. This hypertrophy is presumed to be an attempt by the myocardium to maintain SV and $\dot{Q}$ in the face of an increased afterload.

With ageing and sedentary lifestyle, the myocardium is compromised. Increased cardiac amyloid, collagen, fat, and elastic tissue are all deposited throughout the myocardium

with these stimuli (Zwolinski et al., 1976; Wei, 1992). The invasion of these agents into the myocardium decreases compliance and functional quality of the tissue. This in turn affects molecular transport and conduction. These factors may also be implicated in the age-related decline in LVDF by way of compromised compliance and relaxation.

β-adrenergic responsiveness has been shown to decline with ageing (Lakatta, 1993). Enhanced β-adrenergic stimulation has been shown to increase venous return, thereby improving LVDF, heart rate, and contractility (Leenen & Reeves, 1987). The sympathetic nervous system has been considered to be a predominant vasoconstrictory force in blood flow regulation. However, β-adrenergic stimulation has been shown to be selective in its effect and non-selective β-adrenergic stimulation increases blood flow to the exercising tissue while increasing venous return from non-essential areas (Leenen & Reeves, 1987). β-adrenergic blockade has been studied in an investigation of muscle mass, cardiac output, and peripheral blood flow which showed that after an initial β-adrenergic blockade-mediated vasoconstrictory response, peripheral metabolic controls appear to take over and vasodilate blood flow to the exercising tissues (Hughson & Kowalchuk, 1991). Training has also been shown to improve β-adrenergic responsiveness just as pharmacologic stimulation has. Impaired β-adrenergic responsiveness may also play a role in the age-related decline in aerobic performance and LVDF.

Impaired intra-cellular calcium (Ca^{+}) re-uptake of the myocardial sarcoplasmic reticulum (SR) may in part be responsible for LVDF abnormalities associated with normal ageing as well as cardiac pathological states. With depolarization, Ca^{+} is released from the SR, binds with troponin and allows the actin-myosin cross-bridge stroke or contraction.

Upon completion of contraction, Ca^+ must be actively resequestered back into the SR (Thomas & Weyman, 1991b; Petrella, 1996). Relaxation of the myocardium is impaired if this Ca^+ resequestration is hampered in any fashion. Isovolumic relaxation time is slowed with ageing and has been attributed to impairment of Ca^+ resequestration. Calcium channel blocker (CCB) studies have shown improved IVRT and LVDF in older persons (Arrighi et al., 1994; Petrella et al., 1994). This illustrates the importance of the concept of impaired Ca^+ resequestration in aged myocardium.

In summary, it is important to note that although ageing influences a decline in aerobic performance, deconditioning has a similar impact. Exercise training and higher fitness do in fact partially stave off age-related decline of aerobic performance. The purpose of the present investigation was to determine the effect of alterations in PV elicited by short-term training and/or diuresis on $\dot{V}O_2$ and LV diastolic function.

Figure 1: Modelling of Oxygen Uptake Kinetics.

TD1 The time delay from the onset of exercise to the beginning of the cardiodynamic response.

TD2 The time delay from the onset of exercise to the end of the cardiodynamic response.

TD3 The time delay from the onset of exercise to either the beginning of the steady state response of a sub-ventilatory threshold workload or the beginning of drift in oxygen uptake for a supra-ventilatory threshold workload.

G0 The baseline oxygen uptake from which the gain in oxygen uptake is estimated for each component fit to. In this instance it is approximately 700 ml/min.

G1 The gain in oxygen uptake from the onset of exercise to the end of the cardiodynamic phase or first component. In this instance it is approximately 950 ml/min - 700 ml/min = 250 ml/min.

G2 The gain in oxygen uptake from the onset of exercise to steady state or the second component. In this instance it is approximately 2250 ml/min - 700 ml/min = 1550 ml/min.

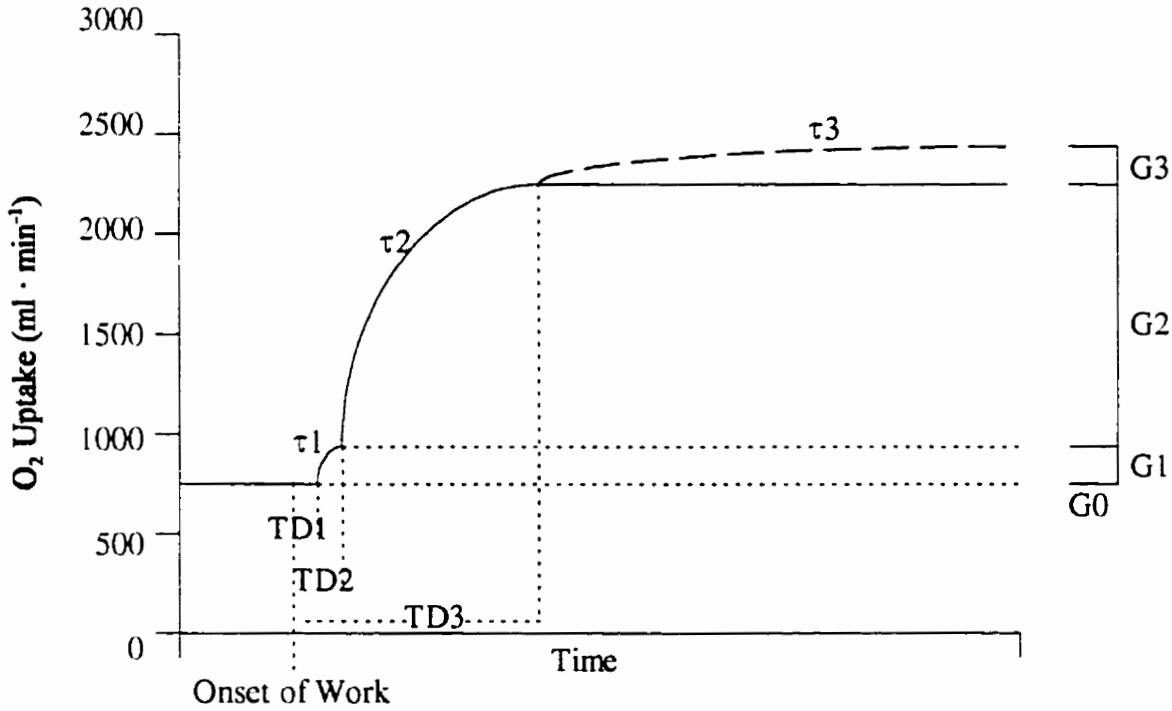
G3 The gain in oxygen uptake from the onset of exercise to the beginning of steady state or the third component. In this instance, although it continues to drift, it is approximately 2400 ml/min - 700 ml/min = 1700 ml/min at the last time it was fit to.

$\tau 1$ Tau or "τ" represents the rate of response or the time it takes to get to 63% of the gain being fit. In this instance it is the time it to 857.5 ml/min [G0 + (0.63 X G1)].

$\tau 2$ Tau or "τ" represents the rate of response or the time it takes to get to 63% of the gain being fit. In this instance it is the time it to 1676.5 ml/min [G0 + (0.63 X G2)].

$\tau 3$ Tau or "τ" represents the rate of response or the time it takes to get to 63% of the gain being fit. In this instance it is the time it to 1771 ml/min [G0 + (0.63 X G3)].

Estimation of Model Parameters for Oygen Uptake Kinetics



$$VO_{2\,(ml\ min^{-1})} = G_0 + G_1 [\ 1 - e^{-(t - TD1)/\tau 1}\ ] + G_2 [\ 1 - e^{-(t - TD2)/\tau 2}\ ] + G_3 [\ 1 - e^{-(t - TD3)/\tau 3}\ ]$$

Chapter 3

Methods

3.1 Subjects

14 older (67 ± 5 y) male subjects were recruited from participants at the Centre For Activity and Ageing (CAA) and the Retirement Research Group at the University of Western Ontario. Subjects were healthy and moderately active (i.e. regularly attended a walking program).

3.2 Screening

All aspects of the study were explained both verbally and in a letter of information (Appendix I.2). The study was approved by The University of Western Ontario's Ethics Review Board For Health Sciences Research Involving Human Subjects (Appendix I.1).

Inclusion criteria:

1. Healthy older males aged > 55 years
2. Not currently taking cardioactive medications (e.g. β-blockers, Ca^{+}-blockers, digoxin)[1]
3. Provision of informed consent

Exclusion criteria:

---

[1] Once it was deemed safe and non-confounding by the medical supervisor (RJP), prospective research participants who were on antihypertensive medications at the time of initial screening were weaned off those medications prior to beginning the study and for its duration.

4. Individuals with diagnosed respiratory diseases
5. Individuals with diagnosed cardiovascular disease other than hypertension
6. Smokers
7. Exercise limited by physical disability
8. Individuals with an irregular cardiorespiratory response to exercise during the initial screening or during any part of a testing procedure

Subjects meeting the entry criteria were given a medical and physical examination, followed by a maximal exercise stress test and echocardiographic examination to rule out silent cardiovascular disease. Blood pressure was monitored regularly throughout the testing and training sessions.

3.3 Study Design

Following screening, subjects were assigned a random order of the three different treatment conditions. The three conditions were exercise only (EXER), diuretic only (DIUR), and exercise plus diuretic condition (EXDI). Each condition of the study was separated by a three-week washout period. To avoid any possible order or carryover effect the three-week washout may not have accounted for, a counterbalanced design was used. The three conditions (EXER, EXDI, DIUR) have six different possible orders (Table 4). If six subjects were randomly assigned to each of these six different condition orders, this would constitute a perfectly counterbalanced design. Since, according to our sample size calculation (Appendix I.3), a minimum of 9 subjects were required, two sets of the six counterbalanced

condition orderings were utilized to accommodate a possible 12 subjects. Subjects were required to visit the CAA laboratory on seven different occasions including baseline screening, first baseline maximal oxygen uptake ($\dot{V}O_{2max}$) test, second baseline $\dot{V}O_{2max}$ test, baseline oxygen uptake kinetics ($\dot{V}O_2$ kinetics) test, and one visit for a $\dot{V}O_2$ kinetics and $\dot{V}O_{2max}$ test at the end of each of three conditions. Subjects also reported on four different occasions for echocardiography; one was during screening, one during Day 7 for each of the three conditions. Data collected during screening and initial testing were considered the control condition or baseline (BASE) measures. A schematic representation of the study time line is included in the "Letter of Information" (Appendix I.2, page 93).

3.4 Treatments

Subjects performed their exercise training in an air conditioned room with an average room temperature of 21 degrees Celsius, average relative humidity of 36 %, and an average barometric pressure of 739 mm Hg for the duration of the study. A fan was used to cool subjects during training if requested. The EXER condition consisted of five consecutive days with 60 minutes of accumulated cycling each day at a pre-determined cycling intensity. At any time during a training session, the subject was allowed to stop for a rest period (potentially due to fatigue or discomfort) with the rest time recorded to ensure the completion of a total of 60 minutes cycling. Their accumulated cycling time, less the rest time, had to total 60 minutes of exercise. On Day 1, the intensity was set at 70% of the subject's BASE $\dot{V}O_{2max}$; Day 2, the load was 80% of BASE $\dot{V}O_{2max}$; Days 3-5 were at 90% of BASE $\dot{V}O_{2max}$. Heart rate and blood pressure were monitored before, during, and after exercise. Training

was performed on either an American Echo electrically braked cycle ergometer or a Monark Model 824E friction braked cycle ergometer. Subjects used the same bicycle for the duration of the study. The DIUR condition consisted of daily ingestion of one 100 mg tablet of the K^+-sparing diuretic spironolactone for 5 consecutive days. During the EXDI condition, the subject took the diuretic as in the DIUR condition and completed the training as outlined in the EXER condition. The diuretic intervention was introduced to attenuate the short-term training-induced PV increase and determine if factors other than PV increase were influencing $\dot{V}O_2$ and LV diastolic filling.

3.5 Testing

All testing was performed in Lab B of the CAA which was also air-conditioned with a mean room temperature of 23.8 ± 1.2 degrees Celsius, a mean relative humidity of 43.1 ± 11.4 %, and a mean barometric pressure of 741 ± 4 mm Hg. Study duration was from April 1998 to January 1999. All subjects were asked to abstain from alcoholic, caffeinated, or any other suspected confounding beverages or foodstuffs for at least the four hours preceding all testing. Body weight was collected on the same Continental Health-O-Meter scale immediately prior to all testing with the subjects wearing only shorts and a T-shirt. All repeat measures were done within two hours of the original time of day to account for possible circadian variation in performance.

3.6 Outcome Measures

1. %ΔPV
2. $\dot{V}O_{2max}$
3. $\dot{V}O_2$ kinetics
4. LVDF

1. %ΔPV

Approximately 5 ml of venous blood was drawn from the antecubital space of the forearm 10 to 15 minutes prior to the second baseline $\dot{V}O_{2max}$ test as well as prior to exercise testing at the end of each of the three conditions (Day 7). Subjects were required to sit for 10 minutes prior to blood samples being drawn from the antecubital space of the forearm. These four sets of blood samples were analyzed for Hb and Hct by a commercial lab. Percent change in plasma volume (%ΔPV) was calculated for each of the three conditions in comparison to BASE using the following method (Van Beaumont, 1973):

$$\% \text{ PV}=[100*(Hb_{pre}/Hb_{post}((1-Hct_{post}/100)/(1-Hct_{pre}/100)))]-100;$$

where Hb_{pre} and Hct_{pre} are baseline hemoglobin and hematocrit measures, and Hb_{post} and Hct_{post} are measures for each condition.

2. $\dot{V}O_{2max}$

Initial testing, consisting of two $\dot{V}O_{2max}$ tests to ensure a true maximum effort, was performed by all of our subjects who were predominantly novices to the testing environment.

The two $\dot{V}O_{2max}$ tests were separated by 48 to 72 hours and the highest of the two was used to represent BASE $\dot{V}O_{2max}$ and individual BASE T_{vent} was determined from this data.

The $\dot{V}O_{2max}$ test was preceded by two minutes of loadless cycling (to ensure proper calibration and data acquisition before capturing data to disk) followed by four minutes of loadless cycling (to establish a baseline) before progressing with the test protocol which consisted of a cycle ergometer ramp test (15 watts/min) to volitional fatigue for the determination of $\dot{V}O_{2max}$, maximal heart rate (HR_{max}), maximal work rate (WR_{max}), and ventilatory threshold (T_{vent}).

Ventilatory threshold was determined from graphs of ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$), ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$), end-tidal oxygen partial pressure ($P_{ET}O_2$), end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$), carbon dioxide output ($\dot{V}CO_2$), and minute ventilation ($\dot{V}_E$) versus $\dot{V}O_2$. Ventilatory threshold was estimated to be the point where $P_{ET}O_2$ increased with no concomitant decrease in $P_{ET}CO_2$ and $\dot{V}_E/\dot{V}O_2$ increased with no concomitant increase in $\dot{V}_E/\dot{V}CO_2$. Three investigators' graphical interpretations were used to determine T_{vent} with discrepancies re-analyzed to obtain agreement.

The $\dot{V}O_2$ versus WR linear regressions used in the determination of T_{vent} and $\dot{V}O_2$ kinetics workloads (pg 34) were used in the determination of a plateau in $\dot{V}O_2$. A plateau was considered attained when the highest $\dot{V}O_2$ occurred before the end of the $\dot{V}O_{2max}$ test or when the rise in $\dot{V}O_2$ at the end of the test differed from the previous $\dot{V}O_2$ by less than 50% of the expected change according to the sub-maximal $\dot{V}O_2$ work rate relationship (linear regression analyses)(Taylor et al., 1955; Babcock et al., 1992). For the purposes of the present study,

a $\dot{V}O_{2max}$ was considered attained when a plateau was achieved or when any two of the following conditions were achieved: 1) an RER > 1.1; 2) a HR > 220-age, or 3) an RPE of > 8 on a 10 point scale.

3. $\dot{V}O_2$ kinetics

The BASE $\dot{V}O_2$ kinetics protocol was performed two to three days post BASE $\dot{V}O_{2max}$ testing. The $\dot{V}O_2$ kinetics protocol workload was determined during this time. The $\dot{V}O_2$ kinetics test was preceded by two minutes of loadless cycling (to ensure proper calibration and data acquisition before capturing data to disk) followed by four minutes of loadless cycling (to establish a baseline) before progressing with the test protocol. The workload chosen for the $\dot{V}O_2$ kinetics test protocol was set at a work rate that elicited an oxygen uptake ($\dot{V}O_2$) corresponding to 80% of BASE $\dot{V}O_{2max}$ ventilatory threshold (T_{vent}).

A linear regression analysis was performed on the $\dot{V}O_2$ versus WR data to determine individual sub-threshold workloads for the $\dot{V}O_2$ kinetics protocol. Zero to four minutes $\dot{V}O_2$ data were averaged to determine individual loadless oxygen consumption. A regression analysis was performed on $\dot{V}O_2$ data (5 - 8 min) vs work rate to determine each individual's sub-threshold $\dot{V}O_2$ vs WR relationship. Loadless O_2 consumption and 80%T_{vent} $\dot{V}O_2$ values were then substituted into the individual's sub-threshold $\dot{V}O_2$ vs WR relationship to determine the workload approximating 80%T_{vent} $\dot{V}O_2$. This workload was used in all $\dot{V}O_2$ kinetics testing for that individual for the duration of their participation in the study.

Day 7 post treatment testing consisted of an echocardiographic examination one to two hours prior to $\dot{V}O_2$ kinetics testing. Oxygen uptake kinetics testing was followed, after

a 10 to 20 minute rest, by a $\dot{V}O_{2max}$ test. The $\dot{V}O_2$ kinetics testing protocol consisted of two 6-min square wave rides, separated by a 10 to 20 minute rest, from loadless pedaling to a load approximating 80% of T_{vent} (Figure 3). This was performed to collect $\dot{V}O_2$ data in response to the onset of a sub-ventilatory threshold workload and assess it for rate of oxygen uptake. A workload below T_{vent} was chosen to avoid the confounding affects of lactate production on $\dot{V}O_2$ kinetics (Wasserman, 1994a).

Each of the two $\dot{V}O_2$ kinetics data files collected from the two six-minute square wave rides were: 1) one second interpolated; 2) split into two on-transient and two off-transient files; 3) the two on-transient files were averaged into one on-transient file and the two off-transient files were disregarded. The resultant on-transient file $\dot{V}O_2$ kinetics were fit using a single exponential model:

$$Y(t) = a\,(1 - e^{-((t-TD)/\tau)})$$

where Y represents any cardiorespiratory variable ($\dot{V}O_2$, HR) at time t, and a, TD, and τ are the amplitude, time delay, and time constant respectively. The variables measured were (Figure 1) Tau, total gain (TG), TD, and total lag time (TLT) for a one component model fit from the end of the cardiodynamic component to steady state. TG represents the amplitude of change, TD the time delay, and Tau the time constant (Figure 1). Phase three was ignored as it is representative of a non-steady state phase and phase one was ignored as it is considered the cardiodynamic component and not representative of what is happening at the muscle (Grassi et al, 1996). To ensure there was no appreciable drift or phase three

component and that steady state had been achieved, all BASE $\dot{V}O_2$ kinetics tests had the average $\dot{V}O_2$ from three and a half to four minutes compared to that from five and a half to six minutes. No drift was detected.

4. LVDF

Echocardiographic indices of the apical four-chamber view at rest in the left lateral decubitus position were collected approximately two hours preceding Day 7 testing in each of the three conditions using a Hewlett Packard Sonos 1000 ultrasound unit and a 2.5 mhz transducer. Measurements were made according to the standards and methods of the American Society of Echocardiography (Feigenbaum, 1994). The echocardiography technician was blind to the treatment condition. IVRT is the period of time between closure of the aortic valve and opening of the mitral valve during which there is a fall in intraventricular pressure with very little to no change in ventricular volume. Peak early trans-mitral flow velocity (E) is measured at the tips of the mitral valve leaflets and represents the peak blood flow velocity from the left atrium to the left ventricle during early diastole. Deceleration time represents the time taken for E to drop to zero or for atrial/ventricular pressures to equilibrate. Peak atrial trans-mitral flow velocity (A) represents the final filling of the left ventricle due to atrial contraction. E:A is the ratio of the peak early and peak late trans-mitral flow velocities. E, A, IVRT, DT, and E:A were measured using pulsed trans-mitral Doppler echocardiography and averaged over five cycles (Figure 2).

3.7 Safety/Monitoring

The following data were collected to ensure proper subject renal function and electrolyte balance (Appendix I.4 Tables A and B):

1. Hematology
2. Urinalysis

1. Hematology

The aforementioned four sets of blood samples were also analyzed for Na^+, K^+, Cl^-, creatinine, albumin, renin, aldosterone, and ACTH by a commercial laboratory. They were compared to age-matched norms and monitored to ensure proper renal function in response to the study interventions.

2. Urinalysis

Twenty-four hour urine collection was performed on Day 1 and 4 of each of the three conditions of the study and analyzed for Na^+, K^+, Cl^-, creatinine, and volume by a commercial laboratory. Electrolytes and creatinine were monitored to ensure proper renal function and urine volume was monitored to ensure level of hydration and response to the study interventions.

3.8 Data Acquisition

Respired gases of oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2) were sampled continuously at the mouth (1 $ml \cdot s^{-1}$) and analyzed using a Perkin Elmer 1100 Series Medical Gas Analyzer (MGA) mass spectrometer calibrated daily using precision calibration gases. An Alpha Technologies VMM-110 turbine flowmeter (TF) was used to measure ventilatory

($\dot{V}_E$) volumes and rates. The turbine was calibrated using a calibration syringe of a known volume (990 ml). Changes in gas concentrations were aligned with inspired and expired volumes by measuring the time delays for a square wave bolus of gas passing the turbine to a resulting change in O_2, CO_2, and N_2. Breath-by-breath $\dot{V}O_2$ was determined using the algorithms of Beaver et al., (1981). Heart rate (HR) data were collected and 3-lead electrocardiogram (ECG) monitored using a Tektronics Neonatal Heart Monitor (HM). A MetraByte DAS16 data acquisition board, installed in a PC-AT computer, was used to convert the analog input signals of the ventilatory measures and HR (MGA, TF, HM) to digital data. The DAS16 also output the ramp and square wave protocol workload wattage as an analog signal to properly control the Lode Corival 400 electrically braked cycle ergometer. Breath-by-Breath Inc.'s respiratory data collection software version 2.0 on DOS 6.0 was used to collect the ventilatory and HR data (MGA, TF, HM) to disk co-ordinated with the automatic cycle ergometer workload.

3.9 Statistics

A minimum n of 9 was calculated as being required to achieve significant power for this study design (Appendix I.3). All data was tabulated with Microsoft Excel version 5.0 and statistical analyses were performed using Jandel Scientific Sigma-Stat version 2.0 software. Means of each dependent variable (primary and secondary outcome measures) for each condition were compared using a one-way repeated measures analysis of variance. Significant results ($p<0.05$) were further analyzed for pairwise comparisons using Tukey's HSD. Pearson product-moment correlations were run between $\%\Delta PV$, $\%\Delta\dot{V}O_{2max}$, $\%\Delta E{:}A$, and $\%\Delta\tau\dot{V}O_2$

for each intervention. Change was measure as a percentage difference from BASE for each of the three interventions (EXER, EXDI, and DIUR). Values reported in the tables are the mean $\pm$ the standard deviation of the mean (mean $\pm$ SD).

Figure 2: Pulsed Wave Doppler Echocardiogram LVDF Indices. Relationship between electrocardiogram (ECG), phonocardiogram (phono), and venous and ventricular inflow Doppler recordings. S1 = first heart sound; S2 = second heart sound; S = systolic venous velocity; D = diastolic venous velocity; AR = atrial reversal; E = early diastolic velocity; A = velocity with atrial contraction; IVRT = isovolumic relaxation time; AT = acceleration time; DT = deceleration time.

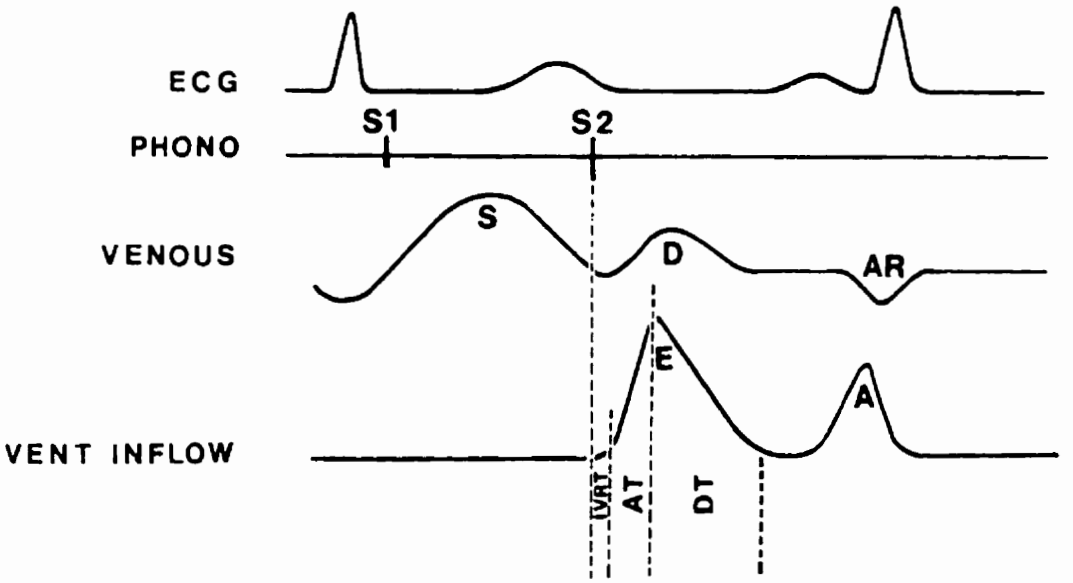

ECG
S1
S2
PHONO
S
D
AR
VENOUS
E
A
VENT INFLOW
IVRT
AT
DT

Figure 3: Oxygen Uptake Kinetics Sub-Ventilatory Threshold Protocol. All testing protocols are preceded by two minutes loadless pedaling to confirm subject acclimatization and proper equipment operation prior to commencement of hard data collection. This plus the loadless four minutes of each protocol makes up six minutes in total of loadless cycling prior to the $\dot{V}O_2$ kinetics square wave on-transient loading or prior to the $\dot{V}O_{2max}$ 15 watt/min ramp protocol.

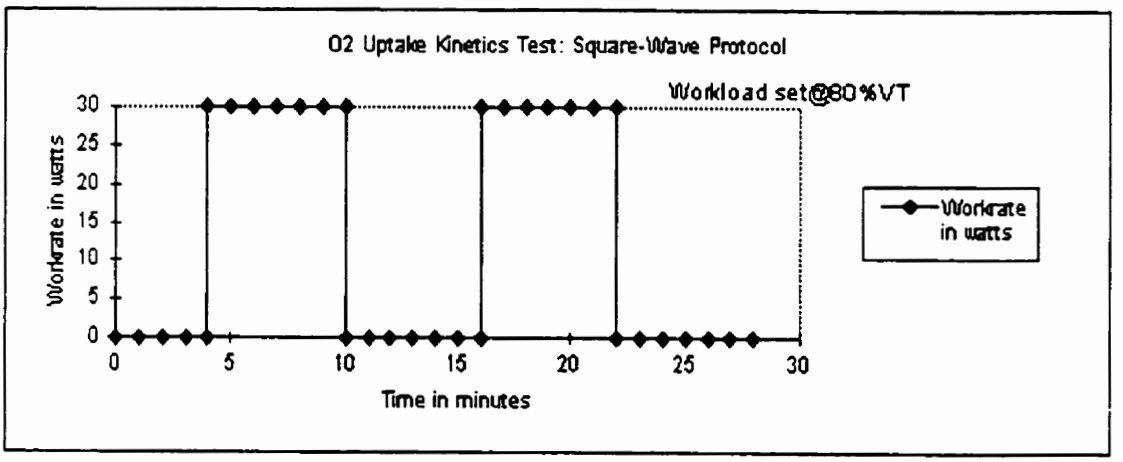

O2 Uptake Kinetics Test: Square-Wave Protocol
Workload set@80%VT
Workrate in watts
30
25
20
15
10
5
0
0
5
10
15
20
25
30
Time in minutes
Workrate in watts

Table 1: Counterbalanced Design*

Subjects	Condition One	Condition Two	Condition Three	Counterbalance
1	Exercise Only	Diuretic Only	Exercise & Diuretic	Set#1
2	Exercise Only	Exercise & Diuretic	Diuretic Only	Set#1
3	Diuretic Only	Exercise & Diuretic	Exercise Only	Set#1
4	Diuretic Only	Exercise Only	Exercise & Diuretic	Set#1
5	Exercise & Diuretic	Exercise Only	Diuretic Only	Set#1
6	Exercise & Diuretic	Diuretic Only	Exercise Only	Set#1

* = Every possible order of the three conditions is satisfied.

Chapter 4

Results

4.1 General and Subject Information

Fourteen subjects were recruited and 11 subjects completed the study with three dropouts from either the initial exercise testing or the first training condition. Reasons for dropout were due to minor overuse injuries of the knee or coccyx. The 11 subjects were 68 ± 5 years of age, 175 ± 6 cm tall, 85.7 ± 10.9 kg in mass, and had a $\dot{V}O_{2max}$ of 25.9 ± 3.6 ml · kg^{-1} · min^{-1}.

4.2 Body Mass

Body mass was measured immediately prior to Day 7 exercise testing for each condition. There was no significant ($p > 0.05$) difference in body mass between conditions. Body mass (mean ± SD) was 85.7 ± 10.4 kg for EXER, 83.9 ± 11.3 kg for EXDI, 84.0 ± 10.6 kg for DIUR, and 85.7 ± 10.9 kg for BASE (Table 3).

4.3 Training Indices

4.3.1 Duration

Subjects cycled for 60 min of accumulated exercise on each of the five days of EXER and EXDI and took brief rests when required. The total time taken each day is shown in Table 2. The time ranged from 61 min to 71 min for EXER and from 64 min to 68 min for EXDI.

4.3.2 Intensity

Individual training work rate (WR) for Day 1 (70% of BASE $\dot{V}O_{2max}$ WR), Day 2 (80%), and Days 3 - 5 (90%) was determined from the highest of the two baseline $\dot{V}O_{2max}$ tests and resulted in an average training WR which increased from 113 watts on Day 1 to 154 watts Days 3 through 5 (Table 2).

4.4 Cardiorespiratory Indices

4.4.1 Oxygen Uptake Kinetics

$\dot{V}O_2$ kinetics testing showed no significant differences from BASE when compared to any of the three treatment conditions (Table 3).

4.4.2 Maximal Heart Rate

HR_{max} was significantly ($p < 0.05$) lower for EXER (-10.7 bpm, $p = 0.050$) and EXDI (-11.1 bpm, $p = 0.041$), and not significantly different for DIUR (-6.9 bpm) compared to BASE (Table 3). In the present study, the age-predicted HR_{max} (HR > 220-age) was 152 bpm. Maximal HR averaged 155 bpm with BASE, 144 bpm with EXDI, 145 bpm with EXER, and 148 bpm with DIUR (Table 3).

4.4.3 Maximal Work Rate

WR_{max} showed no significant differences for any treatment condition compared to BASE (Table 3).

4.4.4 Maximal Oxygen Uptake

Neither absolute $\dot{V}O_{2max}$ ($ml \cdot min^{-1}$) nor relative $\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) showed significant differences for any of the treatment conditions compared to BASE (Table 3). Maximal RER averaged 1.17 with BASE, 1.14 with EXDI, 1.11 with EXER, and 1.13 with DIUR. The Borg index of RPE averaged 10.0 with BASE, 9.82 with EXDI, 9.73 with EXER, and 9.73 with DIUR. Plateaus were confirmed in 55% of BASE, 45% of EXDI, 82% of EXER, 45% of DIUR, and 60% overall of the $\dot{V}O_{2max}$ tests.

4.5 Hematological Indices

Percentage change in plasma volume (%ΔPV) in EXER (+6.2%, $p = 0.069$) approached being significantly higher than BASE and decreased significantly for the EXDI (-6.8%, $p = 0.038$) and DIUR (-11.5%, $p < 0.001$) conditions compared to BASE (Table 4). Hemoglobin showed no significant differences in any treatment condition compared to BASE (Table 4). Hematocrit was not significantly different for EXER compared to BASE (Table 4). Hematocrit was significantly higher for the EXDI (+2.2%, $p = 0.035$) and DIUR (+3.3%, $p < 0.001$) conditions compared to BASE (Table 4).

4.6 LV Doppler Indices

Peak early trans-mitral flow velocity (E) was significantly higher for EXER (76.9 cm $\cdot$ sec^{-1}, $p = 0.043$) and not significantly different for EXDI and DIUR when compared to BASE (67.9 cm $\cdot$ sec^{-1}) (Table 5). No significant differences in peak late trans-mitral flow velocity (A), isovolumic relaxation time (IVRT), peak early flow deceleration time (DT), or

cardiac filling index (E:A) were observed for EXER, EXDI, or DIUR compared to BASE (Table 5).

4.7 Correlations

No significant correlations were found between %ΔPV, $\%\Delta\dot{V}O_{2max}$, $\%\Delta\tau\dot{V}O_2$, or %ΔE:A.

Table 2: Training Indices - Duration and Intensity

Condition	Day 1 70%$\dot{V}O_{2max}$	Day 2 80%$\dot{V}O_{2max}$	Day 3 90%$\dot{V}O_{2max}$	Day 4 90%$\dot{V}O_{2max}$	Day 5 90%$\dot{V}O_{2max}$
EXER (min)	61.3 ± 2.3	64.2 ±6.3	70.8 ± 9.7	69.0 ± 8.1	67.6 ± 8.9
EXDI (min)	64.1 ± 7.5	65.6 ± 7.0	68.4 ± 6.0	68.2 ± 6.9	68.0 ± 7.0
WR (watts/min)	113 ± 23.8	133 ± 25.7	154 ± 30.2	154 ± 30.2	154 ± 30.2

$\dot{V}O_{2max}$ = oxygen uptake in ml · kg^{-1} · min^{-1}; EXER = exercise only condition; EXDI = exercise and diuretic condition; WR = work in watts per minute. Exercise times include rest and exercise time. Values are means±SD.

Table 3: Cardiorespiratory Indices - Oxygen Uptake Kinetics, Maximal Heart Rate, Rating of Perceived Exertion, Maximal Work Rate, and Maximal Oxygen Uptake

Variable	Conditions			
	BASE	EXER	EXDI	DIUR
Tau(s)	40.34 ± 9.5	33.78 ± 12.2	35.44 ± 11.0	36.96 ± 13.8
G0(ml)	748.8 ± 77.7	750.7 ± 82.7	713.4 ± 59.4	718.5 ± 81.8
HR_{max}(bpm)	155 ± 18.9	145 ± 15.0*	144 ± 10.9*	148 ± 15.3
RPE (Borg scale)	10 ± 0	9.73 ± 0.47	9.82 ± 0.4	9.73 ± 0.47
WR_{max}(watts)	178 ± 38.1	187 ± 37.0	184 ± 28.5	181 ± 40.8
$\dot{V}O_{2max}$($ml \cdot min^{-1}$)	2226±439	2289±499	2288±320	2269±452
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	25.91 ± 3.6	26.64 ± 4.6	27.59 ± 3.8	27.06 ± 4.7

Tau = time to 63% of steady state gain; G0 = VO_2 @ 20sec; HR_{max} = maximal heart rate; RPE (Borg scale) is a 10 point scale; WR_{max} = maximal work rate; $\dot{V}O_{2max}$ = maximal oxygen uptake; EXER = exercise only condition; EXDI = exercise and diuretic condition; DIUR = diuretic only condition; BASE = baseline condition. Values are means±SD. * = $p \leq 0.05$, statistically significant difference from BASE following repeated measures analysis of variance.

Table 4: Hematological Indices

Variable	Condition			
	BASE	EXER	EXDI	DIUR
%ΔPV	0	6.17 ± 4.4	-6.80 ± 8.6*	-11.47 ± 7.1*
Hb(g/l)	152.4 ± 9.1	146.9 ± 10.6	157.6 ± 13.8	162.3 ± 11.5
Hct	0.445 ± 0.022	0.432 ± 0.033	0.466 ± 0.042*	0.477 ± 0.038*

From blood samples taken just prior to Day 7 exercise testing. %ΔPV = percentage change in plasma volume; Hb = hemoglobin; Hct = hematocrit. EXER = exercise only condition; EXDI = exercise and diuretic condition; DIUR = diuretic only condition; BASE = baseline condition. Values are means±SD. * = $p \leq 0.05$, statistically significant difference from BASE following repeated measures analysis of variance.

Table 5: LV Doppler Indices

Variable	Condition			
	BASE	EXER	EXDI	DIUR
E($cm \cdot sec^{-1}$)	67.9 ± 11.0	76.9 ± 13.3*	64.5 ± 10.0	67.2 ± 13.3
A($cm \cdot sec^{-1}$)	64.7 ± 20.6	69.4 ± 20.5	68.6 ± 18.8	68.5 ± 19.0
IVRT(ms)	111.1 ± 25.1	113.2 ± 23.4	128.0 ± 25.3	125.4 ± 31.4
DT (ms)	247.2 ± 67.1	293.1 ± 88.5	259.8 ± 56.5	293.9 ± 67.4
E:A	1.13 ± 0.34	1.20 ± 0.38	1.01 ± 0.29	1.02 ± 0.34

From Doppler echocardiography averaged over five cycles performed the morning of Day 7 prior to exercise testing. E = early trans-mitral flow velocity peak, A = late trans-mitral flow velocity peak, IVRT = isovolumic relaxation time, DT = early peak deceleration time, E:A = ratio of early to late flow velocity representative of cardiac filling. EXER = exercise only condition; EXDI = exercise and diuretic condition; DIUR = diuretic only condition; BASE = baseline condition. Values are means±SD. * = $p \leq 0.05$, statistically significant difference from BASE following repeated measures analysis of variance.

Chapter 5

Discussion

The finding of no significant differences across conditions for either sub-maximal $\dot{V}O_2$ kinetics or $\dot{V}O_{2max}$ does not support the initial hypothesis that $\dot{V}O_2$ is related to short-term training-induced PV expansion in healthy older men. However, the finding that %ΔPV appeared to be increased with EXER, HR_{max} appeared to be decreased with EXER, and E was faster with EXER support the second hypothesis that cardiac filling (E:A) may be related to a short-term training-induced PV increase in older men.

5.1 Ageing-Related Decline in Aerobic Performance

5.1.1 Oxygen Uptake Kinetics

In the present study there were no significant differences across conditions in rate of O_2 uptake ($\tau\dot{V}O_2$). Oxygen transport is influenced centrally by HR and SV, and peripherally by vascular resistance (Hughson, 1990). Other studies of ageing, fitness, and $\tau\dot{V}O_2$ support a "slowing" of $\tau\dot{V}O_2$ with ageing that can be improved with aerobic endurance training (Babcock et al., 1994a; Babcock et al., 1994b; Chilibeck et al., 1996). Babcock et al. (1994b) found slower $\dot{V}O_2$ kinetics with no observed differences in HR kinetics when comparing younger and older men. They suggested that this may indicate older male $\dot{V}O_2$ kinetics are not centrally limited. However, a follow-up study by the same research group trained older men and found a high correlation between the speeding of the HR and $\dot{V}O_2$ kinetics after training (Chilibeck et al., 1996). This follow-up study contended that central blood flow limitations may play a part in the slowed $\dot{V}O_2$ kinetics seen in ageing (Chilibeck et al., 1996).

The present study was unable to analyze HR kinetics due to technical difficulties in collecting reliable HR data. However, the HR_{max} data was considered accurate.

Previous studies of $\dot{V}O_2$ kinetics have investigated the effect of different levels of fitness and age with long-term training (Babcock et al., 1994b; Chilibeck et al., 1996). However, Petrella et al. (1996) have investigated $\dot{V}O_2$ kinetics following short-term training with older men and have found improvement in $\dot{V}O_2$ kinetics similar in response to those seen with CCB (verapamil SR) administration in the same study. The present study (5 day training protocol) was likely too short for changes in $\dot{V}O_2$ kinetics to have been altered by mitochondrial enzymatic potential (Green et al., 1989; Green et al., 1991b). Dependent upon the degree of change, changes in hemoconcentration may or may not effect any changes in $\dot{V}O_2$ kinetics of a sub-maximal workload because oxygen supply may not be limiting during a sub-maximal workload demand. Although not investigated in the present study, it may be that manipulation of PV modulates $\dot{Q}$ and $\dot{V}O_2$ kinetics of the cardiodynamic phase (Yoshida et al., 1993). However, the lack of any significant difference in $\dot{V}O_2$ at 20 seconds into the onset of the sub-maximal square-wave workload in the present study does not support this hypothesis.

5.1.2 Maximal Oxygen Uptake

The absence of any significant difference in $\dot{V}O_{2max}$ across conditions in comparison to BASE in the present study is in contrast to the 12 % increase in $\dot{V}O_{2max}$ seen in response to the same exercise protocol in a previous study of older males and LVDF (Petrella et al., 1997). The subjects of the previous study were similar in number (n=12) and age (68 y) but

they were less fit ($\dot{V}O_{2max}$ of 23.5 ml $\cdot kg^{-1} \cdot min^{-1}$) than the present study. The lower initial fitness of the previous study possibly allowed a greater potential for change in response to training than the present study. Another possible explanation for the disparity in $\dot{V}O_{2max}$ results between the present and previous (Petrella et al. 1997) studies is the question of the $\dot{V}O_{2max}$ reliability of the present study. However, maximal oxygen uptake measures of the present study appear to be valid.

There is some disparity in the literature with respect to young male $\dot{V}O_{2max}$ increases in response to short-term training (Green et al., 1987b). The disparity may be related to the duration of the short-term training study. Convertino et al. 1991 observed that there is an initial increase in PV immediately after exercise which accounts for nearly all of the change seen in blood volume up to 10 days, followed by an increase in erythrocyte count thereafter (Convertino, 1991). The $\dot{V}O_{2max}$ results observed in 8 to 10 day studies (Convertino, 1983; Nadel, 1985) may be reflective of the increase in red cell mass and O_2 carrying capacity seen in longer training studies rather than a change in PV. This may in part account for the lack of significant change observed in $\dot{V}O_{2max}$ in the present and other 3 to 5 day training studies in contrast with studies of 10 days and longer duration (Convertino, 1991). However it should be noted that Petrella et al., 1996 did observe a significant increase with older and less fit men and this may indicate the response is fitness and age dependent.

5.2 Short-Term Training Adaptation

5.2.1 Plasma Volume Expansion

The imposition of a diuretic stimulus in the present short-term training study provided a means by which to determine whether changes observed in $\dot{V}O_2$ and LV diastolic filling were related to PV changes observed in older men. The EXER PV expansion of 6% was moderate in comparison to a similar 5-day training study which reported a 10% increase (Petrella et al., 1997). Leutkemeier et al. (1994) used the same diuretic and dosage as the present study, a 3-day training stimulus, and achieved a similar PV differential of 10% between their EXDI and EXER subjects, but had a 10% greater PV increase in EXER compared to BASE.

Spironolactone administration (EXDI and DIUR) produced higher aldosterone levels compared to BASE in the present study. The high EXDI renin secretion may indicate a combined response to maintain renal blood flow through the influence of both the diuretic and post-exercise hypotension (?). The elevated EXDI and DIUR aldosterone are in response to spironolactone and an attempt to regain renal blood flow and pressure. Aldosterone normally causes excretion of K^+ and retention of Na^+ and water at the site of the renal distal tubule. However, spironolactone competes with aldosterone for receptor sites on the distal renal tubule and thereby retains K^+ and allows the excretion of Na^+ and water.

Antidiuretic hormone (ADH) is secreted by the posterior pituitary in response to high blood osmolality subsequently acting upon the collecting duct cell membranes of the kidneys to promote water retention. Blood plasma osmolality and ADH have been shown to be unaffected by short-term training-induced PV expansion (Leutkemeier et al., 1994; Zappe et

al., 1996). This suggests that ADH does not play a role in short-term training-induced PV expansion, although ADH was not measured in the present study.

Hence, the results of this study indicate that short-term training does induce a PV expansion, and diuretic intervention does attenuate that PV response. The RAAS plays a predominant role in short-term training-induced PV expansion as reflected by blood plasma electrolytes. The role of albumin remains uncertain.

5.3 Cardiac Filling and Ageing

5.3.1 Left-Ventricular Diastolic Dysfunction and Exercise

Left ventricular diastolic function is known to decline with ageing (Tables 1 and 2). Specifically, left ventricular diastolic function is compromised by a lower total blood volume, degradation of biochemical processes, or mechanical function affecting a diminished early filling (E), increased atrial systolic (A) contribution, and a longer isovolumic relaxation time (IVRT) (Lakatta, 1993; Wei, 1992). In terms of ageing, the degradation of biochemical processes refers to the decline in β-adrenergic sensitivity or the rate of Ca^{++} re-uptake (resequestration) by the sarcoplasmic reticulum. Decline in mechanical function refers to the loss of compliance of the myocardium due to an increase in collagen and other constituents (fat, amyloid) in the myocardial matrix. Although the SV of older hypertensive people appears to increase in an attempt to maintain $\dot{Q}$, the loss of compliance and decline in LVDF may contribute to the lower SV that persists with ageing in a healthy population (Lakatta, 1993). Stroke volume decline can be attenuated with improved fitness. In the face of an age-related decline in maximum heart rate, the age-related decline in maximum cardiac output, and

thus aerobic performance, can be further delayed as well through exercise training (Ehsani et al., 1991; Petrella et al., 1996).

Subjects' baseline mean early (E) and late (A) peak filling velocities were well within the normal healthy age range (Feigenbaum, 1994). The ratio of early/late peak filling velocities (E:A) is considered to be an index of LVDF and was also in a normal healthy range (Feigenbaum, 1994; Petrella et al., 1996; Thomas & Weyman, 1991b). Early trans-mitral flow velocity with EXER (76.9 cm/s) was greater than BASE (67.9 cm/s)(Table 9). Previous investigations of E:A have reported that older males, when trained (Ehsani et al., 1991; Petrella et al., 1997) or already fit (Hagberg et al., 1998; Petrella et al., 1996), have shown increased E:A. In the present study, the changes in E:A appear to be mediated in part by the changes in PV or total blood volume and this is borne out by the positive correlation shown between %ΔPV and E:A.

Changes in LV relaxation and compliance can be interpreted by IVRT and DT. In aged myocardium, both IVRT and DT increase in duration. This may be attributed to a decrease in the compliance and relaxation of the myocardium due to mechanisms such as increased cardiac amyloid, increased collagen, decreased β-adrenergic sensitivity, and decreased Ca^{++} resequestration. In the present study, IVRT did not differ from BASE. This is in conflict with the findings of a previous similar study which found IVRT to be decreased with short-term training in older men (Petrella et al., 1997). Both studies found no significant changes in DT. In the present study, it is has been shown that the subjects were more fit than the previous study by Petrella et al., (1997) and it may be this improved fitness that accounts for the differences observed in IVRT response to the short-term training protocol. The

natural history of LV filling abnormalities described by Appleton and Hatle (1992) may explain the age and fitness dependence of the degree of response in E, A, E:A, IVRT, and DT to short-term training. In the present study, the subjects were moderately fit and this was reflected by LVDF measures similar to those that appear early in the hypothetical progression of changes in mitral and pulmonary flow velocity that occur with ageing and cardiac disease. Impaired relaxation and decreased compliance are not yet evident.

β-adrenergic responsiveness and Ca^{++} resequestration have been studied in terms of their effect upon LVDF. Changes associated with the impairment of LVDF include ageing and a loss of fitness, and these may in part be responsible for any changes in LVDF observed in the present study (Arrighi et al., 1994; Harrison et al., 1991; Petrella et al., 1994; Spina et al., 1998). Although not investigated in the present study, the short duration of our training protocol should not have resulted in changes to the myocardial matrix influencing mechanical function. It may be that any changes in IVRT observed in the present study or other similar research protocols were due to changes in either preload, β-adrenergic responsiveness, or Ca^{++} re-uptake by the SR. However, it is possible that short-term training-induced PV expansion and increased preload alone may cause the mitral valve to open sooner, thereby decreasing IVRT.

5.4 Summary

The present study was undertaken in an attempt to determine whether short-term training-induced PV expansion or diuresis influenced $\dot{V}O_{2max}$, $\dot{V}O_2$ kinetics, and cardiac filling in older men. Diuretic intervention was implemented alone (DIUR) and with exercise (EXDI)

and compared to EXER to discern whether or not any observed changes in LVDF and aerobic performance were in fact due strictly to the PV expansion, or some other yet unidentified mechanism such as adaptations in β-adrenergic sensitivity or Ca^{++} transport influence upon cardiac filling. If, during EXDI and DIUR, there were significant improvements observed in cardiac filling and aerobic performance, these improvements would have to have been caused by some influence other than PV expansion. With respect to the results of the present study, it appears that alterations in plasma volume following short-term exercise training and diuretic intervention do not effect significant changes in $\dot{V}O_{2max}$ or $\dot{V}O_2$ kinetics in moderately fit older men. However, the present study's observations of improved LVDF for EXER compared to BASE, when combined with the observed PV changes, suggest that improvements seen in short-term training-stimulated LVDF would appear, at least in part, due to PV-mediated changes in cardiac filling.

Chapter 6

Conclusions and Limitations

6.1 Conclusions

This present study's objective was to determine the effect of alterations in blood volume on $\dot{V}O_{2max}$, $\dot{V}O_2$ kinetics, and Doppler echocardiographic indices of cardiac filling following short-term exercise training and/or diuretic intervention. Although $\dot{V}O_{2max}$ and $\dot{V}O_2$ kinetics findings were insignificant, an effect of PV change upon cardiac filling can not be ruled out. It appears that short-term training-induced PV expansion improves cardiac filling and removal of the PV response by diuretic use attenuates those improvements. The initial level of fitness of the study participants may have played a role in the lack of any significant differences in $\dot{V}O_{2max}$, $\dot{V}O_2$ kinetics, or the lack of strength in the indices of LV diastolic filling and function, however, the observed changes in PV, improved E with EXER, and the positive correlation of %ΔPV with E:A suggest that these older men were still amenable to improvements in LV diastolic filling.

6.2 Potential Limitations

1. The power of this study was based upon an expected 3.5% increase in $\dot{V}O_{2max}$ in response to the short-term training protocol. However the fitness characteristics of the group may have limited our ability to achieve the expected changes.
2. It should be noted again that the Doppler echocardiographic indices are non-invasive representations of left ventricular diastolic function and are not direct measures of relaxation or compliance (Thomas & Weyman, 1991a). The present method of

interpreting Doppler echocardiographic indices as left ventricular diastolic function are only speculative without measures of intraventricular pressure.

3. The difficulties observed in achieving a $\dot{V}O_{2max}$ plateau may be attributable to the assignment of a standard ramp WR of 15w/min. The standard ramp WR allowed some subjects to exercise well beyond the suggested 8 to 12 minutes for a $\dot{V}O_{2max}$ test (Buchfuhrer et al., 1983). Making the ramp protocol specific to each person to yield a $\dot{V}O_{2max}$ test duration of 8 to 12 minutes may have improved the ability to yield a plateau.

4. It could be argued that this protocol was too long and demanding and this is a valid concern, however, the study's strength over other similar studies lies in its longitudinal design. This may have affected motivation during training and testing and compliance to the dietary and activity guidelines described upon initial subscription to the study.

6.3 Future Research

Although beyond the scope of the present study, acute β-adrenergic or Ca^{++} transport-mediated changes, as a result of short-term training, may have contributed to the observed changes in LV diastolic filling. Developing a method of non-invasively or invasively determining β-adrenergic or Ca^{++} transport activity in the myocardium may provide greater insight into short-term training-induced improvements in LV diastolic function and its ageing related decline.

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Appendices

Appendix I.1 - Certificate Of Ethics Approval

REVIEW BOARD FOR HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS

1997-98 CERTIFICATION OF APPROVAL OF HUMAN RESEARCH

ALL HEALTH SCIENCES RESEARCH INVOLVING HUMAN SUBJECTS AT THE UNIVERSITY OF WESTERN ONTARIO IS CARRIED OUT IN COMPLIANCE WITH THE MEDICAL RESEARCH COUNCIL OF CANADA "GUIDELINES ON RESEARCH INVOLVING HUMAN SUBJECT."

1997-98 REVIEW BOARD MEMBERSHIP

1) Dr. B. Borwein, Assistant Dean-Research - Medicine (Chairman) (Anatomy/Ophthalmology)
2) Ms. S. Hoddinott, Director of Research Services (Epidemiology)
3) Dr. R. Gagnon, St. Joseph's Health Centre Representative (Obstetrics & Gynaecology)
4) Dr. F. Rutledge, London Health Sciences Centre - Victoria Campus Representative (Critical Care - Medicine)
5) Dr. D. Bocking, London Health Sciences Centre - University Campus Representative (Physician - Internal Medicine)
6) Dr. L. Heller, Office of the President Representative (French)
7) Mrs. E. Jones, Office of the President Representative (Community)
8) Ms. S. Fincher-Stoll, Office of the President Representative (Legal)
9) Dr. D. Freeman, Faculty of Medicine & Dentistry Representative (Clinical)
10) Dr. D. Sim, Faculty of Medicine & Dentistry Representative (Basic)(Epidemiology)
11) Dr. M.I. Kavaliers, School of Dentistry Representative (Dentistry-Oral Biology)
12) Dr. H. Laschinger, School of Nursing Representative (Nursing)
13) Faculty of Health Sciences Representative
14) Ms. R. Bullas, London Clinical Research Association Representative
15) Research Institutes Representative
16) Mrs. R. Yohnicki, Administrative Officer
Alternates are appointed for each member.

THE REVIEW BOARD HAS EXAMINED THE RESEARCH PROJECT ENTITLED:
"VO2 responses to changes in blood volume in young and older men."

REVIEW NO: 6237

AS SUBMITTED BY: Dr. R.J. Petrella, Kinesiology, Thames Hall

AND CONSIDERS IT TO BE ACCEPTABLE ON ETHICAL GROUNDS FOR RESEARCH INVOLVING HUMAN SUBJECTS UNDER CONDITIONS OF THE UNIVERSITY'S POLICY ON RESEARCH INVOLVING HUMAN SUBJECTS.

APPROVAL DATE: 15 January 1998 (UWO Protocol, Letter of Information & Consent)

AGENCY:

TITLE:

Bessie Borwein, Chairman

c.c. Hospital Administration

Appendix I.2 - LETTER OF INFORMATION

Effect of Blood Volume on VO_2 in Older Men

Investigators: Dr. Rob Petrella, M.D., Ph.D.

S. Kelly Harris, M.Sc. Candidate

Introduction:

You are being asked to participate in a human research study to examine the effect of blood volume on maximal oxygen uptake (VO_{2max}). Interventions used in the study are exercise training, oral diuretic administration, and training and diuretic treatments combined. The study is divided into three randomly-ordered phases, with exercise tests repeated after each phase. All exercise will be done on a cycle ergometer in the laboratory at the Centre for Activity & Ageing, and will be monitored by one of the investigators.

Issues For Study:

1. Short-term high-intensity exercise lasting 2-10 days has been shown to increase blood volume.

2. The changes in blood volume is associated with an increase in VO_{2max}, which is often regarded as the best indication of an individual's cardiovascular fitness.

3. The changes in blood volume and VO_{2max} are rapidly lost when training ends.

4. Diuretics or "water pills" decrease blood volume in clinical situations and may lower or blunt the increase in VO_{2max} and fitness in patients during training. This may be particularly important in the elderly who are already sensitive to changes in blood volume.

5. High-intensity training and the related increase in blood volume may offset the diuretic effect and help us understand the relationship between blood volume and exercise performance.

The purpose of this study is to address these issues and to determine the relative effects of each treatment (exercise and diuretic) in older men. This information will be used to improve our knowledge of exercise limitations in this group.

Procedures:

Each participant will complete all three phases of the study, but the order in which the phases are completed will be randomly assigned. The three phases are (in random order):

- Training Phase
- Diuretic Phase
- Combined Training and Diuretic Phase

A description of each phase is included below.

During each phase you will be asked to maintain a dietary record. Throughout the study you are encouraged to maintain your normal eating, drinking, and activity habits.

Baseline Screening:

You will be screened for your participation and safety by a complete medical exam including an exercise stress test on a cycle ergometer under the supervision of a physician. This test will determine your maximal exercise performance or $VO_{2\ max}$.

A small blood sample (2 tbsp) will be drawn from an arm vein and analysed for electrolyte (i.e., potassium) balance for baseline screening and at the end of each phase. You will also be given a large jug in which to collect your urine over 24 hours at the start and finish of each phase. The urine and blood tests will be done to monitor your blood volume changes during the study.

The Study:

To help understand the complete study, 10 different "times" have been identified. A description of what each time entails is included in the Appendix.

Training Phase:

The training phase will be 5 consecutive days, with 60 minutes of accumulated cycling each day at a pre-determined cycling intensity. On Day 1, the intensity will be set at 70% of your $VO_{2\ max}$ (as determined from the greater of two preliminary maximal exercise tests); Day 2, the load is 80% of $VO_{2\ max}$; Days 3-5 will be at 90% $VO_{2\ max}$. At any time during the training, you may stop for a rest period (potentially due to leg fatigue or pain) with the rest time recorded to ensure you complete a total of 60 minutes of cycling. Your heart rate and blood pressure will be monitored regularly during the training.

Diuretic Phase:

The diuretic phase will consist of taking a 100 mg tablet of the diuretic Aldactone (spironolactone) daily (every morning) for 5 consecutive days.

Combined Training and Diuretic Phase:

During this phase, you will take the diuretic as in the diuretic phase and complete the training as outlined in the training phase.

Risks Associated With The Study:

Any intensity of exercise carries a slight risk of heart attack, or may be uncomfortable if you are unfit or not used to exercise. There may be some discomfort during the exercise testing. You may experience an increased awareness of breathing, muscle pain and/or fatigue, increased sweating, general feeling of fatigue and/or nausea. You will be required to wear a face mask (which will prevent you from breathing through your nose while enabling the measurement of the air you breathe) during the exercise tests and this may offer some initial discomfort. Muscle fatigue may be experienced for a few days after the exercise tests and/or training rides (especially in those not accustomed to exercise).

During the exercise testing, surface electrodes will be attached to your chest in order to provide information regarding heart rate and evidence of coronary artery insufficiency. These electrodes may irritate the skin during removal.

Side effects of the Aldactone are the same as those associated with any diuretic antihypertensive agent. The most common side effects (which occur in <1% of patients) are volume depletion, electrolyte imbalance, gastrointestinal symptoms (including nausea, vomiting, cramping, diarrhea, gastric bleeding, gastritis and ulceration), dizziness, lightheadedness, dry mouth or fatigue. Adverse reactions are usually reversible upon discontinuation of the study. This agent is designed to spare potassium loss and hence electrolyte and gastrointestinal symptoms are minimized.

At each visit during the study your condition will be monitored to best prevent these adverse effects.

Benefits:

You may wish to consider this study as a "boost" for a regular fitness program. The short-term high-intensity training will provide a rapid increase in your $VO_{2\ max}$ that would normally take much longer to acquire through a normal exercise program; and you may wish to initiate an exercise program after completion of the study to maintain and further improve your new level of fitness.

You will incur no cost as a direct result of your study participation - you will not be responsible for paying for physician fees, medication, or test procedures.

Confidentiality:

Records from the study are confidential and securely stored. Any publications as a result of this study will in no way identify you by name.

Voluntary Participation:

Your decision to participate in this study is completely voluntary. You are free to choose either to enter the research study or not to enter the study. There will be no penalty of any sort should you decide not to participate.

Should you agree to participate, you may voluntarily withdraw from the study at any time without penalty. Before withdrawing from the study, you should notify one of the investigators of your intent to do so.

Further Information:

You are encouraged at all times to ask questions regarding the purpose of the study and the outcome of your exercise tests. If you have any questions concerning your participation in this study, contact one of the investigators:

Dr. Rob Petrella at 661-1610 or 661-1637 or S. Kelly Harris at 661-1636(w), 858-3609(h).

Do not sign the consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

APPENDIX - EFFECT OF BLOOD VOLUME ON VO_2 PROTOCOL

TIME 0: This is the initial visit. You will have the study described in detail by one of the investigators, repeating all information on this letter of information and further detailing all aspects of training, the diuretic, urine collection and recording a dietary history. At this point, you will have bloodwork drawn and a physical examination to determine if you are a candidate for the study.

You will then set up a schedule for completing the study.

This visit should last no longer than one half hour.

TIME 1: This visit (1st baseline max) will consist of a progressive exercise test to voluntary fatigue, where the intensity of exercise gradually increases until you are unable to continue. This test is called the "$VO_{2\,max}$ Test," and provides an indication of your aerobic fitness, and will be conducted under medical supervision to ensure future exercise tests are performed safely. Expect to spend about 15 minutes riding the bike - the entire visit should last one half hour.

TIME 2: A one hour echocardiographic assessment is scheduled prior to testing. This visit (2nd baseline max) will consist of a progressive exercise test to voluntary fatigue, where the intensity of exercise gradually increases until you are unable to continue. This test is called the "$VO_{2\,max}$ Test," and provides an indication of your aerobic fitness, and will be conducted under medical supervision to ensure future exercise tests are performed safely. Expect to spend about 15 minutes riding the bike - the entire visit should last one half hour.

TIME 3: A kinetics test will be performed at this time, where you will instantaneously switch from loadless pedaling to a resistance representing a moderate intensity ride and then back to zero resistance. You will repeat this sequence two times, riding for a total of six minutes at each intensity before the load changes. A 10 minute break will then be given before you repeat the same ride (repeat the sequence two more times). This visit will last a maximum of 90 minutes, with approximately 60 minutes actually spent riding at a low intensity.

TIME 4: You will start one of the three intervention phases. If you are randomised to either the training phase or the combined training and diuretic phase, you must visit the lab for approximately 60 minutes per day to complete the training; otherwise, in the diuretic phase you will take the diuretic (as outlined in the diuretic phase) at this time. Training, diuretic, or the combination last for 5 days each. All three phases last 7 days in total each.

TIME 5: Two days after the previous phase ends (i.e.,"Day 7" of the preceding phase.), (Day 6 is a recovery day for each of the three phases.), you will have a one half hour echo assessment, perform a $VO_{2\,max}$ and a kinetics test, as well as having blood drawn for analysis. You will then have three weeks rest before beginning the next phase of the study.

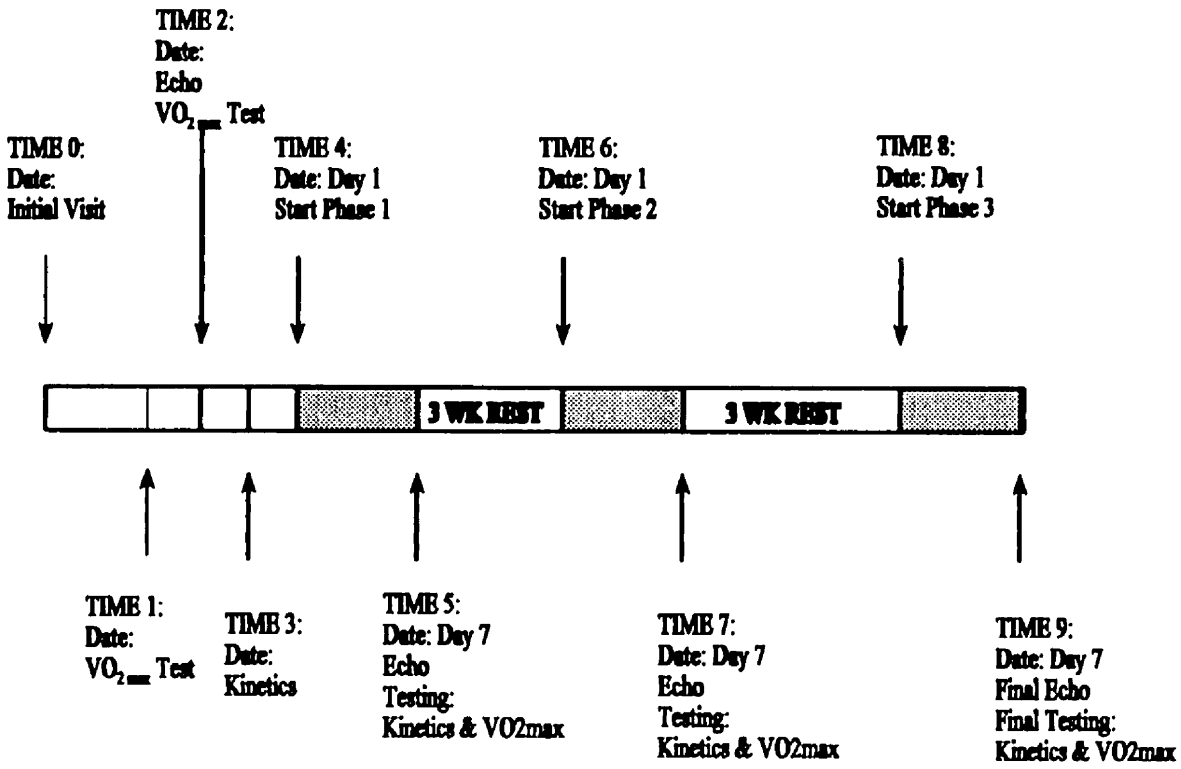
TIME 6: You will begin phase 2. (Days 1-5)

TIME 7: Day 6 is a recovery day and then all tests (echo, blood sample, kinetics, & VO_{2max}) are repeated two days after phase 2 ends on Day 7.

TIME 8: You will start the final phase.

TIME 9: Two days after finishing the final phase, all tests are repeated.

Effect of Blood Volume on VO_2 Protocol



"Testing" includes VO_{2max} test, Kinetics test, and blood volume.

Appendix I.3 - LETTER OF INFORMED CONSENT

Effect of Blood Volume on VO_2 in Older Men

I, _____, have read the attached letter of information and I agree to participate in this study.

All questions have been answered to my satisfaction.

_____ _____
Date Participant Signature

_____
Print Name

_____ _____
Date Witness Signature

_____
Print Name

INVESTIGATOR STATEMENT: One of my delegates or I have carefully explained to the subject the nature of the above protocol. I hereby certify that to the best of my knowledge the subject signing this consent form understands the nature, demands, risks, and benefits involved in participating in this study and that a medical problem or language or educational barrier has not precluded a clear understanding of the subject's involvement in this study.

_____ _____
Date Investigator Signature

_____
Print Name

09-08-98, skh

Appendix I.4 - Sample Size Calculation

For $\alpha = 0.05$, power = 0.8, and using a mean difference of 3.5 ml $\cdot \cdot kg^{-1} \cdot \cdot min^{-1}$ and a standard deviation of 3.5 ml $\cdot kg^{-1} \cdot \cdot min^{-1}$ (Petrella et al., 1997) with the following sample size equation for paired data (Friedman et al., 1992):

$$N_d = (Z_\alpha + Z_\beta)^2 \times SD^2/\Delta^2$$

where $Z_\alpha = 1.96$, $Z_\beta = 0.84$

$$N_d = (1.96 + 0.84)^2 \times (3.5)^2 / (3.3)^2$$

$$N_d = 8.82;$$

where Z_α represents the , Z_β the , SD the standard deviation, and Δ the expected change.

Due to the intensity of the exercise training and the duration of the study, we expected dropouts. Using a dropout rate of 25% and the following equation for non adherence (Friedman et al., 1992):

$$N^* = N_d / (1 - R_o - R_i)^2$$

where $R_o = 0.25$, $R_i = 0$ (no drop-ins)

$$N^* = 8.82 / (1-.25)^2$$

$$N^* = 15.68$$

N* would be rounded to 16 people per group.

Therefore, no fewer than 16 older men were to be recruited.

Appendix I.5 - Safety/Monitoring Indices

Table A: Safety/Monitoring Indices - Hematological

Variable	Condition			
	BASE	EXER	EXDI	DIUR
K^+(mmol/l)	4.7 ± 0.8	4.9 ± 2.0	5.1 ± 0.7	4.7 ± 0.3
Na^+(mmol/l)	140.1 ± 3.1	139.9 ± 3.5	139.8 ± 3.0	140.0 ± 3.6
Cl^-(mmol/l)	103.5 ± 2.1	103.7 ± 3.8	102.2 ± 3.8	101.2 ± 3.0
Creatinine (umol/l)	86.64 ± 7.1	86.00 ± 7.1	92.36 ± 9.2	96.91 ± 9.6*
Renin(ng/l/s)	0.85 ± 0.52	0.72 ± 0.97	3.67 ± 6.82*	1.17 ± 0.75
Aldosterone (pmol/l)	492.5 ± 639.5	466.7 ± 696.9	1148.6±1149.8*	986.6 ± 538.9*
ACTH(pmol/l)	11.0 ± 7.27	11.1 ± 8.61	13.2 ± 9.63	14.4 ± 8.33
Albumin(g/l)	41.64 ± 2.0	40.09 ± 2.2	43.82 ± 2.8	44.18 ± 3.3*

From blood samples taken just prior to Day 7 exercise testing. K^+ = serum potassium; Na^+ = serum sodium; Cl^- = serum chloride; ACTH = adrenocorticotropic hormone. EXER = exercise only condition; EXDI = exercise and diuretic condition; DIUR = diuretic only condition; BASE = baseline condition. Values are means±SD. * = $p \leq 0.05$, statistically significant difference from BASE following repeated measures analysis of variance.

Table B: Safety/Monitoring Indices - Urinary

Variable	Condition					
(24 hour)	EXER	EXER	EXDI	EXDI	DIUR	DIUR
	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
K^+ (mmol/dl)	88.8 ± 22.6	74.2 ± 21.0	72.8 ± 17.5	82.5 ± 23.2	81.5 ± 40.0	90.2 ± 29.5
Na^+ (mmol/dl)	156.2 ± 59.8	138.5 ± 46.0	198.1 ± 47.2	188.9 ± 56.4	259.8 ± 100.5*	213.2 ± 77.8*
Cl^- (mmol/dl)	157.5 ± 66.6	138.2 ± 48.6	187.0 ± 59.6	195.9 ± 60.0*	242.6 ± 104.0*	217.5 ± 81.6*
Creatinine (mmol/dl)	14.4 ± 3.8	14.4 ± 2.4	14.4 ± 2.4	15.6 ± 3.5	15.4 ± 4.7	15.7 ± 2.8
Volume (ml)	1552.3 ± 607.5	1361.8 ± 486.8	1800.0 ± 393.9	1774.5 ± 507.6*	2080.9 ± 613.0	2141.5 ± 650.1*

K^+ = urinary potassium; Na^+ = urinary sodium; Cl^- = urinary chloride; EXER = exercise only condition; EXDI = exercise and diuretic condition; DIUR = diuretic only condition. From separate 24 hour urine collection performed on Days 1 and 4 of each condition. Values are means±SD. * = $p \leq 0.05$, statistically significant difference versus EXER for the same day following repeated measures analysis of variance.