Supplemental Dietary Calcium Attenuates the Development of Ventricular Hypertrophy in Borderline Hypertensive Rats

by

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ABSTRACT

Hypertension affects 1 in 4 American adults (A.H.A, 1993). Hypertension is one risk factor of heart disease, along with heredity, gender and age. Researchers have developed the Spontaneously Hypertensive Rat (SHR) genetically hypertensive model, but investigative quandaries are limited due to secondary pressure-related changes. The Borderline Hypertensive Rat (BHR) succeeded the SHR in 1980 (F_1 generation of SHR x WKY) and is an early predictor of future established hypertension. Arterial hypertension is studied at its inception in the BHR along with associated myocardial alterations.

Hypertensive SHR experience left ventricular hypertrophy as an adaptive reaction to increased pressure overload/afterload. Studies have linked restricted dietary calcium intake to hypertension and hypertrophy in subpopulations with defective calcium metabolisms (low extracellular calcium) such as the SHR. The condition is reversed by high calcium, ameliorating hypertension and hypertrophy in certain human and animal subpopulations. This study tested the hypothesis that increased dietary calcium suppresses or reduces the development of hypertension and hypertrophy in the BHR. Tail-cuff plethysmography and scanning electron microscopy were used to evaluate systolic blood pressure (SBP) and ultrastructural myocardial morphology respectively.

Results of this study demonstrated that dietary calcium may have influenced SBP in the BHR. Cardiac morphology suggested that calcium also influenced the BHR heart architecture. Heart weight/body weight ratio and hypertrophy index in BHR and BHR calcium hearts were greater than WKY, indicating hypertrophy. Left ventricular wall and interventricular wall thickness were greater in BHR than BHR $CaCl_2$ or WKY. This data suggests that while dietary calcium did not consistently influence SBP, it did modulate the development of hypertrophy in the BHR.

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CHAPTER I

INTRODUCTION

BHR (Borderline Hypertensive Rat) Etiology.

J.E. Lawler and his corroborators developed the Borderline Hypertensive Rat (BHR) in1980 as a genetic model for environmentally induced hypertension paradigms. The BHR is the F_1 generation offspring of female salt sensitive Spontaneously Hypertensive Rat (SHR-S) Okamoto-Aoki strain and male normotensive Wistar Kyoto (WKY) rat (Lawler, Barker, Hubbard, and Schaub, 1980).

i. *SHR-S Substrain*:

The SHR-S strain is the preferred model employed in paradigms involving the study of human essential hypertension. Okamoto and Aoki, two Japanese collaborators, developed the SHR-S substrain in 1962 and 1963. The SHR model's systolic blood pressure (SBP) has been consistently recorded above 150 mmHg by 20 weeks of age. Conversely the normotensive WKY rat's SBP climaxed at 135 mmHg (Okamoto, 1969). In contrast to the SHR-S (Taconic River salt sensitive) substrain, a SHR-R (Charles River salt resistant) substrain was originated genetically through backbreeding of the S and R substrains until a model was produced with an attenuated genetic NaCl sensitivity; salt sensitivity is exhibited chiefly by pressor effects (Oparil et al., 1988).

High salt intake in the SHR-S substrain exacerbates hypertension via various neurally-mediated inputs. Sodium chloride supplementation in the SHR-S model induces elevated peripheral sympathetic nervous system activity and neurogenic peripheral vascular tone and perturbations in intracellular calcium levels with changes in storage, retention, and reuptake of catecholamines (Oparil et al., 1988). The SHR-S strain's

sympathetic neural activity is augmented during the developing phase of hypertension (3 months) and is further exacerbated in the presence of NaCl loading. Therefore the BHR offspring inherit a genetic predisposition to both hypertension and, alternatively, developing left ventricular hypertrophy (LVH) as conveyed by the maternal SHR-S parent (Vulpis et al., 1994).

Lawler and his cohorts generated the BHR model in 1980 for preliminary research in determining triggers responsible for environmentally induced hypertension. Moreover Lawler localized his study on two categories of environmental factors: psychological stressors and physiological stressors/dietary intake. Lawler proposed such investigations would allude to facets of the etiology and control of human essential hypertension and hypertrophy (Lawler et al., 1984, 1987, 1989; Sanders et al., 1980).

ii. *Borderline Hypertension Background*:

Borderline hypertension (BHT) is an early predictor of future established hypertension and its sequelae. BHT is a condition of elevated blood pressure with measured readings not significant enough to warrant immediate treatment. Circulation abnormalities in the borderline hypertensive attribute to the BHT's augmented blood pressure. In borderline HT peripheral resistance and vasomotor tone is increased and inappropriately adjusted to compensate for increased "hyperkinetic" cardiac output, blood flow, heart rate, stroke volume, excessive α-adrenergic stimulation, and decreased baroreceptor sensitivity. Other noted conditions in the BHT include decreased plasma volume, enhanced pressor responsiveness, and exacerbated plasma renin activity. Borderline hypertension is speculated to be of neurogenic origin, however abnormal

autonomic control of circulation is not discounted (Julius and Esler, 1975; Lawler et al., 1988).

The BHR prototype displays a sensitive hypertensive cardiovascular response, especially to environmental stressors, due to the genetic contribution of the hypertensive maternal parent. The SHR-S parent acquires a systolic blood pressure above 150 mmHg by the twentieth week of life in contrast to the paternal WKY normotensive parent that sustains a SBP of 135 mmHg during the same time period (Okamoto, 1969).

Although Lawler did not disclose why females genetically represented the SHR strain, Woodworth et al. performed extensive research on this subject in 1990. Woodworth designed two BHR variant generations, (SHR/F_1) with a SHR mother and (WKY/F_1) with a WKY mother. Both variants were genetically equivalent with the same genetic contribution from each parent. However the (SHR/F_1) variant was more reactive to milder stress and the (WKY/F_1) variant maintained a higher threshold to the same stress paradigm. Woodworth and his colleagues concluded that the (SHR/F_1) variant generation have a slightly higher predisposition to developing hypertension than its counterpart (Woodworth et al., 1990). The BHR, (SHR/F_1) variant, offspring's mixed genetic history of hypertension elicits spontaneous borderline hypertension. The BHR's resting/non-stressed tail-cuff SBP usually is within a range of 140 to 160 mmHg at 13 to 14 weeks (4 months) of age. The BHR's normal mean blood pressure measurement is 161±2 mmHg (systolic)/105±2 mmHg (diastolic) (Sanders and Lawler, 1992; Lawler et al., 1988).

Whether hypertension is manifested in the animal is dependent upon two variables: the strength of familial aggregation/genetic history and the influence of

environmental factors. The genetic history of the BHR promotes a spontaneous BHT; therefore the onset of hypertension is dependent upon environmental variables in the BHR (Lawler et al., 1988). Lawler and his fellow researchers demonstrated in several studies in the 1980's that environmental conflict precipitates BHR systolic blood pressures to reach the hypertensive range. Several conflict moieties were tested in these studies: shock-conflict schedule, high sodium diet, stress cages, and crowding paradigms. All of the studies educed hypertensive BHR animals with SBPs comparable in measurement to its maternal SBP values. The conflict schedules engaged in these studies eventually succumbed the BHR animal to permanent hypertension. In Lawler's original study, the stressed BHR animal's systolic blood pressure peaked in a 10 week period to a hypertensive level of 186 mmHg (Sanders and Lawler, 1992; Lawler, Barker, Hubbard, and Schaub, 1980). Several cardiac pathological alterations were inflicted in the BHR due to its hypertensive state. Elevated heart weight. body weight and ventricular weight: body weight ratios, myofibrillar degeneration, fibroblast infiltration, fibrosis, and an accumulation of inflammatory cells in the myocardium were all compensatory outcomes found in two parallel conflict studies (Lawler et al.,1981; Lawler, Barker, Hubbard, and Schaub, 1980; Lawler and Cox, 1985).

Overall the BHR animal has provided a viable model in hypertension research in which genetics and environmental vehicles serve as a means in determining factors in stress induced hypertension, which has become the focal point of essential hypertension. The BHR strain provides a direct link into investigating human essential hypertension, which constitutes 90-95% of all forms of hypertension in man (Okamoto, 1969).

The Relationship between Blood Pressure and Calcium (Chloride).

i. *Calcium and its Functions*:

Calcium is a signaling element essential to cellular function. Muscle serves as one of calcium's primary targets. Calcium is essential in myofibril contraction in the skeletal and heart muscles as well as smooth muscle contraction; activates and modulates myosin light chain kinase with calcium-binding protein. In addition to muscle and nonmuscle motility, calcium is involved in several other cellular processes: regulating metabolic pathways, synthesis and release of hormones, and multiple membrane-linked processes. Complexation of calcium with specific proteins dictates a structural mediatory role within cells and permits cytoplasmic solubility and is intrinsic to membranes. The sarcoplasmic reticulum and plasma membrane have calcium pumps or ATPases incorporated into their structure which enables the movement of calcium across the membrane barrier; the sarcoendoplasmic reticulum is located in muscle myofibrils and the plasma membrane encompasses cells and maintains a calcium gradient between the cell and extracellular space (Carafoli, 1988).

An inverse relationship exists between supplemental dietary calcium and (systolic and diastolic) blood pressure in human and animal models of essential hypertension. The inverse correlation between calcium intake and blood pressure has been established in numerous genetic and experimental models of hypertension, namely the salt sensitive Spontaneously Hypertensive Rat (SHR-S). Human paradigms have established that two-thirds of calcium supplementation studies induced a reduction in the subject's blood pressure (Harlan and Harlan, 1995). Normotensive models, the Wistar Kyoto (WKY), are the least responsive to dietary supplementation due to their genetic variability.

Ayachi was the first investigator to research the effects of calcium supplementation in the SHR and WKY models in a 1979 metabolic study (Ayachi, 1979). Since this landmark study, a plethora of dietary calcium corollary studies in the SHR and WKY models have succeeded (Hatton and McCarron, 1994; Huie et al., 1987; Wyss et al., 1989).

ii. *Abnormal Calcium Metabolism*:

Restricted dietary calcium intake increases the risk of elevated blood pressure in a subset of the essential hypertensive population and is mirrored in experimental SHR models due to an impaired calcium metabolism. This defective metabolism is manifested as reduced serum ionized calcium, hypercalciuria, elevated levels of parathyroid hormone, and cellular defects in calcium handling (Huie et al., 1987).

Complications on the systemic level allude to augmented circulating parathyroid hormone (PTH) levels. PTH has vasodilating or vasodepressive properties, which directly contributes to blood pressure control and cardiovascular homeostasis. Other systemic alterations include reduced serum phosphorus, decreased intestinal calcium absorption, an increase in urinary calcium excretion (hypercalciuria), and decreased basal and stimulated 1, 25-dihydroxyvitamin D_3 (1, 25 $(OH)_2$ vitamin D_3) production (Hatton and McCarron, 1994; Hatton et al., 1987, 1994; McCarron et al., 1981).

On the cellular level there are multiple modifications in the membrane's mono- and divalent ion handling. These adaptations serve as markers accounting for the global disturbance of the cell membrane's lipid composition, thus altering the physiochemical structure of the cell membrane. Abnormal calcium handling in human and animal hypertension is attributed to this modified cell membrane. Cellular abnormalities of calcium binding and transport result in a reduction in calcium binding and the number of

binding sites on the inner erythrocyte membrane surface, attenuated calcium-dependent ATPase activity in erythrocytes, and decreased calmodulin-stimulated calcium transport (Bing et al., 1988).

Another cellular biochemical defect present in hypertensive subpopulations is low serum ionized calcium concentrations induced by abnormal extracellular (EC) calcium binding at the plasma membrane, especially of vascular smooth muscle cells. Although the EC calcium and serum ionized calcium concentration values are abridged, the total serum calcium concentration remains within normal range and equivalent to the normotensive counterpart (McCarron et al., 1981; Stern et al., 1984). Intuitively the intracellular free calcium levels of the hypertensive subjects are abnormally elevated with increased calcium uptake and decreased intracellular calcium removal to compensate for depressed extracellular levels. Hence with the calcium concentrated intracellularly, the cell lacks the "membrane-stabilizing effect on vascular smooth muscle tissue by increased extracellular calcium" which alleviates the vascular smooth muscle tone (McCarron et al., 1981). Therefore there is a positive correlation between intracellular calcium and blood pressure (Hatton and McCarron, 1994; Hatton, Young, Bukoski and McCarron, 1995; Resnick, 1995).

Kestleloot and Geboers (1982) established specific correlations between serum and urinary calcium and systolic and diastolic blood pressure in their 1982 study that further validates the positive correlation between calcium and systolic blood pressure. Conversely the urinary calcium concentration significantly correlates with the diastolic blood pressure (Kesteloot and Geboers, 1982).

iii. *Inverse Relationship of Calcium & Blood Pressure & Genetic Variability*:

Genetic variability exists in the blood pressure response to alterations in dietary calcium in both human and rodent populations. Both human and rodent paradigms reveal differential blood pressure responses from the normotensive and hypertensive subjects, theoretically due to a shared common genetic characteristic and an impaired calcium metabolism. Various studies have discussed that dietary calcium or dairy products are closely associated with blood pressure and have a protective effect on the blood pressure of adult Americans, lowering the blood pressure or risk of hypertension (McCarron and Morris, 1985). Analogous studies confirm this inverse, antihypertensive effect or relation between high calcium supplementation and reduced blood pressure in the SHR-S animal model (Huie et al., 1987; Morris and McCarron, 1987).

Dietary calcium has a physiological role in blood pressure management which influences the baseline blood pressure and blood pressure reactivity primarily in the SHR model as well as in other hybrids of genetically mediated and environmentally induced hypertension (Wyss et al., 1989; Hatton et al., 1987). The efficacy of dietary calcium's hypotensive effects is dependent upon two factors: the age of the animal and composition of the diet. The earlier supplemental calcium is introduced to the SHR, the more immediate the attenuation of hypertension (Muntzel et al., 1989). Several SHR-S blood pressure studies by Hatton et al. (1989) and Wyss et al. (1989) demonstrated that SHR-S on a normal sodium chloride and high calcium diet were more responsive at a younger age. McCarron originated the "Natriuresis hypothesis" based upon these findings in which enhanced sodium excretion invokes a reduction in arterial pressure with

high calcium intake; hence dietary sodium amplifies supplemental calcium's hypotensive effects (Hatton et al., 1995; Stern et al., 1987).

Calcium diets are often manipulated via $CaCO_3$ (calcium carbonate) in dry food chow or $CaCl_2$ (calcium chloride) incorporated in the drinking water. The latter method was chosen in this study's protocol. The normal accepted dietary calcium level is within the range of 0.5% to 1%; 1% is the concentration in most rat chow brands. A high supplemental calcium diet is 2% $CaCl_2$, as established in Hatton et al.'s 1989 study. Hatton and his corroborators initiated many supplemental dietary calcium studies on the SHR and WKY animal models in the late 1980's and mid 1990's. Three levels of dietary calcium were consistently investigated: low (0.1% $CaCl_2$), intermediate or normal (1.0% $CaCl_2$), and high (2.0% $CaCl_2$). High calcium loading attenuated the development of hypertension in the SHR. The normotensive subjects experienced a negligible, small reduction in blood pressure (Morris and McCarron, 1987). There is a significant "diet effect" only in the SHR-S strain where restricted dietary calcium elevates the blood pressure and supplemental calcium (primarily 2%) ameliorates hypertension (Hatton et al., 1989; Stern et al., 1984).

iv. *Mechanisms of Antihypertensive Actions of Calcium*:

The cellular membrane is stabilized due to elevated extracellular calcium levels, increased calcium binding to the plasma membrane, and a reduction in membrane permeability to mono- and divalent cations resulting in diminished vascular contractility and membrane depolarization. A reciprocal function exists between intracellular calcium and Na, K-ATPase activity. A decline in intracellular calcium and increase in Na, K-ATPase activity is attributed to this inverse relationship. An abnormal external calcium

balance/metabolism decreases calcium-adenosine triphosphatase activity. A normal external calcium concentration, induced by dietary calcium, lowers intracellular free calcium levels and raises Ca^{2+}-ATPase activity. Calmodulin has a pivotal role in intracellular calcium regulation and may be implicated in secondary diet-induced Ca^{2+}-ATPase variations. Calmodulin levels are upregulated by dietary calcium alleviating SHR's deficit in calmodulin. Supplemental calcium ameliorates the calcium imbalance by lowering intracellular calcium and heightening extracellular and serum ionized calcium concentrations cellularly, reversing the abnormal calcium levels (Hatton and McCarron, 1994; Hatton et al., 1995; Wyss et al., 1989; Bukoski and McCarron, 1986; Sallinen et al., 1996).

Calcium is integrated in the control and maintenance of vascular smooth muscle tone. Bukoski et al. reported that the endothelium modulates vascular smooth muscle calcium metabolism as a function of extracellular calcium concentration. Supplemental calcium blunts the pressor response, reduces vascular resistance, and lessens vascular reactivity through cell membrane normalization. Overall the vascular smooth muscle experiences subsided contractility and increased relaxation or vasodilation (Kageyama et al., 1986; Bukoski and McCarron, 1986; Ayachi, 1979).

Calcium has a pivotal role in the upregulation and suppression of calcium-regulating hormones. Parathyroid hormone (PTH) suppression is provoked by calcium loading which reduces the blood pressure through a vague vasodilatory role. Lewanczuk et al. (1990) have purported PTH to be mediated by a circulating putative parathyroid hypertensive factor, calcium-sensitive hormone, which is inversely related to calcium (Lewanczuk et al., 1990; Hatton et al., 1989). PTH stimulates the synthesis and release

of 1, 25 $(OH)_2$ vitamin D_3, calcitriol. When PTH is inhibited, calcitriol is also repressed; calcitriol is inversely related to calcium (Hatton et al., 1995).

Calcitonin is a thyroid hormone that influences blood pressure through the central nervous system. Calcitonin possesses vasoactive properties and has been found to lower blood pressure; calcitonin concentrations are directly proportional to calcium (Hatton and McCarron, 1994).

Calcium-sensitive hormones are influenced directly by calcium levels. Post-supplemental calcium induces elevated circulating atrial natriuretic peptide, ANP, which promotes natriuresis and causes peripheral vasodilation and attenuates blood pressure. Diminished renin-angiotensin system activity and suppressed plasma renin activity may facilitate a reduction in blood pressure. Blood pressure alterations inflicted by calcium manipulation alters calcitonin gene-related peptide (CGRP) levels in a dependent manner. CGRP is vasoactive and a potent vasodilator, when under the influence of supplemental calcium it reduces the total peripheral resistance resulting in hypotension (Hatton and McCarron, 1994; Hatton et al., 1995).

Central outflow of sympathetic nervous system activity is reduced by calcium causing a diminution in blood pressure. Circulating catecholamine levels of dopamine, norepinephrine, and epinephrine, all vasoconstrictors, are decreased by high calcium diets (Hatton, Young, Bukoski and McCarron, 1995). The α_1-adrenergic receptor is inversely modified by calcium intake. The α_1-adrenoceptor mediated vasoconstrictor pressor responses are dampened by calcium (Sallinen et al., 1996). Natriuresis is potentiated through calcium's effects on the sympathetic nervous system. Reduced sympathetic

nervous system outflow and α_1-adrenergic activity account for enhanced sodium excretion that reduces the blood volume and, in effect, blood pressure (Stern et al., 1987).

Natriuresis is facilitated on the renal level by improved renal hemodynamics and calcium's direct effect by increasing sodium excretion and inhibiting sodium reabsorption in the proximal tubule (Hatton et al., 1995). Prostaglandin release is augmented by calcium supplementation (Hatton and McCarron, 1994).

Several calcium-induced natriuresis mechanisms were outlined in the previous paragraphs. The following is a complete overview of all plausible natriuresis mechanisms: increased ANP, reduced sympathetic nervous system outflow, diminished α_1-adrenergic receptor activity, decreased angiotensin II receptor expression, attenuated circulating PTH, and renal hemodynamics at the proximal tubule (Hatton and McCarron, 1994).

Calcium excretion may be 8 to 18-fold higher under the influence of high calcium (Stern et al., 1987). In chronic calcium diets renal and fecal calcium excretion is significantly increased. Sodium chloride potentiates calciuresis by the kidney due to an inverse renal relationship. Serum phosphate levels are depleted by calcium, but it is unclear if phosphate has a direct role on blood pressure. Systemic magnesium levels decline in the presence of calcium due to lowered intestinal absorption and enhanced renal excretion. It is unknown if magnesium deficiencies effect blood pressure (Hatton and McCarron, 1994; Hatton et al., 1995).

Calcium has a paramount role in several nonvascular mechanisms. The HSP70 gene expression is suppressed by calcium. The abnormal gene expression linked to experimental hypertension is corrected by calcium intake (Hatton and McCarron, 1994).

Hatton et al. (1988) found blood viscosity, hematocrit, to be inversely related to calcium. The hematocrit variations were due to iron absorption differences. Calcium is thought to compete with iron for intestinal absorption, therefore high calcium levels repress iron absorption and cause iron-deficiency anemia. Iron-deficiency anemia is often evoked in high calcium diet paradigms (Hatton et al., 1988).

High calcium supplementation and its effects are paramount in overcoming the defective calcium metabolism in the SHR model. The BHR hybrid of the SHR x WKY models may inherit this defective calcium metabolism, or hypocalcemic state, from its SHR parent and the associated hormonal and systemic implications. As Julius and Esler (1975) and Lawler (1980) have alluded to, BHR's abnormalities correlate with related irregularities in the SHR originated by its abnormal calcium metabolism. Henceforth, due to the genetic relationship between the SHR and BHR, this study hypothesizes that dietary calcium supplementation will attenuate systolic blood pressure in the BHR. Calcium loading would then correct the defective calcium metabolism and the associated system sequelae that ensue from elevated intracellular calcium and its repercussions (Kesteloot and Geboers, 1982).

Hypertension and Hypertrophy.

Essential hypertension, hypertension of unknown origin, comprises 90-95% of all types of hypertension in man (Okamoto, 1969). Okamoto and Aoki developed the SHR as a working model of essential hypertension in 1962/63. Since SHR's inception, extensive hypertension studies have been performed, laying the foundation in essential hypertension studies. In 1980, Lawler and fellow colleagues originated the BHR model for preliminary psychosocial and environmental stressor studies. Research has been

limited to environmental paradigms for the BHR model. Conversely the SHR model has provided broad insights into essential hypertension and its interplay in man.

i. *Causes of Spontaneous Hereditary Hypertension*:

The etiology of essential hypertension is unknown, however several biochemical and physiological phenotypic differences exist between genetically normotensive and hypertensive rats. Genetic hypertension models, such as the SHR, characteristically have an elevated total peripheral resistance with normal cardiac output. The cause of this condition is due to structural and functional changes in the vasculature associated with an abnormal calcium metabolism (Hatton, Young, Bukoski and McCarron, 1995).

The cell membrane manifests abnormal activity and transport in genetically hypertensive subjects. Na-H exchange activity is amplified in SHR cells with a profound effect at the renal level, resulting in defective sodium excretion. The abnormal calcium transport is central to several aforementioned homeostasis complications in the SHR metabolism. Inadequate control of intracellular calcium and increased monovalent cation permeability is the cell membrane's expression of abnormal calcium transport causing higher blood pressure in SHR and essential hypertensive subjects (Coleman et al., 1980).

Renal maladaptations further exacerbate hypertension. Nephrons in the SHR kidney experience structural changes attributing to hypertension maintenance: increased preglomerular renal arterial resistance, decreased permeability of glomerular membranes (glomerular filtration rate), and a reduced renal blood flow. Human essential hypertensives manifest parallel renal adaptations such as: decreased renal blood flow, increased renal resistance, elevated renal resistance: total peripheral resistance ratio, and lowered glomerular filtration rate (Guyton and Hall, 1996; Coleman et al., 1980).

Irregular humoral factor and hormonal levels contribute to the development of spontaneous hypertension. SHR and essential hypertensive human subjects have a circulating parathyroid hypertensive factor, PTH-related protein (PTHrp) paracrine factor, present in their plasma. This factor is absent in WKY and normotensive plasma and dependent on the parathyroid gland, but a separate entity from PTH. Cardiovascular and cellular activities are altered by the circulating factor through abnormal cellular calcium handling in platelets and neutrophils, elevates calcium uptake, and augmented blood pressure (Lewanczuk et al., 1990; Pang and Lewanczuk, 1989).

Elevated aldosterone and corticosterone levels are ascribed to the SHRs adrenal hyperresponsiveness to adrenocorticotropic hormone (ACTH). Hyperfunctional adrenocorticotroph cells also elicit heightened plasma vasopressin (antidiuretic hormone) concentration in the SHR, which supports a hypertensive state. A circulating Na-K pump inhibitor plasma factor is theorized to be present in both SHR and essential hypertensives and has a role in sustaining hypertension. The plasma renin activity (PRA) levels in borderline hypertensive and BHR subjects is often elevated and is thought to be an indication of increased sympathetic activity. In contrast, PRA concentrations in SHRs and essential hypertensives are lowered which may reflect stimulated steroidogenesis (Page, 1987; Oparil, Chen, Berecek, Calhoun and Wyss, 1995; Victor and Mark, 1995).

Neural traffic and hormonal activity deviate from normotensive values and are augmented in essential hypertension. The SHR is subject to increased sympathetic nerve traffic and adrenergic drive with elevated myocardial contractibility and exaggerated vasoconstrictor response. Central catecholaminergic mechanisms are modified and contribute to hypertension development. Tyrosine-hydroxylase, dopamine-beta-

hydroxylase (DBH), and phentolamine-N-methyl-transferase (PNMT) are elevated in SHR pups while matured SHR animals experience elevated norepinephrine (NE) turnover (Victor and Mark, 1995).

SHR hypertension may also be attributed to a primary defect in the baroreceptors that is reflected in dampened baroreflex sensitivity with age affecting the baroreceptor feedback mechanism (Guyton and Hall, 1996). Young BHR models' neural mechanisms have been investigated on a limited basis but reveal a spectrum of adaptations entwined with vascular hyperresponsiveness: exacerbate sensitivity to central stimuli, increased release of peripheral catecholamines, abnormal vascular wall structure, and altered adrenoceptor mechanisms at effector organs (Oparil, Chen, Berecek, Calhoun and Wyss, 1995; Victor and Mark, 1995).

ii. *Stages of Hypertension and Hypertrophy*:

The SHR traverses through several stages of hypertension during its lifetime. The former, initial hypertension phase from birth to 2 months of age is the first of these stages in which the animal will encounter a prehypertensive stage between days 40 to 50. The latter, fully developed hypertension phase applies to animals over 3 months in age. This phase encompasses two stages: the early hypertensive stage (4-6 months) and the terminal advanced hypertensive stage (12-14 months) (Okamoto, 1969).

The SHR model is subject to four phases of cardiac hypertrophy, which are alleged to directly correlate with the degree of hypertension and its associated stages. The developmental hypertrophy phase comprises the first six months and is characterized by hypertrophied myocyte cross-sectional area (MCSA) measurements in SHR models vs. WKY. During the maturation phase, 6 to 12 months, the myocardium undergoes

significant pathological changes in which the MCSA is significantly elevated while the left ventricular wall thickness is relatively stable. In the aging phase, 12 to 18 months, blood pressure measurements reach a plateau while the left ventricular wall thickness and MCSA continue to rise. The final hypertrophy phase, senescence, occurs between 18 to 24 months of age in the SHR. The SHR's ventricular myocardium encounters myocardial remodeling which allude to degenerative morphological and morphometric characteristics; reduced left ventricular wall thickness associated with myocyte loss and constant MCSA dimensions (Engelmann et al., 1987).

Vliegen et al. (1987) noted in their article that heart weight and ventricular weight provide global information and insight into hypertrophy (Vliegen et al., 1987). Okamoto recorded the SHR-S (Okamoto-Aoki) strain's myocardial weight at the various stages of hypertension. The 1969 study revealed that in the early hypertensive stage, 4 to 6 months, there was an increase in the cardiac weight. However the advanced hypertensive stage, 12 to 14 months, elicited a marked elevation in cardiac weight within this strain. Therefore prolonged hypertension in the SHR-S animal leads to a substantial increase in myocardial weight (Okamoto, 1969).

iii. *Pressure vs. Volume Hypertrophy*:

Established (long term) cardiac hypertrophy, initiated by pressure or volume overloading, is exhibited through gross alterations in anatomical ventricular geometry mediated by irregularly shaped myocytes. Myocyte, cellular, and ventricular remodeling occurs to aid in restoring normal wall stress; the wall stress is directly proportional to pressure and chamber radius and inversely proportional to wall thickness (LaPlace - Gerdes, 1992).

The SHR is an established model for longitudinal studies of the natural progression of left ventricular hypertrophy (LVH) as an adaptive reaction to pressure overload. Pressure overload is link to two pathophysiological stresses: a chronic increase in afterload and increased systolic wall stress. The pathological repercussions of pressure overloading are collectively referred to as a state of concentric hypertrophy. Concentric hypertrophy is pathologically implicated in increased wall thickness, elevated MCSA (diameter), augmented protein kinase C (PKC) activity, exacerbated myofibril: mitochondria ratios, weight gain, and minimal/no increased adjustment in intracavitary volume or chamber size of the left ventricle (Pfeffer et al., 1979). The left ventricular wall dimensions increase out of proportion to the normal SHR body weight growth eliciting concentric hypertrophy. Myofibers hypertrophy, connective tissue elements undergo hyperplasia, both the right and left ventricles hypertrophy, and fibrous tissue content is elevated all attributing to ventricular: body ratios at values nearly two times beyond normotensive controls in concentric hypertrophy (Imamura, 1978; Gerdes, 1992).

Normal left ventricular growth undergoes normal growth increases in left ventricular volume, external diameters, and cardiac mass in proportion to the peak pumping ability relative to the ventricular weight. In the SHR, cardiac hypertrophy, specifically LVH due to pressure overload, progresses naturally and concomitantly with blood pressure elevation and is a common complication associated with hypertension (Gu and Bishop, 1994).

Volume overload is implicated as another cause of hypertrophy. Volume overload is associated with two physiological stressors: increased preload and elevated diastolic wall stress. The pathological indicators of volume overload hypertrophy are

proportional increases in chamber radius and wall thickness with an equivalent increase in myocyte length and diameter (Gerdes, 1992).

iv. *Causes of Hypertrophy*:

Cardiac hypertrophy is a complex biological process that involves remodeling of the heart's architecture due to specific stimuli. The principle cause of hypertrophy (LVH) in the SHR is not clear and the precipice of several diverging pathophysiological theories.

The most accepted origin of hypertrophy in both the SHR model and essential hypertensives is pressure overload cardiac hypertrophy. Researchers have alluded to an increase in afterload present in the uterus or soon after birth in SHR pups; the SHR heart may be unusually responsive to afterload differences during this period (Devereux and Roman, 1995). The compensatory changes of pressure overload in the ventricular myocardium closely correspond to age-associated myocardial modifications but occur at a younger age. The myocyte and vascular components are prone to accelerated aging, which contributes to the deteriorating hypertrophied ventricular pump (Engelmann et al., 1987; Van Der Laarse, 1989; Frenzel and Feimann, 1984).

Circulating growth factors, cardiac myocyte loading, and cardiac fibroblast levels are all elevated and directly affect the cardiovascular system and myocardial hypertrophy. Myocyte loading is potentiated by growth factors which tend to govern myocyte growth and myocardial mass. An increase in cardiac fibroblast levels alters the adaptive myocardial hypertrophic remodeling and renders the heart to pathological hypertrophy and ventricular dysfunction (Brilla et al., 1990). An uncharacterized soluble "growth"

factor also exists in a hypertrophied myocardium that initiates myosin and protein synthesis (Sen, 1987).

Several other causes, neural and homeostatic, are implicated as hypertrophic stimuli. The sympathetic outflow to the myocardium is enhanced causing a hypertensive state, which is in direct correlation to the degree of hypertrophy in the SHR. Abnormal cellular calcium handling constitutes another hypertrophic stimuli. The defective calcium metabolism in this subpopulation may have a role in eliciting an enhanced myocyte sensitivity to mechanical/physical, humoral, ionic, non-hemodynamic, and/or neural factors which modulates myocyte hypertrophy (Vulpis et al., 1994; Hatton, Young, Bukoski and McCarron, 1995).

Calcium loading in the SHR has been found to subdue pathological hypertrophy of the myocardium by restoring a higher normalized concentration of extracellular calcium. This produces membrane stabilization, thus limiting depolarization and diminishing vascular tone, therefore subduing the degree of cardiac hypertrophy in the SHR (Hatton et al., 1995; Bukoski and McCarron, 1986). Dietary calcium supplementation also slows the progression of hypertension in the SHR, which is thought to act as an impetus in myocardial hypertrophy. Therefore dietary calcium directly inhibits the development of hypertrophy. It is then theorized that supplemental dietary calcium in the BHR attenuates the development of cardiac hypertrophy acting through the same mechanisms as outlined above.

v. *Hypertrophic Remodeling of the Myocardium.*

Cardiac hypertrophy elicits architectural remodeling of ventricular components,

muscle cells, connective tissue, and vasculature which exhibit altered morphology and morphometry in the hypertrophic state. The myocardium's hypertrophic response affects a broad horizon of (ultra)structural factors. The most prominent modifications involve elevated cardiomyocyte size and number, which increases the ventricular mass in humans and rodents. Both normal and pathological hypertrophies are ascribed to myocyte hypertrophy, or a general increase in myocyte size (specifically MCSA) in both the right and left ventricle (Engelmann et al., 1987; Van Der Laarse et al., 1989; Campbell et al., 1989). Vliegen et al. established in a 1987 study that myocyte width, area, length, volume in the subepicardial and subendocardial left ventricular tissue is significantly greater in hypertrophic hearts than a normal myocardium. Cardiomyocyte hyperplasia is another myocardial hypertrophic compensatory response encountered in hypertensive subjects (Vliegen et al., 1987; Kawamura et al., 1976).

The hypertrophic response of the myocardium triggers elevated amounts of connective tissue elements. Collagen concentrations increase causing abnormal interstitial fibrillar collagen accumulation, 40 to 150% increase, and thickening, which is implicated as a major factor in physiologic and pathologic hypertrophy (Caulfield and Janicki, 1997). The heart's hypertrophic response to ventricular pressure overload instigates a plethora of collagen synthesis, especially types I and III, with no change or slight reductions in collagen degradation. An overall proliferation results in the extracellular collagen content (McCarron and Morris, 1985). The myocardium is physically more rigid and experiences left ventricular diastolic dysfunction when indiscriminate amounts of collagen burgeon in the heart (Kawamura et al., 1976).

Cardiac hypertrophy incites compensatory neovascularization and increased ventricular vasculature in essential hypertensives and SHRs (Devereux and Roman, 1995). Other ventricular components are varied due to pressure overload in left ventricular hypertrophy: non-myocyte hyperplasia and non-muscle cell volume fraction increases (Long et al., 1991). Progressive hypertrophy elicits hyperplasia of cellular organelles, regressive modifications in subcellular structures, and an elevation in sarcomere number in cardiomyocytes (Fraticelli et al., 1989; Kawamura et al., 1976).

A significant amount of research has been devoted to the effects of calcium on hypertrophy and hypertension in WKY and SHR models representative of normotension and essential hypertension. The BHR model has not been extensively investigated in this field and has been limited to psychosocial and environmental stressor paradigms since its inception in 1980. Investigators have induced permanent hypertension and pathological myocardial hypertrophy in a multitude of conditioned stressor experiments in the BHR animal. Lawler, among others, have placed a principle emphasis on stress-diet interactions in the BHR model, with minimal deviation from his focus on environmental stimuli. The purpose of this study was to investigate dietary calcium's interaction on systolic blood pressure and cardiac pathology in the BHR model. Therefore two hypotheses were originated in this investigation: (1) supplemental dietary calcium attenuates systolic blood pressure in the BHR, and (2) supplemental dietary calcium attenuates the development of hypertrophy in the BHR. These studies were conducted in the absence of an environmental stressor paradigm. Several parameters were explored, all pertaining to hypertension and hypertrophy in the BHR in this study: (1) resting/baseline systolic blood pressure, (2) body, heart, and ventricular weight (used to

derive a heart: body weight ratio and ventricular: body weight ratio), (3) right, left, and interventricular septal wall thickness, and (4) myocyte cross-sectional area (MCSA). In interpreting dietary calcium's effects on BHR's blood pressure and myocardial morphology, a dietary effect may be established in the BHR animal. This information may be employed in future congruent study and treatment regimes in the human borderline hypertension subpopulation.

CHAPTER II

MATERIALS AND METHODS

Experimental Design

Female SHR (Taconic Farms-Germantown, NY) were bred with male normotensive Wistar Kyoto (WKY) rats to yield an F_1 generation Borderline Hypertensive Rat (BHR). Male age-matched pups sampled from each litter were weaned and randomly placed in one of two groups, high calcium experimental group or normal calcium control group (n=12). All animals were given tap water and standard rat chow (.26% Na, .98% Ca) ad libitum upon weaning. An initial systolic blood pressure (SBP) was recorded as a standard on all animals at week five. After the pre-diet SBP was recorded, the BHR $CaCl_2$ experimental group was started on a high 2% calcium chloride water diet (Kageyama et al., 1986). An additional control group, WKY normotensive rats (Taconic Farms), and a BHR control group were included in the study. All animals were housed at a constant humidity and ambient room temperature of 22°C with a 12-hour photoperiod. These experiments were approved by the Animal Care and Use Committee at Youngstown State University (Protocol #97-007).

Systolic Blood Pressure Measurements

Body weight and indirect tail-cuff plethysmography measurements were performed weekly for 18 weeks following the week of weaning. Conscious, unanaesthetized rats were confined in a cylindrical plexiglass restraint on a preheated baseplate set to the animal's body temperature of 37°C. The animal was placed in the Narco Biosystems sphygmomanometer chamber 5 to 10 minutes prior to the onset of the SBP determinations. Tail-cuff systolic blood pressure measurements were recorded

weekly from 5 weeks of age up to 22 weeks. SBP was monitored at a rate of one every 30 seconds. A minimum of three artifact-free recordings were obtained for each subject and averaged to attain the weekly systolic blood pressure value for each animal.

Tissue Collection and Fixation

At 23 weeks of age the animals were weighed and sacrificed. Animals were in a carbon dioxide chamber until drowsy, and then decapitated. The myocardium was excised from the thoracic cavity and dissected free of connective and fatty tissue. The aortic remnant attached to the heart was measured and recorded. In order to clear the heart of any remaining blood, a blunt 5cc syringe was used to force room temperature 0.1M PBS, phosphate buffered saline pH 7.4, manually through the aorta and under the auricles. The heart was also submerged into a beaker of room temperature PBS to clean the exterior.

The heart was blotted dry with Kimwipes, weighed and recorded (Vulpis et al., 1994; Engelmann et al., 1987; Kawamura et al., 1976). The auricles/atria were detached and the isolated ventricles were blotted dry, weighed and recorded (Pfeffer et al., 1979). Ventricular length was measured from apex to aortic insertion and recorded. The myocardium was then transversely cut at a right angle at 50% the length for later SEM ventricular wall thickness morphometric measurements.

The posterior ventricles preserved in Karnovsky's fixative were refrigerated at 4°C for a minimum of 72 hours to ensure development of a rigid ultrastructure before ethanol dehydration and carbon dioxide critical point drying.

Scanning Electron Microscopy Tissue Preparation

Prior to dehydrating, the tissue was bisected at right angles to the long axis to include the right and left ventricles and interventricular septum (Engelmann et al., 1987). The superior ventricular section was used to determine wall thicknesses (Yang et al., 1997). The remaining inferior ventricular tissue was sagittally sectioned into two parts: 1) the right ventricle, left ventricle and interventricular septum, and 2) the remaining left ventricular mass, which was used to measure the myocyte cross-sectional area (MCSA).

Tissue was washed twice in 0.1M PBS, pH 7.3, for five minute intervals, and then followed with a series of graded alcohol washes at 10 minutes each (50, 75, 85, 95% ethanol). Another change at 95% ethanol was made as well as two changes in absolute ethanol respectively. The tissue was then stored in 100% absolute ethanol until further prepared for scanning electron microscopy. Specimens were dehydrated further by critical point drying. Critical point drying was performed as described in the Winter 1999 Scanning Electron Microscopy Manual (Walker, 1999). After the tissue was fixed, dehydrated, and dried it was applied to a metallic aluminum stub using Avery Spot-O-Glue double-sided sticky tape. Tissue specimens were coated with a 10 nm layer of conductive gold metal using a BioRad SC-510 Sputter Coater, as described in the Winter 1999 SEM Manual (Walker, 1999).

Operation of the Hitachi S-450 Scanning Electron Microscope

At least three micrographs were taken of each 2 mm transverse section at a fixed magnification ranging from (Engelmann et al., 1987). This provided a relatively gross observation of the thickness parameter of three walls: the left ventricle, right ventricle, and interventricular septum. Myocyte cross-sectional area (MCSA) data was collectively

drawn from three micrographs taken of the sagittal section at a fixed magnification of 1.3 kX or 1300X. Two micrographs were captured of non-overlapping areas of the left ventricular lumen edge myocardiocytes. The last micrograph contained left ventricle myocytes at a 50% width area in the same aforementioned sagittal section.

Measurement and Quantification of Morphology

ADOBE Photoshop. The positive micrograph prints were scanned and stored into a JPEG formatted file on disc via the ADOBE Photoshop software program. The JPEG format was changed to a TIFF format in order to adapt to specifications rendered in analyzing the data in the National Institutes of Health (NIH) Image v1.5 program.

Wall thickness dimensions were measured using NIH Image v1.5 program. Ten equidistant perpendicular measurements were made of the left ventricle, right ventricle, and interventricular septal wall thickness within a 1500 μm area demarcated by a reference line (Engelmann et al., 1987). The same protocol was used for all specimens. Mean thickness was calculated for each wall parameter.

In sagittal sections, myocyte cross-sectional area (MCSA) dimensions of the left ventricular myocardiocytes were determined by tracing the cursor around the periphery of myocardiocytes enveloped by a well-developed, continuous and intact basal membrane (Vulpis et al., 1994). An individual micrograph would contain five to ten of the characterized myocytes on average. In order to assess differences in regional myocyte size, three micrographs were taken of each subject: two micrographs were taken sampling the lumen edge surface and one micrograph was taken representing myocardiocytes at 50% of the wall thickness. Individual specimen's mean myocyte cross-sectional areas and respective standard errors were calculated for the lumen edge

and 50% width myocardiocytes. An overall mean lumen edge MCSA and 50% MCSA and standard error values were projected for each of the three experimental or control group strains.

Statistical Analysis

All data acquired are presented as the mean ± SEM and was statistically analyzed with the SigmaStat program by Jandel Scientific. Assessment of the pre and post diet systolic blood pressure (SBP) changes within each group was analyzed by one-way repeated measures analysis of variance (ANOVA), and all pairwise multiple comparison procedures were executed by Dunnett's method. Any significant changes in the systolic BP between the three strains were evaluated by one-way ANOVA, and all pairwise multiple comparison procedures were performed by the Student-Newman-Keuls method. An overall mean systolic blood pressure assessment between the three groups was analyzed by one-way ANOVA, and all pairwise multiple comparison procedures were evaluated by Dunn's method. Hypertrophic indexes (ventricular weight/body weight ratio) and heart weight/body weight ratios were compared among the three strains by one-way ANOVA, and all pairwise multiple comparison procedures were examined by Dunn's method. Heart, ventricular, and body weight were assessed among the three group strains by one-way ANOVA, and all pairwise multiple comparison procedures were ascertained via the Student-Newman-Keuls method. Strain-related changes in the three wall thicknesses and MCSA were investigated by one-way ANOVA, and all pairwise multiple comparison procedures were completed via the Student-Newman-Keuls method. Pairwise comparisons of the lumen edge and 50% width MCSA was performed

with the paired t-test in order to confirm if a significant difference exists between these two parameters.

CHAPTER III

RESULTS

Independent Variables

Body Weight.

As Figures 1 and 5 illustrate, the body weight was significantly different among all treatment groups (one-way ANOVA; Student-Newman-Keuls, $p<0.05$, $n=12$). WKY had the highest body weight among the three groups throughout the entire study (Cierpal and McCarty, 1987). BHR on a 2% calcium chloride diet weighed the least (392±13.21g). This finding parallels previous literature where the body weight was significantly less in animals administered high 2% calcium diets and food consumption significantly greater in the calcium supplemented animal (Hatton et al., 1989; Stern et al., 1984, 1987). It is noted, however, that food intake was not monitored in the present investigation. BHR control remained at an intermediate body weight (508±11.33g), not surpassing the WKY (610±9.71g) or declining below BHR $CaCl_2$. Finally, Figure 2 illustrates the significant differences between WKY, BHR control and BHR $CaCl_2$ at the conclusion of the study and reaffirms the collective findings in body weight differences ($p<0.05$, $n=12$).

Systolic Blood Pressure.

In Figure 3, WKY revealed a hypotensive trend that was substantiated by statistical analyses. WKY established a 5.35% overall decrease in the systolic blood pressure over the course of the experiment, week 5 to week 22. WKY control animals exhibited minimal SBP fluctuation throughout the study. Weeks 13, 14, 17 and 20 were significantly elevated in comparison to the established pre-diet week 5 (one-way repeated

measures ANOVA; Dunnett's Method, $p<0.05$, $n=12$). The WKY SBP was significantly lower than both BHR group's SBP in over half of the study.

In contrast, as Figure 3 illustrates, BHR control demonstrated an increase between SBP and time. Systolic blood pressures in the BHR control group were not significantly different from the pre-diet week (week 5) from weeks 6 through 10 and at week 14. During the remaining weeks of the experiment systolic blood pressures were significantly elevated (one-way repeated measures ANOVA; Dunnett's Method, $p<0.05$, $n=12$). At week 22, BHR control had a 23.85% increase in systolic blood pressure versus week 5.

As demonstrated in Figure 3, BHR on calcium chloride experienced a 49.51% increase in SBP during the course of the study, from weeks 5 to 22. The larger increase in BHR $CaCl_2$'s systolic blood pressure in comparison to BHR control is due to an initial difference in SBP of the two BHR groups; at 5 weeks, the SBP of BHR $CaCl_2$ is 12.2% lower than BHR control (refer to Figure 3). In BHR $CaCl_2$, all post-diet SBP weeks were significantly greater than the pre-diet SBP week except for week 17 (one-way repeated measures ANOVA; Dunnett's Method, $p<0.05$, $n=12$). These results confirm that post-diet systolic blood pressure changes occur in BHR animals on a high 2% calcium chloride diet.

In Figure 3 the comparison of BHR control and experimental groups suggest that dietary calcium had a delayed, but significant effect on systolic blood pressure. During the first 10 weeks on the diet, 15 weeks of age, there was no significant difference in systolic BP between BHR control and BHR $CaCl_2$, with one exception at week 12. However, after 10 weeks on a high calcium diet, the SBP of BHR $CaCl_2$ was less than

BHR control. During weeks 16, 17, 18 and 19 the SBP of BHR $CaCl_2$ was less than BHR control ($p=0.0531$, $p=0.0512$, $p<0.05$, $p<0.05$ respectively, $n=12$). This effect was reversed during the last weeks of the experiment. At weeks 20, 21 and 22, after 17 weeks on the high calcium diet, there was no significant difference in SBP of BHR and BHR $CaCl_2$.

The SBP of WKY was significantly less than BHR and BHR $CaCl_2$ throughout most of the experiment. With the exception of weeks 5, 6, 13, 14, 16, 17 and 20, the SBP of WKY was less than both BHR groups (one-way ANOVA; Student-Newman-Keuls, $p<0.05$, $n=12$).

Figure 4 illustrates the overall mean systolic blood pressure of all three groups during the course of this investigation. The overall mean systolic BP was significantly different between all treatment groups (one-way ANOVA; Dunn's method, $p<0.05$, $n=12$). Normotensive WKY had the lowest overall mean SBP among the three groups (93.6±1.24mmHg). Calcium supplemented BHR represented the intermediate overall mean SBP with its BHR control counterpart manifesting the highest overall mean systolic BP (103.8±1.13mmHg and 111±1.19mmHg respectively).

Gross Morphology of the Heart

Heart Weight.

Figure 5 outlines the myocardial weights of the three experimental and control groups at the conclusion of the study, 23 weeks. The heart weight of BHR $CaCl_2$ was significantly lower than WKY and BHR control (1.27±0.0571g, 1.63±0.0459g, 1.61±0.0720g respectively; one-way ANOVA; Student-Newman-Keuls, $p<0.05$, $n=12$). Control BHR was not significantly different from WKY in cardiac weight.

Ventricular Weight.

As outlined in Figure 5, the ventricular weight of BHR control and WKY were significantly greater than BHR $CaCl_2$ (1.44±0.0513g, 1.47±0.0388g, 1.17±0.0568g respectively; one-way ANOVA; Student-Newman-Keuls, $p<0.05$, $n=12$).

Heart Weight: Body Weight Ratio.

As Figures 5 and 6 illustrate, the heart weight: body weight ratios of BHR control and $CaCl_2$ were significantly greater than WKY (0.00316±0.0001249, 0.00324±0.0000553, 0.00267±0.0000512 respectively; one-way ANOVA; Dunn's Method, $p<0.05$, $n=12$), but not statistically different from one another. Heart weight-to-body weight ratios of BHR resembled Lawler's reported values for normal BHR hearts in maturation (Lawler et al., 1981). The WKY group, which held the highest values for both cardiac and body weight, resulted in the lowest ratio value when corrected with body weight.

Ventricular Weight: Body Weight Ratio (Hypertrophy Index).

Referring to Figures 5 and 7, hypertrophy indexes of all three groups paralleled the heart-to-body weight ratios. The hypertrophy index of BHR control and $CaCl_2$ were significantly greater than WKY (0.00284±0.0000913, 0.00296±0.0000600, 0.00240±0.0000461 respectively; one-way ANOVA; Dunn's Method, $p<0.05$, $n=12$), but not statistically different from each other.

Ultrastructure of the Heart

Wall Thickness – Right Ventricle.

As illustrated by Figure 8, dietary calcium did not appear to affect right ventricular wall thickness. There were no significant differences in the right ventricle

between BHR control and BHR $CaCl_2$ (528±34.7μm, 454±29.2μm respectively). However, the right ventricular wall in WKY (634±60.3μm) was significantly greater than BHR CaCl2 (one-way ANOVA; Student-Newman-Keuls, $p<0.05$, n=12). Right ventricular wall photomicrographs of WKY, BHR control and BHR $CaCl_2$ are located in Figures 12, 16 and 20 respectively.

Wall Thickness – Interventricular Septum.

In Figure 9, septal wall thickness in the BHR control was significantly greater than BHR $CaCl_2$ and WKY (1779±54.5μm, 1445±71.2μm, 1423±44.7μm respectively; one-way ANOVA; Student-Newman-Keuls, $p<0.05$, n=12). Dietary calcium did influence the interventricular septal wall thickness. The interventricular septum was not significantly different between the BHR $CaCl_2$ and WKY groups. Photomicrographs of the interventricular septum in WKY, BHR control and BHR $CaCl_2$ groups are respectively depicted in Figures 13, 17 and 21.

Wall Thickness – Left Ventricle.

In this study, as demonstrated by Figure 10, the LV wall thickness of BHR control was significantly greater than BHR $CaCl_2$ (1934±48.5μm, 1623±88.1μm respectively; one-way ANOVA; Student-Newman-Keuls, $p<0.05$, n=12). There was no significant difference in the left ventricle of WKY (1739±82.9μm) vs. BHR $CaCl_2$ or WKY vs. BHR control. WKY, BHR control and BHR $CaCl_2$ left ventricular photomicrographs are represented in Figures 14, 18 and 22 respectively.

MCSA (Myocyte Cross-Sectional Area).

The present study investigated the effects of dietary calcium on cardiomyocyte morphology of the left ventricle by measuring myocyte cross-sectional area. In order to

ensure that myocardiocyte location did not influence diameter, the myocyte cross-sectional area was measured at two locations: 1) ventricular lumen edge, and 2) 50% ventricular width. MCSA values from the lumen edge and 50% width myocardiocytes were not significantly different (BHR $CaCl_2$ 172±11.7μm^2, 195±13.4μm^2; WKY 201.8±10.6μm^2, 191.7±12.2μm^2; BHR 203.1±20.2μm^2, 242.4±20.6μm^2 respectively; paired t-test, $p>0.05$, $n=12$). In all subsequent analysis, MCSA values from both areas were pooled for morphometric analysis.

Figure 11 illustrates no significant difference in MCSA measurements between WKY, BHR control, or BHR $CaCl_2$ (197±10.15μm^2, 223±17.92μm^2, 184±6.90μm^2 respectively; one-way ANOVA; $p>0.05$, $n=12$). MCSA photomicrographs of WKY, BHR control and BHR $CaCl_2$ are illustrated respectively in Figures 15, 19 and 23.

Figure 1. Mean (±*SEM*) body weight (grams) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The body weights of all three groups are significantly different from one another at the conclusion of the study ($n=12$, $p<0.05$).

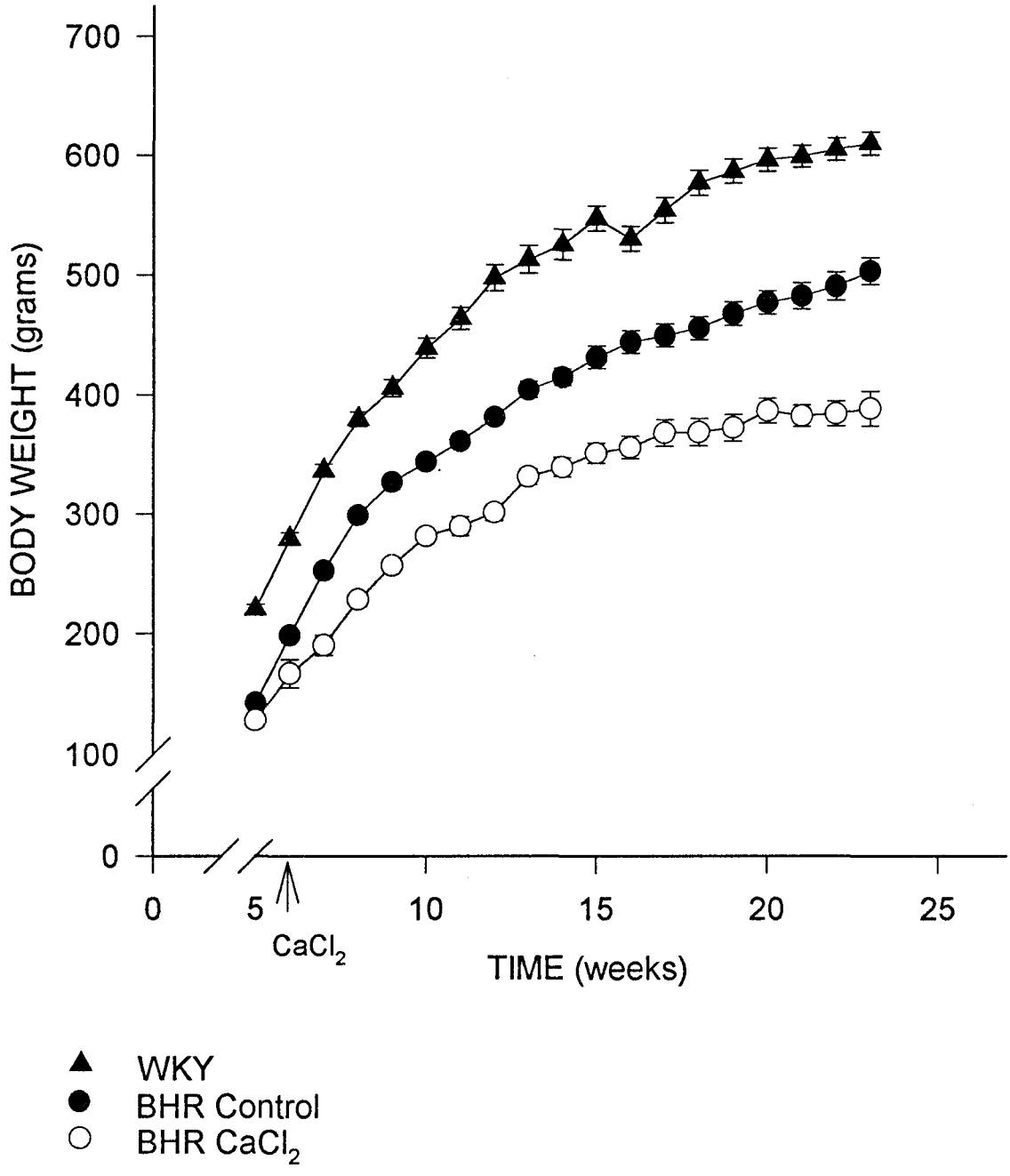

700
600
500
400
300
200
100
0
BODY WEIGHT (grams)
0
5
10
15
20
25
CaCl2
TIME (weeks)
WKY
BHR Control
BHR CaCl2

Figure 2. Mean (±*SEM*) body weight (grams) at the conclusion of the study, 23 weeks, in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The final body weights of all three groups were significantly different from one another (one-way ANOVA, *=$p<0.05$, n=12).

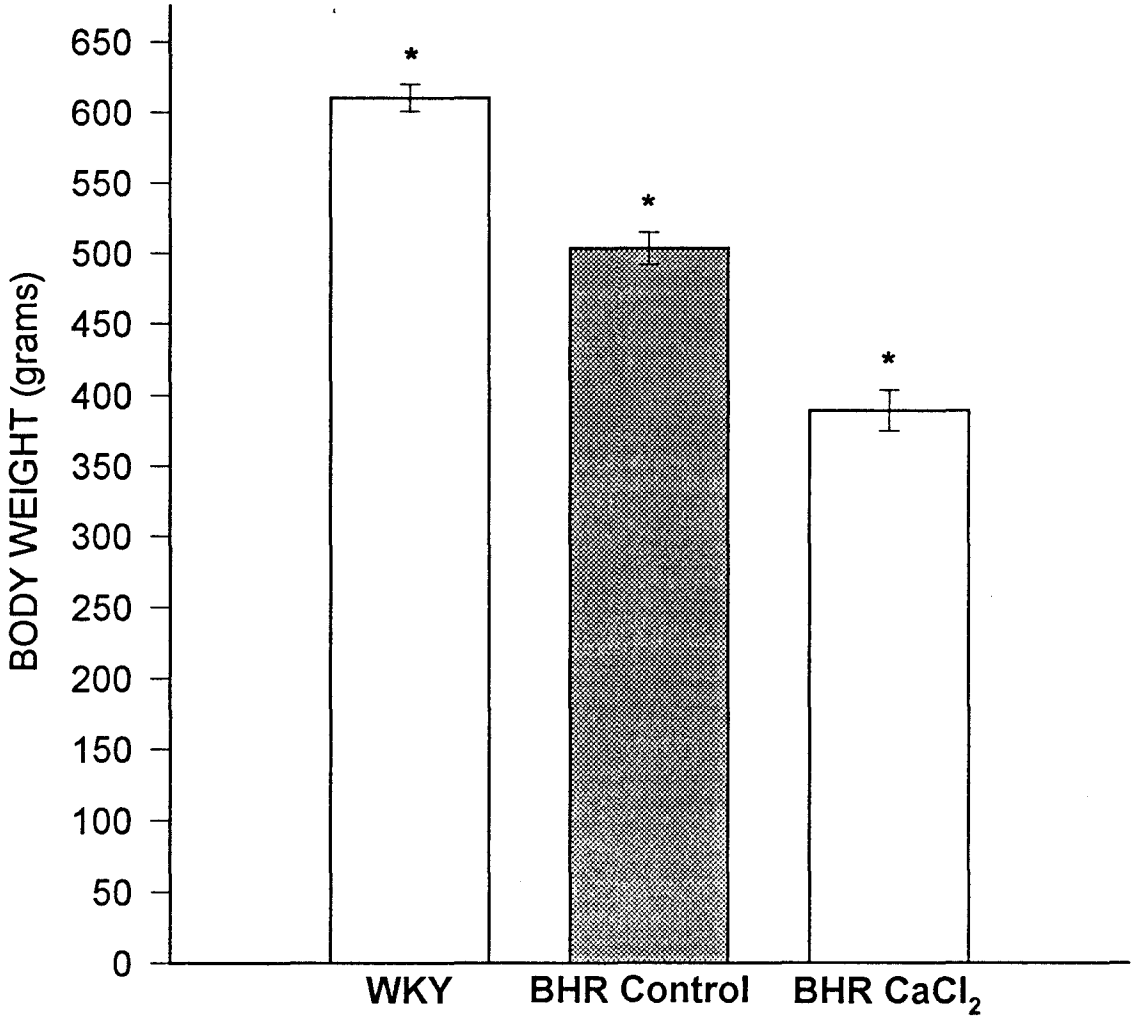

BODY WEIGHT (grams)
650
600
550
500
450
400
350
300
250
200
150
100
50
0
*
*
*
WKY
BHR Control
BHR CaCl2

Figure 3. Mean (±*SEM*) systolic blood pressure (mmHg) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The (*) symbol represents a significant difference between WKY and BHR control, BHR $CaCl_2$. The (+) symbol signifies a significant difference between WKY and BHR control. The (**) symbol represents significant differences among all three groups ($n=12$, $p<0.05$).

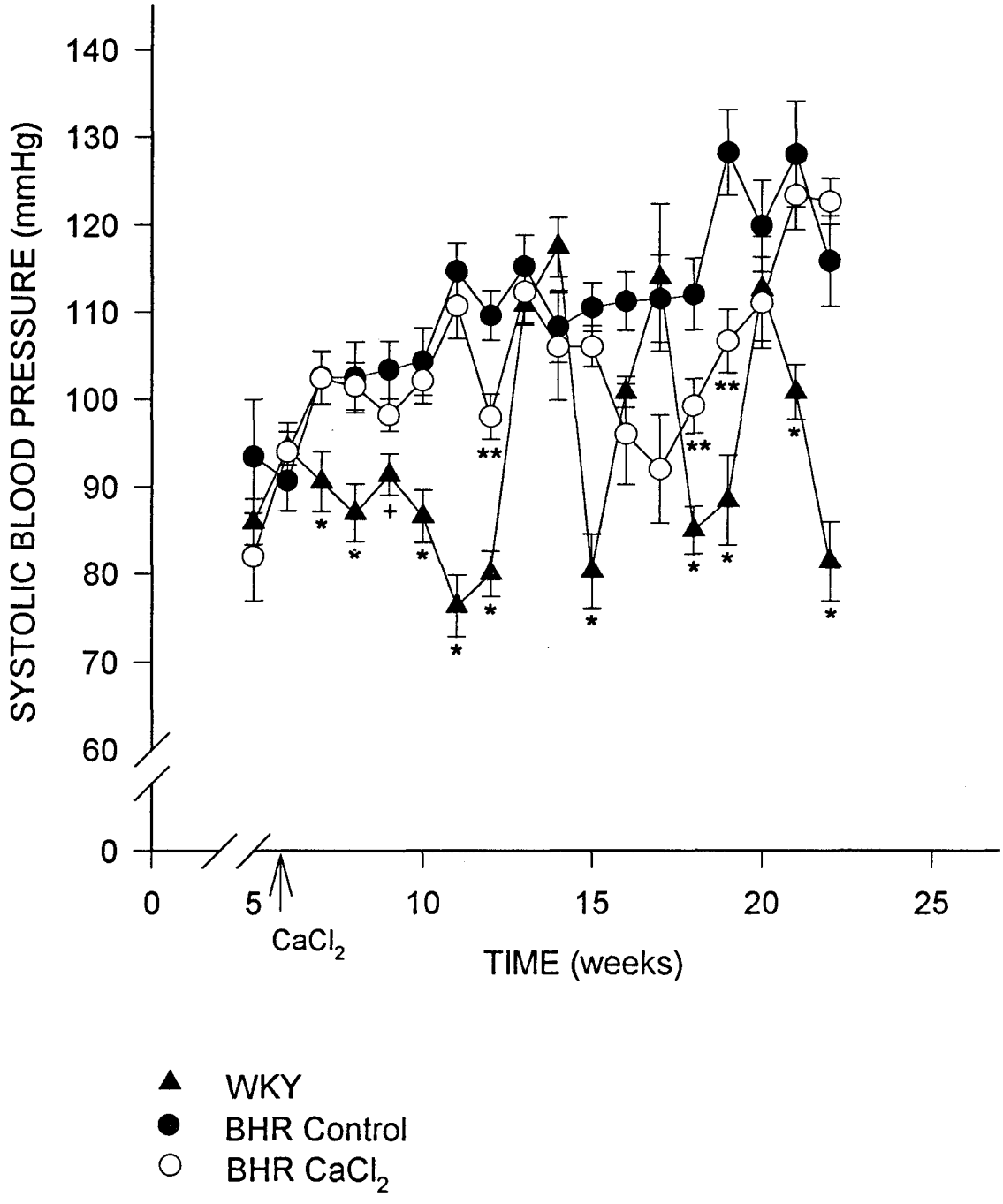

SYSTOLIC BLOOD PRESSURE (mmHg)
140
130
120
110
100
90
80
70
60
0
0
5
10
15
20
25
CaCl2
TIME (weeks)
WKY
BHR Control
BHR CaCl2

Figure 4. Overall mean (±*SEM*) systolic blood pressure (mmHg) during the course of the entire study in WKY (control), BHR control, and BHR $CaCl_2$ animals. The overall mean systolic blood pressures of all three groups were significantly different from one another (one-way ANOVA, *=$p<0.05$, n=12).

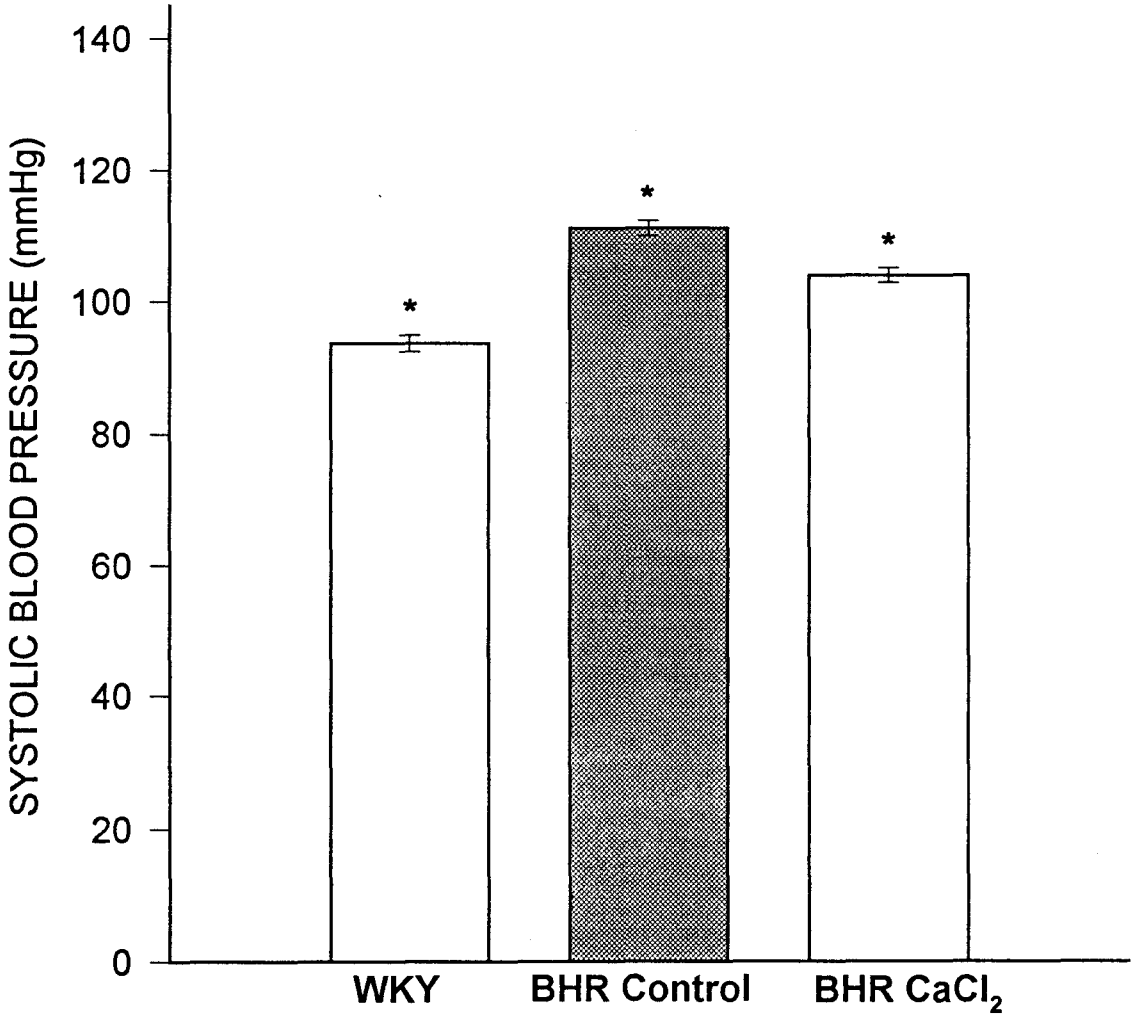

SYSTOLIC BLOOD PRESSURE (mmHg)
140
120
100
80
60
40
20
0
*
*
*
WKY
BHR Control
BHR CaCl2

Figure 5. Mean (±*SEM*) body weight (grams), heart weight (grams), ventricle weight (grams), heart weight-to-body weight ratio and hypertrophy index at the conclusion of the study, 23 weeks, in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. All statistical analyses were performed by one-way ANOVA (*=$p<0.05$, n=12).

Experimental and Control groups	WKY	BHR Control	BHR $CaCl_2$
Body weight (g)	610±9.71*	508±11.33*	392±13.21*
Heart weight (g)	1.63±0.0459	1.61±0.0720	1.27±0.0571*
Ventricle weight (g)	1.47±0.0388	1.44±0.0513	1.17±0.0568*
Heart weight: Body weight (10^{-3})	2.67±0.0512*	3.16±0.1249	3.24±0.0553
Hypertrophy Index (10^{-3})	2.40±0.0461*	2.84±0.0913	2.96±0.0600

Figure 6. Mean heart weight-to-body weight ratios (±*SEM*) in age-matched WKY control, BHR control, and BHR $CaCl_2$ animals. The WKY heart weight-to-body weight ratio is significantly lower than BHR control and $CaCl_2$ as represented by the (*) symbol ($n=12$, $p<0.05$).

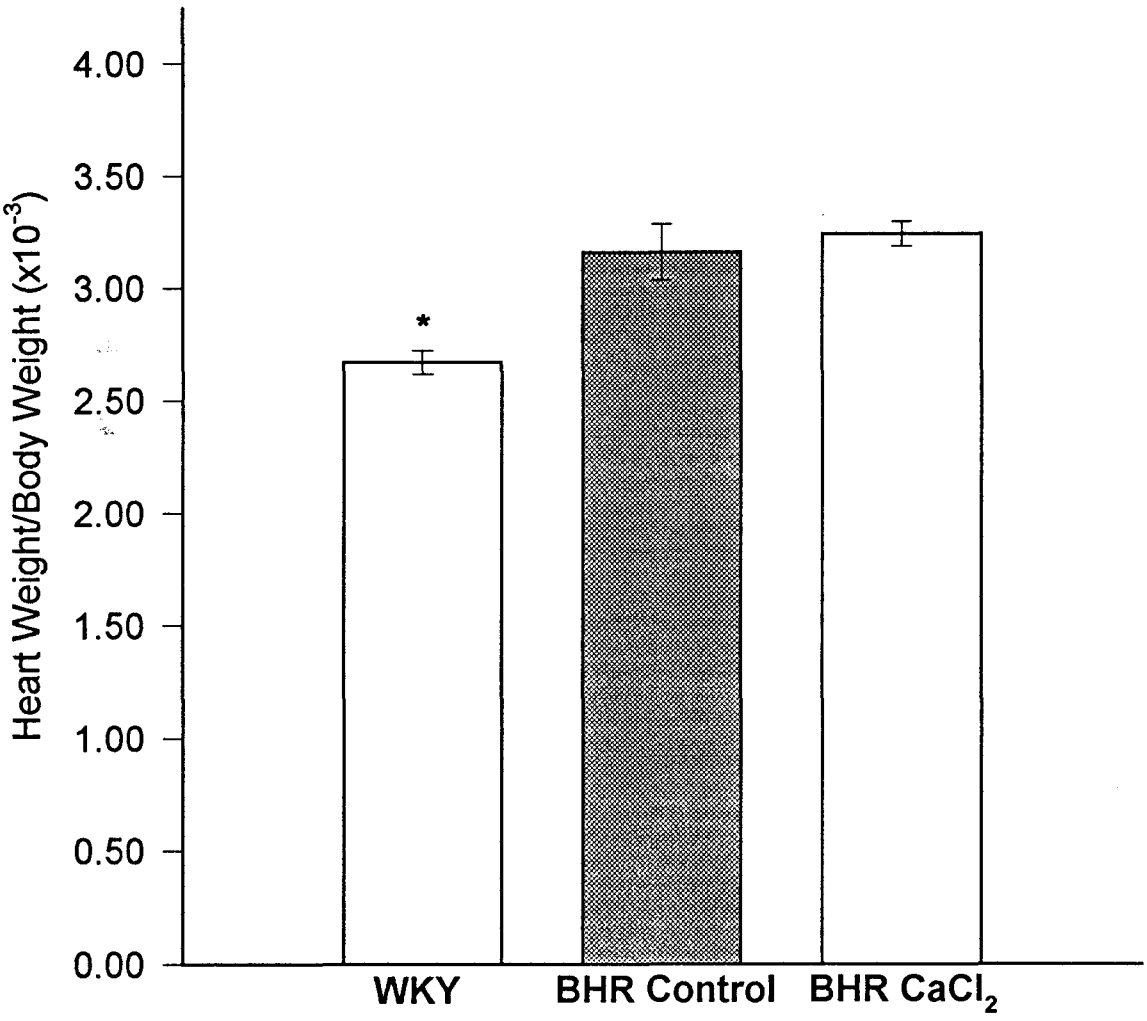

4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Heart Weight/Body Weight (x10-3)
*
WKY
BHR Control
BHR CaCl2

Figure 7. Mean hypertrophy index (ventricular weight-to-body weight ratios) (±*SEM*) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The WKY hypertrophy index is significantly lower than BHR control and $CaCl_2$ as represented by the (*) symbol ($n=12$, $p<0.05$).

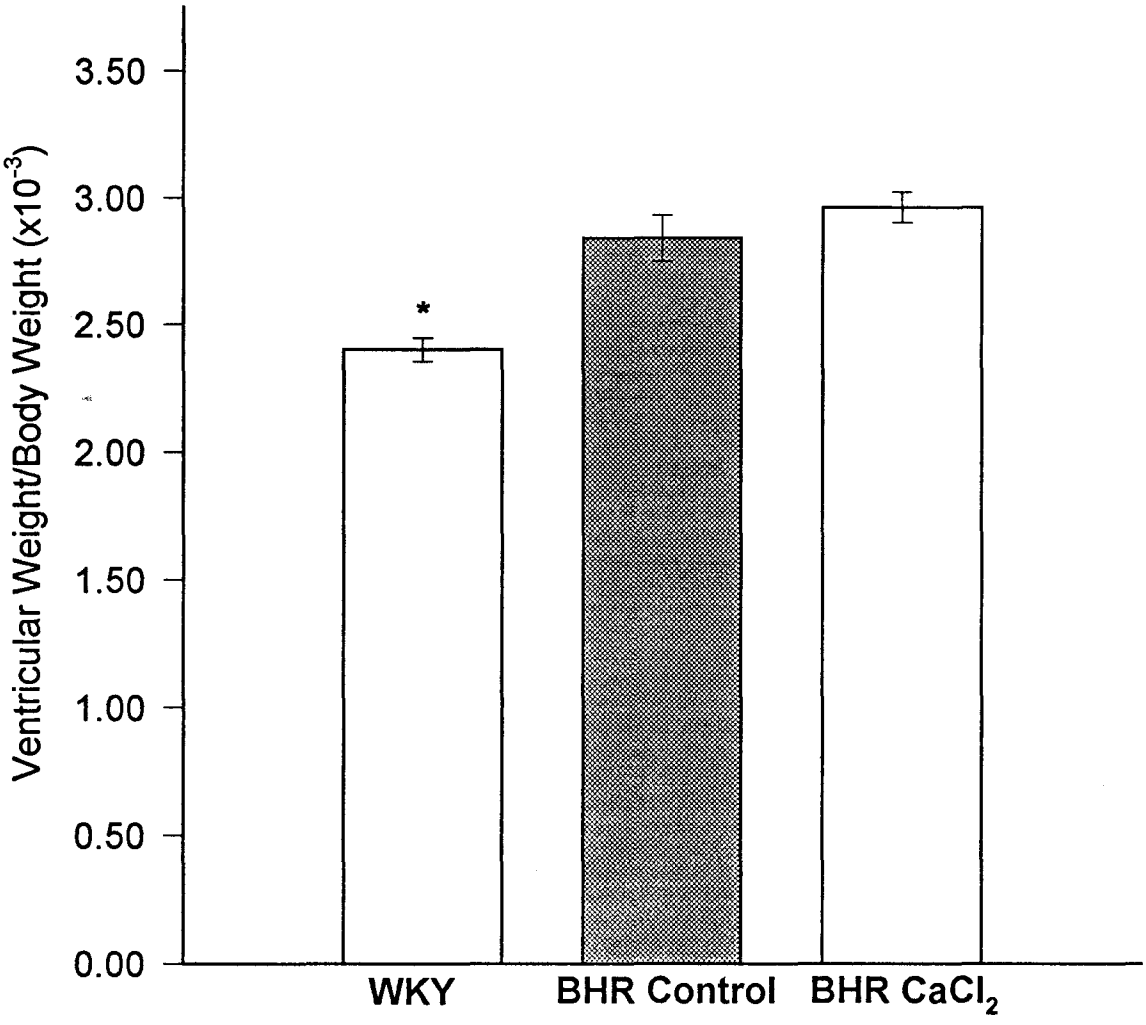

Ventricular Weight/Body Weight (x10^{-3})
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
*
WKY
BHR Control
BHR CaCl$_2$

Figure 8. Mean right ventricular wall thickness (±*SEM*) (μm) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The (+) symbol compares right ventricular wall thickness in WKY and BHR $CaCl_2$ ($n=12$, $p<0.05$).

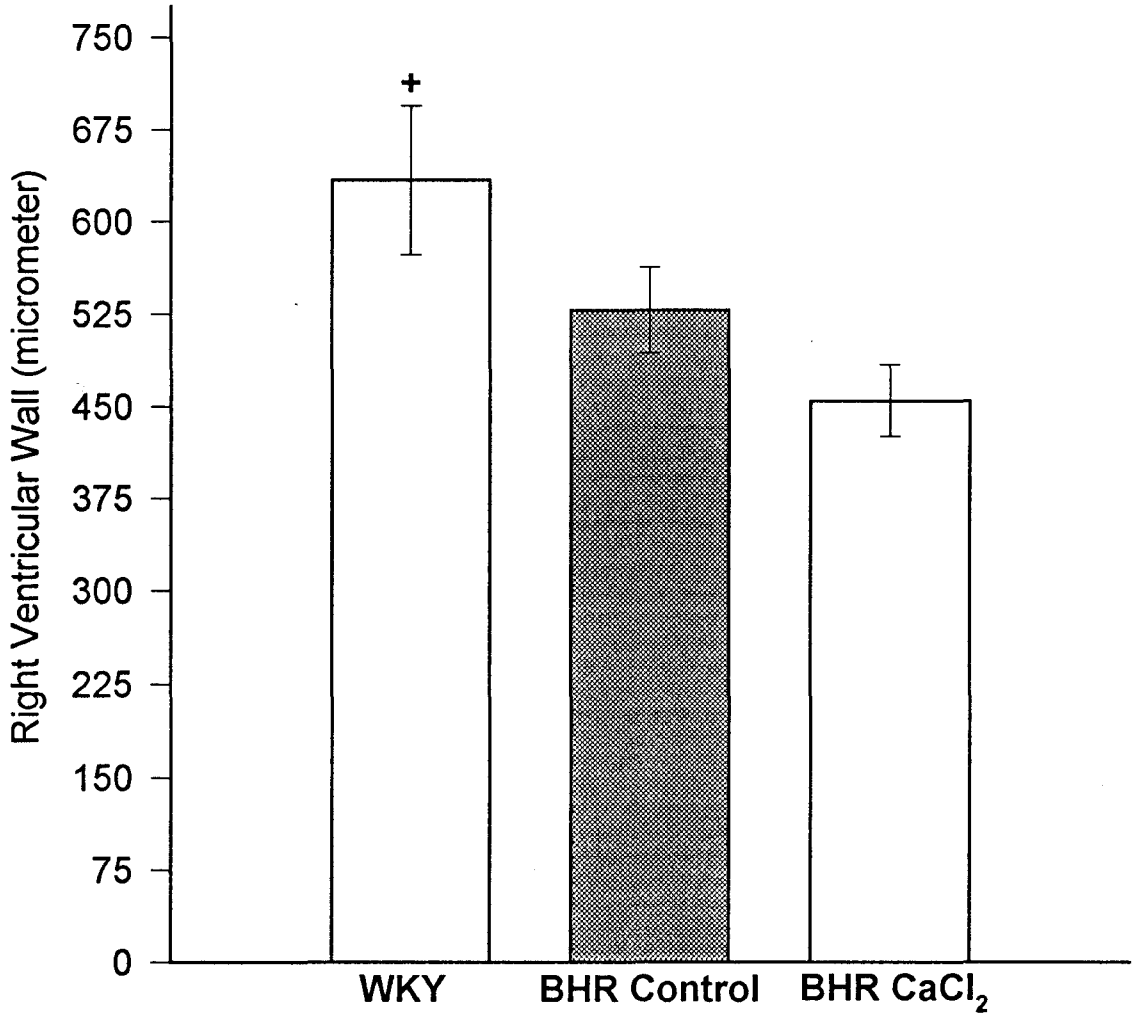

Right Ventricular Wall (micrometer)
750
675
600
525
450
375
300
225
150
75
0
+
WKY
BHR Control
BHR CaCl2

Figure 9. Mean interventricular septal wall thickness (±*SEM*) (μm) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The BHR control septal wall thickness is significantly greater than both BHR $CaCl_2$ and WKY as denoted by the (*) ($n=12$, $p<0.05$).

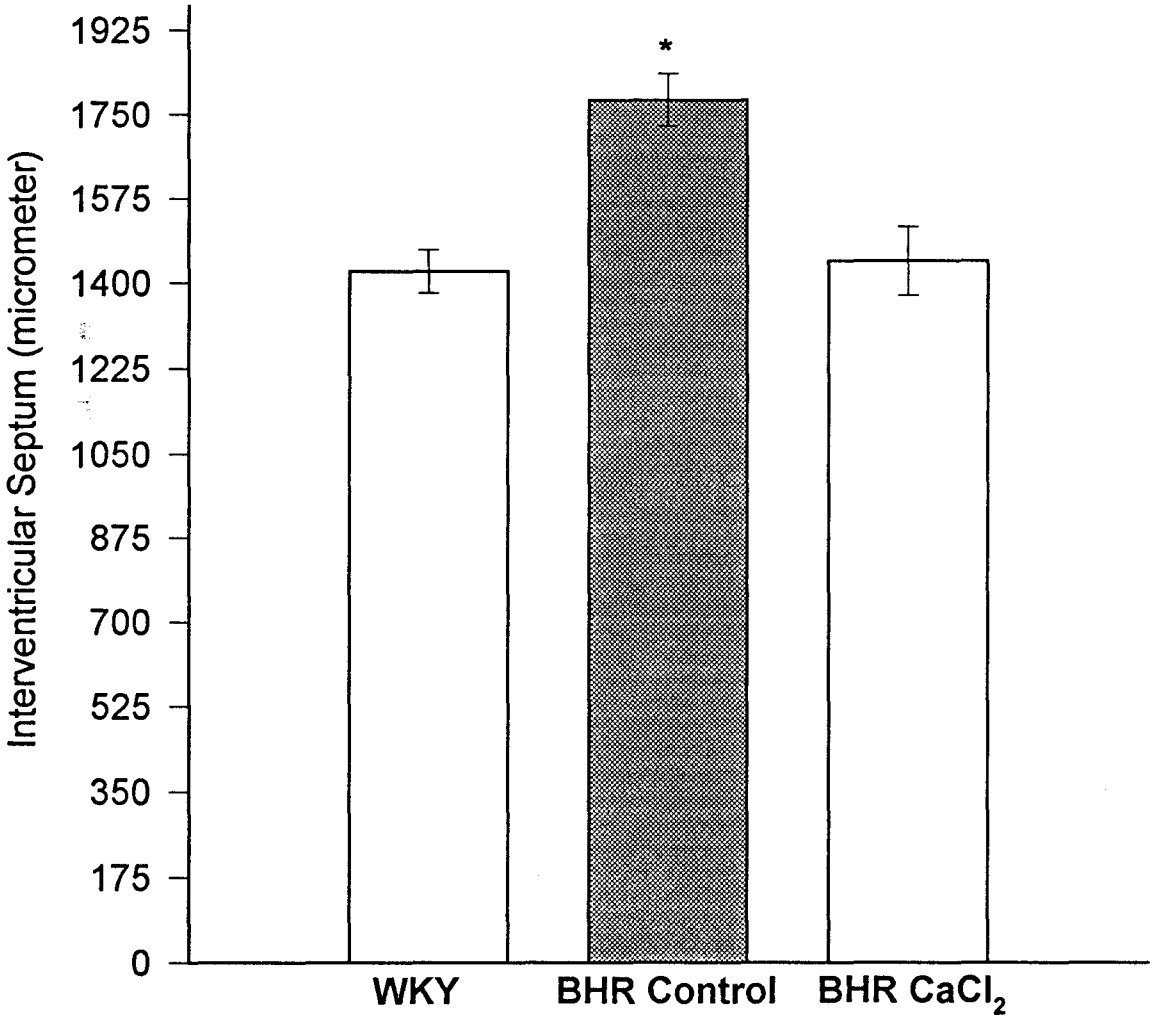

Interventricular Septum (micrometer)
1925
1750
1575
1400
1225
1050
875
700
525
350
175
0
*
WKY
BHR Control
BHR CaCl2

Figure 10. Mean left ventricular wall thickness (±*SEM*) (μm) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. The (+) symbol signifies that left ventricular wall thickness is significantly greater in BHR control as compared to BHR $CaCl_2$ ($n=12$, $p<0.05$).

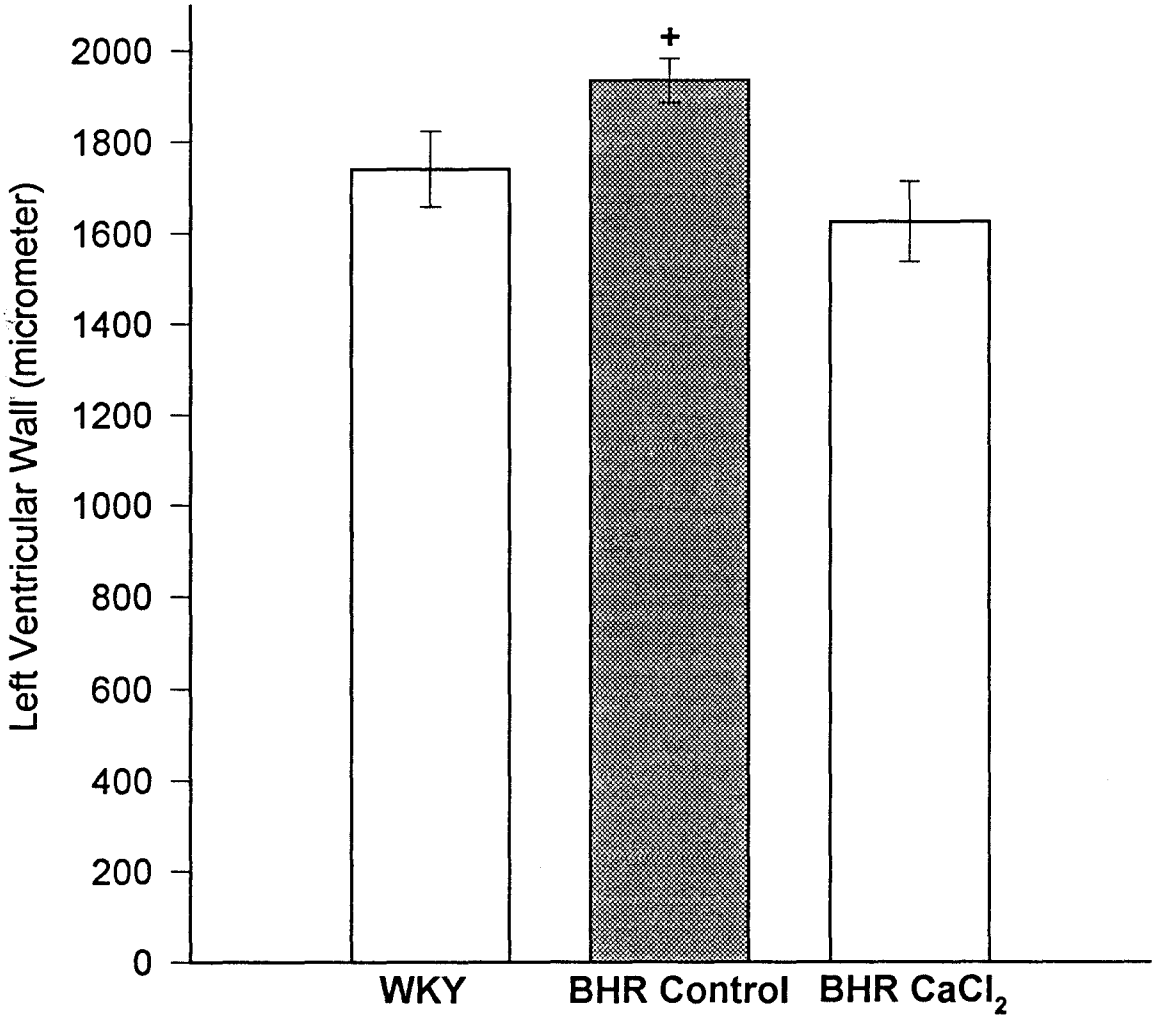

Left Ventricular Wall (micrometer)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
+
WKY
BHR Control
BHR CaCl2

Figure 11. Mean myocyte cross-sectional area (±*SEM*) (μm^2) in age-matched WKY (control), BHR control, and BHR $CaCl_2$ animals. There is no significant difference among the three experimental and control groups in myocyte cross-sectional area (n=12, $p>0.05$).

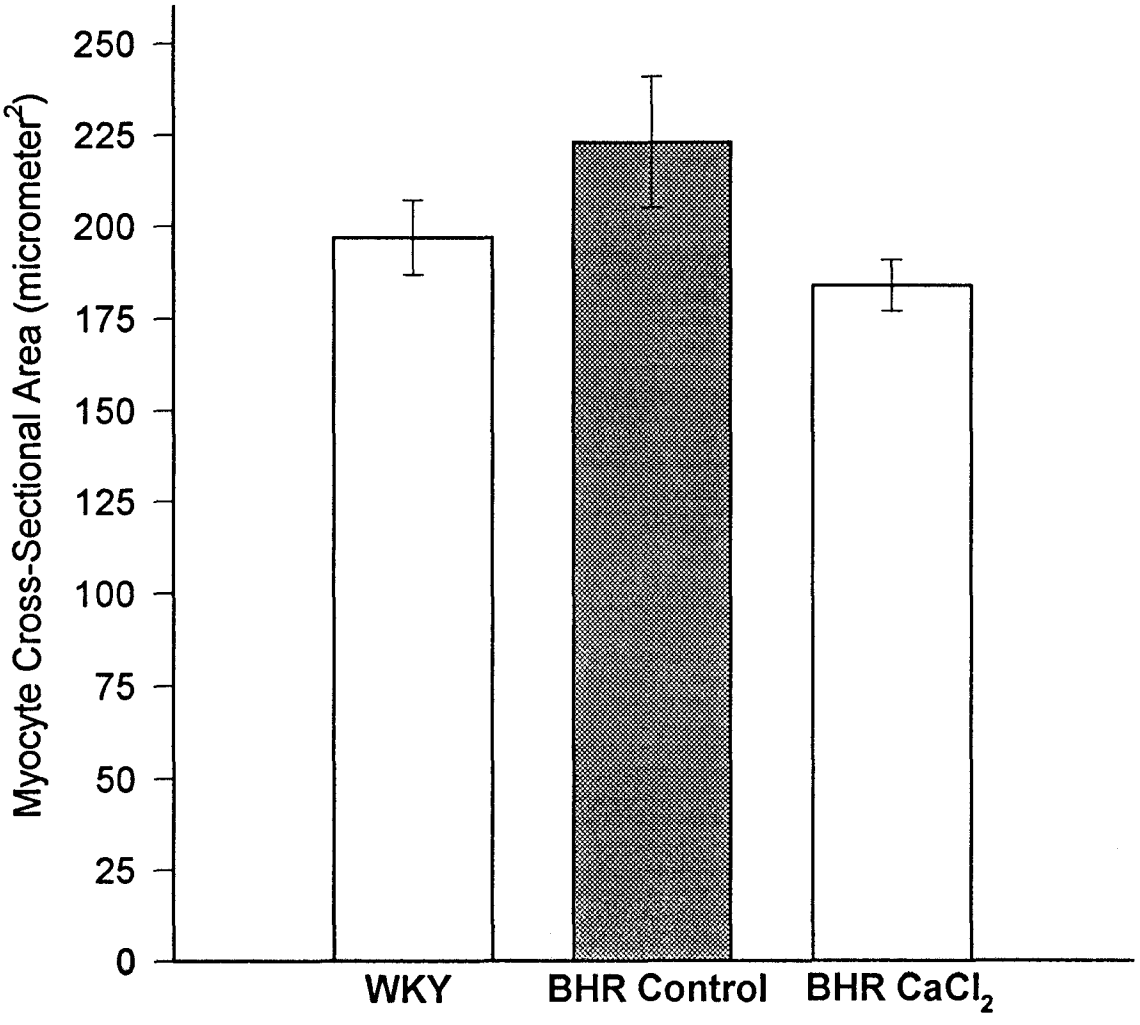

Myocyte Cross-Sectional Area (micrometer^2)
250
225
200
175
150
125
100
75
50
25
0
WKY
BHR Control
BHR $CaCl_2$

Figure 12. Photomicrograph of a transverse section of the right ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a WKY (control) animal (x29).

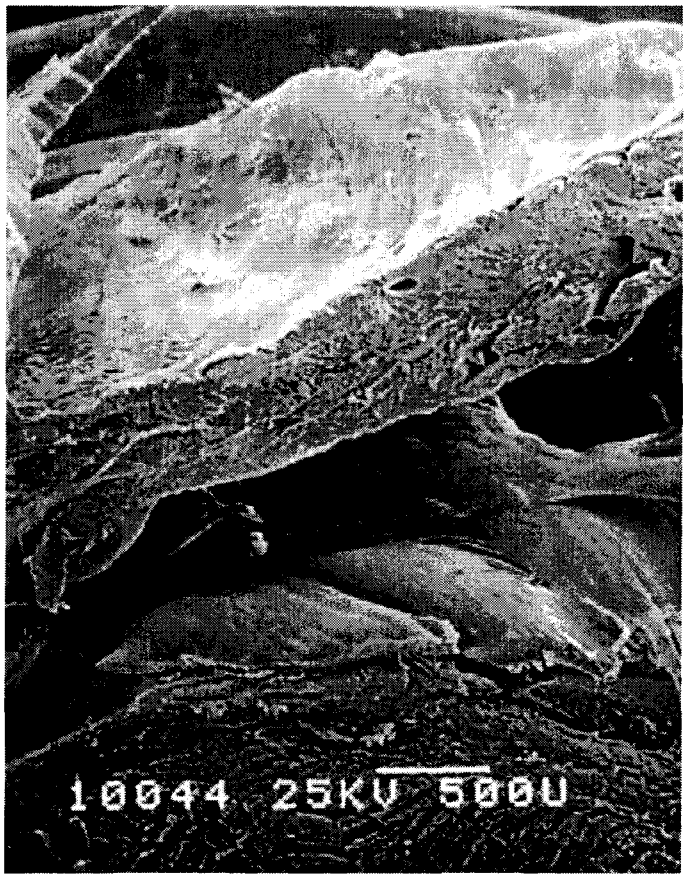

10044 25KV 500U

Figure 13. Photomicrograph of a transverse section of the interventricular septum taken at mid-point (50%) of myocardial length, between the apex and base, in a WKY (control) animal (x29).


10045 25KV 500U

Figure 14. Photomicrograph of a transverse section of the left ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a WKY (control) animal (x29).

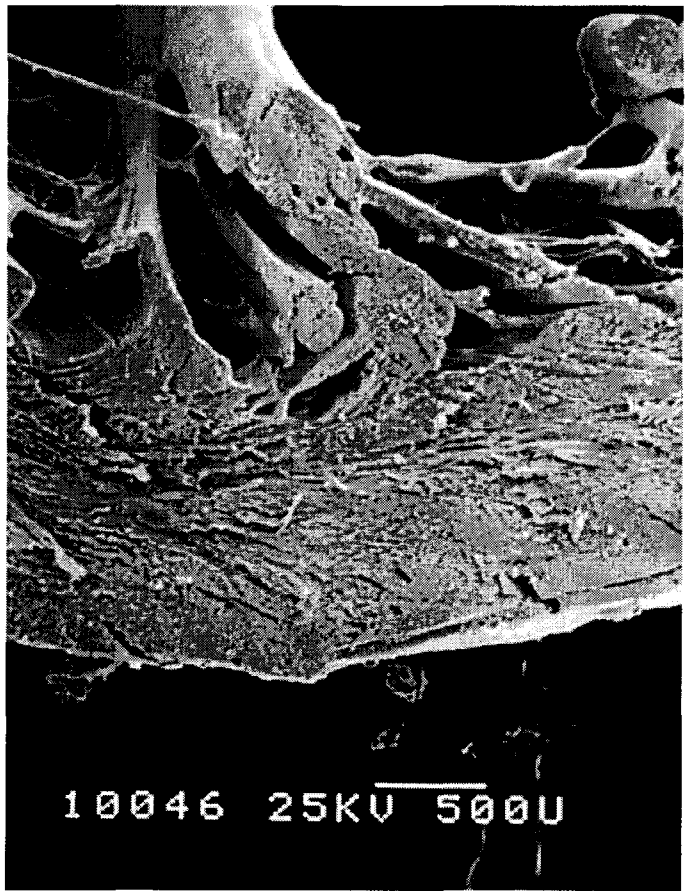

10046 25KV 500U

Figure 15. Photomicrograph of left ventricular myocytes from a sagittal section through the left ventricle, mid-point (50%) of length, in a WKY (control) animal (x1300).

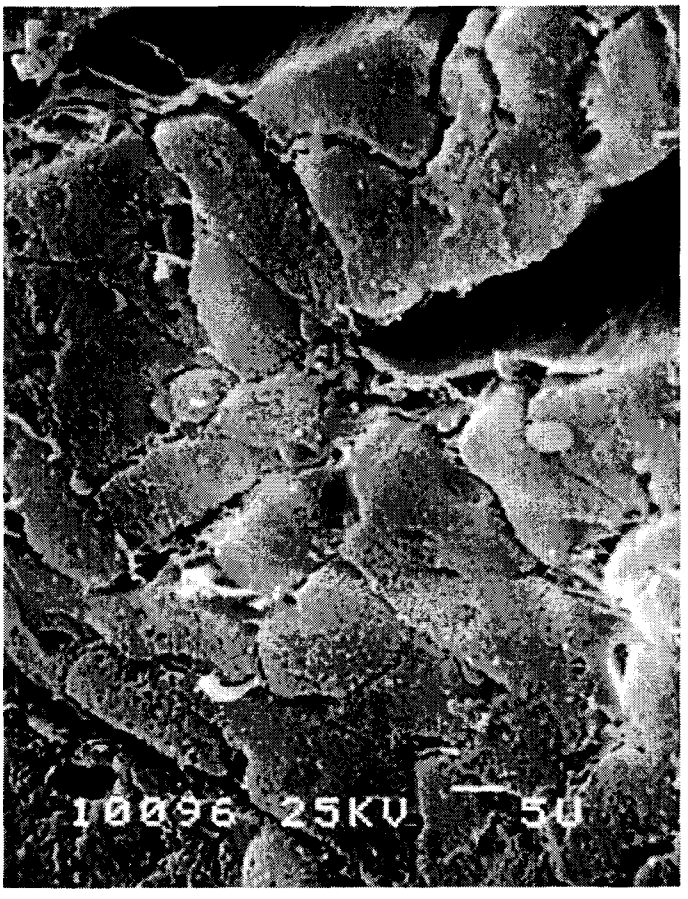

10096 25KV 5U

Figure 16. Photomicrograph of a transverse section of the right ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR control animal (x28).

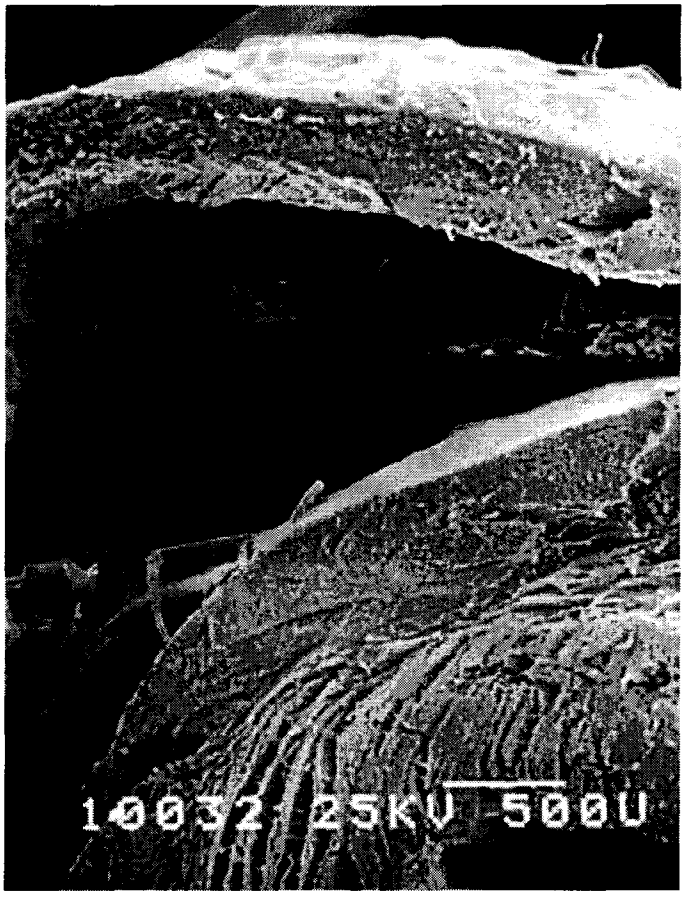

10032 25KV 500U

Figure 17. Photomicrograph of a transverse section of the interventricular septum taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR control animal (x28).

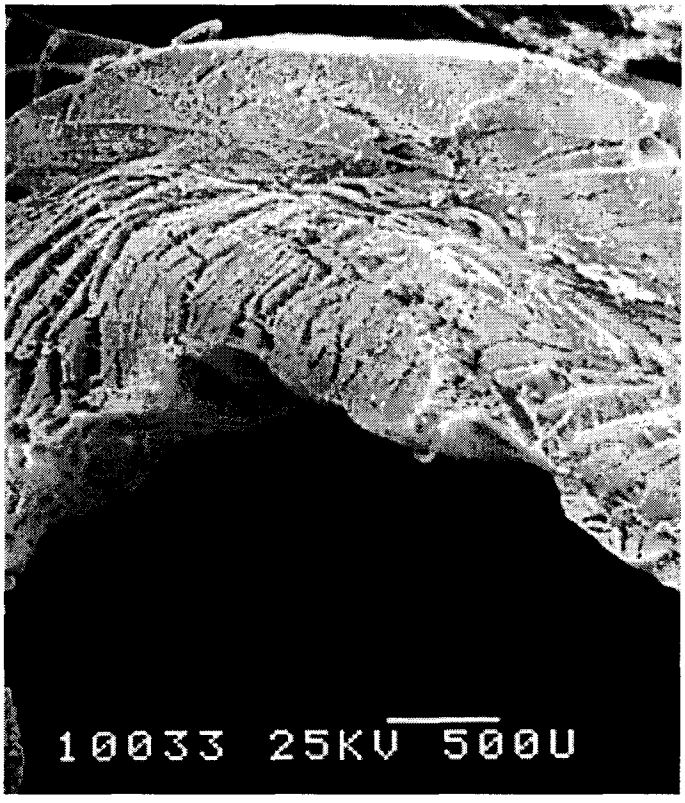

10033 25KV 500U

Figure 18. Photomicrograph of a transverse section of the left ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR control animal (x29).

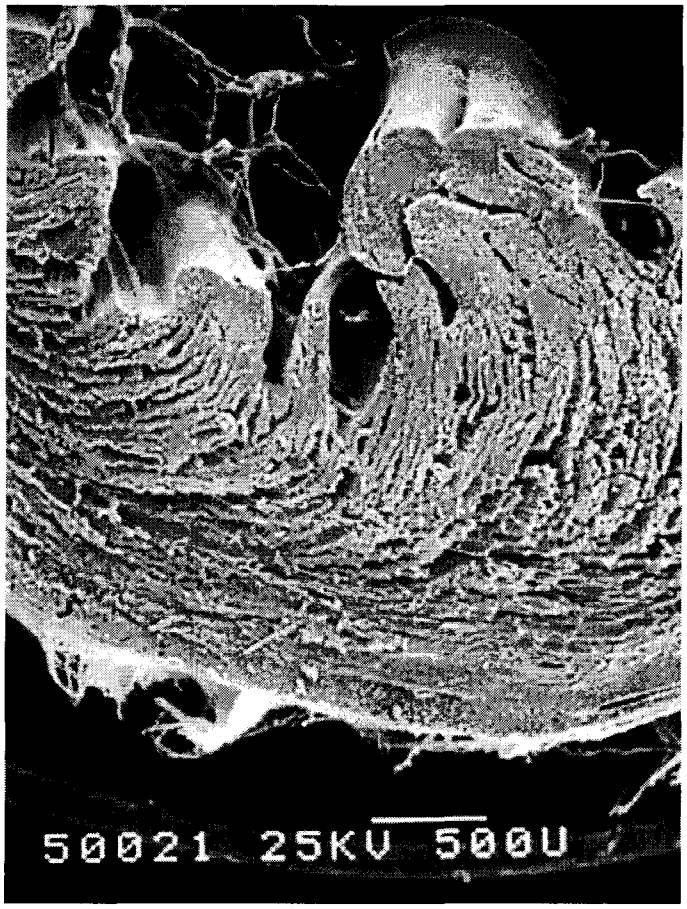

50021 25KV 500U

Figure 19. Photomicrograph of left ventricular myocytes from a sagittal section through the left ventricle, mid-point (50%) of length, in a BHR control animal (x1300).

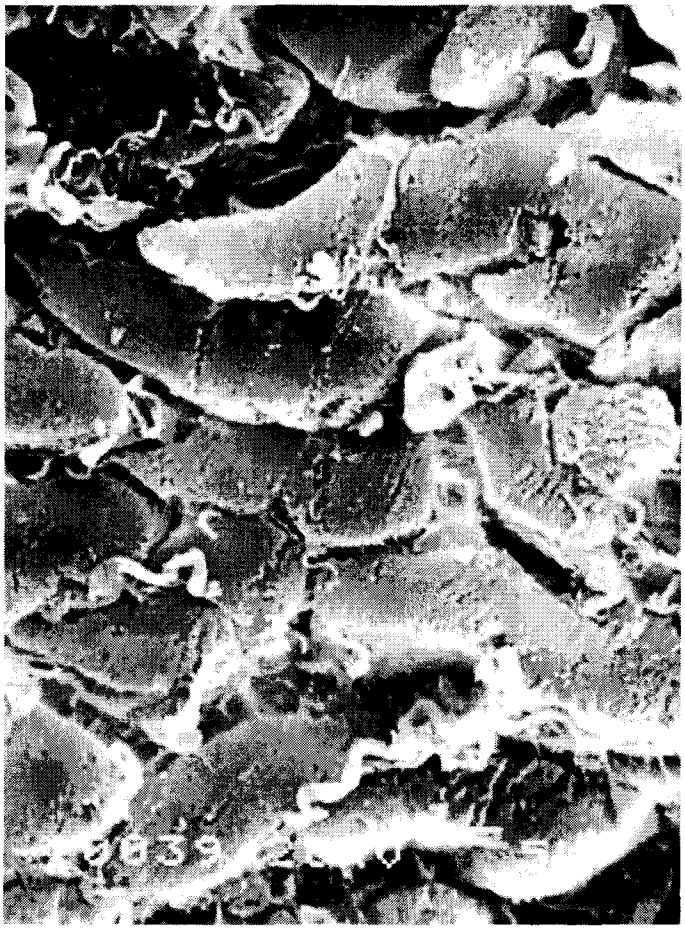


Figure 20. Photomicrograph of a transverse section of the right ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR $CaCl_2$ animal (x29).

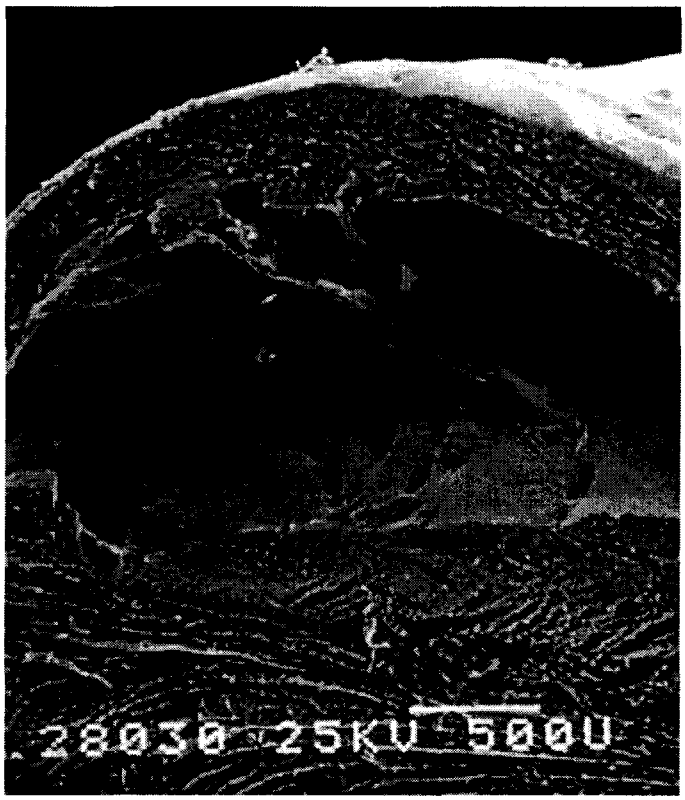

28030 25KV 500U

Figure 21. Photomicrograph of a transverse section of the interventricular septum taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR $CaCl_2$ animal (x29).


28031 25KV 500U

Figure 22. Photomicrograph of a transverse section of the left ventricle taken at mid-point (50%) of myocardial length, between the apex and base, in a BHR $CaCl_2$ animal (x30).

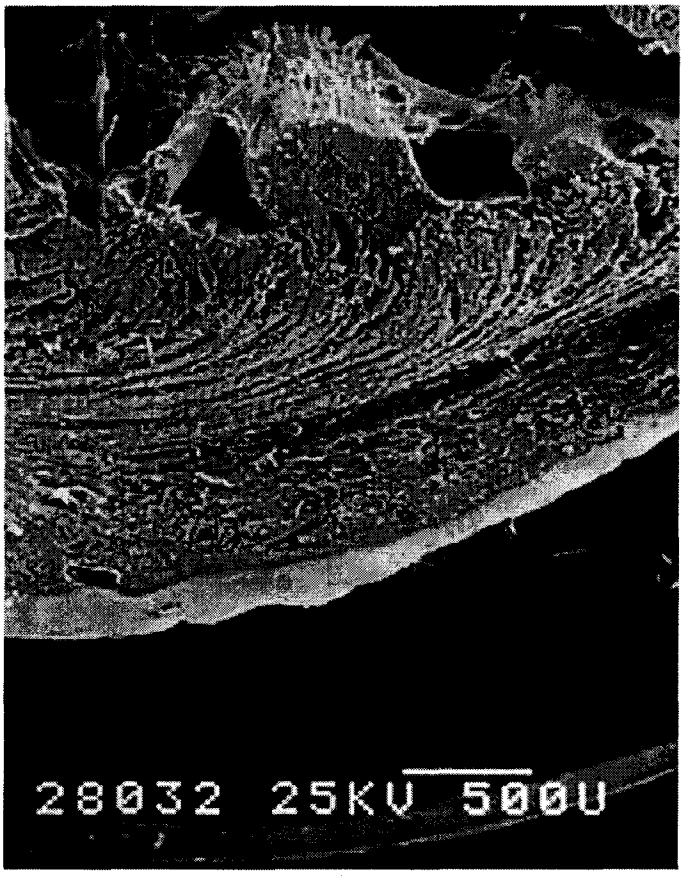
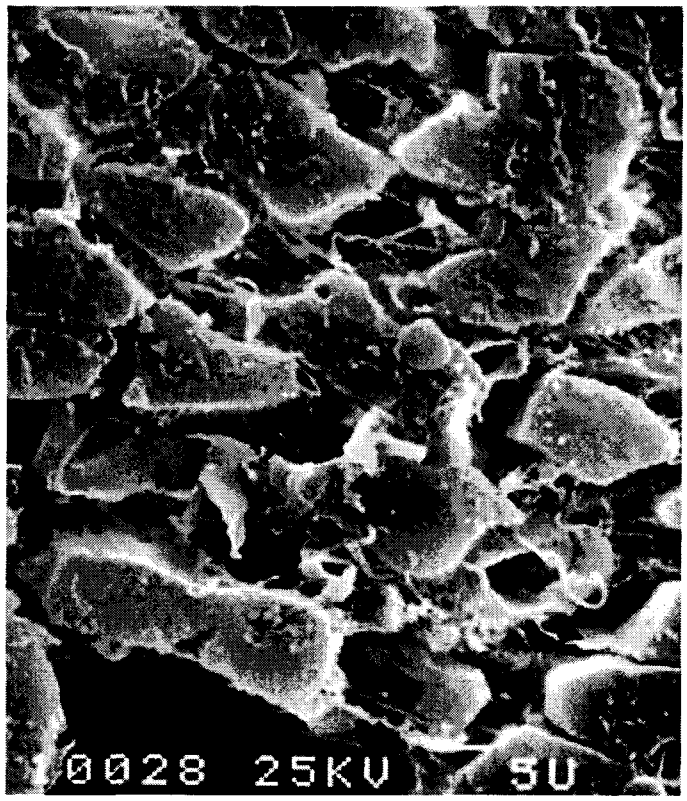

28032 25KV 500U

Figure 23. Photomicrograph of left ventricular myocytes from a sagittal section through the left ventricle, mid-point (50%) of length, in a BHR $CaCl_2$ animal (x1300).


0028 25KV 5U

CHAPTER IV

DISCUSSION

The present study examined the effects of supplemental dietary calcium on hypertension and hypertrophy in the borderline hypertensive rat hybrid. Dietary calcium affected BHR's systolic BP for at least 5 weeks (weeks 12, 16-19); the SBP of BHR $CaCl_2$ was attenuated compared to BHR control. Systolic blood pressure recordings between WKY and both BHR groups were significantly different throughout most of the study. Standard, quantitative morphometrical parameters of hypertrophy were analyzed to provide lateral evidence of disparities in the three strains' values of: heart weight: body weight ratio, ventricular weight: body weight ratio (hypertrophy index), right, left and interventricular septal wall thicknesses, and the myocyte cross-sectional area (MCSA). Overall, calcium had an affect on the body, heart and ventricular weight, systolic blood pressure, interventricular septal thickness, and left ventricular thickness in the BHR; BHR control values were significantly greater than BHR $CaCl_2$ in the above parameters.

INDEPENDENT VARIABLES

i. *Body Weight*:

The body weight among the three models assayed varied significantly from each other. BHR on 2% $CaCl_2$ gained the least amount of weight during the study (refer to Figures 1, 2 and 5) while BHR control on a normal calcium diet (0.98% calcium) represented an intermediate weight class. Normotensive WKY were the heaviest in weight at the conclusion of the 23 week study.

A study conducted by Lawler in 1981 reported 17 and 29 week old BHR control body weights at 402g and 461g respectively; the present study reports BHR control body weight at 17 and 23 weeks to be similar (449g and 508g respectively) (Lawler et al., 1981). BHR control subjects in this study were 12% and 10% heavier than Lawler's BHR group at 17 and 19 weeks respectively. Thus, BHR control was larger in mass than other age-matched BHR previously investigated.

Low BHR $CaCl_2$ body weights were supported by Karanja et al. (1987) and Stern et al. (1984) who documented comparable findings in SHR animals on high calcium diets, reporting a lower net weight gain in these animals than its SHR counterpart on a normal calcium diet. Although a calcium loaded animal weighs less than an animal on a normal calcium diet, Metz et al. (1988) have observed high calcium animals consuming up to 30% more than the control group; this parameter was not measured in the present study. Notably, however, the variations in body weight do not determine any diet-related alterations in blood pressure (Hatton and McCarron, 1994).

Lawler et al. (1987) conducted a study in which body weights were documented in both the WKY and BHR animals as a control parameter. Throughout the study's course the normotensive animal's body weight was consistently heavier than the BHR. Coincidentally, WKY in this study are larger than age-matched WKY in previous studies. WKY body weight was 21% and 71% greater in this study than Ayachi's and Lawler's WKY animals recorded weights at weeks 8 and 18 respectively (Ayachi, 1979; Lawler et al., 1987).

ii. *Systolic Blood Pressure*:

The BHR $CaCl_2$ group was hypothesized to have attenuated systolic blood pressure mitigated by the supplemental calcium diet. Collectively, BHR $CaCl_2$ overall mean systolic BP during the entire study was significantly attenuated in comparison to the BHR control group's overall mean SBP (refer to Figure 4). Dietary calcium may have provoked an attenuation in BHR $CaCl_2$ systolic blood pressure for at least 5 weeks, weeks 12 and 16-19, in comparison to the BHR control. However, supplemental calcium in the BHR did not sustain this hypotensive "diet effect" after week 19. A few more weeks on the calcium diet may be needed to establish a constant, attenuated SBP in the BHR $CaCl_2$. The BHR $CaCl_2$ SBP was not attenuated during weeks 5-11, 13-15 and 20-22; both BHR groups SBP were greater than WKY. Despite this fact, neither group attained the "borderline hypertensive range" (between 140 and 160 mmHg systolic blood pressure) at 15 weeks of age (Lawler et al., 1984).

The WKY established the lowest normotensive SBP range and tendency amid all three groups. Generally, WKY systolic BP readings were significantly lower than the two BHR groups (refer to Figure 3). The WKY normotensive trend has been confirmed in several papers involving BHR comparison trials performed by: Sanders et al. (1988), Lawler et al. (1984, 1987, 1988) and Lawler and Cox (1985). Hallback's 1975 paper documented WKY's systolic BP between one to seven months of age. The present study replicated WKY's SBP readings between weeks 5 through 22, which were analogous to Hallback's published values (Hallback, 1975). Both BHR counterpart's systolic BP's were significantly elevated in comparison to WKY. Lawler et al. (1984) established that 13 to 15 week old BHR animals have higher systolic blood pressures than age matched

WKY animals. During the course of the investigation WKY overall mean SBP was significantly lower than both BHR groups as depicted in Figure 4.

Previous SHR studies may provide some insight into the absence of calcium's effects on BHR's systolic BP. McCarron et al. (1981) alluded to a time-dependent relationship between dietary manipulation, blood pressure changes, and an animal's age (Hatton and McCarron. 1994). A strong correlation is thought to exist between these three dependent factors such that if supplemental dietary calcium was initially administered at 6 weeks of age, more than 2 months may transgress without a change in blood pressure (McCarron et al., 1981). Bukoski and McCarron (1986) suggest a minimum of 15 weeks is needed on a calcium-fortified diet to establish the "diet effect" in SHR animals. Several researchers concede that experimental data presents a plausible relationship between calcium and blood pressure that may be weak and applicable only in specific subsets of populations, both human and animal (Morris and McCarron, 1987). Hence one of the present study's main objectives was to test the BHR on a supplemental calcium diet. Perhaps SHR's defective calcium metabolism is not inherited by its BHR offspring, is manifested in the BHR to a lesser degree, or a longer duration of dietary calcium manipulation should be instigated in younger animals.

GROSS MORPHOLOGY OF THE HEART

i. *Heart Weight: Body Weight Ratio*:

Elevated heart weight: body weight ratios have been employed as an indirect parameter in assessing hypertrophy in an animal. Lawler and his colleagues have conducted two studies validating the correlation between hypertrophy, hypertension, and heart weight: body weight ratios as measures of cardiac pathology in hypertrophy

(Lawler et al., 1981). Heart weight-to-body weight ratio results from both of Lawler's studies, in comparison to the present study, allude to normal BHR morphology in both BHR control and $CaCl_2$ groups.

BHR control and BHR $CaCl_2$ heart weight: body weight ratios are not significantly different (0.00316 and 0.00324 respectively). Neither BHR control nor $CaCl_2$ heart-to-body weight ratio is at a hypertrophic range, approximately 0.00372 at 29 and 39 weeks (Lawler et al., 1981), but is representative of normal BHR morphology due to maturation (Lawler et al., 1981; Lawler, Barker, Hubbard and Schaub, 1980). Previous studies reported the ratio at approximately 0.0035 for the control BHR group at 29 and 39 weeks of age. Calcium failed to have a dietary effect in reducing the BHR heart weight: body weight ratio parameter over the course of this study.

As our data demonstrates, WKY had the lowest heart: body weight ratio at 0.00267. The WKY heart weight-to-body weight ratio in this study is similar to previously reported data (0.00253) published in a 1994 study (Vulpis et al., 1994). The WKY group's heart: body weight value was significantly less than both BHR groups (refer to Figures 5 and 6).

ii. *Ventricular Weight: Body Weight Ratio (Hypertrophy Index)*:

Vulpis et al. (1994) reported a ventricular weight: body weight ratio in their investigation as an indirect parameter of hypertrophy for SHR and WKY animals. This ratio was used to coin the term "index of hypertrophy" or "hypertrophy index". The hypertrophy indexes of all three groups followed the identical trend as heart weight: body weight ratios.

The present study is unprecedented in reporting hypertrophy indexes in both the BHR control and BHR $CaCl_2$. BHR control and $CaCl_2$ indexes are not significantly different and parallel the heart: body weight trend. It is then deduced that both BHR control and $CaCl_2$ have normal cardiac morphology consistent with their hypertensive genetic background, age and natural maturation process. WKY had the lowest hypertrophy index value and was significantly different from both BHR control and BHR calcium (refer to Figures 5 and 7). This hypertrophy index value was quantitatively equivalent to Yang et al.'s findings in their 1997 inquiry of 24 week old WKY animals; these findings authenticate the control group's data in the present study (Yang et al., 1997).

Lawler and Cox (1985) established a positive correlation between systolic blood pressure and the heart weight-to-body weight ratio in BHR animals in their investigation, this correlation has since been extrapolated to include the hypertrophy index (ventricular weight: body weight ratio). The present study corroborates their findings further in establishing a dependent relationship between blood pressure and cardiac pathology. Indeed hypertrophy did not take place in WKY due to the normotensive SBP range and typical heart and/or ventricular: body weight ratios. Both BHR counterparts did maintain significantly higher systolic BP's than WKY throughout the study's duration. Intuitively, based on the higher systolic BP's of the BHR animals, the heart weight: body weight ratios and hypertrophy indexes are elevated in the BHR hybrids due to the dependent nature of blood pressure and cardiac pathology and blood pressure and heart-to-body weight ratios (Lawler et al., 1981).

ULTRASTRUCTURE OF THE HEART

Regardless of the origin of cardiac hypertrophy, pressure or volume overload, both conditions elicit a change in myocyte shape and morphometry. Ultimately the hypertrophy is manifested in gross anatomical alterations in the ventricular geometry and an increase in ventricular wall thickness (Gerdes, 1992). Campbell et al. confirm in their 1989 study the presence of hypertrophy in both the right and left ventricles in association with pressure overload (Campbell et al., 1989). Another study in 1989 performed by Cuspidi and his cohorts reaffirm the presence of hypertrophy in both the right and left ventricles and a direct correlation in both due to hypertension (Cuspidi et al., 1989). Thus this study evaluated and measured the ventricular wall thickness in the right and left ventricles, as well as the interventricular septum.

i. *Right Ventricle*:

Cardiac hypertrophy in the SHR, and the BHR, is due to pressure overloading which elicits an increase in wall thickness and myocyte cross-sectional area (MCSA) with minimal to no alterations in chamber volume (Gerdes, 1992). This hypertrophic response to increased afterload is thought to affect not only the left ventricle, but also concomitantly alters the right ventricle in human and SHR subjects (Campbell et al., 1989; Cuspidi et al., 1989). Preceding studies have not measured the right ventricular wall thickness in the WKY or BHR, however right ventricular weight has been documented by Pfeffer et al. (1979) in the WKY at 13 and 25 weeks of age. Previous reports have shown that the WKY right ventricle weight significantly surpassed the age-matched SHR weight at 13 and 52 weeks of age; at 25 weeks both strain's right ventricle weight were equivalent and increased proportionately with the 12 weeks of maturation

(Pfeffer et al., 1979). Later in Pfeffer's inquiry, 90 weeks, the SHR right and left ventricles showed disproportionate hypertrophy in contrast to the normotensive WKY. Ventricular weight is directly proportional to ventricular thickness, thus implying WKY right ventricular wall thickness surpasses SHR in weeks 13 and 52, and is equivalent at week 25.

The present study compared the right ventricular wall thickness among the three groups at 23 weeks of age. WKY's right wall thickness was significantly greater than BHR $CaCl_2$ (refer to Figure 8). There was no significant difference between BHR control and BHR $CaCl_2$ or BHR control and WKY right ventricular wall thickness.

ii. *Interventricular Septum*:

The interventricular septum was measured among all three groups as a control amid the three wall thicknesses evaluated. Pfeffer et al. (1979) exhibited in their study that the septal thickness does not experience a concomitant increase with elevated left ventricular wall thickness in the SHR animal. Still, the septal thickness is greater in the SHR model and may play a small role in eliciting decreased ventricular compliance. BHR control septal wall thickness was significantly greater than BHR $CaCl_2$ and WKY, while septal wall measurements were nearly equivalent between the normotensive WKY and BHR calcium experimental group (refer to Figure 9). It is plausible that the septal wall thickness was proportionally higher in the BHR control in association with a thickened left ventricular wall and increased overall wall rigidity.

iii. *Left Ventricle*:

The left ventricular wall is a common pathological marker of hypertrophy due to

pressure overloading, specifically in the SHR. An increased afterload expedites the architectural remodeling of the left ventricle. The outcome is expenditure of more energy and compensation by hypertrophying/thickening of the LV or left ventricular hypertrophy, LVH (Frenzel and Feimann, 1984). Several longitudinal and lateral morphometric studies of the heart have employed left ventricular wall thickness as a standard measure of pathological pressure overload hypertrophy. Engelmann et al. (1987) measured longitudinal variations among the SHR and WKY groups in left ventricular wall thickness up to 2 years of age. The left ventricular wall thickness of WKY animals at 24 weeks quantitatively matched the LV wall thickness of 23 week old WKY specimens in the present study. In this study, the WKY LV wall thickness was 1.7 mm compared to 1.65 mm found in Engelmann's study.

The BHR control left ventricular wall thickness was significantly greater than BHR $CaCl_2$. The BHR $CaCl_2$ had the smallest LV wall, but was not significantly different from WKY (refer to Figure 10). The left ventricular wall thickness was significantly elevated in BHR control (1.93 mm) and was similar to age-matched SHR LV wall thickness, 2.13 mm (Engelmann et al., 1987). The BHR control group may have thickened LV wall morphometry due to augmented size and/or number of myocytes, the amount of vasculature, or connective tissue elements (i.e. interstitial fibrillar collagen types I or III) (Engelmann et al., 1987). A possible rise in myocardial fibrillar collagen may account for the borderline hypertensive control animals having a significantly thicker left ventricle and interventricular septum. The structural integrity of the BHR control's myocardium, specifically the phenotypic collagen of the left ventricle and septum, may have differentiated from the other two groups investigated due to an

independent relationship between blood pressure and collagen phenotypes (Mukherjee and Sen, 1993).

BHR control and $CaCl_2$ myocardial gross morphology ratios lack significant differences between their heart weight-to-body weight ratios and hypertrophy indexes; both parameters were significantly greater than normotensive WKY. However, BHR $CaCl_2$ is significantly lower than BHR control and WKY in both cardiac and ventricular weight. These results suggest that calcium normalized BHR's gross myocardial pathology. Ultrastructurally, BHR $CaCl_2$ interventricular septal wall and left ventricular wall thickness were in the same range as the normotensive WKY, suggesting normal cardiac ultrastructure in BHR $CaCl_2$. Alternatively, the BHR control demonstrated two ultrastructural ventricular characteristics of a hypertrophied heart that are also manifested in SHR: 1) elevated septal wall thickness, and 2) thickened left ventricle (Pfeffer et al., 1979; Engelmann et al., 1987). In summary, the gross morphological and ultrastructural findings in this study allude to a hypertrophied BHR myocardium and normal BHR $CaCl_2$ heart with calcium representing the only confounding variable between the two groups.

iv. *Myocyte Cross-Sectional Area*:

Vliegen and his colleagues indicated that heart and ventricular weight provide global information in research. Inversely Vliegen established that individual myocyte size discloses specific cellular information in a study paradigm (Vliegen et al., 1987). Kawamura et al. were forerunners in ultrastructural cardiac hypertrophy investigations in the SHR model in 1976. In this Japanese inquiry the degree of cardiac hypertrophy was assessed upon cross-sectional estimations of the muscle/myocyte fibers; augmented

cross-sectional areas are indicative of hypertrophy (Kawamura et al., 1976). The myocyte cross-sectional area (MCSA) of both ventricles has been proven to naturally increase with age in SHR and WKY models (Engelmann et al., 1987; Fraticelli et al., 1989). Engelmann et al. found that during the maturation (6-12 months) and aging (12-18 months) phases of cardiac hypertrophy the SHR specimen experienced distinctive elevation in the MCSA index in the left ventricle as a corollary of pressure overloading (Engelmann et al., 1987). Myocyte morphometry has since been established as a landmark in elucidating the development and progression of left ventricular hypertrophy (LVH) in correlation with pressure overload; pressure overload is the primary cause of LVH in the SHR model (Pfeffer et al., 1979; Campbell et al., 1989; Cuspidi et al., 1989). In 1992 Gerdes reported that an increase in the myocyte cross-sectional area is a fundamental feature exploited in distinguishing the origin of hypertrophy as pressure overload in contrast to volume overload (Gerdes, 1992).

Pressure overload (increased afterload/systolic wall stress) has been directly associated with concentric hypertrophy and an elevated myocyte cross-sectional area (MCSA) as the foremost feature of LVH (Gerdes, 1992; Campbell et al., 1989). Moreover, growth in LV wall thickness during the first 6 months of life in WKY and SHR animals have been primarily attributed to a general increase or hypertrophy of individual cardiomyocyte cells (Engelmann et al., 1987). Thus both normal and pathological ventricular hypertrophy is elicited by an increase in MCSA (Engelmann et al., 1987). Hence, one of this study's main objectives was to measure and report unprecedented MCSA values for high and normal calcium BHR subjects; MCSA values

are a standard used in ascertaining the presence of normal or pathological ventricular hypertrophy.

MCSA morphometrical data is considered to be a direct assessment of cardiac hypertrophy, explicitly LVH. Morphometric MCSA measurements were assayed in the left ventricles of all animals investigated in this study. All three groups' MCSA values failed to demonstrate any statistical significance from one another (refer to Figure 11).

The hypertrophied left ventricle and interventricular septum in the BHR control may induce the following hypertrophic response as manifested in the SHR: increased collagen concentration, thickening of existing fibrillar collagen, addition of newly synthesized collagen, and/or a gradual reduction in type I to III collagen ratio (Caulfield and Janicki, 1997; Yang et al., 1997). In addition, the hypertrophied ventricular walls may be the product of an accumulation of connective tissue elements, inflammatory cells, and/or fibrosis. This summation may be the most inclusive in elucidating the origin of the BHR control hypertrophied left ventricular and septal wall in genetic correlation to cardiac hypertrophy in its SHR maternal parent.

SUMMARY

In summary, results of this study suggest that supplemental dietary calcium does attenuate hypertrophy in the BHR animal. The influence of dietary calcium on systolic blood pressure appears to be variable with time. Perhaps the "diet effect" requires a longer time course to significantly attenuate the SBP in BHR $CaCl_2$ animals. However, BHR $CaCl_2$ animals manifested septal and left ventricular wall thicknesses and myocyte cross-sectional areas similar to the non-pathological WKY heart, while BHR control exhibited characteristics of the hypertrophied heart. While the heart weight-to-body

weight ratio and hypertrophy index value of BHR $CaCl_2$ were similar to hypertrophic values, this may be due to the animal's low body mass disproportionately weighting the ratios. Presumably, the calcium is able to act independently of the systolic BP in myocardial morphometric remodeling. This independent relationship explains the BHR $CaCl_2$ animal's attenuated hypertrophy; the calcium enables each myocyte to generate more force per unit muscle. Whereas the BHR control myocytes produce less force per unit muscle, therefore more myocardial tissue mass was needed to contrive the required force for contraction. Calcium and its antihypertrophic, as well as antihypertensive effects may provide a potential solution to hypertension and all of its associated sequelae.

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May 12, 1997

Dr. Johanna Krontiris-Litowitz
Department of Biological Science
UNIVERSITY

Dear Dr. Krontiris-Litowitz:

The Institutional Animal Care and Use Committee of Youngstown State University has approved the modifications to your Protocol #97-007 entitled, "Supplemental Dietary Calcium Attenuates the Development of Stress-Induced Hypertension." Please note that Dr. Horne, our consulting veterinarian, made and initialed one change on page 6, 5.a. from (ii) to (iii), "No anesthetic, analgesic, or tranquilizer drugs will be administered to eliminate or minimize pain."

You must adhere to the procedures described in your approved request; any modification must first be authorized by the Institutional Animal Care and Use Committee.

Sincerely,

Dr. Peter J. Kasvinsky
Dean of Graduate Studies

PJK:cc

c: Dr. Paul Peterson, Chair
Department of Biological Sciences
IACUC Committee