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The relationship of blood pressure, calcium, and exercise in normotensive women

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THE RELATIONSHIP OF BLOOD PRESSURE, CALCIUM, AND EXERCISE IN NORMOTENSIVE WOMEN

by

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APPROVAL PAGE

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This study assessed possible relationships between exercise, blood calcium, and blood pressure in women. Serum total calcium (STCa), serum ionized calcium (SICa), platelet ionized calcium (PCa), and blood pressure were compared in trained (n=20) and untrained (n=21) normotensive women (18-40 yrs.). Serum sodium, potassium, magnesium (SMg), albumin (SAlb), and total protein (STpro), plus dietary intakes of total calories, calcium, protein, fat, and carbohydrate were also measured. Training was defined as running at least 20 miles/week for the six months prior to the study, with untrained defined as less than 10 miles/week.

Three blood pressure measurements were obtained from fasted subjects in the supine position, after a ten minute stabilization period, utilizing a Dinamap vital signs monitor. A blood sample for analysis of the serum parameters and PCa was taken after the blood pressure measurements. Subjects were asked to keep a seven-day food record which was analyzed with the Nutripractor 6000 dietary analysis software package.

Resting heart rate (HR), mean arterial blood pressure (MAP), and diastolic blood pressure (DBP) were significantly lower in the trained than in the untrained women ($p=0.0005$, $p=0.023$, and $p=0.001$, respectively). ANCOVA adjustment for HR removed the significance in MAP but not DBP between the groups. SMg, STpro, SAlb, STCa, and PCa levels were significantly higher in the trained than in the untrained group, while SICa levels were significantly lower. ANCOVA

adjustment of the blood calcium parameters for SAlb concentration removed the significant difference for STCa and PCa levels between the groups but not SICa. No associations were observed between calcium indices and blood pressure; however, SAlb was negatively correlated with DBP ($r = -0.35$, $p = 0.02$), and SICa ($r = -0.54$, $p = 0.0003$), and positively correlated with STCa ($r = 0.53$, $p = 0.0004$), and PCa ($r = 0.50$, $p = 0.0009$). The trained women had a higher caloric intake resulting primarily from higher protein and carbohydrate intakes, and a higher calcium intake, than the untrained group. Dietary total calcium did not correlate with either blood pressure or any of the blood calcium parameters.

The results of this study suggest that the hypotensive effect of training is not related to alterations in either blood calcium parameters or to dietary calcium. The results further confirmed that training increased SAlb and STpro concentrations and altered calcium binding kinetics within the various blood calcium fractions. The significant relationships between SAlb and all of the blood calcium parameters and between SAlb and DBP suggest a role for SAlb in blood pressure regulation.

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CHAPTER I

INTRODUCTION

Cardiovascular disease reportedly accounts for more than 50 percent of all deaths in the United States (Gotto, 1986). Research from clinical and epidemiological studies on cardiovascular morbidity and mortality has indicated that smoking, hypertension, and hypercholesterolemia are the three primary risk factors in the development of the disease. The identification of these risk factors has prompted the treatment of cardiovascular disease through risk factor intervention.

Epidemiological studies observing the association between blood pressure, morbidity, and mortality suggest that resting pressure is a major indicator for cardiovascular morbidity and mortality (Fagard et al., 1990). This research supports the earlier assessment of the Framingham study that of the three primary risk factors, hypertension is the most prevalent contributor to cardiovascular morbidity and mortality (Kannel, 1975). Data from the Framingham Study (Kannel, 1975) suggest that hypertensives in the 45-74 age range have approximately a three-fold greater chance of developing coronary heart disease, a four-fold greater chance of congestive heart failure, and suffer seven times as many strokes as their normotensive counterparts. Hypertensive health care costs account for approximately ten to twelve percent of the estimated eighty billion dollar annual national health care costs associated with cardiovascular disease (The 1988 Joint National Committee, 1988).

Hypertension is traditionally defined as a systolic/diastolic pressure reading of 160/95 mm Hg or above, with borderline hypertension identified as a reading between 140/90 - 160/95 mm Hg. It has been estimated that approximately sixty million Americans are either hypertensive or borderline hypertensive (The 1988 Joint National Committee, 1988).

Although great advances have been made in the treatment and understanding of hypertension in the past fifteen years, it is still not possible to discuss the mechanisms involved in its development with a high degree of certainty. Only secondary hypertension, which accounts for between five to ten percent of all hypertension cases, has known causes. Since so many systems are involved in the regulation of vascular tension and vary between acute, intermediate, and long-term control, it can reasonably be assumed that there is no one single cause of hypertension. Alterations in plasma volume, renal function, cardiac output, peripheral resistance, the renin-angiotensin system, and intra- and extracellular cation levels all play a role in vascular tension, making blood pressure regulation highly complex and sometimes confusing.

Whatever alterations there are in the pressure mechanisms, the effect is normally a change in the contractile state of the vascular smooth muscle. Changes in electrical activity of the sarcolemma and the interaction of agents with membrane receptors alter the permeability of the membrane, which alters the intracellular concentration of various cations. Alterations in the cellular mechanisms which regulate cytosolic cation concentrations may therefore contribute to abnormal vascular tension observed in various hypertensive states.

Ionic abnormalities, involving mainly sodium and calcium, have been well documented in hypertensive animal and human subjects (Erne, Bolli, Burgisser, & Buhler, 1984; Folsom, Smith, Prineas, & Grimm, 1986; Le Quan Sang & Devynck, 1986; McCarron, 1982; Postnov & Orlov, 1984). While alterations in sodium handling initially received the most attention, research in the past decade has identified an association between sodium sensitivity and cellular calcium handling (Stoy,1988). This has led to the proposal that alterations in cell membrane function result in abnormal cellular calcium handling and subsequent alterations in blood pressure (Postnov & Orlov, 1984). This proposal is supported by the fact that any physiological changes resulting in either an increased influx of ionized calcium from the extracellular medium, or a decreased efflux of calcium ions from vascular smooth muscle cells will lead to an increased cytosolic free calcium and increased vascular tension (Rubin, 1970). Thus, treatment for hypertension resulting from ionic abnormalities must be geared to the restoration of ionic balance both intra- and extracellularly.

The two principal approaches to hypertensive treatment are pharmacologic therapy (also called anti-hypertensive drug therapy), and nonpharmacologic therapy, which includes such interventions as weight reduction, sodium restriction, dietary potassium and calcium manipulation, relaxation, and exercise. Applications of the current knowledge of differences in calcium parameters in relation to blood pressure are present in the use of pharmacological therapies such as beta-blockers, which block the activation of receptor operated channels, and calcium-channel blockers, which block the

influx of ionized calcium into the cytosol (Lees & Reid, 1990). Calcium channel blockade results in a decreased influx of extracellular ionized calcium, decreased cytosolic ionized calcium, and decreased blood pressure. While the effectiveness of anti-hypertensive drugs in reducing blood pressure has been well established, recent evidence suggests that their efficacy may be enhanced, and some side effects mitigated when combined with nonpharmacologic treatments (Kaplan, 1984).

Exercise training has proven useful in blood pressure reduction in both hypertensive and normotensive subjects (Duncan et al., 1985; Kiyonaga, Arakawa, Tanaka, & Shindo, 1985; Saar, Chayoth, & Meyerstein, 1986; Seals & Hagberg, 1984). It has been suggested that the decreases in blood pressure observed with aerobic exercise training are consequences of a decrease in peripheral vascular resistance, possibly due to a decrease in peripheral sympathetic tone (Saar et al., 1986). Although changes in sympathetic nervous system activity have been suggested, only a few studies have assessed changes in blood parameters associated with exercise training. Two studies have observed decreased plasma catecholamine levels with exercise training (Duncan et al., 1985; Kiyonaga et al., 1985), while another observed no change in concentration despite a significant reduction in blood pressure (Seals & Hagberg, 1984). Increased plasma prostaglandin E_2 levels, decreased plasma insulin levels, and increased urinary excretion of sodium have also been observed with exercise training (Kiyonaga et al., 1985). While some of these changes may be associated with a decrease in sympathetic tone, no

mechanism has yet been identified as being responsible for effecting these changes. Peripherally circulating catecholamines and other vasoactive substances affect the permeability of various cell membranes allowing alterations in ionic fluxes across them (Nishizuka, 1989). The alteration of extra- and intracellular ionic calcium concentrations play a critical role in muscular contraction, in the release of catecholamines from adrenergic nerves, and in vascular tension (Rubin, 1970). If changes in blood pressure observed with exercise training are due to a peripheral effect of altered concentrations of vasoactive substances or membrane sensitivity to them, changes in the serum and/or cellular calcium parameters should correlate with changes in blood pressure. Current research, however, is limited in the assessment of a possible relationship between exercise training and blood calcium parameters and its correlation with blood pressure.

Since chronic aerobic exercise appears to have a beneficial blood pressure lowering effect and alterations in smooth muscle cytosolic calcium concentrations appear to correlate with changes in vascular tension, there may be an effect of exercise on the permeability of the smooth muscle membrane affecting cellular calcium handling. The purpose of this study, therefore, was to assess whether differences exist in resting blood calcium parameters, specifically serum total calcium, serum ionized calcium, and platelet ionized calcium, between trained (defined as running a minimum of 20 miles/week for at least six months prior) and untrained (defined as running less than 10 miles/week for at least six months prior) normotensive premenopausal females,

and if a significant relationship existed between these parameters and blood pressure. Additional parameters (serum albumin, total protein, magnesium, sodium, and potassium concentrations) were assessed to determine if there were significant differences between the trained and untrained subjects and if these parameters might be associated with possible changes in blood calcium parameters.

The following hypotheses were proposed:

H1: Mean arterial pressures (MAP) for the trained group will be significantly lower ($p < 0.05$) than for the untrained group.

H2: There will be a mean difference of at least 2 mm Hg diastolic and 5 mm Hg systolic between trained and untrained subjects.

H3: Serum ionized calcium concentration will be significantly greater ($p < 0.05$) in the trained vs. untrained group.

H4: Platelet ionized calcium concentration will be significantly lower ($p < 0.05$) in the trained vs. untrained group.

H5: Serum total calcium and albumin modified serum total calcium concentrations will not be significantly different ($p > 0.05$) between the two groups.

H6: Serum magnesium, sodium, and potassium will not be significantly different ($p > 0.05$) between the two groups.

H7: There will be a significant positive relationship between platelet ionized calcium and diastolic, systolic, and mean arterial pressures.

H8: There will be a significant positive relationship between serum albumin and

diastolic, systolic, and mean arterial pressures.

H9: There will be a significant inverse relationship between serum ionized calcium and diastolic, systolic, and mean arterial pressures.

H10: There will be a significant inverse relationship between dietary calcium intake and diastolic, systolic, and mean arterial pressures.

CHAPTER II

REVIEW OF LITERATURE

Blood Pressure Regulation

The pressure of the blood as it flows through the vasculature is determined by the nature of the blood (volume, viscosity, etc.), the heart (stroke volume and cardiac output), and the resistance in the vessels. The nature of the blood will only affect pressure when its viscosity changes, which rarely happens in humans, except with polycythemia, hyperglobulinemia, or macroglobulinemia (Guyton, 1980). The effect of the heart and vascular resistance on blood pressure is mediated by the factors of cardiac output, blood volume, arterial wall elasticity, and peripheral resistance. These factors interact to regulate blood pressure at rest and in response to internal and external influences such as changes in temperature, physical or emotional stress, and the metabolic demands of the body.

Regulation of the factors affecting blood pressure is accomplished through intrinsic and extrinsic control processes. The intrinsic process, called autoregulation, is a system of mechanisms which directly alter arterial diameters in response to local tissue needs. The mechanisms involved in this process are stress relaxation, which enables blood vessels to gradually dialate in response to increasing blood pressure, and capillary fluid shift, which moves plasma between the vessels and the extravascular compartments (Guyton, 1980). The intrinsic process of autoregulation is one of many non-neural processes involved

in arterial pressure regulation. Mechanical and chemical, rather than nervous, stimulation of stretch receptors in the vascular tissue is the force behind autoregulatory induced vascular relaxation (Burnstock & Griffith, 1988; Guyton, 1980). In most tissues of the body the requirement for oxygen appears to be the most important initiator of autoregulatory vasodilation, with other nutrients such as glucose, amino acids, and vitamins playing a minor role (Burnstock & Griffith, 1988; Guyton, 1980). Locally released non-neural vasodilators which may be related to the autoregulatory process include histamine from mast cells, bradykinin, prostaglandins, and leukotrienes (Burnstock & Griffith, 1988). Adaptations in the autoregulatory process develop over a period of weeks, months, or possibly longer, resulting in a fine tuning of the blood flow to meet the needs of the vascular tissue (Guyton, 1980), and a possible contributing mechanism in chronic pressure regulation.

Extrinsic control processes, so called because they are initiated outside the circulatory system, regulate cardiac output, peripheral resistance, and blood volume through a complex, highly coordinated process of central nervous system and endocrine responses, called the neuroendocrine response. This response involves centrally located centers in the brain stem, hypothalamus, and spinal cord which regulate the responses of the heart and peripheral vasculature to maintain cardiovascular homeostasis.

To maintain this continuous control of arterial pressure the body has developed a hierarchy of controls. Intrinsic and extrinsic pressure control systems can be grouped into three major categories based on their reaction

time and duration of effect. Acute mechanisms, which react and dissipate rapidly, are primarily cardiovascular reflexes, such as the carotid baroreceptors, mediated by the nervous system (Guyton, 1980). Intermediate mechanisms, which are slower to react and more sustained than acute mechanisms, include the autoregulatory mechanisms of stress relaxation and capillary fluid shifts. Both acute and intermediate mechanisms, however, are not capable of returning the arterial pressure to its baseline levels following pressure changes, their primary function being pressure buffering rather than establishing baseline pressure (Guyton, 1980).

Guyton (1980) suggested that the systems that maintain baseline blood pressure are those which regulate blood volume (i.e. the kidneys) and vascular tone. The primary importance of acute and intermediate control mechanisms is in their response to the external effects on arterial pressure. The mechanisms which mediate long-term pressure control seem to be the most important in chronic hypertension, and are also those about which the least is known (Guyton, 1980).

The aim of this review is to provide a general overview of the roles played by the central nervous system, autonomic nervous system, and the primary cardiovascular organs involved in blood pressure homeostasis. Particular attention will be given to the ionic alterations in vascular smooth muscle relating to changes in blood pressure, and to the effect of exercise training on blood pressure.

Nervous Control of Blood Pressure

Central Nervous System Regulation.

The control of cardiovascular function is maintained by mechanical, chemical, and neural mechanisms whose contribution to circulatory regulation varies in different vascular beds. The global task of integrating these regulatory mechanisms to ensure that all cardiovascular functions are adjusted according to the current circulatory situation rests with the central nervous system. While the role of the central nervous system in the moment-to-moment control of arterial pressure is fairly well accepted, its role in chronic regulation is less clear.

The medulla area of the brain stem is the primary region that influences the cardiovascular system. Nuclei in this region are connected to regions in the spinal cord, the hypothalamus, and the cardiovascular organs, providing a highly complex, central control network for the maintenance of cardiovascular homeostasis. Information is transmitted back and forth along this network by means of chemical messengers such as catecholamines, opioid and other *neuropeptides, and by direct electrical stimulation.*

Regulatory centers in the brain stem rely on the input they receive from the periphery for the current state of cardiovascular function. Baroreceptors, primarily involved in acute regulation mechanisms, sense changes in blood pressure and relay the information to the nucleus tractus solitarius in the dorsal medulla, which is the primary site of termination for impulses from all arterial and cardiopulmonary baroreceptor afferent neurons (Wyss, Oparil, & Chen, 1990). The neurotransmitters released from these baroreceptor afferent neurons at the

nucleus tractus solitarius have not been firmly identified. Studies involving microinjections of epinephrine, norepinephrine, dopamine and other adrenergic agonists into the region of the nucleus tractus solitarius have been shown to result in bradycardia and arterial hypotension similar to that observed with baroreceptor activation (Guthrie & Kotchen, 1984).The stimulated nucleus tractus solitarius, in turn, stimulates sympathetic preganglionic neurons projecting to the paraventricular nucleus of the hypothalamus, where it modifys the activity of vasopressin-releasing neuron projections to the pituitary, and inhibits parasympathetic neurons to the heart, resulting in an increased heart rate (Wyss et al., 1990). Other areas of the medulla also stimulated by the nucleus tractus solitarius include the dorsal motor nucleus of the vagus, the nucleus ambiguus, the ventrolateral medulla, and the area postrema. Innervation of the neurons in the dorsal motor nucleus of the vagus and the nucleus ambiguus results in increased parasympathetic stimulation of the heart. The nucleus ambiguus region appears to be the major center of parasympathetic depressor projections to the heart (Wyss et al., 1990). The importance of the nucleus tractus solitarius in blood pressure regulation was demonstrated by Dampney and Moon (1980), whose experiments showed that lesions in the nucleus tractus solitarius of laboratory animals results in hypertension.

Immediately dorsal to the nucleus tractus solitarius is the area postrema, which also receives messages from the peripheral baroreceptors. Its uniqueness lies in its lack of a blood-brain barrier, which allows it to react to

peripherally circulating vasoactive substances such as angiotensin II (Wyss et al., 1990). This addition of peripheral activation to the signals being relayed to the nucleus tractus solitarius results in a more comprehensive control of vasomotor sympathetic activity and a more intergrated control of arterial pressure.

The two areas of the ventrolateral medulla involved in cardiovascular pressure regulation are the C1 and A1 areas (Wyss et al., 1990). C1 neurons project to sympathetic autonomic nuclei in the spinal cord. Stimulation of C1 neurons results in significant increases in heart rate and blood pressure, release of adrenomedullary catecholamines, and release of the vasoconstrictor vasopressin from the posterior pituitary (Wyss et al., 1990). C1 neurons may play a role in the long-term regulation of blood pressure through their action on the sympathetic nervous system. It has been suggested that these neurons maintain background input to the vasomotor centers in the spinal cord, thereby continuously regulating sympathetic nervous control of arterial pressure (Wyss et al., 1990).

The A1 area of the ventrolateral medulla contains noradrenergic neurons, stimulation of which inhibits sympathetic discharge and lowers blood pressure (Imaizumi, Granata, Benarroch, Sved, & Reis, 1985). In contrast to the C1 area, release of norepinephrine from stimulated A1 neurons seems to have an inhibitory effect on tonic vasomotor neurons (Imaizumi et al., 1985).

Although the medulla appears to contain the majority of the centers involved in the regulation of arterial pressure, other brain stem areas also play a

role. Signals from the nucleus tractus solitarius and the hypothalamus stimulate the parabrachial nucleus of the pons producing an increase in heart rate and a rise in arterial pressure (Mraovitch, Kamada, & Reis, 1982). The periaqueductal region of the midbrain also appears to have a role, as yet unspecified, in cardiovascular regulation (Wyss et al., 1990).

Autonomic Nervous System Regulation.

The spinal cord contains the autonomic nervous link between the cardiovascular regulatory centers in the brain and the peripheral vascular system. The information received in the brain centers on the current cardiovascular situation is intergrated and transmitted to various areas of the cardiovascular system via the autonomic nervous system. The autonomic nervous system regulates cardiac output, blood volume and composition, and blood pressure through dual antagonistic innervation of its two efferent divisions, the sympathetic and parasympathetic nervous systems. Both systems commonly act on all cardiovascular organs, but produce opposite responses. Vasoconstrictor activity is normally associated with the sympathetic nervous system and vasodilator activity is normally associated with the parasympathetic system (Clark, Queener, & Karb, 1982). Thus, the activity of cardiovascular organs is the result of the stimulation of two opposing systems directed by the regulatory systems in the brain and the hypothalamus.

The common pathway for brain center regulation of sympathetic nervous activity is through the preganglionic neurons in lamina seven of the thoracic spinal cord, with most of the neurons located in the intermediolateral cell column

(Guthrie & Kotchen, 1984; Wyss et al., 1990). The activity of these neurons is controlled by direct sensory innervation from the periphery and via descending projections from the regulatory centers in the brain.

Information from the regulatory centers in the brain passed along autonomic neurons to the appropriate cardiovascular organs is mediated by the release of neurotransmitters from the neurons. Norepinephrine, the predominant neurotransmitter released from sympathetic neurons, has an intense vasoconstrictor effect upon arterial smooth muscle, mediated primarily by alpha-1 (A_1) receptors on the vascular membrane (Burnstock & Griffith, 1988). Other acute hemodynamic effects of sympathetic stimulation include stimulation of the heart, an increased pre- to postcapillary resistance ratio, and venous constriction (Guyton, 1980). Sympathetic stimulation also indirectly contributes to peripheral vasoconstriction by stimulating the release of vasoactive substances such as renin, from the kidney, and vasopressin, from the pituitary (Guyton, 1980). At rest the sympathetic nervous system is the primary effector of the tone of the cardiovascular system. This tone is maintained by a minimal but constant release of neurotransmitters to the components of the cardiovascular system, with the sympathetic nervous system dominant over the parasympathetic system at rest (Clark et al., 1982). The magnitude of the initial response of arterial pressure to changing cardiovascular situations is therefore a reflection of the degree of sympathetic stimulation which, in turn, is determined and coordinated by regulatory centers in the central nervous system. Although there is still no concensus on whether or not

measurements of plasma catecholamines provide an index of sympathetic tone in the resting state, there does appear to be a positive correlation between plasma catecholamine concentrations and sympathetic activity in response to work intensity (Guthrie & Kotchen, 1984).

Parasympathetic stimulation is generally associated with vasodilation and decreased heart rate and blood pressure (Clark et al., 1982). Parasympathetic preganglionic neurons originate primarily from the dorsal motor nucleus of the vagus and the nucleus ambiguus, with projections to the heart extending from both areas (Guthrie & Kotchen, 1984). Although there is documented evidence that parasympathetic stimulation of the heart results in a decreased heart rate there is very litte evidence for active neurogenic vasodilation in the systemic vessels of most species (Burnstock & Griffith, 1988). Acetylcholine released from parasympathetic neurons has been shown to cause dilation of systemic arteries by acting on muscarinic receptors on the endothelium (Burnstock & Griffith, 1988). This action causes local release of endothelium derived relaxing factor (EDRF) which results in local vasodilation (Furchgott & Zawadzki, 1980). The chemical components of EDRF are currently unknown, however, early research has ruled out any prostaglandin, adenosine, and adenosine monophosphate (AMP) as the possible active factor (Furchgott, 1983). The EDRF mechanism is presently the only recognized path for parasympathetic innervated vasodilation of the peripheral vasculature. Furchgott (1983) has suggested that the relaxation observed with the release of EDRF may be due to a combined effect of other non-neural mechanisms acting with the EDRF and not solely to the action of EDRF.

Hypothalamic Regulation.

The hypothalamic nuclei involved in cardiovascular regulation receive their sensory input primarily from the regulatory centers in the brain stem. Stimulation of these nuclei elicit either increases or decreases in heart rate, cardiac output, and blood pressure depending on the origin of the signal and which branch of the autonomic nervous system is stimulated (Varner, Barman, & Gebber, 1988). The lateral and posterior hypothalamic areas, as well as the ventrolateral hypothalamic nucleus contain sympathoexcitatory regions. Stimulation of these regions results in increased arterial pressure, heart rate, and vascular resistance (Wyss et al., 1990). Nuclei in the lateral and posterior hypothalamus contain neurons which project directly to the medulla and the spinal cord, providing a link between the brain centers and the peripheral vascular system. The paraventricular nucleus of the hypothalamus is also tied to the medulla and to the autonomic nervous system in the spinal cord. Its difference lies in the fact that it also regulates secretion of vasopressin by the pituitary and projects to the vagal parasympathetic nuclei as well as the thoracic sympathetic neurons (McKeller & Loewy, 1981).

Research suggests that both vagal and sympathetic outflow are controlled by the anterior hypothalamus (McKeller & Loewy, 1981). Blood pressure changes observed with stimulation of the anterior region suggest a strong role in sympathoinhibitory control of arterial pressure, while activation of the posterior hypothalamus increases sympathetic activity leading to increased vascular tension (Wyss et al., 1990).

Central Nervous Control and Hypertension.

Subtle alterations in the organization of neural mechanisms involved in cardiovascular regulation or in the neurochemistry of the central nervous system may result in hypertension. Further, these neural imbalances may manifest at the central and/or the peripheral level. The central nervous system regulates cardiovascular homeostasis through a widely distributed network of neural circuits integrating regulating brain centers with the spinal cord and effector organs, such as the heart and vascular tissue. Alterations in the sensory input to the brain centers, in the control signals these centers send to the effector organs, or in the responsiveness of these organs may result in hypertension. In some hypertensives the baroreflex system is reset, requiring an increased arterial pressure for stimulation of the appropriate brain centers (Sealy & Laragh, 1990). Sympathetic nervous activity, a primary pathway in brain center control, is altered in nearly all forms of human and experimental hypertension (Goldstein & Kopin, 1990; Wyss et al., 1990). The normal response to increased arterial pressure is a reduction in sympathetic nervous activity, however, this activity may be normal or increased in hypertensives (Wallin, Morlin, & Hjemdahl, 1987). Decreasing sympathetic activity by either surgical or chemical means reduces arterial pressure in many hypertensives (Wallin et al., 1987). Wyss et al. (1990) have suggested that some of the drugs used to lower arterial pressure do so by direct action on areas of the central nervous system.

In addition to observed differences in sensory input and sympathetic nervous activity, alterations in cardiac output, organ vascular resistance, and

total peripheral resistance have been noted in hypertensive individuals (Frohlich, 1987). The increased cardiac output observed in young individuals in the early stages of hypertension can be explained in part by an observed redistribution of the intravascular volume to the central circulation, a faster heart rate, and increased myocardial contractility (Frohlich, 1987). Increased peripheral and organ vascular resistance may be related to altered vascular membrane ion transport or to structural alterations in the vascular tissue (Postnov & Orlov, 1985). In the majority of adults with mild, moderate or severe hypertension an increased peripheral and organ vascular resistance and a normal cardiac output are present (Frohlich, 1987).

The Role of Opioid Peptides

Opioid peptides are chemical transmitters which perform a role similar to that of catecholamines. Numerous opioid peptides and their receptors have been found centrally in various brain nuclei involved in cardiovascular control, and peripherally in the heart, blood vessels, sympathetic nerves, and adrenal gland (Feuerstein & Siren, 1987). These peptides have at least two functionally different amino acid sequences; an active site that binds with a specific receptor to initiate a specific response, and an auxiliary amino acid sequence which may contribute to the affinity of the peptide for a specific receptor and protect it from degradation (Burnstock & Griffith, 1988). These peptides can be grouped into three main categories, each with its own characteristic precursors, distribution, and receptor affinities; beta-endorphin (B-endorphin), enkephalins, and dynorphins (Grossman & Sutton, 1985).

Opiates from each of the major categories have been found in the central nervous system, with both endorphins and enkephalins present in measurable levels in the circulation (Grossman & Sutton, 1985). Enkephalins have been identified in nerve terminals surrounding the nucleus tractus solitarius, the dorsal vagal nucleus, and the nucleus ambiguus, all areas involved in cardiovascular regulation (Thoren, Floras, Hoffmann, & Seals, 1990). These opiates in the central nervous system are thought to influence cardiovascular function in much the same manner as catecholamines, i. e. stimulation via receptor activation (Burnstock & Griffith, 1988). Opioid peptides may influence the peripheral cardiovascular system via peptidergic innervation of end organs of blood pressure control, receptor activation by circulating opiates, or there may be local generation of opiates in the peripheral vasculature which moderate its activity (Burnstock & Griffith, 1988).

Several opiate actions in cardiovascular regulation have been documented. It has been firmly established that opioid peptides exert an inhibitory effect on the release of posterior pituitary hormones such as vassopressin (Grossman & Sutton, 1985; McArthur, 1985). Injection of B-endorphins into the nucleus tractus solitarius region of laboratory animals produces vascular hypotension (Guthrie & Kotchen, 1984). Likewise, injection of dynorphins into the periventricular hypothalamic nucleus decreases arterial pressure (Guthrie & Kotchen, 1984). Peripherally, stimulation of opioid receptors located on systemic vessels and other cardiovascular structures appears to inhibit peripheral sympathetic transmission (Thoren et al., 1990). In

general, research has shown that both central and peripheral administration of opiates produces bradycardia and vascular hypotension through inhibition of sympathetic outflow and the enhancement of parasympathetic responses (Thoren et al., 1990). Caution has been indicated however, in the assumption that peripheral circulating levels of opiates is a reflection of central opiate activity. Since the blood-brain barrier is relatively impermeable to opioid peptides, the relationship between central and peripheral levels of opiates remains conjecture until research has established the specific brain site(s) of action mediating the effect of opiates on the periphery.

Role of the Kidneys

The kidneys affect blood pressure primarily through two mechanisms, regulation of blood volume, and the vasoconstrictor action of angiotensin II on the smooth muscle vasculature. The ability of the kidneys to affect the long-term regulation of arterial pressure by controlling the volume of body fluids, particularly blood volume, has been known for some time (Guyton, 1980). The basic mechanism is as follows: when the arterial pressure rises beyond a certain point, the kidneys increase their output of water and electrolytes causing a drop in blood volume and a return to normal pressure. One very important feature of this mechanism, which distinguishes it from buffering mechanisms like those involved in acute and intermediate pressure control, is its potential to return arterial pressure to its original value.

Cardiac output is directly influenced by the kidney's control of blood volume. This can be explained by a more in-depth discussion of the regulating

mechanism. The steps are as follows:

1. Increases in the arterial pressure result in an increased volume output from the kidney (i.e. increased urinary output).
2. The decrease in extracellular fluid volume results in a decreased blood volume.
3. This decrease in blood volume leads to a decrease in the mean circulatory filling pressure (a measure of the compactness of the blood in the circulation).
4. This decrease leads to a decrease in the venous return to the heart which causes a decrease in the cardiac output and thus, a decrease in the arterial pressure.

As stated earlier, this mechanism has the potential to return arterial pressure to normal values, this has been demonstrated in dogs into which a 30 percent increase in blood volume was induced by blood transfusion (Dobbs, Prather, & Guyton, 1971). The unique factor in this study however, was that the dogs had their nervous system destroyed so they were devoid of nervous reflexes, leaving the kidneys as the major regulator of arterial pressure. When cardiac output and blood volume values have been measured in hypertensive individuals, lower than normal blood volumes (Ulrych, 1973) and either normal or below normal cardiac output (Frohlich, 1987) have been observed. These observations are contradictory to what would be expected if the regulation of blood volume were the primary factor in essential hypertension. These results also lend strength to the hypothesis that hypertension is primarily a disfunction

in peripheral resistance since a higher vascular tension must be present to counter the lower cardiac output and produce the observed hypertension.

In addition to affecting arterial pressure through blood volume regulation, the kidneys also affect pressure by acting on peripheral resistance through the renin-angiotensin-aldosterone system. Renin is a proteolytic enzyme formed in the juxtaglomerular cells located primarily in the walls of the afferent arterioles of the kidneys. When there is a decrease in arterial pressure, which can result from sodium depletion, dehydration, or sympathetic stimulation, the baroreceptors in the kidney's afferent arterioles are stimulated, and renin is formed and released into the blood. Renin acts on renin substrate (angiotensinogen), formed in the liver, to form angiotensin I, which is acted on by converting enzyme in the lungs and kidneys to form angiotensin II. Angiotensin II exerts a powerful, direct, and immediate vasoconstrictive effect on the arterial bed by increasing the cytosolic ionized calcium levels of the smooth muscle cells. It also acts on the adrenal cortex to increase secretion of aldosterone which, in turn, acts on the renal tubules to increase reabsorption of sodium and water.

Renin, unlike other plasma enzymes, has no inhibitor, only its secretion is regulated. Therefore, once renin is in the blood it can continuously produce angiotensin I as long as the liver provides sufficient amounts of substrate. Research has indicated that amounts of angiotensinogen produced by the liver and angiotensin converting enzyme produced in the lungs are not rate limiting in the production of angiotensin II (Sealey & Laragh, 1990). Negative feedback

from the levels of angiotensin II, vasopressin, and atrial natriuretic factor is the only limitation on the rate of renin secretion.

Renin release is stimulated by the action of epinephrine on the Beta-1 (B_1) receptors on the juxtaglomerular cell membrane, and by the circulating levels of prostaglandin I_2, prostaglandin E_2, kallikrein, and other kinins. Available evidence suggests that all of the factors which stimulate renin release do so by changing the intracellular concentration of ionized calcium in the juxtaglomerular cells, with an increase in concentration suppressing release and a decrease stimulating it (Sealey & Laragh, 1990).

Since renin is primarily secreted when blood volume is reduced, the low blood volumes observed in some hypertensives (Ulrych, 1973) may be countered to some extent by the high renin profiles observed in certain groups of hypertensives (Weber & Drayer, 1984). Marked elevation in plasma renin concentration has also been observed in normal individuals after 20 minutes of continuous exercise (Goldstein & Kopin, 1990). At one time it was proposed that individuals with high renin profiles had a "vasoconstricted" form of hypertension while those with low profiles had a more volume-dependent form (Frohlich, 1987). Although there is little evidence to justify classification based solely on renin profiles, differences in these profiles have been incorporated into a broader and more widely accepted classification proposal. Stoy (1988) has proposed two classifications: The Type I defect is characterized by low serum ionized calcium levels, increased 1, 25 dihydroxycholecalciferol levels, and decreased plasma renin activity compared to that of normotensives; the Type II

defect, on the other hand, is characterized by increased serum ionized calcium, decreased 1, 25 dihydroxycholecalciferol, and increased plasma renin activity. Since renin secretion is positively associated with vasoconstriction and low levels of renin activity are observed in some forms of hypertension, it may be assumed that while alterations in renin secretion are a factor in the increased vascular tension observed in some hypertensives, they are not the sole factor.

The Role of Calcium in Vascular Tension

It has been suggested that hypertension is a disorder of cell function and that this dysfunction resides in the cells of one or more of the tissues primarily involved in pressure regulation: neural synapses, adrenal glomerulosa, juxtaglomerular apparatus, or the vascular smooth muscle cells (Rasmussen, 1983). Since the major means by which the secretion of norepinephrine, renin, and aldosterone are regulated, and by which norepinephrine and angiotensin II affect the contractile state of the vasculature is by altering the vasculature or the cellular organelles to allow either an increased influx of ionized calcium into the cell or a decreased efflux from the cell, the proposed cellular dysfunction in hypertension possibly involves a disorder of cellular calcium metabolism.

Force generation in the vascular smooth muscle is produced in a manner similar, but not identical, to the sliding filament model present in skeletal muscles (Bohr & Webb, 1984). In both types of muscle, force is developed as a result of the formation of myosin cross-bridges alternately attaching and detaching from the actin molecule sliding one filament over the other. This cycling, in both muscle types, is initiated by an increase in the intracellular

concentration of ionized calcium (Bohr & Webb, 1984). The main difference between the two muscle types is in the control of the cycling process. In skeletal and cardiac muscle, the process is controlled by a troponin-based system present on the actin filament. Smooth muscle contractility, on the other hand, is regulated by the myosin light chain, which is a component of the head of the myosin molecule. It has been fairly well established that in smooth muscle, tension production is a function of the rate of cross-bridge cycling, which in turn is determined by the degree of phosphorylation of the myosin light chain (Bohr & Webb, 1984; Rasmussen, 1983). The degree of myosin light chain phosphorylation is directly proportional to the cytosolic concentration of ionized calcium, making cellular calcium metabolism an important component of vascular tension. If hypertension does involve a dysfunction of cellular calcium metabolism, it may manifest itself in the mechanisms which regulate the cytosolic concentration of ionized calcium or the phosphorylation of myosin light chain.

Regulation of Extracellular Calcium Concentration.

Entry of ionized calcium from the extracellular space into the cytosol of the vascular smooth muscle cell initiates vascular contraction (Khalil, Lodge, Saida, Gelband, & van Breeman, 1990). The regulation of extracellular calcium concentration is dependent upon the net effect of intestinal absorption, skeletal accretion and resorption, renal filtration and resorption, and the degree of calcium binding to anionic sites in the extracellular medium (McCarron, 1985). These processes are in turn affected by systemic pH, temperature, and the

concentrations of serum protein, parathyroid hormone, calcitonin, and vitamin D in the circulation. The end result is a total calcium concentration in the extracellular fluid, fairly rigidly maintained in the range of 2-5 millimolar (mM). The actions of vitamin D in regard to calcium concentration include: a) intestinal absorption of calcium, b) the mobilization of calcium from bone, and c) renal reabsorption of filtered calcium. Parathyroid hormone, secreted when the plasma ionized calcium concentration falls below the parathyroid setpoint of approximately 4.5 mg/100 ml (approximately 1.12 mM), also acts to stimulate the mobilization of calcium from bone and renal reabsorption of calcium. Along with these functions parathyroid hormone also stimulates the conversion of 25-hydroxycholecalciferol to 1, 25-dihydroxycholecalciferol in the kidney, which is the form of vitamin D necessary for the intestinal absorption of calcium. Also stimulated by the level of plasma calcium is the secretion of calcitonin from the thyroid gland. While parathyroid hormone is secreted in response to low levels of plasma calcium, calcitonin is released in response to rising levels, due either to an increase in intestinal absorption of calcium or increased calcium resorption from bone. Calcitonin acts to inhibit calcium resorption from bone, thereby reducing its plasma concentration toward normal. It is primarily the actions of parathyroid hormone, calcitonin, and vitamin D which maintain the extracellular ionized calcium levels within the narrow 2-5 mM range.

The total concentration of extracellular calcium can be divided into three main fractions, a protein bound fraction, a complexed fraction, and an ionized fraction. Generally, the protein bound and the complexed fractions are in

equilibrium with the ionized fraction comprising approximately 40, 10 and 50 percent of the total calcium concentration, respectively (Robertson, 1988). Although there is likely to be a role for the complexed or protein bound fractions, no physiologically important role has presently been identified. The ionized fraction is generally regarded as the factor of primary physiological importance in extracellular calcium homeostasis. The ionized calcium fraction is maintained under strict hormonal control with the mean normal plasma ionized calcium concentration at 4.7 mg/100 ml (Robertson, 1988). The plasma ionized calcium concentration changes very little over the life span with an intra-individual variation of normally not more than 0.1 mg/100 ml, which is close to the detection limits of most analytical techniques. Further, no significant sex difference in plasma ionized concentrations has been observed (Robertson, 1988).

Total calcium is often used as an index of calcium homeostasis since a correlation between total and ionized calcium in plasma has been suggested when the full range of plasma calcium values are computed (Marshall, 1976). This measure, however, is not completely valid if the plasma protein concentrations or plasma pH are abnormal since the concentration of albumin-bound calcium may vary with blood pressure (Fogh-Andersen, Hedegaard, Thode, & Siggaard-Andersen, 1984). Although some studies have reported no differences in total or ionized calcium concentrations between groups of hypertensives and sex- and age-matched normotensives (Strazzullo et al., 1983), others have reported significant correlations between total calcium and

blood pressure (Kesteloot & Geboers, 1982) and ionized calcium and blood pressure (Fogh-Andersen, et al., 1984). Although these studies reported the total or ionized plasma calcium concentrations, no information on the contributions of the protein bound or complexed fractions were mentioned. Pottgen and Davis (1977) reported a significant inverse relationship ($r = -0.99$), based on linear regression, between plasma ionized calcium and plasma albumin regardless of whether the ionized calcium was measured directly or calculated. Since albumin-bound calcium is a component of the protein bound calcium fraction, it is possible that alterations in the concentration of the protein bound fraction may affect the concentration of the ionized calcium fraction. In contrast to the inverse relationship reported by Pottgen and Davis (1977), two independent studies, utilizing different methods of collecting and storing samples and different apparatus for measuring ionized calcium concentrations and pH, reported significant positive correlations ($p \leq 0.001$) for ionized calcium, adjusted for pH, with albumin concentration (Butler, Payne, Gunn, Burns, & Paterson, 1984). The authors concluded that there was a definite association of low plasma ionized calcium concentrations and low plasma albumin concentrations in individuals with hypoalbuminemia. Butler et al. (1984) further indicated that this positive association may have been due to a high prevalence of malnutrition in their subjects or, more likely, to a positive interference by plasma proteins with the calcium measuring instruments. Identification of a relationship between serum albumin and serum ionized calcium concentrations has led to the development of several formulas, utilizing a mean value of 40 or

42 grams/liter (g/L) for serum albumin (Butler, et al., 1984; Hvarfner, Bergstrom, Morlin, Wide, & Ljunghall, 1987). These formulae adjust the total calcium concentration so that a clearer picture of the relationship between serum ionized calcium and serum total calcium can be viewed without serum albumin as a confounding variable. Although these studies have indicated both positive and negative correlations between serum ionized calcium and serum albumin concentrations, and that alterations in the serum ionized calcium concentration are possible without being reflected in the total calcium values, no measurement of blood pressure was reported. Further, no measurements or estimates of the protein-bound or complexed calcium fractions and their relationship to the total calcium concentration were made in these studies.

When the relationship between blood pressure and calcium indices has been examined, no significant relationships between any blood pressure variable and total serum calcium or albumin modified total serum calcium were reported (Hvarfner et al., 1987). However, Hvarfner et al. (1987) did report a significant inverse relationship ($p \leq 0.0001$) between plasma ionized calcium and blood pressure, a significant positive relationship between serum albumin concentration and mean arterial ($p=0.001$), diastolic ($p=0.002$), and systolic pressures ($p=0.005$), and indicated that there was an inverse association between serum ionized calcium and serum albumin.

Regulation of Intracellular Calcium Concentration.

While the extracellular ionized calcium concentration is in the millimolar range, its intracellular concentration is generally 100-100,000 times lower.

Because of this vast gradient, activation of such calcium-regulated processes as secretion and contraction only require changes in the micromolar (uM) range. The rate of movement of calcium ions across the plasma membrane is primarily controlled by the relative impermeability of the membrane to calcium and by the action of various calcium channels in the membrane. The plasma membrane may be viewed as a two-dimensional mosaic consisting of integral proteins embedded in a lipid layer with other proteins loosely bound to both the inner and outer surfaces. Calcium ions from the extracellular medium bind to the protein fraction of the membrane causing an increase in protein cross-linking in the lipid layer. This binding by calcium increases the rigidity of the membrane which increases its electrical resistance (Tobias, Agin, & Pawlowski, 1962). In addition, this calcium binding acts to increase membrane surface pressure by condensing the lipid bilayer, decrease the thickness of the bilayer, and reduce the fluidity of the membrane by stabilizing the phospholipid moiety (Robertson, 1988). The stabilization of the phospholipid moiety is achieved through the formation of a calcium-ATP-phospholipid complex. The combined effect of calcium ions on both the phospholipid and protein moieties in the membrane is the creation of "channels" which define the membrane's permeability to various ions (Robertson, 1988).

Vascular smooth muscle cell membranes contain a number of different receptor sites and are electrically connected to one another via nexuses, allowing changes in membrane permeability to be brought about by either agonist application or membrane depolarization. A strong correlation between

influx of extracellular ionized calcium across the plasma membrane and the state of vascular contraction has been suggested by studies utilizing calcium antagonists and agonists. Calcium ion antagonist blockage of calcium-45 (^{45}Ca) influx was observed to be associated with inhibition of vascular force development (van Breemen, Mangel, Fahim, & Meisheri, 1982), while augmented ion influx by the agonist Bay K8644 was observed to enhance force development (Yamamoto, Hwang, & van Breemen, 1984). The combination of these observations and an understanding of calcium's effects on the plasma membrane have led to the postulation of three major pathways of calcium entry into the cell. The first, or voltage-gated channel, has been identified in a variety of different tissues (Rassmussen, 1983) and allows increased calcium ion entry upon membrane depolarization. The second, or receptor-operated channel, allows increased ion influx upon stimulation of specific membrane receptors such as stimulation of alpha-receptors by angiotensin II. The third pathway has been referred to as the leak pathway (Khalil, Lodge, Saida, Gelband, & van Breeman, 1990). In this pathway calcium ions cross the resting plasma membrane in the absence of membrane stimulation by depolarization or receptor activation. The magnitude of this calcium leak, as determined by ^{45}Ca flux measurements in resting rabbit aorta, is approximately 15 umol/kg/min (Khalil et al., 1990). It has been suggested that this leak of calcium ions across the membrane occurs via the sodium-channel (Rasmussen, 1983), and that it is continually compensated for by sequestration into the endoplasmic reticulum and by calcium ion extrusion to the extracellular space (Khalil et al., 1990).

While it has been concluded that sufficient physiologic and biochemical evidence exists to support the presence of sodium-calcium ion exchange (Young, Bukoski, & McCarron, 1990), the extent of its contribution to intracellular calcium regulation is currently unknown. Thus, this pathway may play a greater role in vascular smooth muscle activation if cellular mechanisms responsible for sequestration or expulsion are compromised in any way.

In addition to the three primary pathways of calcium ion influx, it has been suggested that stretch or elevated intravascular pressure can result in a maintained increase in vascular smooth muscle tone (Khalil et al., 1990). Further, evidence indicates that this fourth pathway or stretch-activated channel, is highly influenced by extracellular calcium ion concentration (Bohr & Webb, 1984). Evidence in support of the existence of this fourth pathway comes from observations that stretching of the smooth muscle vasculature stimulates Ca^{45} influx (Laher, Hwa, & van Breeman, 1988) and that it can also open nonspecific cation channels in the smooth muscle membrane (Kirber, Singer, & Walsh, 1987).

Maintenance of calcium ion homeostasis in the cytosol is vital for the prevention of cell intoxication and regulation of the biological systems dependent on changes in the intracellular calcium ion concentration. Cytosolic calcium ion concentration is primarily regulated by the actions of an energy-dependent calcium-magnesium-ATPase (Ca-Mg-ATPase) pump, which actively moves calcium ions back across the plasma membrane, and a calcium-sodium ion exchange system which exchanges intracellular calcium ions for extracellular

sodium ions (Postnov & Orlov, 1985; Rasmussen, 1983). Both systems are located in the plasma membrane and extrude calcium ions from the cell against a concentration gradient. In addition to these two systems, the cell can also regulate calcium ion concentration through its binding in the cytosol to various substrates, such as citrate, phosphate, troponins, and calmodulin; sequestering of the ions into subcellular compartments, such as the endoplasmic reticulum, microsomes, and the mitochondria; and through the stabilizing effect of calcium ions on the permeability of the plasma membrane (Campbell, 1988). The overall effect of these internal systems is to maintain up to 98 percent of the total intracellular calcium concentration either localized in intracellular compartments or bound to membrane structures and cytoplasmic substrates. Since only micromolar changes in ionized calcium concentration are necessary to induce stimulation, shifts in the functioning of these internal regulating systems could lead to alterations in its intracellular concentration, affecting calcium-dependent mechanisms. Systems involved in vascular contraction, neurotransmitter release, and membrane ionic permeability, all factors in vascular tension, are all calcium-dependent mechanisms. Therefore,an association between micromolar alterations in ionic calcium levels and the level of vascular tension has a definite theoretical basis.

In principle, an increase in the intracellular calcium ion concentration could result from an increased release from internal stores, an increased permeability of the plasma membrane, an inhibition of the mechanisms responsible for calcium efflux, or a decrease in the ability of subcellular

components to bind or sequester calcium. A small increase in plasma membrane permeability or a small fractional release of calcium ions from a subcellular component will lead to a large fractional rise in cytosolic ionized calcium levels to a level several fold greater than that of the cell in its non-activated state. In studies on smooth muscle, nerve terminals, endocrine cells, and platelets, where external ionized calcium has been removed, intracellular stores have been depleted or inhibited from releasing calcium, and it has been demonstrated that the bulk of the increase in intracellular ionized calcium necessary for cell activation comes from an internal store, probably a portion of the endoplasmic reticulum (Campbell, 1988).

Mechanisms of Intracellular Calcium Activation.

The association of a calcium-mobilizing agonist with its receptor or depolarization of the plasma membrane, results in an influx of calcium ions from the extracellular medium into the plasma membrane. As calcium ions enter the membrane's lipid bilayer through their respective channels, activation of the phosphodiesterase, phospholipase C, occurs. Phospholipase C preferentially hydrolyses the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2), into 1,2-diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3) (Khalil, et al., 1990). Diacylglycerol is a lipid-soluble compound which acts with phosphatidylserine and the calcium ions entering the membrane to change the conformation of protein kinase C (PKC), exposing its active catalytic site and thus increasing its binding affinity for calcium and its ability to activate other biological systems (Campbell, 1988). The cellular responses elicited by PKC

activation are separate from and synergistic to those activated via an increase in intracellular ionized calcium and include increased catecholamine release from the adrenal medulla and neuronal synapses, increased muscular contraction, and increased parathyroid hormone release (Nishizuka, 1989), all of which can alter vascular tension. Additionally, PKC appears to be involved in the phosphorylation of key actin-binding proteins which may play a role in the regulation of vascular contractile mechanisms (Young et al., 1990).

Inositol trisphosphate is a water-soluble compound which moves from the membrane into the cytosol where it has been shown to be responsible for the release of ionized calcium from internal stores, most likely from a compartment of the endoplasmic reticulum (Streb, Irvine, Berridge, & Schulz, 1983; Suematsu, Hirata, Hashimoto, & Kuriyama, 1984). The mechanism by which IP_3 promotes the release of ionized calcium from internal stores, however, is not presently known. This release of calcium by IP_3 activates the second major calcium messenger pathway, distinct from the PKC pathway activated by DG. This pathway involves the binding of the excess calcium ions to calmodulin or troponin C. The binding of calcium ions to these proteins causes a conformational change in their structure, increasing their affinity for calcium ions and allowing them to complex with other proteins or response elements and initiate various biological events such as muscle contraction and increased activity of ionized calcium efflux mechanisms. The increase in intracellular calcium ion levels acts synergistically with IP_3 increasing the rate of release from the endoplasmic reticulum (Khalil et al., 1990). This synergistic action of

increased calcium levels has been called calcium-induced calcium release and it has also been suggested to induce the release of calcium ions bound to the inner plasma membrane (Rasmussen, 1983).

The increase in intracellular ionized calcium in vascular smooth muscle from 10^{-7} moles/liter (mol/L) to 10^{-6} mol/L is the primary initiator of vascular contraction. The formation of a calcium-calmodulin-response element complex results in activation of the calcium-dependent protein kinase, myosin light chain kinase (MLCK), stimulating the phosphorylation of myosin light chain (MLC), which in turn activates the myosin head of protein filament resulting in sliding of the filaments and contraction. Research has indicated that the state of tonic contraction in vascular smooth muscle is positively correlated with the degree of phosphorylation of MLC (Rasmussen, 1983).

Calcium Metabolism and Vascular Relaxation.

While activation of alpha-1 receptors on the plasma membrane or membrane depolarization results in increases in vascular tension, catecholamine activation of beta-2 (B_2) receptors on the cell surface result in smooth muscle relaxation (Rasmussen, 1983). The activation of B_2 receptors by catecholamines leads to a rise in cyclic adenosine monophosphate (cAMP) concentration which increases protein kinase activity. This increased activity results in protein phosphorylation at three major sites: the plasma membrane, the endoplasmic reticulum, and the cytosol (Rasmussen, 1983). Protein phosphorylation at the plasma membrane results in activation of the sodium/potassium ATPase (Na^+/K^+ ATPase) pump, which upon activation,

lowers the intracellular sodium ion content by actively extruding sodium ions into the extracellular medium, against the concentration gradient. This lowering of the intracellular sodium concentration stimulates the exchange of extracellular sodium ions for intracellular calcium ions through sodium channels in the membrane (Rasmussen, 1983). Thus, intracellular sodium ion concentration returns to normal at the expense of the cytosolic calcium ion concentration. Phosphorylation at the endoplasmic reticulum membrane leads to increased activity of the calcium-ATPase (Ca^{2+}-ATPase) pump resulting in increased transport of cytosolic calcium ions into the endoplasmic reticulum. The overall effect of phosphorylation at both of these membrane sites is calcium ion efflux from the cytosol, resulting in a lowered ionized calcium level, and ultimately a decrease in vascular tension. The third site of phosphorylation is the protein kinase MLCK in the cytosol. Phosphorylation of MLCK decreases its sensitivity to activation by calcium, and since the degree of vascular tension is proportional to the degree of phosphorylation of MLC (Rasmussen, 1983), a lower concentration of phosphorylated MLC leads to a lower level of vascular tension.

Calcium Metabolism and Hypertension

The metabolic pathways by which disturbed calcium homeostasis produces a rise in arterial pressure remain conjectural. Available data suggest that factors which reduce the vascular plasma membrane's exposure to ionic calcium or impair intracellular storage or efflux of calcium ions may lead to increased vascular tension (McCarron, 1985). The presence of these alterations leads to a less stable vascular plasma membrane, increased membrane

permeability to calcium, and a greater influx than efflux of calcium ions. An alteration in the binding kinetics of extracellular bound calcium, making it less available for mobilization, is one possible mechanism whereby an alteration in calcium metabolism could lead to vascular smooth muscle dysfunction. Such an abnormality is a possible explanation for the normal serum total calcium and decreased extracellular ionized calcium levels observed in both humans and experimental animals with high blood pressure (McCarron, 1985). A defect in the transport of calcium across the plasma membrane is another possible mechanism which might alter the intracellular and extracellular calcium levels, thereby adversely affecting vascular smooth muscle function.

Since it is difficult to study human smooth muscle cell function, various models such as the erythrocyte, lymphocyte, and blood platelet have been used. Although no direct relationship between these blood cell models and smooth muscle cells, with regard to calcium metabolism, has been established, there is evidence of calcium mechanisms present in all. Platelets, like smooth muscle cells, have an alpha-2-adrenoceptor-adenylate cyclase system and a calcium-dependent contraction-coupling mechanism (Erne et al., 1984; Rassmussen, 1983). Additionally, the majority of platelet intracellular calcium is associated with cellular mitochondria, storage granules, the plasma membrane, and the dense tubular system, which in platelets is analogous to the endoplasmic reticulum of muscle (Salzman & Ware, 1988). Generation of IP_3, in a manner similar to that in the sarcolemma, acts on the dense tubular system in the platelet to raise the cytosolic calcium level. This intracellular rise in free

calcium may reach two to three micromolar, as indicated by the fluorescent dye indicators fura-2 or indo-1 (Salzman & Ware, 1988). This process in the platelet mirrors that in the vascular smooth muscle cell, resulting in phosphorylation of MLC, activation of calmodulin, and stimulation of the processes which result in increased vascular tension. Higher intracellular ionized calcium levels in platelets have been reported in patients with hypertension and also found to be directly associated with blood pressure (Erne et al., 1984). Erne et al. (1984) suggested that their results may be due to a defect in membrane permeability or to possible alterations in the calcium membrane pump mechanism.

In both human hypertension and hypertension in experimental animals, disorders in calcium metabolism have been reported in a variety of organ functions, biochemical parameters, and subcellular organelles (McCarron, 1982; McCarron, 1985). In the laboratory, vascular tissue from hypertensive animals has been reported to exhibit increased membrane permeability, altered binding kinetics on the cell membranes, and accumulation of calcium in subcellular fractions (McCarron, 1982; McCarron, 1985; Webb & Bohr, 1978). The biochemical disorders associated with calcium metabolism in human and experimental hypertension are varied. Several studies have compared serum and intracellular calcium fractions in hypertensive and normotensive subjects (Bing, Heagerty, Jackson, Thurston, & Swales, 1986; Erne et al., 1984; Folsom et al., 1986; Le Quan Sang & Devynck, 1986; McCarron, 1982). Folsom et al. (1986) found significantly lower mean levels of serum ultrafilterable calcium ($p=0.01$), a combination of the ionized and complexed calcium fractions, and

serum complexed calcium ($p=0.04$), with a trend toward lower levels of serum ionized calcium ($p=0.09$) in their comparison of 28 hypertensives to 28 normotensive controls. Significantly higher platelet ionized calcium levels have been found in hypertensives ($p\leq0.001$) versus normotensive controls (Erne et al., 1984; Le Quan Sang & Devynck, 1986), and a close positive correlation between the intracellular free calcium level and both systolic and diastolic pressures ($r=0.883$ and $r=0.931$ respectively) (Erne et al., 1984). In a study of normotensives with ($n=17$) and without ($n=22$) a family history of hypertension, and of untreated hypertensives ($n=22$), there was a significant correlation between leukocyte intracellular ionized calcium and mean supine blood pressure in normotensives with no history of hypertension ($r=0.73$, $p\leq0.01$) (Bing et al., 1986). Although serum total calcium has been reported to be normal in some studies, free or ionized calcium was decreased (McCarron, 1982; McCarron, 1985). Other alterations observed in hypertensives include elevated parathyroid hormone levels, low serum phosphorus concentrations, abnormal vitamin D precursor levels, and both increased and decreased intestinal calcium transport (McCarron, 1982; McCarron, 1985).

The most commonly studied animal model of genetic hypertension is the spontaneously hypertensive rat (SHR). Although it is not a perfect model, the alterations in calcium metabolism found in the SHR appear to closely approximate those in human hypertension (Young et al., 1990). It has generally been found that genetic models of hypertension are characterized by an increased total peripheral resistance with normal cardiac output, and increased

arterial pressor responses to the presence of contractile agents and psychological stress, when compared to normotensive counterparts (Young et al., 1990). The cellular defects in calcium metabolism observed in the smooth muscle cells of the SHR are characterized by an increased permeability of the cell membrane to calcium, an increased membrane calcium content, an increased mitochondrial calcium content with delayed mitochondrial uptake of cytosolic calcium, and altered calcium-sodium countertransport across the plasma membrane (McCarron, 1985).

Dietary Calcium and Blood Pressure

The functional importance of disordered calcium homeostasis in the pathogenesis of high blood pressure has been suggested by several epidemiological studies. In humans, decreased dietary (Kesteloot & Geboers, 1982; McCarron, Morris & Cole, 1982) and environmental exposure (Ackley, Barrett-Conner, & Suarez, 1983; Stitt, Crawford, Clayton, & Morris, 1973) to calcium has been associated with an increased risk of hypertension. The HANES I data, when controlled for age, race, sex, and weight, also indicates that calcium consumption is lower in individuals with essential hypertension (McCarron, 1985). The assumption drawn from this literature is that reduced intake of calcium from either food or water sources is associated with an increased probability of hypertension, and ultimately, cardiovascular disease.

Studies in the SHR and its genetic, normotensive control, the Wistar-Kyoto rat (WKR), have provided additional evidence that maintenance of calcium homeostasis is a factor in blood pressure control (Ayachi, 1979; McCarron,

Yung, Ugoretz, & Krutzik, 1981). Both Ayachi (1979) and McCarron et al. (1981) reported that supplementation of the SHR's diet with calcium results in a marked attenuation of hypertension ($p \leq 0.05$ and $p \leq 0.0001$, respectively) and that this effect can be observed in as short a period as two weeks. In contrast to diet supplementation, removal or reduction of dietary calcium in the SHR results in an acceleration of the animal's development of hypertension.

Observations in the WKR are consistent with those in the SHR. Blood pressure of this normotensive rat also demonstrates an inverse relationship with the calcium content of its diet. Increased calcium in the diet results in lower pressure in the adult animal, whereas calcium restriction produces borderline hypertension (McCarron, 1985). Also, as in the SHR, dietary calcium manipulation of as short a duration as two weeks will modify the adult WKR's arterial pressure (McCarron, 1985).

Supplementation studies in human subjects have yielded varied, and sometimes conflicting results with regard to blood pressure. Johnson, Smith, and Freudenheim (1985) supplemented 34 hypertensive females with one and a half grams/day of calcium (as calcium carbonate) for a period of three years. Their results indicated significant reductions in systolic blood pressure (mean reduction = 13 mm Hg). Another study of 48 hypertensive men and women (McCarron & Morris, 1985) reported significant reductions in both systolic and diastolic pressures (mean reductions = 5.6 and 2.3 mm Hg for systolic and diastolic respectively) after eight weeks of supplementation with one gm/day of calcium as the carbonate. Although calcium supplementation studies utilizing

hypertensive populations have been consistent in demonstrating reductions in blood pressure (Johnson et al., 1985; McCarron & Morris, 1985), those using normotensive groups have yielded conflicting results. In one such study of 18-35 year old women (Belizan et al., 1983) a significant decrease in diastolic blood pressure (2.85%, 4.19%, and 5.64% in the seated, lateral, and dorsal positions, respectively) was observed following nine weeks of supplementation with one gram/day of elemental calcium. Other investigators however, have failed to observe significant decreases in either systolic or diastolic pressures in normotensive women supplemented with 1.5 gram of calcium per day for a three year period (Johnson et al., 1985) or significant correlations between dietary calcium and blood pressure (Schramm, Cauley, Sandler, & Slemenda, 1986). A major difference between the study by Belizan et al. (1983) and those failing to show significant results is a difference in the mean age of the groups. Belizan et al. (1983) utilized young women age 18-35 (mean = 24.0) while both Johnson et al. (1985) and Schramm et al. (1986) used subjects who were middle aged or older (age 35-65, mean = 52.0 and age 46-66, mean = 57.0, respectively). In genetically hypertensive animals both blood pressure and calcium handling have been demonstrated to be dependent on age (Young et al., 1990). Thus, hormonal differences, alterations in intestinal calcium absorption, or altered cellular calcium handling as a result of age may possibly explain the conflicting results observed in these studies.

The precise mechanism by which dietary calcium intake exerts its antihypertensive effect is not known beyond the finding that supplemental

calcium results in increased serum ionized calcium levels and does not exacerbate calcium loss in the urine (Karanja & McCarron, 1986). It has been postulated that dietary calcium intake may lower blood pressure by acting to stabilize the vascular smooth muscle cell membrane (Karanja & McCarron, 1986; Robertson, 1988).

A major criticism of human calcium supplementation studies is that the levels of calcium used are usually in excess of what is observed in normal dietary intakes (Karanja & McCarron, 1986). Although significant evidence exists that an inverse relationship exists between calcium intake and blood pressure (Kesteloot & Geboers, 1982; McCarron, Morris, & Cole, 1982), it is currently unknown at what level of calcium intake this effect becomes significant, since the literature does not contain studies observing the effects of controlled calcium intake on blood pressure.

Calcium and Pharmacological Treatment of Hypertension

Since alteration of the free intracellular calcium concentration is the ultimate effect of most peripheral vasoconstrictor mechanisms (Kiowski et al., 1989), reductions in this concentration should result in relaxation of the vascular smooth muscle and a concomitant decrease in blood pressure. Pharmacological antihypertensive therapy comprises several classes of drugs which act directly or indirectly to modify the intracellular calcium concentration in vascular tissue. Adrenergic receptor blockers belong to a class of drugs which reduce blood pressure by competitively inhibiting the effects of agonists like catecholamines on central and peripheral adrenergic receptors. Two types of

beta-adrenergic and two types of alpha-adrenergic receptors have been identified. Stimulation of beta-1 (B_1) receptors increases heart rate and heart contractility, resulting in increased blood pressure primarily through increased cardiac output (Somani, 1982). Beta-2 (B_2) stimulation in the periphery results in relaxation of the smooth muscle vasculature and decreased peripheral resistance (Somani, 1982). Alpha-1 (A_1) receptor stimulation in smooth muscle vasculature is the opposite of B_2 stimulation, resulting in vasoconstriction with a concomitant rise in blood pressure, while central A_2 receptor stimulation inhibits presynaptic release of norepinephrine and renin release (Somani, 1982). The precise mechanism by which beta-blocking drugs exert their antihypertensive effects is presently unknown. It has been hypothesized that they regulate blood pressure by inhibiting central mechanisms responsible for regulation of circulation (Meier, Orwin, Rogg, & Brunner, 1980), or by reduction of cardiac output, lowering of plasma renin concentrations, or reduction of peripheral sympathetic activity (Meier et al., 1980; Cohn, 1982; Struthers & Dollery, 1985). Since angiotensin II and norepinephrine are potent vasoconstrictors, reductions in the concentrations of these agonists would mean that the ultimate beta-blocking effect at the cellular level is a reduction in the stimulation of receptor-operated calcium channels and a reduced influx of calcium ions.

Beta-blocking drugs and alpha-antagonists have the ability to exert their effects on both the central nervous system and the periphery (Reid, 1986; van Zwieten, 1986). Drugs affecting alpha receptors are thought to act centrally on the brain stem to reduce sympathetic outflow, reducing the secretion of

catecholamines from sympathetic nerves (Reid, 1986). Peripherally, alpha-antagonists bind the A_1 receptors on the vascular smooth muscle, reducing vasoconstriction through limited stimulation of receptor-operated channels and reducing calcium influx (Reid, 1986).

Adrenoceptor blocking drugs lower blood pressure at the vascular membrane by selectively blocking receptor-operator calcium channels. This blockade inhibits the release of calcium ions from intracellular stores, maintaining a low level of tonic contraction in vascular tissue. Like adrenoceptor blockers, calcium antagonists interfere with the influx of extracellular calcium ions across the vascular membrane. Calcium channel blockers, however, have a voltage-dependent, as opposed to a receptor-dependent, mode of action (Reuter, 1983). The potency of these drugs appears to increase as membrane depolarization increases, as well as during repetitive stimulation (Reuter, 1983). The primary effect then, is a desensitizing of the vascular membrane to depolarization, requiring a greater level of stimulation for voltage-dependent calcium channel activation. Calcium antagonists do not appear to compete with calcium for vascular binding sites but, rather bind to sites that interfere with the channel gating mechanism (van Breemen, Lukeman, & Cauvin, 1984). Although it is presently unknown whether voltage-dependent channels function differently in hypertensives, research has indicated that a reduction of calcium influx brought about by antagonist administration tends to reduce the level of smooth muscle contractility and peripheral resistance (van Breemen et al., 1984).

In addition to adrenoceptor and calcium antagonists, diuretics, vasodilators, and angiotensin converting enzyme (ACE) inhibitors have also proven effective in the treatment of hypertension. These pharmacological regimens, however, are not without their drawbacks. There is potential in these drug therapies for additive and synergistic effects between antihypertensive drugs and for interaction between these drugs and other medications and dietary components (Lees & Reid, 1990). This potential for drug interaction is considerable in middle-aged and elderly individuals where antihypertensive drugs are widely used in long-term therapy (Lees & Reid, 1990). Research has indicated that when left ventricular function is markedly impaired, short- and long-term therapy with calcium channel blockers can increase cardiovascular morbidity and mortality (Packer, 1989). Inhibition of contractility of the smooth muscle of the gut and of atrioventricular conduction in the heart has also been shown with administration of the calcium antagonist verapamil (Lees & Reid, 1990). There is also evidence that calcium antagonists can affect liver metabolism (Bauer et al., 1986).

Other antihypertensive drugs such as beta-blockers and ACE inhibitors which interfere with neurohormonal activity, can favorably modify the long-term outcome of individuals with impaired left ventricular function (Packer, 1989), but can also result in adverse effects. Side-effects of beta-blockers include postural hypotension, fatigue, bradycardia, and reflex tachycardia (Loebl & Spratto, 1983). Adverse reactions from the use of ACE inhibitors include gastric irritation, nausea, anorexia, constipation, and possible precipitous hypotension

when used in conjunction with diuretics (Loebl & Spratto, 1983).

The effectiveness of antihypertensive drug therapy has been proven in both short- and long-term treatment of high blood pressure (van Breemen et al., 1984; Lees & Reid, 1990). The financial outlay and potential for adverse side effects however, make non-drug therapies as alternative or component treatments attractive when choosing a means to regulate high blood pressure.

Exercise and Blood Pressure

Research has indicated that antihypertensive drug treatment of individuals with mild hypertension (defined as a persistent diastolic pressure between 90-105 mm Hg) results in a significantly lower mortality and fewer cardiovascular complications than in individuals who do not receive drug treatment (Kaplan, 1984). In spite of their proven effectiveness, however, the use of antihypertensive drugs in the treatment of high blood pressure has been brought into question with the increasing identification of individuals with mild hypertension in which drug-lowered pressure has not demonstrated the expected degree of protection against coronary heart disease, prompting renewed interest in alternative treatment methods (Kaplan, 1984). Reviews of antihypertensive drug therapies have indicated that they generally tend to make people feel worse, and that they can induce various biochemical aberrations (Kaplan, 1984). This has prompted the recommendation by an expert committee of the World Health Organization and International Society of Hypertension that non-drug therapies should be used for at least three months for individuals with diastolic pressures below 100 mm Hg and indefinitely for

those with diastolic pressures below 95 mm Hg (World Health Organization/International Society of Hypertension, 1983).

Weight reduction, sodium restriction, potassium or calcium supplementation, relaxation, and exercise are a few of the current non-drug therapies for hypertension. Although therapies such as weight reduction, sodium restriction, and exercise have demonstrated significant pressure lowering effects in specific clinical trials, no one non-drug therapy has conclusively proven to result in lower blood pressure in all cases (Kaplan, 1984). The role of exercise as a viable antihypertensive therapy is not as established as that of therapies such as weight reduction and sodium restriction, as reflected by the varied results reported in the current literature (Fagard et al., 1990).

In exploring the possible reasons for an effect of exercise on blood pressure, some basic factors should be reviewed. Since blood pressure is the product of cardiac output (heart rate times stroke volume) and total peripheral resistance (TPR), any intrinsic or extrinsic factors which alter heart rate, stroke volume, blood viscosity, or vascular smooth muscle tone and reactivity, will also affect blood pressure. Dynamic exercise, such as cycling and running, results in an increase in cardiac output and a decrease in TPR during exercise (Lamb, 1984). It is possible that exercise training may modify hemodynamic responses such that this decrease in TPR persists after cessation of exercise, resulting in a concomitant decrease in resting blood pressure. It is also known that in a trained individual, resting heart rate decreases as a result of exercise training and resting stroke volume increases to maintain cardiac output at rest (Lamb,

1984). It is therefore conceivable that a decrease in resting blood pressure with exercise training may be due to a decrease in cardiac output as a result of a decrease in resting heart rate below the compensatory increase in stroke volume. Exercise training may therefore reduce resting blood pressure by either a reduction in cardiac output, TPR, or both. In a comprehensive review of cross-sectional and longitudinal exercise training studies by Tipton (1984), no conclusive support for the cardiac output theory was reported. The majority of investigators, therefore, have attempted to explain the effects of exercise training on blood pressure as a function of changes in TPR (Tipton, 1984).

Significant reductions in blood pressure with exercise training have been reported in both hypertensive and normotensive subjects (Duncan et al., 1985; Kiyonaga et al., 1985; Saar, Chayoth, & Meyerstein, 1986; Seals & Hagberg, 1984). Studies observing the long term effects of endurance exercise training (defined as greater than three months) in human subjects have reported decreases in systolic blood pressure ranging from 10.3-16.0 mm Hg in hypertensives (Boyer & Kasch, 1970; Cade et al., 1984; Choquette, & Ferguson, 1973; Duncan et al., 1985; Nelson, Esler, Jennings, & Korner, 1986) and from 4.0-6.3 mm Hg in normotensives (Choquette & Ferguson, 1973; Duncan et al., 1985). Reported decreases in diastolic pressure ranged from 4.0-11.8 mm Hg in hypertensives (Boyer & Kasch, 1970; Choquette & Ferguson, 1973; Kukkonen, Rauramaa, Voutilainen, & Lansimes, 1982; Nelson et al., 1986) and from 2.0-6.0 mm Hg in normotensives (Boyer & Kasch, 1970; Choquette & Ferguson, 1973). Not all studies, however, have observed significant reductions

in blood pressure (Cleroux, Peronnet, & de Champlain, 1987; Deplaen & Detry, 1980; Johnson & Grover, 1967). It is possible, however, that the conflicting results reported in the literature may be due to small sample sizes, pre-study fitness levels of the subjects, type or duration of the exercise regimen, or dietary factors. It has been suggested that in most cases, results of exercise training studies should be viewed with caution due to problems such as small sample sizes, poor study designs, methodological problems, limited use of adolescent and young adult populations, and differences in screening criteria (Seals & Hagberg, 1984). The inconclusive nature of this area of research makes it difficult to recommend the use of exercise training as a replacement for pharmological intervention in treatment of hypertension. Literature reviews of exercise training and blood pressure have suggested however, that while exercise training cannot replace pharmacological therapy in persons with sustained moderate-to-severe hypertension, it may be a benefical component of the therapy (Fagard et al., 1990; Seals & Hagberg, 1984). Additionally, exercise training may play an even greater role in the treatment of individuals with mild hypertension (Fagard et al., 1990; Seals & Hagberg, 1984).

It has been suggested that the decreases in blood pressure observed with exercise training in certain studies are a consequence of a decrease in peripheral vascular resistance, possibly due to a decrease in sympathetic tone (Saar et al., 1986). This premise comes from research which has indicated that physical training significantly lowers the sympathetic response to dynamic exercise, and that activity and reactivity of the sympathetic system are higher in

labile hypertensive patients (Cleroux et al., 1987). Plasma catecholamine levels are generally regarded as indices of sympathetic activity. Thus, attempts to establish alterations in sympathetic activity as a possible mechanism for the reductions in blood pressure observed after exercise training have included measurement of catecholamine levels. Several studies utilizing dynamic exercise training in both normotensives and hypertensives have reported lower plasma norepinephrine levels in conjunction with lower blood pressure (Duncan et al., 1985; Jennings et al., 1986; Kiyonaga et al., 1985; Nelson et al., 1986; Urata et al., 1987). On the other hand, plasma epinephrine levels have been reported to be decreased in some studies (Hickson et al., 1979; Winder, Hagberg, Hickson, Ehsani, & McLane, 1978), and unchanged in others (Jennings et al., 1986; Nelson et al., 1986; Urata et al., 1987). Cleroux et al. (1987) observed no significant changes in either catecholamine levels or blood pressure in a group of labile hypertensives (n=12) upon completion of a 20 week aerobic training program. Although some of the reported changes in blood parameters with exercise training may be associated with alterations in sympathetic function, alterations at the cellular level with exercise training and the identification of a specific mechanism remain to be established.

Exercise, Calcium, and Blood Pressure

While some studies have correlated alterations in the levels of circulating catecholamines with decreases in blood pressure after exercise training (Duncan et al., 1985; Kiyonaga et al., 1985), few have attempted to observe possible alterations in ionic parameters, specifically calcium. Since calcium

plays a critical role in the release of catecholamines from adrenergic nerves (Rubin, 1970) and in vascular membrane stabilization (Robertson, 1988), changes in the serum or cellular ionized calcium levels affecting the release of catecholamines, or membrane responsiveness to calcium channel activators may be a possible link between the suggested sympathetic response and reductions in blood pressure after exercise training. Along with increases in blood pressure observed during exercise, catecholamine levels increase (Lamb, 1984), and this increased sympathetic activity leads to an increased influx of calcium ions into the vasculature resulting in the observed vasoconstriction. If the platelet is a good model for the vasculature, increases in intracellular calcium levels should be paralleled.

Correlations between platelet intracellular calcium and blood pressure have been documented in several studies (Erne et al., 1984; Le Quan Sang & Devynck, 1986; Ueno et al., 1988), however, these studies only compared blood parameters and made no allowances for factors such as exercise or diet. Since a positive correlation between intracellular ionized calcium concentration and blood pressure has been reported, possibly due to alterations in the cell membrane, any decreases in blood pressure observed with exercise training should be mirrored by decreases in intracellular ionized calcium levels if peripheral smypathetic activity is involved. The few studies that have measured intracellular platelet calcium concentration after exercise have reported no significant changes in intracellular levels even though there were significant elevations in blood pressure, heart rate, and serum catecholamines (Barr et al.,

1988; Haller et al., 1986). Although these studies indicate that intracellular platelet calcium concentrations do not parallel changes in vascular tension during acute bouts of exercise, there is no current research observing the long term effects of exercise training on blood pressure in conjunction with calcium parameters. Since different mechanisms are involved in acute and long term blood pressure regulation, longitudinal studies or cross-sectional studies measuring differences between trained and untrained individuals may demonstrate a correlation between intracellular platelet calcium concentrations and vascular tension not evident in studies observing relationships with acute exercise. Therefore, this study was designed to compare the differences in blood calcium parameters and blood pressure between trained and untrained normotensive, premenopausal females in order to assess possible relationships between exercise, blood calcium, and blood pressure.

CHAPTER III

METHODS

Sample Selection

Forty-one female volunteers (mean age= 26.5, range 18-40) were recruited from the University of Virginia/Charlottesville area. Subjects were screened for participation on the basis of the following criteria:

1) 18-40 years of age.
2) Nonsmoker.
3) No history of renal or hepatic disease, diabetes, or hypertension.
4) No birth control medication for at least six months prior to testing.
5) Mean blood pressure below 140/90 mm Hg.
6) Within 15% of their ideal body weight as indicated by the 1983 Metropolitian Life Height and Weight Tables.
7) Running at least 20 miles/week, or less than 10 miles/week for the past six months.

This study was approved by the Human Investigation Committee of the University of Virginia (HIC #3645). All volunteers were fully informed of the potential risks and benefits of the proposed research prior to giving their consent for participation.

Program Design

Subjects were divided into two groups based on their level of training. The trained group (n= 20, mean age= 27.5, range 18-40) consisted of women

running at least 20 miles per week consistently during the past six months or longer. The untrained group (n= 21, mean age= 25.6, range 18-40) included those women running no more than 10 miles per week or maintaining an activity level equivalent to this for the past six months. Activities of the untrained women included exercise such as one to two hours of aerobic dance class/week, one to one and a half hours of stationary cycling/week, or six to ten miles of walking or jogging/week.

During the initial screening a personal medical history and a physical examination were obtained from each subject at the University of Virginia General Clinical Research Center (GCRC) by qualified medical personnel. The medical history and physical examination were used in determining whether or not the individual fit the physical qualifications for participation and as a basis for determination of menstrual cycle status. Classification of cycle status was based on the number of menstrual cycles, reported by the subject, for the previous year. Classification was as follows:

1) Eumenorrheic - ten to twelve menses per year.

2) Oligomenorrheic - three to nine menses per year.

3) Amenorrheic - fewer than three menses per year.

Oligomenorrheic and amenorrheic women were further tested to rule out the possibility of underlying reproductive function abnormalities or metabolic disorders. This testing included assessment for hyperprolactinemia, thyroid disorders, and excess androgens. Any subjects identified as having any of the aforementioned disorders were excluded from participation. The trained group

consisted of twelve eumenorrheic and eight oligomenorrheic (combined oligo- and amenorrheic) women while all the women in the untrained group were eumenorrheic. Also, an explanation of the testing procedures and instruction on maintaining a seven-day food record, based on estimation of food consumption by household measures, were given at the initial visit.

The testing occurred during two subsequent visits, one to the GCRC for blood pressure measurement and blood sampling, and one to the University of Virginia Exercise Physiology Laboratory for body composition analysis. Dietary food records were started on the day of the visit to the GCRC for testing and continued for seven consecutive days. These records were completed by the subjects on an individual basis and then collected.

Blood Pressure Measurement and Blood Sampling

Subjects were asked to return to the GCRC for testing, after an eight-hour fast and at least a four hour abstention from caffeine, and prior to daily exercise regimens. All blood pressure measurements and blood sampling were conducted between the hours of 6:30 and 9:00 am. Upon arrival at the GCRC and in order to approximate resting conditions as closely as possible, subjects were placed in the supine position in a relaxed atmosphere for a ten minute period prior to testing. After this rest period three different blood pressure measurements were taken, with a two minute rest period between measurements. If three measurements were obtained with a difference of five mm Hg or less among them, the mean of the values was taken. If the three initial measurements yielded values with a greater than five mm Hg difference,

additional measurements were made until a mean of three values with a five mm Hg or less difference was obtained. The positioning, cuff application, time between measurements and number of measurements either met or exceeded the recommendations for blood pressure measurement by the British Hypertension Society (Petrie, O'Brien, Littler, & DeSwiet, 1986).

Blood pressure measurements were made utilizing the Dinamap vital signs monitor, model 845XT (Critikon, Inc., Tampa, FL). This instrument is an indirect, noninvasive, automatic arterial pressure measurement device which utilizes a microprocessor controlled oscillometric method of measurement (Goldthorp, Cameron, & Asbury, 1986). In addition to measuring diastolic and systolic blood pressures, the Dinamap also measures mean arterial pressure and heart rate.

Upon completion of the blood pressure measurement, a thirty milliliter (ml) venous blood sample was drawn from the anticubital vein by GCRC personnel. Twenty ml of this sample were immediately placed in a tube containing two ml of acid citrate dextrose (ACD) to prevent platelet aggregation. Approximately 15 microliters (ul) of the sample were drawn for hematocrit analysis. The 15 ul were drawn into two capillary tubes and then spun in a microcentrifuge (Damon/IEC Division, Needham Heights, Mass.) at 12,500 RPM for three minutes. The spun samples were then read using a micro-capillary reader (Damon/IEC Division, Needham Heights, Mass.) to obtain the hematocrit values. The remaining ten ml of sample were sent to the University of Virginia Medical Center's Clinical Chemistry Laboratory for analysis of the other required serum parameters.

Platelet and Serum Analysis

Human platelet-rich plasma (PRP) was isolated from the twenty ml portion of the sample by a series of three, three to five minute centrifuge spins at 1500 RPM. The platelets were then loaded with Indo-1/AM, a Ca^{2+} binding fluorescent dye (Molecular Probes, Eugene, OR) according to the procedure outlined in Appendix A. Upon completion of the loading procedure, intracellular platelet ionized calcium concentration was measured using a Perkin-Elmer MPF-44A fluorescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) at emission wavelengths of 395 and 480 nm with an excitation wavelength of 335 nm. The value yielded by a ratio of the emission amplitude at 395 nm over the emission amplitude at 480 nm, after correction for the unloaded sample blank, reflects the micromolar concentration of platelet intracellular ionized calcium.

Analyses for serum total calcium, serum sodium, serum potassium, serum albumin and serum total protein were performed utilizing a Technicon SMAC continuous analyzer according the the methods outlined in Appendices B, C, D, E, and F, respectively. Serum total calcium adjusted for serum albumin concentrations was calculated according to the formula: modified total calcium (mmol/l) = total calcium (mmol/l) - 0.019 [albumin (g/l) - 42], (where 42 g/l is the normal mean value of serum albumin) derived by Hvarfner et al. (1987). Analysis for serum ionized calcium was performed using a Nova 2 analyzer as outlined in Appendix G, and for serum magnesium using a Ektachem 700 analyzer as outlined in Appendix H.

Body Composition Analysis

Each subject's percent body fat was assessed by hydrostatic weighing at the University of Virginia Exercise Physiology Laboratory. An Accu-weigh beam scale (accurate to 0.1 kg) was first used to assess the subject's weight in air. Residual volume was measured prior to weighing underwater using the oxygen dilution technique described by Wilmore (1969). Underwater weight was measured at least five times with a Chatillion autopsy scale (accurate to 10 gm) at a water temperature of 37 degrees Celsius. An average of the last three of the five measurements was used as an estimate of the true underwater weight for the determination of body density (Katch, Michael, & Horvath, 1967). Percent body fat was then computed from the formula developed by Brozeck, Grande, Anderson, and Keys (1963).

Dietary Analysis

Subjects were instructed in the use of household measures to estimate food consumption. Seven-day food consumption records were analyzed utilizing the Nutripractor 6000 dietary analysis software package (Practorcare, Inc., CA). Values used for total kilocalories, protein, carbohydrate, fat, and calcium intake were an average of the seven-day food record analysis.

Statistical Analysis

Statistical analysis of the data was performed utilizing the CLINFO software system (BBN Software Products Corp., Cambridge, MA). Initial statistical analysis involved the use of the two-tailed Student's T-Test for unpaired observations. This analysis was performed between the trained and

untrained groups and between individuals in the trained group divided into two groups based on menstrual function (eumenorrheic, n=12 and oligomenorrheic, n=8). The Wilk-Shapiro test for normality was performed on all data sets prior to the performance of the Student's T-Test, all data tested was normally distributed. Correlations between variables were assessed with simple linear regression. Analysis of Covariance (ANCOVA), using regression models, was employed to assess the dependency of serum magnesium and of the calcium parameters on the serum albumin and total protein concentrations. ANCOVA was also used to assess the dependency of the blood pressure variables on heart rate.

Although 22 separate t-tests were performed, it was determined that there was no problem of an inflated alpha level with the performed t-test statistics due to the high degree of association between the variables tested. For this reason no multiple comparison corrections were performed on the data. The level of significance for all statistical tests was set at 0.05.

CHAPTER IV

RESULTS

The characteristics of the study population are presented in Table 1. There were no significant differences between the groups for mean age, body weight, percent body fat, or hematocrit values. Resting heart rates in the trained women were significantly lower ($p = 0.0005$) than those of the untrained women. Heart rates for the combined group ($n = 41$) were positively correlated with mean arterial pressure (Figure 1) and with diastolic blood pressure (Figure 2), but not with systolic pressure (not shown). Both mean arterial pressure and diastolic blood pressure in the trained group were significantly lower than those of the untrained group ($p = 0.023$ and $p = 0.001$, respectively). No difference in systolic blood pressure between the groups were noted. Using ANCOVA to control for the effect of heart rate revealed no significant differences in mean arterial pressure (Appendix I, Table I-1) or systolic blood pressure (Appendix I, Table I-2) between the two groups. However, diastolic blood pressure remained significantly different ($p = 0.012$) between the two groups, with the trained group exhibiting a mean diastolic blood pressure that was lower than the untrained group by a mean of six mm Hg (Appendix I, Table I-3).

The measured blood chemistry parameters for the trained and untrained subjects are listed in Table 2. The mean serum levels of potassium and sodium were similar in both groups. The serum magnesium levels of the trained group were significantly higher ($p = 0.016$) than those of the untrained group.

Table 1. Mean (± SEM) characteristics of trained and untrained women

Characteristic	Trained (n=20)	Untrained (n=21)	P-value
Mean age (yr)	27.55 ± 1.64	25.62 ± 1.53	0.39
Body weight (kg)	57.45 ± 1.07	56.64 ± 1.09	0.60
Body fat (%)	21.90 ± 0.62	23.62 ± 1.05	0.17
Hematocrit (%)	37.52 ± 0.45	37.34 ± 0.50	0.80
Heart rate (beats/min)	57.70 ± 2.30	68.90 ± 1.80	0.0005
Mean arterial pressure (mm Hg)	76.33 ± 1.29	80.53 ± 1.23	0.023
Mean diastolic pressure (mm Hg)	61.64 ± 1.80	68.79 ± 0.80	0.001
Mean systolic pressure (mm Hg)	108.05 ± 1.64	111.45 ± 1.67	0.15

Note. Mean body weight values are within 10% of normal ranges, body fat, hematocrit, heart rate, and blood pressure values are all within normal established ranges.

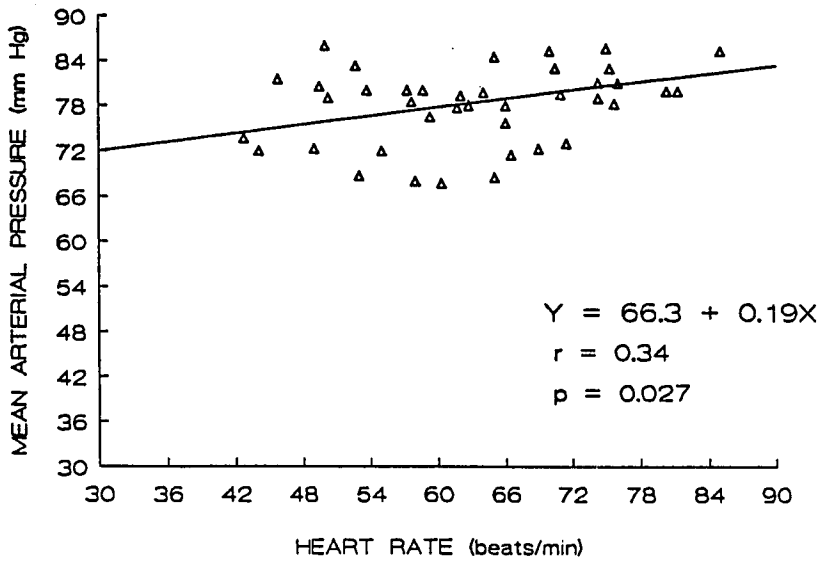

Figure 1. Relationship between heart rate and mean arterial pressure

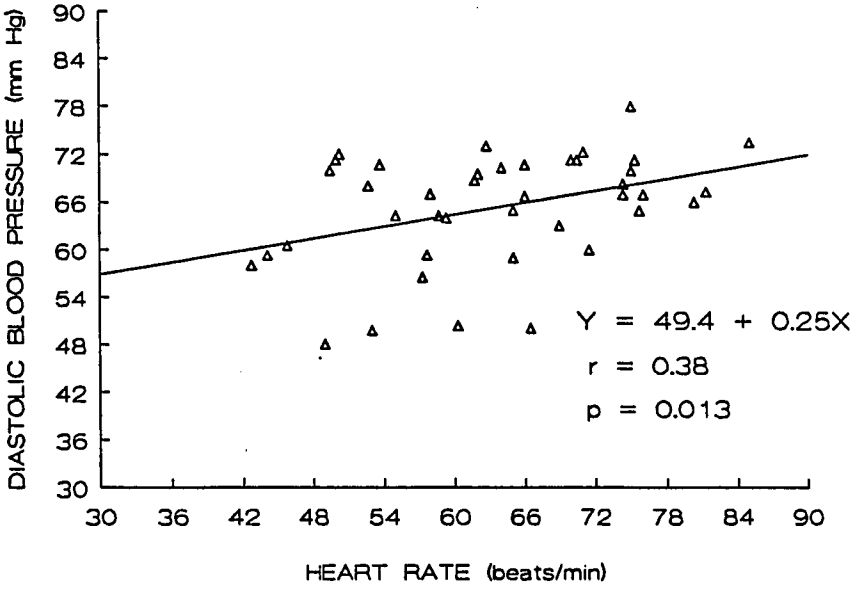

Figure 2. Relationship between heart rate and diastolic blood pressure

Table 2. Mean (± SEM) blood chemistry measurements in trained and untrained women

Parameter	Trained (n=20)	Untrained (n=21)	P-value
Serum potassium (mEq/L)	4.15 ± 0.09	4.14 ± 0.05	0.944
Serum sodium (mEq/L)	140.1 ± 0.4	140.5 ± 0.4	0.513
Serum magnesium (mg/100 ml)	1.86 ± 0.03	1.76 ± 0.03	0.016
Serum albumin (g/100 ml)	4.77 ± 0.15	4.20 ± 0.07	0.0001
Serum total protein (g/100 ml)	7.17 ± 0.62	6.61 ± 0.37	0.002
Serum total calcium (mg/100 ml)	9.68 ± 0.10	9.41 ± 0.08	0.034
Adjusted serum total calcium (mmol/L)*	0.813 ± 0.0002	0.814 ± 0.0002	0.166
Platelet ionized calcium (uM)	0.146 ± 0.009	0.122 ± 0.004	0.020
Serum ionized calcium (mg/100 ml)	4.96 ± 0.05	5.25 ± 0.03	0.0001

Note. All mean values are within normal established ranges for the given parameter.

* - Adjusted serum total calcium (mmol/L) = total calcium (mmol/L) - 0.019 [albumin (g/L) - 42] (where 42 is the normal mean value of serum albumin) (Hvarfner et al., 1987).

The trained women exhibited serum albumin and serum total protein concentrations that were significantly higher than their untrained counterparts ($p = 0.0001$ and $p = 0.002$, respectively). A significant negative relationship was observed between serum albumin concentration and diastolic blood pressure (Figure 3). Significant relationships were also present between serum albumin concentration and all of the measured blood calcium parameters (Figures 4-6). Positive relationships were observed between serum albumin and both serum total calcium and platelet ionized calcium concentrations (Figures 4 and 6, respectively). A significant negative association between serum albumin and serum ionized calcium concentration was also present (Figure 5).

All of the measured blood calcium parameters were significantly different between the two groups. Serum total calcium and platelet ionized calcium concentrations were greater in the trained group ($p = 0.034$ and $p = 0.02$, respectively), while serum ionized calcium concentration was significantly higher in the untrained group ($p = 0.0001$). No significant correlations were observed between any of the individual blood pressure variables (diastolic, systolic, or mean arterial pressure) and the levels of serum total calcium, serum ionized calcium, platelet ionized calcium, serum magnesium, or serum total protein.

Adjustment of the serum total calcium concentrations of the two groups for the effect of the serum albumin concentration removed the significant difference observed when the uncorrected total calcium concentrations were compared (Table 2). This occurred whether the serum albumin adjustment was calculated from the formula of Hvarfner et al. (1987), or computed statistically

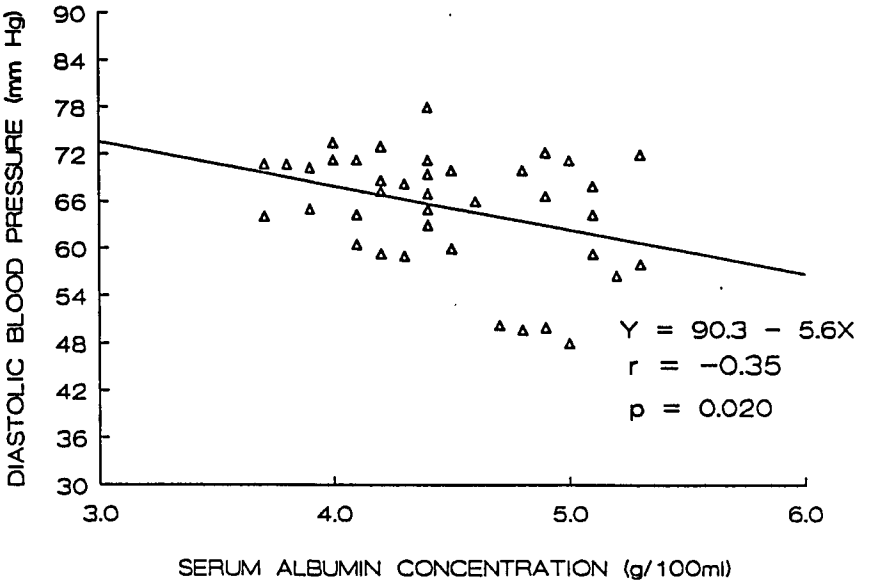

Figure 3. Relationship between serum albumin concentration and diastolic blood pressure

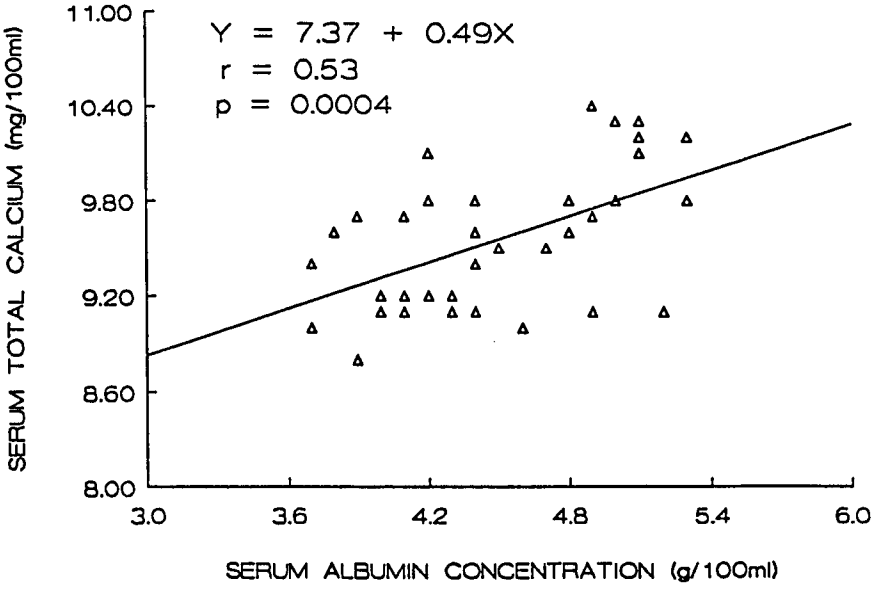

Figure 4. Relationship between serum albumin concentration and serum total calcium concentration

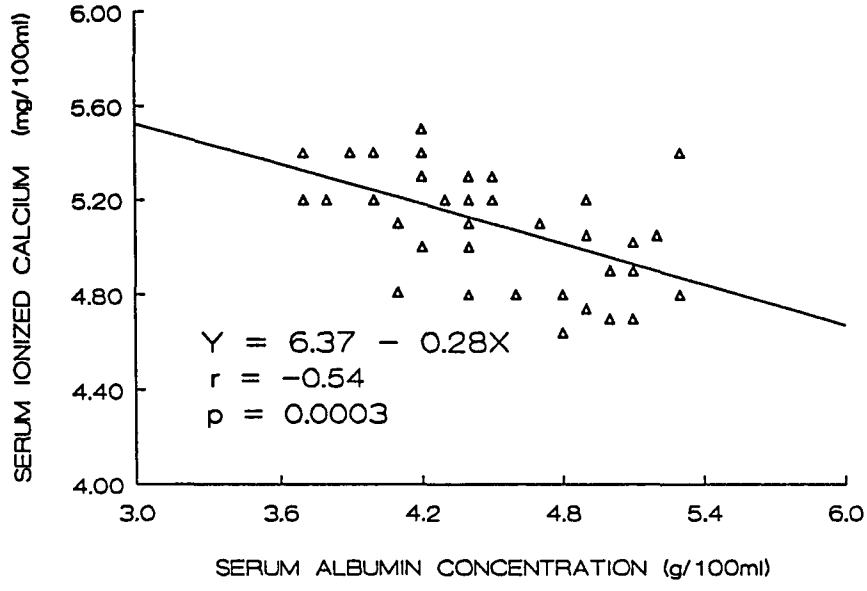

Figure 5. Relationship between serum albumin concentration and serum ionized calcium concentration

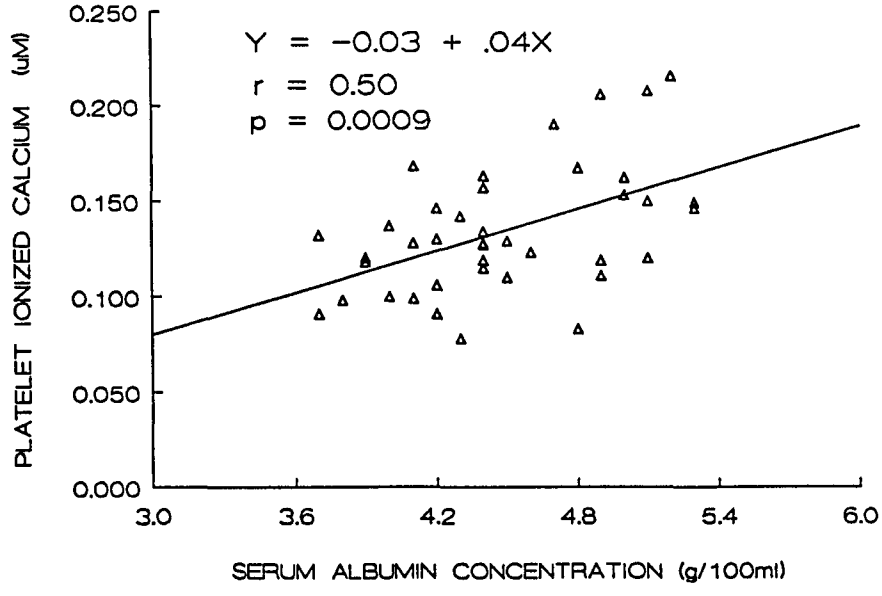

Figure 6. Relationship between serum albumin concentration and platelet ionized calcium concentration

using ANCOVA (Appendix I, Table I-4). Using ANCOVA to adjust for the serum albumin concentration also removed the difference in platelet ionized calcium observed between the groups (Appendix I, Table I-5). Serum ionized calcium concentration was significantly lower in the trained group whether unadjusted ($p = 0.0001$), adjusted for serum albumin concentration ($p = 0.005$) (Appendix I, Table I-6), or adjusted for serum albumin and total protein concentrations ($p = 0.0001$) (Appendix I, Table I-7).

The dietary intake values for calcium, total calories and the macronutrients are presented in Table 3. Mean dietary calcium intake was greater in the trained group by more than 250 mg per day ($p = 0.006$). When calcium intake was expressed as milligrams per 1000 kilocalories (kcal), intake between the groups was similar (503.4 $\pm$ 26.5 mg in the trained group vs. 457.9 $\pm$ 22.3 mg for the untrained group, $p = 0.2$). No significant correlations were observed between total dietary calcium intake and any of the blood pressure variables (diastolic, systolic, or mean arterial pressure), or between dietary calcium and platelet ionized, serum ionized, or total calcium.

Total calorie intake, protein intake, and carbohydrate intake were significantly greater in the trained women ($p = 0.008$, $p = 0.003$, $p = 0.002$, respectively). No difference in fat intake between the groups was observed. Expressing protein, fat, and carbohydrate as percentages of the total calorie intake indicated that there was no difference between trained and untrained subjects in the percentage of calories from protein (14.8% vs. 14.9%), however, the untrained women received a higher percentage of their calories from fat

Table 3. Mean (± SEM) dietary intake values for trained and untrained women

Dietary Parameter	Trained (n=20)	Untrained (n=21)	P-value
Calcium (mg)	979 ± 78	711 ± 47	0.006
Total calories (kcal)	1973 ± 130	1556 ± 68	0.008
Protein (g)	72.8 ± 3.9	57.9 ± 2.3	0.003
Fat (g)	62.3 ± 5.3	61.0 ± 3.1	0.830
Carbohydrate (g)	271.3 ± 22.8	186.0 ± 8.3	0.002

(35.3% vs. 28.4%) and a lower percentage of their calories from carbohydrate (47.8% vs. 55.0%) than their trained counterparts.

Since altered estrogen levels relating to menstrual function is reported to affect calcium absorption and excretion (Heaney, 1982), differences in menstrual function between groups might account for some of the significant results that were observed. All of the subjects in the untrained group were eumenorrheic (had normal menstrual function), however, only twelve of the trained group were eumenorrheic while the other eight had irregular function (oligomenorrheic and amenorrheic). Therefore, the trained group was split into two groups, eumenorrheic and oligomenorrheic (containing both the oligo- and amenorrheic subjects for the purpose of analysis), to determine if menstrual status could account for some of the observed differences between groups. Table 4 contains the results of the comparison of the eumenorrheic and oligomenorrheic trained women. The trained women with normal function were significantly older (31.1 vs. 22.3 yrs., $p = 0.002$), had a lower percentage of body fat (21.0% vs. 23.3%, $p = 0.04$), and a lower concentration of total calcium (9.52 vs. 9.94 mg/100 ml, $p = 0.02$) than the trained women with irregular function. When total calcium concentrations were adjusted for the serum albumin concentration, the mean concentrations were no longer significantly different. No differences between these two groups of trained women were observed in any of the other blood pressure variables, serum parameters, or dietary intake parameters.

Table 4. Mean (± SEM) characteristics, blood chemistries, and dietary intake values for trained eumenorrheic vs. oligomenorrheic women

Parameter	Eumenorrheic (n=12)	Oligomenorrheic (n=8)	P-value
Age (yr)	31.1 ± 1.9	22.3 ± 1.7	0.002
Body weight (kg)	56.3 ± 1.3	59.1 ± 1.8	0.225
Body fat (%)	21.0 ± 0.8	23.3 ± 0.7	0.043
Hematocrit (%)	38.2 ± 0.6	36.6 ± 0.6	0.070
Heart rate (beats/min)	60.4 ± 3.4	53.7 ± 2.2	0.117
Mean arterial pressure (mm Hg)	77.7 ± 1.4	74.4 ± 2.3	0.252
Diastolic blood pressure (mm Hg)	63.7 ± 2.1	58.5 ± 3.1	0.177
Systolic blood pressure (mm Hg)	109.4 ± 2.2	106.1 ± 2.4	0.319
Serum potassium (mEq/L)	4.09 ± 0.13	4.24 ± 0.12	0.412
Serum sodium (mEq/L)	139.9 ± 0.5	140.4 ± 0.8	0.633
Serum magnesium (mg/100 ml)	1.82 ± 0.03	1.93 ± 0.05	0.068
Serum albumin (g/100 ml)	4.67 ± 0.12	4.93 ± 0.10	0.120
Serum total protein (g/100 ml)	6.96 ± 0.15	7.48 ± 0.23	0.081
Serum total calcium (mg/100 ml)	9.52 ± 0.12	9.94 ± 0.11	0.018
Adjusted serum total calcium (mmol/L)*	0.813 ± 0.0004	0.813 ± 0.0002	0.177
Platelet ionized calcium (uM)	0.150 ± 0.013	0.142 ± 0.011	0.653
Serum ionized calcium (mg/100 ml)	4.98 ± 0.07	4.91 ± 0.08	0.508
Dietary calcium (mg)	968 ± 48	996 ± 188	0.891
Total calories (kcal)	1915 ± 186	2059 ± 176	0.582
Protein (g)	74.1 ± 4.5	70.8 ± 7.4	0.704
Fat (g)	61.5 ± 7.9	63.6 ± 6.5	0.841
Carbohydrate (g)	249.0 ± 22.3	304.7 ± 45.8	0.297

Note. Mean body weight values are within 10% of normal ranges, body fat, hematocrit, heart rate, blood pressure values, and blood chemistry values are within normal established ranges for the given parameter.

* - Adjusted serum total calcium (mmol/L) = total calcium (mmol/L) - 0.019 [albumin (g/L) - 42] (where 42 is the normal mean value of serum albumin) (Hvarfner et al., 1987).

CHAPTER V

DISCUSSION

The purpose of this study was to compare the blood calcium parameters and blood pressure variables of trained and untrained healthy women in order to assess possible relationships between exercise, blood calcium parameters, and blood pressure. Serum sodium, potassium, magnesium, albumin, and total protein, and dietary total calories, calcium, protein, fat, and carbohydrate, were also measured and assessed for possible differences between groups.

Resting heart rate, mean arterial pressure, and diastolic blood pressure were significantly lower in the trained women. Since the groups were similar for the characteristics of age, body weight, and percent body fat, these results suggest a depressor effect of prior training on heart rate and blood pressure. The significantly lower resting heart rates observed in the trained women are in agreement with the literature on cardiovascular adaptations to training (Astrand & Rodahl, 1977; Fagard et al., 1990; Lamb, 1984; Tipton, 1984). It has been proposed that this training-induced decrease in heart rate is probably caused by increased activity of the parasympathetic nerves projecting to the heart from the regulatory centers in the brain (Astrand & Rodahl, 1977; Lamb, 1984), or by a decrease in the sensitivity of the heart to sympathetic neurotransmitters (Lamb, 1984). Recent research suggests that training-induced decreases in heart rate may be due, in part, to both an increase in parasympathetic influence and a decrease in sympathetic influence, favoring parasympathetic dominance (Smith,

Hudson, Graitzer, & Raven, 1989). Changes in the electrical properties of the sinoatrial node causing it to slow the heart rhythm, independent of autonomic changes, have also been proposed (Lewis, Nylander, Gad, & Areskog, 1979). The proposed role of the sinoatrial node comes from the observation that a significantly lower resting heart rate is still present in trained versus untrained men when both sympathetic and parasympathetic impulses to the heart are blocked (Lewis et al., 1979). Changes in autonomic activity may be at least partly responsible for the training-induced decrease in resting heart rate, as may changes in the electrical properties of the heart; however, it has not been ascertained to what extent changes in heart rate affect blood pressure (Fagard et al., 1990).

Significant decreases in systolic (Boyer & Kasch, 1970; Choquette & Ferguson, 1973; Duncan et al., 1985) and diastolic blood pressure (Boyer & Kasch, 1970; Choquette & Ferguson, 1973) have been reported with aerobic exercise training. The majority of the training studies in the literature have utilized male rather than female populations (Fagard et al., 1990). The significantly lower diastolic and mean arterial pressures observed in the trained women in this study suggest that training, defined here as running at least 20 miles/week for at least the past six months, has a definite lowering effect on blood pressure in this population.

Significant positive correlations were observed between resting heart rate and both mean arterial pressure and diastolic blood pressure. When heart rate was controlled for statistically, the significant difference in mean arterial pressure

between the groups disappeared. This suggests that the initial significance observed in mean arterial pressure may be related to the lower resting heart rates observed in the trained women. Since heart rate is one of the determining factors in cardiac output, this might mean that the decrease observed in mean arterial pressure is partially dependent on the effect of training on cardiac output as well as on peripheral vascular resistance. This, however, remains conjecture, since cardiac output was not measured in this study. After accounting for the effect of heart rate, the trained women maintained significantly lower diastolic pressure than the untrained women, indicating that the majority of the difference in diastolic pressure observed with training is independent of the training effect on the heart. If this premise is true, then it provides support for the theory that reductions in blood pressure observed with exercise training are more likely due to greater changes in total peripheral resistance than in cardiac output (Tipton, 1984).

It has been suggested that decreases in blood pressure observed with exercise training are a result of decreased sympathetic activity (Saar et al., 1986). Using levels of circulating catecholamines as indices of sympathetic activity, several investigators have reported lower plasma norepinephrine levels in conjunction with lower blood pressure in trained subjects (Duncan et al., 1985; Jennings et al., 1986; Kiyonaga et al., 1985; Nelson et al., 1986; Urata et al., 1987). Opioid peptides, also known to have a role in pressure regulation, have also been reported to be lower in subjects after training (Lobstein & Ismail, 1989). A direct relationship between catecholamine release and plasma beta-

endorphins has been suggested since endorphin binding sites exist in the adrenal medulla (Akil et al., 1984). Measurement of plasma catecholamines and beta-endorphins, therefore, might reflect alterations in sympathetic action on centrally located regulatory centers. Future research from animal studies, providing more information on alterations in central regulation with training and the relationship between centrally and peripherally circulating catecholamines and opiates, should clarify this area of pressure regulation.

Peripherally, catecholamines alter the contractile state of the vasculature by altering the cytosolic concentration of ionized calcium in the smooth muscle cell (Rasmussen, 1983). Activation of alpha-1 receptors on the vascular cell membrane by catecholamines results in an increased influx of calcium ions into the cytosol, increasing activation of the contraction-coupling mechanism and vascular tension. Catecholamines are also known to activate beta-2 receptors on the vascular membrane resulting in a decrease in cytosolic ionized calcium concentration and vascular relaxation. However, the conditions under which sympathetic stimulation acts to increase or decrease vascular tension have not been firmly established. The one factor that has been established is that receptor activation by catecholamines, be they alpha-1 or beta-2 receptors, alters the intracellular ionized calcium concentration of vascular smooth muscle (Postnov & Orlov, 1984). Therefore, studies reporting associations between calcium indices and blood pressure suggest that these associations are evidence that some forms of hypertension are a result of dysfunctions in vascular cell calcium metabolism. Proposed membrane alterations include:

1. Dysfunction of the ionized calcium transport system in the plasma membrane of the smooth muscle cell, resulting in increased cytosolic ionized calcium concentrations and increased vascular tension (Postnov & Orlov, 1984).
2. Dysfunction of the ionized calcium transport system in the plasma membrane of nerve cells, resulting in enhanced neurotransmitter release and altered synaptic transmission of impulses (Postnov & Orlov, 1984).
3. Alterations in the vascular membrane changing both its sensitivity and reactivity to the action of catecholamines and other vasoactive substances (Postnov & Orlov, 1984).

All of the proposed alterations would lead to an increase in intracellular ionized calcium concentrations, resulting in an increase in vascular tension. Significant elevation of platelet intracellular ionized calcium levels has been reported to be positively correlated with both systolic and diastolic blood pressure in hypertensives (Erne et al., 1984; Hvarfner et al., 1988; Le Quan Sang & Devynck, 1986; Ueno et al., 1988). Significant associations between serum ionized calcium, total calcium and blood pressure have also been reported in hypertensives (Folsom et al., 1986; Strazzullo et al., 1983) and in normotensives (Hvarfner, Ljunghall, Morlin, Wide, & Bergstrom, 1986). A relationship between serum ionized and intracellular ionized calcium was proposed by McCarron (1982), who suggested that lower levels of serum ionized calcium would result in increased intracellular ionized calcium concentrations through a membrane destabilizing effect. This theory is supported by Hvarfner et al. (1988) who

reported correlations between lower levels of serum ionized calcium and higher levels of platelet ionized calcium and blood pressure in non-treated hypertensives. Based on the reported association between calcium and blood pressure in the literature, and the effect of sympathetic activity on the vasculature, it was hypothesized that significant differences in blood pressure between trained and untrained individuals would be paralleled by significant differences in blood calcium parameters. This hypothesis assumes that the effect of training is manifested in alterations of the action of catecholamines and other vasoactive substances on the peripheral vasculature. Significant differences observed between the groups for blood pressure were in the hypothesized direction, with the trained group demonstrating the lower mean arterial pressures and lower diastolic blood pressures. The significant differences observed in serum and platelet ionized calcium, however, were opposite of that expected. Based on the literature, it was expected that the group that exhibited the lower blood pressures would also have the lower intracellular and higher extracellular ionized calcium concentrations. The trained women, with the lower blood pressure variables, exhibited lower serum ionized and higher platelet ionized calcium concentrations than the untrained women, in contrast to the results reported in the literature (Bruschi et al., 1985; Erne et al., 1984). Although the results of this study support the fact that there is an association between serum and intracellular ionized calcium levels (McCarron 1982), they do not support the proposed direction of the associations between ionized calcium levels and blood pressure. Since the studies that reported this

association between ionized calcium levels and blood pressure utilized hypertensive populations (Bruschi et al., 1985; Erne et al., 1984; Hvarfner et al., 1988), one possible explanation is that this association may be a result of rather than a cause of elevated blood pressure. Another possiblilty is that training level has a pressor effect on blood pressure that is independent of peripheral alterations in the calcium-dependent, vascular contraction-coupling mechanism. This training-induced depressor effect on blood pressure may therefore be due to either central regulatory changes or alterations in the autoregulatory process which do not rely on changes in vascular cytosolic calcium concentrations to produce vasodilation.

No significant correlations were found between any of the calcium parameters and systolic, diastolic, or mean arterial pressure, which agrees with the results reported by Shore and associates (Shore, Booker, Sagnella, Markandu, & MacGregor, 1987). Several studies have reported a significant positive relationship between intracellular ionized calcium concentrations and blood pressure (Bing et al., 1986; Bruschi et al., 1985; Erne et al., 1984). According to the multivariate statistical model of Hvarfner et al. (1988), the platelet ionized calcium concentrations explained only five percent of the variability in mean arterial pressure, with serum parathyroid hormone concentration, serum ionized calcium concentration, and body mass index all influencing the relationship between platelet ionized calcium and blood pressure (Hvarfner et al., 1988). From these results the authors concluded that smooth muscle contraction may be, but is not necessarily, related to platelet ionized

calcium levels. Questions about the relationship between platelet ionized calcium concentration and blood pressure have also been raised in studies in which the effects of acute bouts of exercise on blood pressure and platelet ionized calcium levels were observed (Barr et al., 1988; Haller et al., 1986). Both of these studies reported significant increases in blood pressure, plasma lactate, and plasma catecholamines without differences in platelet ionized calcium levels from those prior to the exercise bout. Therefore, Barr et al. (1988) suggested that the rapid changes which presumably occur in ionized calcium concentrations of the smooth muscle cell and accompany physiological alterations in blood pressure are not reflected in platelet ionized calcium concentrations. Haller et al. (1986), who also measured platelet ionized calcium levels in conjunction with acute exercise, pointed out that the increase in blood pressure in their study was short-term and that their results did not account for platelet receptor binding or platelet membrane alterations. The results of these studies raise the possibility that either the platelet is not an adequate model for the smooth muscle cell or that the platelet is a sufficient model in the resting state but not during exercise. If the platelet is accepted as an adequate model, then these results suggest that the pressor effect of training on blood pressure is probably due to central or autoregulatory alterations.

Reports on a relationship between serum ionized calcium and blood pressure are varied, with some investigators reporting an inverse relationship (Hvarfner et al., 1988), and others indicating a positive relationship (Fogh-Andersen et al., 1984; Shibata et al., 1987). Hvarfner et al. (1988) suggested

that the inverse relationship they observed between serum ionized calcium and blood pressure, independent of platelet ionized calcium and serum parathyroid hormone concentrations, indicated a direct interaction between serum ionized calcium and blood pressure regulation. Robertson (1976), in a review of measurement procedures for ionized calcium, indicated that there are many factors which may alter measured calcium values. Primary among these are the effect of inactivity and alterations in the pH of the sample. Robertson (1976) states in his review that long-term inactivity, such as bedrest, produces changes in both serum total and ionized calcium concentrations, probably due to an induced imbalance between the rates of bone formation and resorption caused by the absence of weight-bearing activity. Inactivity reportedly produces an elevation in the serum ionized calcium fraction and a concomittent increase in total calcium levels (Robertson, 1976). Those studies, observing the relationship between blood pressure and blood calcium, reporting measurements of serum ionized calcium, did not account for activity level (Fogh-Andersen et al., 1984; Hvarfner et al., 1988; Shibata et al., 1987). Additionally, in studies which assessed the effect of exercise on ionized calcium, only measurements of intracellular ionized calcium were reported (Barr et al., 1988; Haller et al., 1986). Therefore, it is possible that the level of activity in the trained group in this study was sufficient to depress the concentration of serum ionized calcium if activity level indeed has a depressor effect on serum ionized calcium levels. Robertson (1976) also reported that any loss of carbon dioxide from the sample during analysis would result in a rise in the pH of the sample

and a fall in the level of serum ionized calcium. Therefore, the pH of both serum and platelet ionized calcium samples in the present study was maintained between 7.2-7.4. A failure to maintain the serum sample at a pH of between 7.2-7.4 during measurement may be a reason for the varied results in the literature.

The higher total calcium and lower serum ionized calcium concentrations in the trained group suggest that there were alterations in the levels of the protein-bound and complexed calcium fractions. No mention of these fractions was made in many of the studies previously cited (Barr et al., 1988; Erne et al., 1984; Haller et al., 1986; Hvafner et al., 1988). Correction of the serum total calcium concentration for serum albumin in the combined qroup, by the formula of Hvarfner et al. (1987) or by ANCOVA negated the significant difference initially observed between the groups. Since albumin is the principle blood protein to which calcium binds, this suggests that the protein-bound calcium fraction is higher in the trained group. Positive (Butler et al., 1984; Shibata et al., 1987) and negative (Pottgen & Davis, 1977) relationships between serum ionized calcium and serum albumin have previously been reported. A significant negative correlation between serum ionized calcium and serum albumin, and a significant positive correlation between platelet ionized calcium and serum albumin was observed in this study. These correlations suggest that as albumin concentration increases, there is an increase in the extracellular binding of calcium to albumin, lowering the concentration of the serum ionized calcium fraction, which, if McCarron's hypothesis is correct, results in a destabilization of

the vascular membrane and an increased intracellular ionized calcium concentration. Zaloga, Willey, Tomasic, and Chernow (1987), reported that as free fatty acid levels in the blood increase, serum ionized calcium levels significantly decrease. Free fatty acids are carried on the albumin molecule along with calcium. Therefore, Zaloga et al. (1987) suggested that variations in serum free fatty acid concentration may alter calcium binding, lowering serum ionized calcium concentrations by chelating calcium ions. Thus, differences in the blood concentration of free fatty acids between the groups might support the premise of increased binding of the protein-bound calcium fraction.

An interesting finding was that serum ionized calcium concentrations remained significantly lower in the trained group even after adjustment for serum albumin concentration removed the significant differences observed between groups for serum total calcium and platelet ionized calcium concentrations. This finding suggests that there are alterations in the calcium fractions of the blood that cannot be accounted for by increased binding of calcium by albumin. This might indicate an increased binding of ionized calcium by the complexed fraction, whose activity in biological mechanisms is as yet undetermined. Regardless of which serum fraction contains the calcium, the findings of this study indicate that there is an increased concentration of calcium in the blood of trained individuals, possibly due to increased mobilization from bone, increased intestinal absorption, or decreased membrane binding of the calcium ions. Haigh, Fruin, Pinn, and Lea (1988) reported that distance running affects the fatty acid composition of membrane lipids in both platelets and erythrocytes,

which could possibly affect the ability of the membrane to bind calcium. Decreased membrane binding of calcium ions and alterations in the serum calcium fractions so that membrane permeability to calcium ions is increased is a possible explanation for the increased platelet ionized calcium levels observed in the trained women in this study. Measurement of the calcium mobilizing hormones, parathyroid hormone and 1,25 dihydroxycholecalciferol, the levels of membrane bound calcium, and the concentration of calcium in each of the three serum calcium fractions will help to identify the alterations in blood calcium fractions that may be induced by training.

Heaney (1982) reported that lowered estrogen levels, such as those found in altered menstrual function, affects calcium absorption and excretion, thus differences in menstrual status might affect the concentration of the calcium fractions in the blood. Only eight of the trained women demonstrated irregular menstrual function, and when compared to those trained women with normal function, no significant differences were observed in any of the blood pressure variables, dietary variables, or blood chemistry indices, with the exception of total calcium, which, when corrected for serum albumin, lost its significance. This suggests that menstrual function does not confound the results obtained for the blood pressure and blood calcium parameters in this study. However, the sample sizes observed here may have been too small to demonstrate conclusive differences.

Endurance training reportedly causes an approximately eight percent increase in resting blood volume, due primarily to an increased retention of

plasma proteins, specifically albumin (Lamb, 1984). The increased concentration of plasma proteins is due to an increased delivery of proteins from the lymph and a decreased loss of proteins from the plasma to the tissues (Lamb, 1984). This would explain the greater serum concentrations of total protein and albumin observed in the trained women. It might also be an indication of a training-induced alteration in the autoregulatory mechanism of capillary fluid shift.

A significant inverse correlation was observed between serum albumin concentrations and diastolic blood pressure, with no relationship present between serum total protein and blood pressure. This conflicts with the results of Shore et al. (1987), who reported a positive correlation between total protein and both systolic and diastolic blood pressure, but no correlation between any of the three calcium fractions (protein bound, complexed, and ionized) or albumin with blood pressure. Increased concentrations of serum albumin and total protein are related to increases in resting blood volume observed with endurance training (Lamb, 1984). Although mean hematocrits were not significantly different between the two groups, this only accounts for the percentage of red blood cells present, and does not identify alterations in plasma volume. Measurements of plasma volume, therefore, might help to clarify the observed association between serum albumin and diastolic blood pressure.

No differences were observed in the serum sodium and serum potassium concentrations between the groups. This suggests that these ions were

probably not confounding factors in the differences observed in blood pressure and blood calcium parameters between the groups. Serum magnesium concentrations, however, were significantly higher in the trained women. Magnesium can function as a calcium-channel blocker, modulating calcium-channel activity and resulting in vasodilation (Resnick, 1990). Increased levels of serum magnesium reportedly blunt agonist-induced vascular contractions, while intracellular magnesium ions compete with calcium ions in the cytosol for calcium binding sites (Resnick, 1990). The increased levels of serum magnesium reported here may therefore have a role in the observed decrease in diastolic blood pressure through its action as a calcium channel blocker. Additionally, the increased serum levels of magnesium may reflect increased intracellular magnesium concentrations, which, through competitive blockade, may help to explain the increased platelet ionized calcium concentrations observed in the trained group.

The two groups were homogenous for age, body weight, and percent body fat. Since there were no significant differences in body weight or percent body fat between the groups, it may be assumed that the increased caloric intake of the trained group is related to their increased energy expenditure. It would also follow that there would be an increased intake of macronutrients to meet the increased caloric intake. This would explain the increased protein and carbohydrate intake of the trained women. The calcium intakes of the trained group were significantly greater than that of the untrained group. Both groups were close to the recommended daily allowance (RDA) intake of 800 mg/day,

with the mean value for the trained group being higher (979 $\pm$ 78 mg/day), and that of the untrained group lower (711 $\pm$ 47 mg/day) than the RDA. Expressing the calcium intake as milligrams per 1000 kcal instead of a daily total indicated that the groups were similar in their calcium intake on a per 1000 kcal basis. Thus, the higher calcium intakes in the trained group is due to the higher caloric intake.

No correlations were observed between dietary calcium intake and any blood pressure or blood calcium parameter, or serum albumin. Studies which have reported significant associations between blood pressure and dietary calcium have primarily been supplementation studies where one to one and a half grams of calcium were added to the subject's diet (Belizan et al., 1983; Johnson et al., 1985; McCarron & Morris; 1985). This would result in a doubling or possibly a tripling of the intake compared to the RDA. Since in calcium supplementation studies it has been reported that significant decreases in blood pressure occur in hypertensives (Johnson et al., 1985; McCarron & Morris, 1985), and in normotensives (Belizan et al., 1983), and no associations between dietary calcium and blood pressure were observed in this study, it is suggested that the level of calcium intake necessary to induce a hypotensive effect is probably at least double the RDA. Establishment of the dietary calcium level necessary for this effect is an area for future research.

The percent of calories derived from each macronutrient indicated that each group received approximately 15 percent of their calories from protein, and that the untrained group received a higher percentage of their calories from fat

and a lower percentage from carbohydrate than their trained counterparts. It has been reported that increasing the protein content of the diet increases urinary calcium excretion (Schaafsma, 1988). Since there were no differences between the groups in the percent of calories derived from protein, it is unlikely that the protein content of the diet could have significantly affected the serum calcium fractions. However, measurement of urinary calcium would be necessary before it could be concluded that dietary protein was not a confounding factor.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

In comparing the blood calcium parameters and blood pressure variables in trained and untrained normal women, it was hypothesized that the trained women would exhibit lower blood pressure values which would be associated with higher serum ionized and lower platelet ionized calcium concentrations and higher dietary calcium intakes. The results of this study suggest a definite pressor effect of training on blood pressure but failed to establish any relationship between alterations in blood calcium parameters and blood pressure, despite significant differences in the blood calcium parameters between the two groups.

The complexity of blood pressure regulation involving integration of central and peripheral control of specific brain centers, the heart, the kidneys, and the peripheral vasculature, make it hard to identify all of the pathways that training affects. From a hemodynamic standpoint, decreases were observed in resting heart rates, mean arterial pressures, and diastolic blood pressures in the trained women. Measurements of cardiac output, plasma volume, and total peripheral resistance are needed to identify their contributions to the observed decreases in blood pressure.

The significant correlations between serum albumin and the blood calcium parameters, the observed differences in blood calcium parameters between the groups, and the significantly higher serum total calcium

concentrations observed in the trained women suggest that training alters the composition of the calcium fractions in the blood. The results further suggest that measurements of all three calcium fractions (protein bound, complexed, and ionized) should be made before any associations are drawn between blood calcium indices and other parameters, such as blood pressure.

Controlled feeding studies, manipulating the levels of calcium intake, may identify a relationship between dietary calcium and blood pressure, or between dietary calcium and blood calcium parameters. The present study, with no dietary controls, failed to demonstrate any hypotensive effect of calcium intakes close to the RDA, or any relationship between calcium intake and blood calcium parameters.

This study, although failing to identify a relationship between training-induced changes in blood pressure and altered blood and cellular calcium metabolism, has identified several directions for future research in the area of training, blood pressure, and calcium metabolism. Measurement of the hemodynamic parameters of cardiac output, total peripheral resistance, and plasma volume, in conjunction with measurements of opiate and catecholamine concentrations should help to identify whether central or peripheral actions play a greater role in training-induced hypotension. Studies observing the relationship between calcium mobilizing hormones, serum proteins, and serum calcium fractions, should help identify the mechanisms involved in the effect of training on calcium metabolism. Likewise, studies measuring calcium fraction binding and cell membrane binding will help to identify how training may affect

calcium binding kinetics and may further help to establish the role of magensium, which is in competition with calcium for binding sites.

The establishment of the hemodynamic and blood chemistry alterations in response to training in individuals without defects in their blood pressure regulating systems will hopefully provide the medical profession with the knowledge necessary to utilize exercise training, either by itself or in conjunction with pharmacological therapy, as a viable treatment for hypertension.

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APPENDIX A

PLATELET WASHING AND MEASUREMENT PROCEDURE

INDO-AM LABELING:
PLATELET WASHING PROCEDURE

1. Platelet Preparation
   a. To the PRP add extra ACD, @ 0.5ml/10 ml PRP.
   b. Then add apyrase (Grade VII stock = 1 mg protein/ml saline) @ 20 ul/ml PRP.
   c. Then add PGI_2 (stock = 1 mg/ml glycine buffer) @ 0.3 ul/ml PRP.
   d. Then add indomethacin (stock = 1 mg/ml ETOH) @ 1 ul/ml PRP.
   e. Then add indo-1/AM (stock = 1 mM in dry DMSO) @ 2.5 ul/ml PRP.
   f. Mix by swirling. Gas with air/CO_2 (95% to 5% ratio) and then cap tube.

2. Incubate for 30 minutes at 37°C, in a shaker bath for the first 20 min standing for the last 10 min. Let stand at 25°C if desired.

3. Centrifuge 20 minutes @ 2000 RPM.

4. Suspend pellet in 1/2 volume warm ACD containing albumin (fatty acid-free BSA) @ 0.003 g/ml ACD and apyrase @ 20 ul/ml ACD. (Add apyrase just prior to resuspension).

5. Centrifuge 20 minutes @ 2000 RPM.

6. Make up Eagle's/HEPES medium; for 10ml final volume: Eagle's Salts = 0.096 g, $NaHCO_3$ = 0.0176 g, HEPES buffer = 0.012 g, Albumin = 0.03 g

Dissolve Eagle's salts, HEPES, $NaHCO_3$, and albumin in deionized water. Use 0.1N NaOH and 0.1N HCl to adjust final pH to 7.2 - 7.4, add deionized water to make up final volume (10 ml), gas with air/CO_2 (95% to 5% ratio) and cap.

7. Bring up pellet in warm Eagle's/HEPES medium (37° C)adding hirudin (stock = 20 units/ml saline) @ 5 ul/ml PRP.

8. Cover Platelet-rich Eagle's solution (PRE) with foil and let sit at 25°C for 1 hour.

9. Incubate PRE for 15-30 minutes at 37°C. Just prior to measurement, centrifuge a 2 ml aliquot of PRE for 5 minutes at 12,000 RPM. Use supernatant (PPE) for blank in spectrofluorimeter.

10. Immediately measure emission of PRE and PPE.
Perkin-Elmer: Excitation = 335 Emission = 395/480

Reference: Gear, A. R. L. (1982). Rapid reactions of platelets studied by a quenched-flow approach: Aggregation kinetics. Journal of Laboratory and Clinical Medicine, 100, 866-886.

APPENDIX B

MEASUREMENT OF SERUM TOTAL CALCIUM

Test: Calcium

Methodology: Calcium when mixed with the metal complexing dye cresolphthalein complex one in an alkaline medium will produce a pink colored calcium dye complex with a maximum absorbance at 570 nm. Initially a solution of HCl and 8-hydroxyquinoline is added to release protein bound calcium and bind free magnesium ions respectively. The free calcium ions are then dialyzed across a semipermeable (type H) membrane into a stream containing cresolphthalein complex and additional 8-hydroxyquinoline. Lastly, diethylamine is added as a color intensifier.

Protein bound calcium + HCl---> Free Calcium

Free Calcium + 8-hydroxyquinoline---> Free Calcium + Magnesium-8-OH complex

Free Calcium + cresolphthalein complex one + diethylamine---> calcium dye complex (abs @ 570 nm)

Sample: only serum or heparinized plasma

Sample Size: 700 ul

Reagents: 8-hydroxyquinoline, cresolphthalin complex one, diethylamine

Equipment: Technicon SMAC continuous analyzer

Measurement Type: endpoint 630 nm

Expected Values: 8.5 - 10.5 mg/dl

Critical Values: <6.0, >13.0 mg/dl

Dilutions: Any samples greater than 15.0 mg/dl should be diluted with deionized water or saline (9% NaCl) to a level less than 15.0 mg/dl and reanalyzed.

Interferences: Calcium Salts in tap water increase values. Filter paper can contain high calcium concentrations, do not filter reagents. Oxalates coprecipitates calcium with sodium, potassium, and magnesium and will decrease values.

Special Procedures and Comments:
1. Anticoagulants other than heparin are unacceptable for analysis.

2. Calcium reagents have been known to deteriorate tubing. Calcium channel tubing must be replaced periodically.

3. Calcium samples are analyzed in triplicate with the Medlab computer selecting the median value when three results are received, the mean when only two results are received, and the single result when only one result is received.

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX C

MEASUREMENT OF SERUM SODIUM

Test: Sodium

Methodology: In the SMAC II the sodium and potassium channels are combined into one cartridge utilizing a common reference electrode. The sample is mixed with sodium potassium buffer and flows past the glass membrane of the sodium ion-selective electrode. Changes in the concentration of sodium cause changes in the electrical potential that exists between the ionically variable outer surface and the ionically constant inner surface of the sodium electrode. These changes are automatically measured against the potential of the reference electrode. The resulting difference in signal are processed by the computer to determine the sodium concentration. The computer calculates the concentration using the Nernst equation, which states that the potential difference between indicator and reference electrodes is proportional to the logarithm of the activity of the measured ion.

Sample: only serum or heparinized plasma

Sample Size: 700 ul

Reagents: Sodium potassium buffer, electrode filling solution, and sodium ISE etching solution

Equipment: Technicon SMAC continuous analyzer

Measurement Type: potentiometric

Expected Values: 136 - 145 mEq/L

Critical Values: <120, >160 mEq/L

Dilutions: Any samples greater than 160 mEq/L should be analyzed on the Astra and the Astra value reported. No sodium dilutions are to be run on the SMAC II.

Interferences: None known.

Special Procedures and Comments: None.

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX D

MEASUREMENT OF SERUM POTASSIUM

Test: Potassium

Methodology: In the SMAC II the sodium and potassium channels are combined into one cartridge utilizing a common reference electrode. The potassium measurement utilizes a potassium selective valinomycin based electrode which is an excellent complexing agent for potasium. The sample is mixed with sodium potassium buffer and flows past the glass membrane of the potassium electrode. Changes in the concentration of potassium cause changes in the electrical potential that exists between the ionically variable outer surface and the ionically constant inner surface of the sodium electrode. These changes are automatically measured against the potential of the reference electrode. The resulting difference in signal are processed by the computer to determine the sodium concentration. The computer calculates the concentration using the Nernst equation, which states that the potential difference between indicator and reference electrodes is proportional to the logarithm of the activity of the measured ion.

Sample: only serum or heparinized plasma

Sample Size: 700 ul

Reagents: Sodium potassium buffer, electrode filling solution, and sodium ISE etching solution.

Equipment: Technicon SMAC continuous analyzer

Measurement Type: potentiometric

Expected Values: 3.5 - 5.0 mEq/L

Critical Values: <2.5, >6.0 mEq/L

Dilutions: Any samples greater than 10.0 mEq/L should be analyzed on the Astra and the Astra value reported. No potassium dilutions are to be run on the SMAC II.

Interferences: False elevations of potassium are caused by the ammonium ion.

Special Procedures and Comments:
1. Hemolysis will increase potassium in serum. Specimens with slight hemolysis can be analyzed but specimens with moderate or gross hemolysis should not have potassium values reported.

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX E

MEASUREMENT OF SERUM ALBUMIN

Test: Albumin

Methodology: Albumin when mixed with a buffered solution of bromcresol green (BCG) will yield an albumin-BCG complex which absorbs light at 630 nm. The reaction is monitored approximately 1 minute after sample addition for absorbance of the complex at 630 nm.

Albumin + BCG ---> Albumin-BCG complex (abs. @ 630 nm)

Sample: only serum or heparinized plasma

Sample Size: 700 ul

Reagents: BCG reagent

Equipment: Technicon SMAC continuous analyzer

Measurement Type: endpoint 630 nm

Expected Values: 3.0 - 5.2 g/dl

Critical Values: None

Dilutions: Any samples greater than 6.0 g/dl must be diluted with water or saline (.9% NaCl) to a level less than 6.0 g/dl and reanalyzed.

Interferences: Sodium flouride > 500 mg/dl; Transferrin > 500 mg/dl; Triglycerides > 300 mg/dl

Special Procedures and Comments:
1. Samples for only albumin or samples > 700 ul in volume should be analyzed on the RA-1000.

2. No body fluids other than serum or plasma should be analyzed on the SMAC II.

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX F

MEASUREMENT OF SERUM TOTAL PROTEIN

Test: Total protein

Methodology: Total protein is analyzed in the SMAC II by the Biuret reaction utilizing both a test and a blank measurement by two separate channels. In the test channel, sample is added to a stream of biuret reagent. The biuret reagent contains copper sulfate, the copper from which combines with the protein in the sample to produce a purple color. Also contained in the reagent are sodium potassium tartrate, a complexing agent, and potassium iodine to prevent auto reduction. In the blank channel, the sample is added to total protein blank solution which contains all of the constituents of biuret reagent with the exception of the copper sulfate and sodium potassium tartrate. Without these two reagents the biuret reaction does not occur and the absorbance measured is predominatly due to endogenous serum pigments. The absorbance of each channel is determined at 550 nm with the blank subtraction performed by differential colorimetry.

Sample: only serum or heparinized plasma

Sample Size: 700 ul

Reagents: Biuret reagent, Total protein blank solution

Equipment: Technicon SMAC continuous analyzer

Measurement Type: endpoint blank corrected 550 nm

Expected Values: 6.0 - 8.5 g/dl

Critical Values: None

Dilutions: Any samples greater than 10.0 g/dl must be diluted with water or saline (.9% NaCl) to a level less than 10.0 g/dl and reanalyzed.

Interferences: Dextran infusions and hemolysis - increase values; ammonium ions - decrease values.

Special Procedures and Comments: None

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX G

MEASUREMENT OF SERUM IONIZED CALCIUM

Test: Ionized Calcium

Principle: The Nova 2 Ionized Calcium Analyzer combines an ion selective electrode, microcomputer-based electronics, and a simple analytical approach to provide a practical procedure for the measurement of ionized calcium in plasma, serum, or whole blood. The calcium and reference electrodes are a straight-tube design allowing the sample to flow straight through the electrode. The calcium electrode uses an immobilized liquid membrane, while the reference electrodes provide continuous flow of both reference solution and measured solutions (standards and samples). The electrical potential from the calcium/reference measuring circuit is amplified and presented to the computer for calculation.

Sample: plasma, serum or heparinized whole blood (If samples are to be analyzed within one hour of collection, heparinized whole blood or plasma are preferred).

Sample Size: 350 ul

Reagents: None

Equipment: Nova 2 Ionized Calcium Analyzer

Special Procedures and Comments:
1. Samples for ionized calcium analyses must be collected and kept anaerobic. If the sample is exposed to air, some carbon dioxide loss will take place, elevating sample pH. Since ionized calcium varies inversely with pH, a sample with an artificially high pH will have a correspondingly low ionized calcium.

2. Since the electrodes do not respond to intracellular constituents of blood, the instrument will give results on whole blood identical to those obtained on plasma from the same sample.

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX H

MEASUREMENT OF SERUM MAGNESIUM

Test: Magnesium

Principle: The Kodak Ektachem Clinical Chemistry Slide (Mg) contains a dry, multilayered analytical element coated on a clear polyester support. The assay is based on the reaction of magnesium with an indicator dye to form a colored complex. The density of the resulting colored complex is related to the concentration of magnesium in the sample and can be measured spectrophotometrically. Calcium interference is eliminated by using a scavenger layer containing a calcium chelator. Predilution of serum samples is not necessary when the analyte concentration is within the dynamic range of the analyzer.

Procedure: A 10 ul drop of serum sample is deposited on the slide and evenly distributed by the spreading layer into the underlying layers. Solute molecules penetrate the scavenger layer where calcium is chelated from the sample. Magnesium (both free and protein-bound) from the sample then reacts with the formazan dye derivative in the reagent layer; the high magnesium affinity of the dye dissociates magnesium from binding proteins. The resulting magnesium-dye complex causes a shift in the dye absorption maximum from 540 nm to 630 nm. The amount of dye complex formed is proportional to the magnesium concentration and is measured by reflection density at 630 nm.

$$Mg^{+2} + Ca^{+2} \xrightarrow[\text{chelator}]{\text{----------}} Mg^{+2} + \text{Ca-Chelator complex}$$

$$Mg^{+2} + \text{Formazan dye derivative} \xrightarrow[\text{pH 9.75}]{\text{----------}} \text{Mg-Dye complex (abs. @ 630 nm)}$$

Sample: serum

Sample Size: 10 ul

Equipment: Ektachem 700 analyzer

Measurement Type: endpoint 630 nm

Special Procedures and Comments: None

Reference: Kaplan, A., Szabo, L. L., & Opheim, K. E. (Eds.). (1988). Clinical chemistry: interpretation and techniques, 145-350. Philadelphia: Lea & Febiger.

APPENDIX I

ANCOVA TABLES

Table I-1

The effect of training on mean arterial pressure after controlling for the effect of heart rate.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	233.1	116.5	3.64	0.036
Error	38	1217.8	32.0		
Total	40	1450.9			

Parameter	Estimate	SE	t	p
Intercept	72.02	6.76	10.65	0.0001
Group*	-2.82	2.07	-1.36	0.182
Heart rate	0.12	0.10	1.28	0.208

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-2

The effect of training on systolic blood pressure after controlling for the effect of heart rate.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	141.6	70.8	1.24	0.301
Error	38	2172.1	57.2		
Total	40	2313.7			

Parameter	Estimate	SE	t	p
Intercept	105.82	9.03	11.71	0.0001
Group*	-2.49	2.77	-0.90	0.374
Heart rate	0.08	0.13	0.63	0.530

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-3

The effect of training on diastolic blood pressure after controlling for the effect of heart rate.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	562.6	281.3	7.34	0.002
Error	38	1455.6	38.3		
Total	40	2018.3			

Parameter	Estimate	SE	t	p
Intercept	61.47	7.40	8.31	0.0001
Group*	-5.96	2.27	-2.63	0.012
Heart rate	0.11	0.11	1.01	0.320

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-4

The effect of training on serum total calcium concentration after controlling for the effect of serum albumin concentration.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	1.90	0.95	7.41	0.002
Error	38	4.87	0.13		
Total	40	6.76			

Parameter	Estimate	SE	t	p
Intercept	7.34	0.70	10.53	0.0001
Group*	-0.01	0.15	-0.07	0.488
Serum Albumin	0.49	0.17	2.99	0.005

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-5

The effect of training on platelet ionized calcium concentration after controlling for the effect of serum albumin concentration.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	0.011	0.005	6.43	0.004
Error	38	0.032	0.001		
Total	40	0.043			

Parameter	Estimate	SE	t	p
Intercept	-0.013	0.057	-0.236	0.814
Group*	0.006	0.012	0.481	0.633
Serum Albumin	0.032	0.013	2.411	0.021

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-6

The effect of training on serum ionized calcium concentration after controlling for the effect of serum albumin concentration.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	2	0.94	0.47	14.19	0.00003
Error	38	1.26	0.33		
Total	40	2.21			

Parameter	Estimate	SE	t	p
Intercept	5.76	0.36	16.20	0.0001
Group*	-0.22	0.07	-3.00	0.005
Serum Albumin	-0.12	0.08	-1.44	0.158

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).

Table I-7

The effect of training on serum ionized calcium concentration after controlling for the effect of serum albumin and total protein concentrations.

ANCOVA Summary Table

Source of Variation	df	SS	MS	F	p
Model	3	0.95	0.32	9.35	0.0001
Error	37	1.25	0.03		
Total	40	2.21			

Parameter	Estimate	SE	t	p
Intercept	5.61	0.47	11.94	0.0001
Group*	-0.23	0.08	-3.00	0.005
Serum Albumin	-0.13	0.09	-1.51	0.141
Serum Total Protein	0.03	0.06	0.50	0.622

* - Members of the untrained group were given a value of zero (0) and members of the trained group were given a value of one (1).