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BLOOD PRESSURE IN RELATIONSHIP TO DIETARY AND WHOLE BLOOD IONIC MAGNESIUM IN NORMOTENSIVE SUBJECTS

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ABSTRACT

The primary purpose of this investigation was to examine the relationship between dietary and whole blood ionic magnesium (iMg) and measures of blood pressure (BP) (SBP-systolic BP; DBP-diastolic BP; MAP-mean arterial pressure) in a normotensive sample. A secondary purpose was to examine the relationship between dietary and whole blood ionic calcium (iCa), sodium (iNa), and potassium (iK) and measures of BP. Comparisons between BP and PAS (physical activity score) and BMI (body mass index) were also made. Subjects (119 females; 82 males) were physically active students with an average age of 21.1 years. Participants underwent three BP measurements and whole blood samples were collected and analyzed for iMg, iCa, iNa, and iK. Physical activity levels were assessed by a physical activity questionnaire (PAQ) and mineral intakes were determined by analysis of a three-day dietary record. Examination of each factor with BP was performed in a 2X2 fashion using the McNemar chi-square and kappa test statistics. Relative Risks (RR) and Odds Ratios (OR) were also determined. No protective influence was observed between BP and dietary or ionic measures of magnesium. Negative associations between BP and iNa, dietary Na, and BMI were demonstrated. It was concluded that in normotensive subjects, dietary and ionic magnesium does not demonstrate a relationship with BP.

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CHAPTER ONE: INTRODUCTION

1.1 PURPOSE

The primary purpose of this investigation was to examine the relationship between dietary and whole blood ionic magnesium (iMg) and measures of blood pressure (BP) (SBP-systolic BP; DBP- diastolic BP; MAP-mean arterial pressure) in a normotensive sample. A secondary purpose was to examine the relationship between dietary and whole blood ionic calcium (iCa), sodium (iNa), and potassium (iK) and measures of BP. Comparisons between BP and PAS (Physical Activity Score on Canadian Physical Activity Fitness and Lifestyle Assessment questionnaire) and body mass index (BMI) were also made.

1.2 IMPORTANCE OF THE STUDY

Cardiovascular diseases (CVD) such as coronary heart disease (CHD) and stroke are the leading cause of death in all industrialized countries (Kaplan, 1986). Since hypertension is the most prevalent risk factor for CVD (Whelton & Klag, 1989), it has been recognized by the World Health Organization (WHO) as a major public health problem (Yamori et al., 1990). Recommendations of the WHO have placed emphasis on a community-wide strategy for the primary prevention of hypertension and CVD based on dietary improvement (Yamori et al., 1990). Whelton and Klag (1989) comment that our traditional approach to the prevention of blood pressure (BP) related cardiovascular diseases is to detect and treat those at highest risk of having high blood pressure. They further suggest that this strategy is an inadequate response to the current epidemic of blood pressure related CVD and should be coupled with a population-based approach that seeks to reduce blood pressure in the entire community. The WHO advocates dietary improvement as such an approach (Yamori et al., 1990).

Over the years, there has been a belief that a diet high in sodium or low in magnesium potassium, or calcium predisposes people to high BP (Burgess et al., 1999). This belief is supported by epidemiological, physiologic, and clinical studies that indicate that the mineral elements magnesium (Mg), potassium (K), calcium (Ca), and sodium (Na) play a central role in vascular smooth muscle contraction and the normal regulation of BP

(Karpannen, 1991; Burgess et al., 1999). This is of particular importance as it is an increased vascular resistance that accounts for raised BP in most patients with hypertension (Ives, 1989; Karpannen, 1991). These regulatory agents (Mg, K, Ca, Na) are usually derived from various foods and drinks, and are occasionally administered as drugs (Karpannen, 1991). Once in the blood, the transport and interaction of these ions (Na+, K+, Ca2+, Mg2+) across the vascular smooth muscle membrane influences the intracellular calcium ion concentration and contractile activity of the cell (Ives, 1989). A rise in intracellular Ca2+ increases the formation of the calcium-calmodulin complex, triggering vascular smooth muscle contraction and increasing blood pressure (Karpannen, 1991). Most hypothesis linking abnormalities in ion transport to hypertension considers intracellular Ca2+ as the final common mediator of vascular tone (Ives, 1989).

Among the cationic regulators of vascular smooth muscle contraction, Mg2+ has a major role as it directly competes for Ca2+ binding sites at the cell membrane (Altura, Altura, Carella & Turlapaty, 1981a). According to Altura et al. (1981a), the higher the extracellular magnesium concentration, the more effective it is at blocking a rise in intracellular calcium. This has become the physiologic rationale for the reported associations between hypertension and sub-optimal magnesium intakes and ionic blood concentrations. (Dyckner and Wester, 1983; Altura, 1984; Seelig, 1994; Whang & Whang, 1994; Shirey, 1995; Altura & Altura, 1996;). This may also counteract the hypertensive effects of excess sodium intake (Karpannen, 1991).

Most studies involving mineral intakes and BP have involved the clinical supplementation of Mg, K, or Ca, (Kaplan, 1986; Burgess et al., 1999) and/or dietary restriction of Na (Muntzel & Drueke, 1992) in hypertensive (symptomatic) individuals. The results of epidemiological studies of the relationship between dietary minerals and BP in normotensive (asymptomatic) individuals have been inconsistent (Burgess et al, 1999). Consistency is important in order to establish dietary recommendations on mineral intakes for the prevention of hypertension. This study similarly examined the relationship between dietary mineral intakes and BP in normotensive individuals. Of primary interest was the relationship between Mg and BP in view of the potentially beneficial and protective influence suggested by Whelton and Klag (1989), Shirey (1995), and Altura and Altura (1996). Additionally, the ionic concentration of Mg2+ and other blood

cations (Ca2+, Na+, K+) was determined in whole blood, using an ion selective electrode (ISE) (Nova Biomedical, Canada: model 11-3C). This allowed for the examination of Mg2+, Ca2+, Na+, and K+ in the form in which they exert influence on vascular smooth muscle contraction and blood pressure (Shirey, 1995). As dietary estimates may not necessarily reflect the cationic blood levels of these minerals, the precise determination of ionic concentrations improved comparisons with BP by providing a direct physiologic measure.

1.3 DEFINITIONS

(1) Ionic Magnesium (iMg): a measure of that fraction of whole blood magnesium which is physiologically active; neither bound to other ions or protein (Elin, 1992).

(a) *Normal iMg range (mmol/L):* 0.460 - 0.600 mmol/L (Ising, Bertschat, Gunther, Jeremias, & Jeremias, 1995)

(b) *Deficient iMg range (mmol/L):* $\leq$ 0.450 mmol/L (Ising et al., 1995)

(2) Total Magnesium (tMg): a measure of whole blood magnesium that includes ionic magnesium, complexed magnesium, and protein bound magnesium (Elin, 1992).

(3) Ion Sensitive Electrode (ISE): allows for the precise determination of ions such as magnesium, without interference from other cations, enabling the determination of independent blood ionic (iCa, iNa, iMg, iK) profiles (Altura, 1996).

(4) Diastolic Blood Pressure (DBP): Pressure of blood (mmHg) against the vasculature, during the relaxation phase of the heart (Guyton, PG 165.).

(5) Systolic Blood Pressure (SYS): Pressure of blood (mmHg) against the vasculature, during the contractile phase of the heart (Guyton, pg.165).

(6) Mean Arterial Pressure (MAP): The average blood pressure during the cardiac cycle determined from DBP and SBP by the equation MAP = DBP + 1/3(SBP-DBP) (Guyton, pg165).

(7) Normotensive BP: A DBP< 90 *and* a SBP<140 (Tomiak & Gentleman, 1993).

(8) Hypertensive BP: A DBP $\geq$95 *or* a SBP $\geq$160 (Tomiak & Gentleman, 1993).

1.4 ABBREVIATIONS

ATP	- Adenosine Triphosphate
BMI	- Body Mass Index (kg/m^2)
BP	- Blood Pressure
Ca	- Mineral Calcium (dietary)
Ca2+	- Ionic Calcium (extracellular or intracellular calcium ions)
DBP	- Diastolic Blood Pressure
EC	- Extracellular
Hct	- Hematocrit
iCa	- Intracellular
iCa	- Ionic Calcium (referring to the calcium measure in blood)
iK	- Ionic Potassium (referring to the potassium measure in blood)
iMg	- Ionic Magnesium (referring to the magnesium measure in blood)
iNa	- Ionic Sodium (referring to the sodium measure in blood)
ISE	- Ion Sensitive Electrode
K	- Mineral Potassium (dietary)
K+	- Ionic Potassium (extracellular or intracellular potassium ions)
MAP	- Mean Arterial Pressure
Mg	- Mineral Magnesium (dietary)
Mg2+	- Ionic Magnesium (extracellular or intracellular magnesium ions)
Mg2+-ATP	- Magnesium-Adenosine Triphosphate
Na	- Sodium (dietary)
Na+	- Ionic Sodium (extracellular or intracellular sodium ions)
Na+/K+-ATPase	- Sodium-Potassium Pump
OR	- Odds Ratio
PAS	- Physical Activity Score
RR	- Relative Risk
SBP	- Systolic Blood Pressure
tMg	- Total Magnesium

1.5 LIMITATIONS

(1) The dietary analyses depended on the consistency and accuracy of subjects' self-reported journals. Food scores were generated using software and food database by NutriQuest (McGraw-Hill, 1998). Scores were estimates based on the average micronutrient and macronutrient composition of common food items. As such, it is acknowledged that the assessment of dietary intakes were inherently limited in their degree of accuracy. Attempting to improve this accuracy, dietary records were taken for three-day periods (2 weekdays and 1 weekend day) and averaged.

(2) The accuracy of blood pressure measurements was a source of potential error. Minimizing this error and improving blood pressure estimates, SBP and DBP measurements were taken three times by the same technician and reported as the mode score. (If no modal score resulted, the median score was then taken).

(3) The analysis of ionic magnesium and other hematological variables was dependent on the accuracy of the ion selective electrode (ISE). The ISE used was the multi-test Stat Profile Ultra Analyzer model 11-3c (Nova Biomedical Canada Ltd., Mississauga, ON).

1.6 DELIMITATIONS

This study was delimited to 201(82 male; 119 female) Lakehead University Kinesiology students. These students were predominantly in their 3rd year of study, having an average age of 21.4 years for males and 20.9 years for females. This was a physically active and asymptomatic sample.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

This literature review examines the physiologic roles, uptake and metabolism of magnesium with a primary focus on the implications for the regulation of blood pressure. Clinical, epidemiological and experimental evidence regarding magnesium(iMg and dietary Mg) and blood pressure (BP) will be presented. Additionally, current theories as to the role of magnesium deficiency in the etiology of hypertension will be stated. Included in this section will be a discussion of other important and relevant blood ions, Ca2+, K+, and Na+. The final section of this review will examine dietary and lifestyle factors that have been found to correlate to BP.

2.2 MAGNESIUM: AN OVERVIEW OF ITS PHYSIOLOGIC ROLES

According to Altura and Altura (1996), magnesium (Mg2+) is a cofactor for over 300 enzymes, which play central roles in the control of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, and vasomotor tone. These physiologic roles are the consequence of biochemical activities in which Mg2+ is involved in cellular energy production, protein synthesis, adenylate cyclase synthesis, and electrolyte transport. These biochemical functions occur intracellularly, with Mg2+-ATP acting as an obligate substrate for a wide variety of enzymes (Altura & Altura, 1996).

The involvement of Mg2+ in the storage and utilization of energy is through the activation of phosphatases and phosphokinases (Altura & Altura, 1984; 1991a). In turn, these phosphatases exert control on the intermediates of metabolism through such rate-limiting enzymes as hexokinase, pyruvate dehydrogenase, and enolase. In total, Mg2+ is involved in the regulation of 7 enzymes in glycolysis, and 4 enzymes in the citric acid cycle. As a consequence, it is integral to the metabolism of fat, carbohydrate, and protein (Altura & Altura, 1984; 1991a).

Mg2+ is also involved in the regulation of deoxyribonucleic acid(DNA) and ribonucleic (RNA) synthesis. Within the cell's nucleus, Mg2+ is bound to the pentose phosphate backbone of DNA, regulating DNA synthesis and cell growth (Altura & Altura, 1996). Additionally, Mg2+ controls hemoglobin function as most of the ATP in

red blood cells is bound to the mineral in the Mg2+-ATP complex. There is also evidence that the synthesis of erythrocyte 2,3-diphosphoglycerate (2,3-DPG) is Mg2+ dependent through the activation of hexokinase. Thus, the delivery of oxygen to working muscles may be controlled by Mg2+, implying that the maintenance of adequate Mg2+ levels is important for the efficient delivery of oxygen (Casoni et al.,1990).

Magnesium also exercises control over electrolyte balance across the cell membrane through its obligate substrate association with the sodium-potassium (Na+-K+)-pump through Mg2+-ATP. This, in turn, "fuels" the pump so that K+ can be pumped into the cell, and Na+ out of the cell, establishing the electrical gradient necessary for the conduction of action potentials(Shirey, 1995; Altura & Altura, 1984; 1991a) Similarly, the Ca2+-ATPase pump is associated with Mg2+-ATP, and is involved in pumping Ca2+ out of the cell, lowering intracellular levels of Ca2+, and allowing for muscle relaxation (Altura & Altura, 1996). Additionally, Mg2+ competes with Ca2+ for extracellular membrane binding sites on the muscle cell (smooth, cardiac, or skeletal), decreasing Ca2+ binding, entry into the cell, and release from the sarcoplasmic reticulum(Altura & Altura, 1996). In the presence of a decreased extracellular Mg2+ there is a greater uptake of Ca2+ into the cell (Shirey, 1995). As calcium initiates muscle contractile processes, elevated calcium levels can lead to increased contraction and hypertension, tachycardia, neuromuscular irritability and muscle cramps (Shirey, 1995). Conversely, if one has an elevated extracellular Mg2+, intracellular Ca2+ will decrease, resulting in hypotension and bradycardia(Shirey, 1995). These processes, however, will be elaborated on in the discussion of magnesium's role in the etiology of hypertension.

Finally, Mg2+ is an antioxidant, inhibiting the formation of free radicals, which oxidize essential molecules and cause cellular damage (Rice, 1983). Damage can occur, for example, when cholesterol is oxidized, availing it for easier attachment to the arterial wall, increasing the risk for heart disease. Mg2+ also acts as a sedative on nerves and muscles by decreasing or blocking the release of acetylcholine at nerve endings and has also been found to influence temperature regulation, wound healing, and immunocompetence(Rice, 1983).

2.3 MAGNESIUM: INTAKE, UPTAKE & METABOLISM

2.3.1 Dietary Intake

Legumes, whole grains, nuts, meat, fish, eggs, peanut butter, cheese, milk, and fruit are rich sources of Mg(Rice, 1983). Lukaski (1995) reports that examples of foods with high Mg content include spinach (1cup cooked = 150mg), beans and black-eyed peas (1cup cooked = 100mg) and tofu (1 cup = 300mg). With a normal diet, 30-40% of the ingested Mg is absorbed through the jejunum and ileum (Altura & Altura, 1991a). Urinary analysis has revealed that waterborne Mg2+ is absorbed 30% better than dietary Mg. Additionally, there is considerable diurnal variation in tMg status, with morning values being 50-70% lower. (Lowik, Groot, & Binnerts, 1982).

The absorption of Mg2+ may be influenced by other dietary factors. Rice (1983) states that Vitamin D assists in the uptake of Mg2+. Lukaski (1995) reports that excessive fibre intake may decrease Mg2+ due to a rapid intestinal passage time, resulting in a negative Mg2+ balance. Typical western diets, which are high in protein and/or fat, do not seem to adversely affect Mg2+ balance despite the fact that these diets are typically lower than the recommended daily intake (RDI) of 350mg of Mg/day (Rice, 1983). If an individual is not able to acquire sufficient Mg2+ from their diet, Mg2+ supplementation may be beneficial. Supplemental Mg2+ may be taken as gelatin capsules or tablets with Mg2+ salts. In general, Mg2+ salts have a fractional absorption of about 20% (Lukaski, 1995).

2.3.2 Absorption, Excretion & Metabolism

The absorption of Mg2+ is dependent on intake, intestinal transit time and the absorption of water. Normally, approximately 40% of the dietary Mg2+ is taken in at the jejunum and ileum. This fractional values changes, however, with intake. At extremely low intakes, the fractional absorption of Mg2+ is increased to 65%; meanwhile, at extremely high intakes, the fractional absorption of Mg2+ can be as low as 11% (Altura & Altura, 1991a).

Under normal circumstances, the kidney is the prime regulator of Mg2+ balance in the body (Altura & Altura, 1984). Approximately, 70-80% of the plasma Mg2+ is filtered at the glomerulus, and 20-30% is reabsorbed in the proximal convoluted tubule. This reabsorption is reduced with antibiotics, diuretics, thyroxine, calcitonin, and hypercalcemia (Rice, 1983). Repetitious use of antacids will increase Mg2+ levels

beyond 2.5mmol/L, becoming toxic. Several disease processes such as chronic diarrhea, diabetes mellitus, and renal tubular disorders may decrease Mg2+ (Elin, 1992).

Serum Mg2+ is maintained with a narrow range by the kidney and small intestine. In instances of magnesium depletion, both organs increase Mg2+ absorption to counter the imbalance. If depletion persists, bone stores give up Mg2+ to the plasma (Elin, 1992).

Serum magnesium levels (tMg or iMg) reflect not only kidney function, but also acute dietary changes. Either an increased/decreased intake and/or excretion are reflected in serum measures (Elin, 1992; Whang et al., 1994). For example, during a three-week period, 24 normal subjects consumed a low Mg diet and became Mg deficient (less than 0.6mmol/L tMg in serum)(Whang et al., 1994). Additionally, magnesium status measures may not reflect direct intake, as levels are influenced by fat and calcium intakes. These serve to reduce Mg2+ levels in the blood (Seelig, 1994). Also, increases in insulin levels result in the shifting of extracellular Mg2+, intracellularly (Altura & Altura, 1996).

Finally, ethnicity and sex have been postulated to play a role in Mg homeostasis. Differences in iMg and tMg have been observed between both white and black hypertensive and normotensive populations (Resnick, Bardicef, Altura, Alderman, & Altura, 1997) suggesting that ethnicity may influence Mg metabolism. As well, Yamori et al. (1990) reported an inverse relationship between magnesium and blood pressure in women only. This might be attributable to differences in magnesium metabolism between men and women. Preuss (1993) reported that women retain magnesium better than men, and proposed estrogen as a possible explanation.

2.4 MAGNESIUM: DISTRIBUTION & ASSESSMENT

There is still much uncertainty as to the exact proportion and distribution of magnesium throughout the body. Total body stores are estimated between 21-28g (Elin, 1992). Approximately 60% is stored in bone, 20-27% in skeletal muscle, and less than 1% of total body magnesium is in the blood (of which. 0.3% is present in the serum) (Elin, 1992). These values are estimations as there is some flow between magnesium stores and the circulating extracellular environment. Extracellularly, magnesium is 33% protein bound, 12%complexed to anions, and 55% is in the free ionized form. Ionized magnesium (iMg) is the most important fraction as it represents the form of magnesium that is physiologically active or available (Altura & Altura, 1991c; Elin, 1992). Previously, the assessment of magnesium has typically been based on total magnesium measures (tMg). Now, iMg can be distinguished from tMg (Altura &Altura, 1984).

Additionally, it should be mentioned that iMg levels do not always correlate with tMg levels in serum (Altura & Altura, 1996). Altura & Altura (1996) noted that even borderline hypertensives (140/90 to 150/95) usually exhibited significantly depressed iMg, but not tMg levels. This is a further argument for the use of iMg in the diagnostic assessment of magnesium status as tMg levels are more consistently maintained. Al-Ghamdi et al. (1994) reports that tMg is maintained in a narrow range by the kidney and can be normal in the presence of Mg2+ deficiency. If depletion is severe enough, the Mg2+ will result in altered tMg values. The normal reference interval for the concentration of tMg in adults is 0.75-0.96mmol/L (Elin, 1992). Values below this are considered to be Mg2+ deficient. Ionic Mg2+, however, is a more representative indicator of magnesium status with intracellular values corresponding to those found in whole blood, plasma, and serum. As well, iMg appears to cross the cell membrane relatively quickly which would suggest that the two reservoirs are in dynamic equilibrium (Shirey, 1995). This has important implications when trying to assess whole body Mg2+ status, since, as stated previously, tMg measures are not reflective of intracellular concentrations. However, normal serum iMg values are not well established as ranges from 0.55-0.67 mmol/L have been reported by Elin (1992), and Ising et al. (1995) report a normal range of 0.46-0.60 mmol/L. Ionic magnesium deficiency has also been defined

by Elin (1992) as those less than 0.55mmol/L (Elin, 1992); whereas, Ising et al. (1995) states that values less than 0.45mmol/L are deficient.

Magnesium levels can be assessed in various tissues such as serum, red blood cells, muscle, and mononuclear blood cells. Total magnesium content in one tissue may not relate to another. Also, magnesium can be assessed physiologically with balance, renal excretion, retention, and isotope studies. Now, most commonly, is the assessment of serum iMg. This can be determined using an ion-selective electrode (ISE), fluorescent indicators, or nuclear magnetic resonance spectroscopy (Elin, 1992). Use of ISE has become preferable in that accurate linear measurements of iMg can be determined rapidly, without interference from other cations or trace metals (Altura & Altura, 1991c). Serum is generally used rather than plasma since the anticoagulant in plasma can contaminate or affect the assay (Elin, 1992).

2.5 MAGNESIUM AND BLOOD PRESSURE(BP): THE EVIDENCE

Recently, evidence from epidemiological, clinical, and experimental studies has accumulated that indicates that Mg has an important role in regulating ion transport and vascular tone and reactivity. As such, Mg deficiency may be an important etiologic factor in the development and treatment of hypertension. These mechanistic processes will be discussed in section 2.6. Presented here, however, is a review of some of the evidence that links magnesium to blood pressure.

2.5.1 Epidemiological

Interest in magnesium and its role in cardiovascular disease first arose in the 1950's as ecologic studies around the world found an inverse relationship between water hardness and the incidence of cardiac fatalities (Altura & Altura, 1991b; Whelton & Klag, 1989). At that time, it was reasonable to think that a "common factor" would explain such a trend. In recent years, the observation that magnesium contributes to water hardness and has known physiologic roles related to cardio-protection, has led to it becoming the link or "common factor" in the "water-story" (Anderson & Le Rich, 1971; Marier, 1985; Whelton & Klag, 1989).

Furthering these observations, comparisons between water hardness and blood pressure have been made. In 24 West Texan communities, Dawson et al. (1978) demonstrated an inverse relationship between drinking water magnesium, and hypertension (as cited in Whelton & Klag, 1989). While numerous investigations have been done at the community level, few studies have investigated the relationship between magnesium intake and blood pressure within individuals. The Honolulu Heart Study was performed at the individual level and found that 24 hour intake of magnesium (as measured by dietary recall) was inversely related to blood pressure (Joffres, Reed, & Yano, 1987 as cited in Whelton & Klag, 1989). On a larger scale, an internationally cooperative study (CARDIAC: Cardiovascular Diseases and Alimentary Comparison), examining the relationship between diet and blood pressure in 20 different countries concluded that dietary factors influence the genesis of hypertension. In this study, it was noted that magnesium had a beneficial effect on blood pressure (Yamori et al., 1990).

2.5.2 Clinical

Altura & Altura (1996) report that in 28 independent clinical studies, patients with hypertension exhibited decreased serum tMg. On average, the reduction in tMg was 15% from normal. They further report that even borderline hypertensives (SBP/DBP=140/90-150/95) usually exhibit significantly depressed iMg, but not tMg. A variety of such clinical studies, as well as observations from clinical experiences, support the idea that magnesium deficiency is linked to hypertension. Nevertheless, in clinical practice, oral magnesium therapy is still controversial as some studies fail to support this idea

(Widman, Wester, Stegmayr, & Wirell, 1993). Outlined in the following are some of these studies and their findings.

Lind, Lithell, Pollare, & Ljunghall (1991) performed a double blind, placebo controlled study, adding 15mmol of Mg to a free diet of 71 subjects over six months. These subjects were classified as mildly hypertensive, or having high-normal blood pressure. They reported that the treatment, which elevated urinary magnesium excretion by 30%, induced no general effects on blood pressure. However, when the changes in blood pressure in the actively treated group were related to their pretreatment magnesium status, a correlation was found. In subjects with low pretreatment urinary excretion of magnesium, blood pressure was reduced. It was concluded that in general, magnesium supplementation does not seem to be effective in reducing blood pressure in unselected subjects with mild hypertension or high-normal blood pressures. However, in subjects with a low urinary excretion of magnesium, probably representing magnesium deficiency and/or a low magnesium intake, a hypotensive effect was seen.

In a study performed by Witteman et al. (1994), a reduction in blood pressure was observed with oral magnesium supplementation in women with mild to moderate hypertension. In a double blind fashion, 91 women were assigned randomly to either a treatment or placebo group. The treatment consisted of 20mmol Mg/day of magnesium-aspartate. At the end of the study, SBP had fallen by 2.7mmHg ($p=0.18$), and DBP had fallen 3.4mmHg ($p=0.003$). In a similar study, Widman et al. (1993) performed a double-blind cross-over study of magnesium supplementation with 17 patients with DBP over 90mmHg. Treatment consisted of receiving 15 mmol Mg2+/d for 3 weeks, 30 mmol Mg2+/d the following 3 weeks, and ended with 3 weeks of 40 mmol Mg2+/d. A significant decrease in SBP was observed such that mean levels went from 154+/-10.7 mmHg to 146+/-16.9 ($p=0.031$). DBP was also observed to significantly decrease from 100.2+/- mmHg to 92+/-6.6 mmHg ($p=0.0001$). (It should be noted that 15mmol Mg2+/d represents the RDA for men, while 13 Mg2+/d represents the RDA for women.)

The aforementioned studies support the idea that magnesium has a beneficial effect on blood pressure. Nevertheless, there are numerous studies which fail to demonstrate this relationship. Emerging from these investigations is a focus on initial magnesium status and the dosages of magnesium used. In those studies showing a beneficial influence of

magnesium supplementation, it may be likely that initial magnesium status was deficient, or that a pharmacologic dose of magnesium was used. In those studies failing to demonstrate a relationship, it may be that subjects were initially magnesium replete, or that non-pharmacologic (or "nutritional") doses of magnesium produce no effect. For example, Plum-Wirell, Stegmayr, & Wester (1994) performed a double-blind cross-over study, randomly assigning thirty-nine untreated hypertensive patients to either a treatment or placebo group. Treatment consisted of 15 mmol/d of oral magnesium. No significant changes in blood pressure were observed and although urinary magnesium rose in the treatment group, no changes were seen in serum or muscle magnesium levels. While Plum-Wirell et al. (1994) noted that a high number of patients had low muscle and serum magnesium at the start of the study, there was, nevertheless, an obvious lack of effect with the dose used. When comparing this to the work performed by Widman et al. (1993), it seems that "nutritional doses" of magnesium (15 mmol/day) are insufficient to elicit an effect, as Widman reports blood pressure decreases with a treatment regimen that consisted of progressively higher Mg2+ doses (15, 30, & 40 mmol/day across 3 weeks.) Contrary to this, however, is the work of Zemel et al. (1990) wherein pharmacologic doses of 40mmol Mg-aspartate were administered. No effect was observed on blood pressure, however, it was indicated that these patients were magnesium replete at the start of treatment. Similarly, Yamamoto (1995) reports a lack of blood pressure effect with magnesium supplementation in adults with high-normal blood pressure in a TOHP (Trial of Hypertension Prevention) study involving 698 adults. In this study, nutritional doses of 360mg/day of magnesium were used. In 1999, Burgess et al. investigated clinical trials, meta-analyses and review articles (taken from 1966-1999 on Medline) regarding the supplementation and consumption of magnesium as treatment or prevention for hypertension. Their evidence-based recommendations concluded that magnesium supplementation is not recommended as a means of preventing or treating an increase in blood pressure. They do, however, comment that most studies thus far have been short-term and that no long-term studies have evaluated the effect of increased intake of magnesium on morbidity or mortality rates. Additionally, most of these studies have involved dietary manipulations of more than one cation, making it difficult, if not impossible, to determine the influence of one cation alone.

Results from a variety of clinical studies are difficult to compare as different doses of magnesium have been used and patients are divergent in both their degree of hypertension and initial magnesium status. It is important to consider all of these factors when evaluating the effectiveness of magnesium as an anti-hypertensive agent. Nevertheless, most studies report finding a suppressed iMg level in hypertensive individuals. The following table outlines representative iMg and tMg measures for normotensive and hypertensive individuals, taken from Resnick et al. (1994) as cited in Altura & Altura (1996).

Table 1 : iMg and tMg measures in normotensive and hypertensive individuals:

	N	*iMg(mmol/L)*	*tMg(mmol/L)*	*iCa/iMg*
Normotensive	*61*	*0.62 ±0.007*	*0.88 ±0.01*	*1.96 ±0.03*
Hypertensive	*23*	*0.584 ±0.011**	*0.82 ±0.02*	*2.13 ±0.04*

**significant difference from normotensive controls ($p<0.01$)*

2.5.3 Experimental

Altura & Altura (1974) (as cited in Altura & Altura, 1985) performed in vitro experiments on rat aorta in order to determine whether graded changes in external Mg2+ concentration can affect the baseline tension of isolated arteries and whether or not these changes also influence drug and hormone induced maximal contractile responses of vascular smooth muscle. Results from this study show a graded-response, wherein a greater reduction in Mg2+ extracellulary was observed with a greater mechanical response. Increasing Mg2+ was found to decrease basal tone and reduce contractile activity. This observation is explained on the basis of Mg2+ competing for Ca2+ binding sites on the surface of the vascular smooth muscle cell. (These processes will be elaborated upon in Section 2.6). In 1996, Altura & Altura report that further experimentation with rats demonstrated that dietary deficiency of Mg produced an increased arterial blood pressure, decreased arteriolar, venular, and pre-capillary lumen sizes with a simultaneous reduction in blood flow. Within the same study, iCa/iMg ratios were seen to increase in vascular wall, increasing vascular tone and enhancing reactivity to endogenous vasoconstrictors. Berthelot & Esposito (1983) report that a diet normal in magnesium content supplied to spontaneously hypertensive rats slowed the rate of progression of hypertension as compared to those rats receiving a magnesium deficient diet.

2.6 MAGNESIUM, CALCIUM, SODIUM & POTASSIUM: ROLES IN VASCULAR HEALTH

2.6.1 Mechanism by which Mineral Elements affect Blood Pressure

Increased vascular resistance accounts for raised blood pressure in most patients with essential hypertension (Karppanen, 1991). Calcium, sodium, potassium and magnesium play an important part in regulating this tension and the resistance to blood flow. These mineral elements interact extracellularly, at the membrane, and intracellularly to influence the vascular smooth muscle. Those interactions which result in a rise in intracellular calcium ion concentration account for the increased resistance observed in hypertensive individuals as there is a greater association of calcium with calmodulin (Karpannen, 1991). This, in turn, triggers a cascade of events as the calcium-calmodulin complex activates myosin light chain kinase to phosphorylate the myosin head, enabling the binding of actin and the subsequent contraction of the vascular smooth muscle (Ives, 1989). The following outlines the main mechanisms by which an increase in intracellular Ca2+ is brought about and influenced by other ions.

1. Ca2+ Channels at the Membrane

One factor increasing the level of calcium in the vascular smooth muscle cell is the action potential. This opens voltage operated calcium channels and allows the entry of calcium ions from the extracellular space into the intracellular space. Vasoconstrictor agents such as noradrenaline, angiotensin II and serotonin increase the concentration of intracellular calcium ions by opening receptor operated calcium channels (Fig.1) (Karpannen, 1991). A rise in extracellular calcium concentration will increase intracellular concentrations, and Karpannen (1991) reports that this is observed with a concurrent increase in blood pressure. Antihypertensive drugs (such as nifedipine) act to block Ca2+ binding and entry into the vascular smooth muscle cell. Evidence has accumulated to indicate that extracellular Mg2+ can regulate Ca2+ binding sites, and thus, influence the control of arterial tone and blood pressure. Altura & Altura (1996) elaborate on direct and indirect experimentations that have shown Mg2+ is a Ca2+ antagonist, competing for both membrane and intracellular binding sites. This decreases Ca2+ binding, entry and translocation in the vascular smooth muscle cell, causing vasodilation and lowering blood pressure. Conversely, it has been observed that a

reduction in extracellular Mg2+ corresponds to an increase in intracellular Ca2+ content and elevated blood pressure (Altura & Altura, 1984, 1985).

In addition to influencing basal tone, Mg2+ also affects the reactivity of smooth muscle to catecholamines, as well as the release of catecholamines. Seelig (1994) reviewed experimental findings where the removal of Mg2+ from the external environment enhanced the reactivity of small and large coronary vessels to catecholamines, angiotensin II, serotonin, and acetylcholine. Conversely, the action of coronary relaxants such as vasopressin and prostanoids, were greatly attenuated as Mg2+ was reduced. Seelig (1994) reported that when immersed in solutions with high or low Mg2+/Ca2+ ratios, catecholamine secreting granules from adrenal medulla or nerve endings, release less or more catecholamine, respectively. This makes Mg2+ an important player in stress reactions as deficiency promotes such a release. Elevations in catecholamines will result in increased lipolysis, availing free fatty acids (FFA) to the blood. These FFA bind to Mg2+, soaking it up in a sponge-like fashion, reducing iMg levels and furthering exacerbating Mg2+ status (Seelig, 1994). From the aforementioned observations of Mg2+'s influence on basal tone, reactivity and catecholamine release, it has been compared to verapamil, a pharmacologic Ca2+-channel blocker. This makes Mg2+ a physiologic Ca2+-channel blocker (Seelig, 1994).

Figure 1. Ion Transport in the Vascular Smooth Muscle Cell

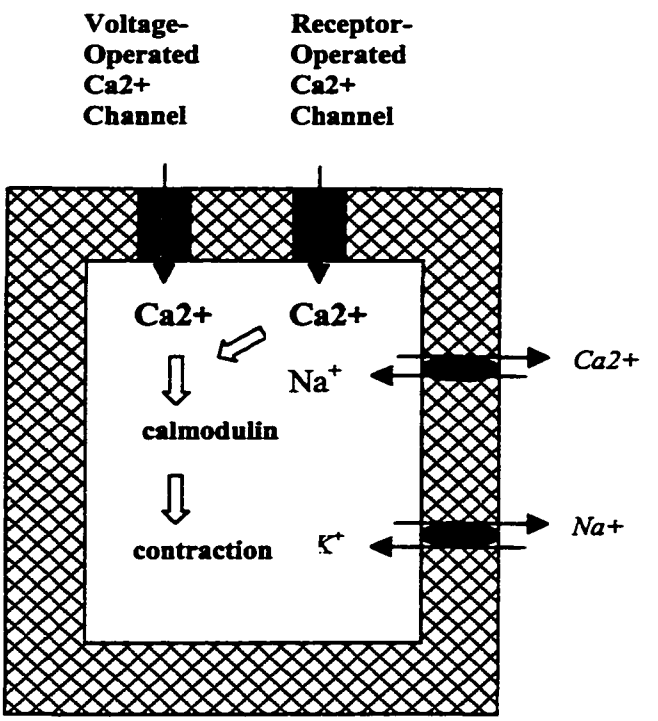

Figure 1: The role of calcium (Ca2+), potassium (K+), and sodium (Na+) ions, as well as the Na+-K+-pump, Ca2+-Na+ exchange mechanism, and Ca2+ channels in the regulation of vascular smooth muscle contraction. The sites of action of magnesium ions (Mg2+) as activators of the Na+-K+-pump and as antagonists of Ca2+ channels are not shown. Diagram adapted from Karpannen (1991).

2. Na+-K+ ATPase Pump and Na+-Ca2+ Exchange Sites (refer to preceding Fig.1)

Sodium excess in the body, due to a high intake or impaired elimination, may increase the level of endogenous Na+-K+ATPase inhibitors, also called digitalis like agents (Karpannen, 1991). Similarly, a decrease in Mg2+ will reduce the activity of Na+-K+ATPase as it has an obligate dependence on Mg2+-ATP (Karpannen, 1991). Inhibition of Na+-K+ATPase in either instance, will increase the intracellular Na content. As a consequence of raised intracellular sodium content, the activity of the Na+-Ca2+ exchange mechanism falls, increasing intracellular Ca2+ content (Karpannen, 1991). Therefore, the inhibition of the Na+-K+ATPase, either thru increase Na+ or decreased Mg2+, results in increased intracellular Ca2+ levels, inducing contraction of the vascular smooth muscle, and raising blood pressure. A rise in potassium can reactivate the Na+-K+ATPase, exerting an anti-hypertensive effect (Karpannen, 1991).

3. CAMP levels and Ca2+-ATPase Extrusion of Ca2+

Altura & Altura (1985) reported that the influence of Mg2+ on vascular muscle tone and reactivity could also be explained in terms of an effect on adenosine 3',5'-monophosphate (cAMP) formation with the cells. The synthesis of cAMP is dependent on the activity of adenylate kinase, an enzyme that is activated by Mg2+. They further report that some experimental evidence suggests that increased and decreased cAMP levels participate in vasodilation and vasoconstriction, respectively. A decreased cAMP level, resulting from an insufficient Mg2+ activation of adenylate kinase, could result in an increase in the concentration of Ca2+ in the cytoplasm as there is less cAMP mediated Ca2+ sequestration. Altura & Altura (1985) report that an alternative mechanism has been proposed wherein a Mg2+ dependent Ca2+-ATPase could be inhibited with low available Mg2+. This Ca2+-ATPase would be responsible for extrusion of Ca2+ at the membrane.

4. Mg2+-Induced Alterations in Vascular Muscle Ion Permeability

Altura & Altura (1985) report that evidence has accumulated that Mg2+ not only acts as a regulator of the entry and exit of Ca2+, but can also alter membrane permeability to other ions. Experimentation with the removal of Mg2+ from the extracellular environment has caused the membranes to become "leaky", allowing divalent and trivalent cations (Be2+, Fe3+, Al3+) with atomic radii smaller than Mg2+, to gain access to the cytoplasm. These, in turn, promote the release of intracellular Ca2+.

2.6.2 An Integrated Hypothesis for the Magnesium/Hypertension Relationship

Altura & Altura (1985) propose a hypothetical schema for the development of hypertension in the presence of dietary and metabolic Mg2+ deficits. This schema integrates the aforementioned mechanisms for the regulation of vascular tone. In particular, it focuses on Mg2+-Ca2+ and Na+-Ca2+ exchange sites as regulators of vascular tone, blood pressure, and local blood flow. The following (Fig. 2) is a representation of this integrated hypothesis.

Figure 2. Hypothetical Schema for Development of Hypertension

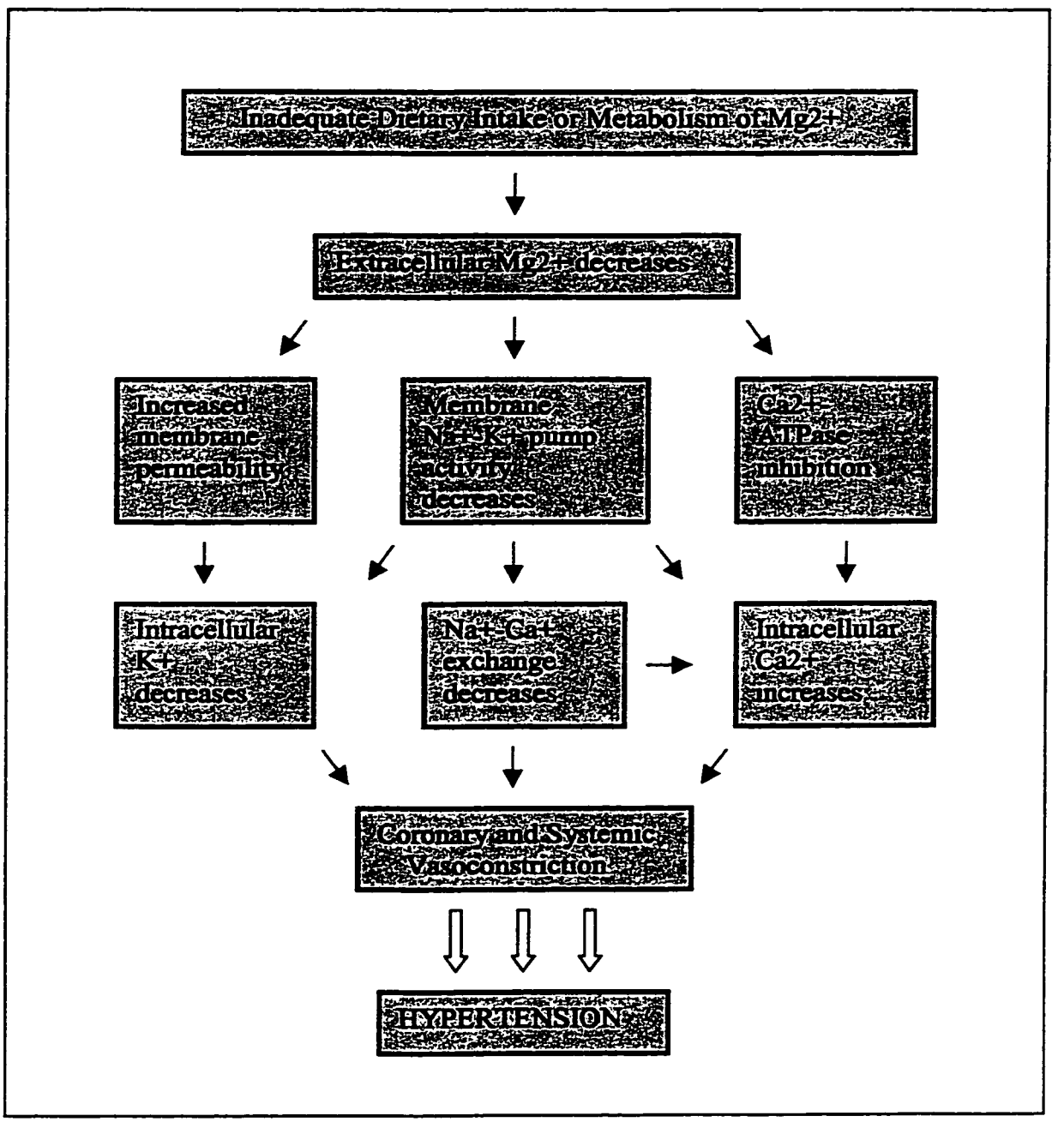

Figure2: Hypothetical schema for the dysfunction of normal cardiovascular tone by deficits in dietary Mg intake, or, inadequate metabolism of Mg2+. Diagram adapted from page 134, Altura & Altura (1985).

2.6.3 Magnesium and Arteriosclerosis: Lipid Hypothesis

Morrill et al. (1997) commented that the accumulated epidemiological data that relates geographic regions of low magnesium content (soft water and Mg-poor soil) with high incidences of ischemic heart disease (IHD) and sudden cardiac death (SCD,) might be explained by a Mg2+ etiology for arteriosclerosis and cardiovascular disease. This is supported by the observation that the coronary arteries of such patients often show deficits of 30-40% in total magnesium content and a simultaneous elevation in Ca2+ content(Morrill et al, 1997). Altura & Altura (1985) also report that several studies, in both animals and man, indicate an inverse relationship between cholesterol concentration and magnesium level. It is known, that at least experimentally, it is possible to prevent atheroma formation by increasing the dietary intake of Mg (Altura & Altura, 1985). Furthermore, magnesium salts have been shown effective in the clinical treatment of several cardiovascular diseases (arrhythmias, hypertension, myocardial infarction) (Morrill et al.,1997).

Etiologically, Altura & Altura (1985) comment that the development of atherosclerotic plaques is Ca2+ dependent. Since Mg2+ is a competitive inhibitor of Ca2+ on vascular smooth muscle, one must consider that elevations in dietary Mg may aid in preventing arteriosclerosis. Morrill et al. (1997) also found that lipid oxidation of vascular smooth cells was increased with decreasing Mg2+ concentrations extracellularly. This suggests Mg2+ acts as an anti-oxidant, protecting endogenous membrane lipids from free radical attack. This is important as disruptions to the intimal smooth muscle membrane precedes the development of intermediate atherogenic lesions (Morrill et al., 1997). Haennie et al. (1999) reports that an inverse relationship with atherogenic lipid fractions and magnesium levels, was more evident using iMg than tMg. This indicates that iMg may be a more relevant and important measure when studying the relationship between serum electrolytes and lipoproteins.

2.7 HYPERTENSION: LIFESTYLE AND DIETARY RISK FACTORS

2.7.1 Hypertension Defined

Hypertension is the most prevalent risk factor for premature cardiovascular disease, and since cardiovascular diseases are the leading cause of death in all industrialized countries, hypertension can be called our major public health problem (Kaplan, 1986). The total estimated number of hypertensive people in the United States totaled 30 million as reported in 1986 by Kaplan. In Canada, our 1986 estimate for the number of hypertensive individuals under 70 years of age was 1.25 million as reported by Stephens, Craig & Ferris (1986). Gentleman and Tomiak (1992) further report that large numbers of Canadians have high blood pressure but do not have their condition under control or have not yet identified it. In 1993, Tomiak & Gentleman performed a study of the risk factors for hypertension through the Canada Health Survey. In this report, normal blood pressure was defined as having a DBP <90 and a SBP <140. An elevated blood pressure was defined as having a DBP $\geq$95 or SBP$\geq$160. Intermediate to these two definitions is what is defined as borderline hypertensive. These definitions are consistent with other sources. It should be noted, however, these definition are based on adult norms.

Hypertension can be identified in children and adolescents, although it is less prevalent and the absolute levels of BP differ from those of adults(Falkner & Sadowski, 1995). Falkner & Sadowski (1995) define hypertension as having systolic or diastolic BP measures that are repeatedly above the 95th percentile for age and sex. Table 2 outlines such percentile norms for SBP and DBP in males and females aged 20-29 as determined from the 1981 Canada Fitness Survey (Stephens, Craig, & Ferris, 1981). These percentiles allow for the appropriate consideration of BP values in younger subjects.

Table 2: (adapted from Stephens, Craig, & Ferris, 1981).
Percentile Values for Blood Pressure in 20-29 year olds in Canada

	Percentile	Male	Female
Systolic BP	90	140	124
	75	130	118
	50	120	110
	25	112	102
Diastolic BP	90	90	84
	75	84	80
	50	78	72
	25	70	68

2.7.2 Lifestyle Links

Tomiak and Gentleman (1993) examined risk factors for hypertension, as measured by the Canada Health survey. Their analysis showed that age, sex, genetic history, and body mass index were the most important factors in predicting high blood pressure. For males, degree of physical activity and blood cholesterol levels were other predictive factors. In females, marital status and family income had significant associations with hypertension. The risk for high blood pressure in individuals smoking more than 10 cigarettes a day was found to not be significantly different than those who have never smoked. Tomiak & Gentleman (1993) report that this observation is consistent with other studies, which have shown no relationship between blood pressure and smoking, adding that it is, however, important to recognize smoking is closely related to coronary artery disease. Additionally, the amount of alcohol consumed was shown to have no significant association with BP measures. This observation is countered in Kaplan (1986), reporting that experimental and epidemiological work indicates that blood pressures are progressively higher with increasing alcohol consumption. Kaplan (1986) suggests that chronic alcohol intake may exert chronic pressor actions. Furthermore, it is suggested that alcohol in more than moderate amounts (2 oz/day) may raise blood pressure enough to make it the most prevalent cause of reversible hypertension. Kaplan (1986) also discusses caffeine, indicating that it raises blood pressure acutely, but its intake has not been clearly associated with the induction of permanent hypertension.

2.7.3 Diet as Therapy

Kaplan (1986) states that changes in diet are advocated for those who are hypertensive. This recommendation is not only based on the observation that dietary changes have corresponded to reductions in blood pressure, but due to the fact that the use of anti-hypertensive drugs present additional risks while reducing the elevate blood pressure. Kaplan (1986) also furthers that although evidence for the anti-hypertensive efficacy of dietary change rests largely on epidemiological or methodologically weak studies, these changes should be considered; they "should do no harm to those who do not need them, and may provide a great deal of benefit to those who do." Kaplan(1986) concludes that "non-pharmacologic approaches be used as definitive intervention and as and adjunct to drug therapy." The following section discusses the different dietary targets for blood pressure control, including both micronutrient and macronutrient components.

1. Micronutrient Intakes: Na, K, Ca, and Mg

The experimental and epidemiological evidence relating a high sodium intake to the development of hypertension is strong, showing that a marked reduction in sodium intake decreases blood pressure significantly and vice versa (Kaplan, 1986).The latter has been called Na+-induced hypertension and it results in an expansion of the extracellular volume as Na+ and water accumulate. In turn, there is a concomitant rise in BP. What has emerged in more recent studies, however, is that there is a strong genetic factor relating to sodium sensitivity and the development of Na+-induced hypertension (Preuss, 1993; Muntzel & Drueke, 1992). That is to say that some individuals may be salt sensitive, and others may not. Muntzel & Drueke (1992) report that dietary salt restriction generates variable blood pressure responses in different individuals. These differences are mediated through either intrinsic or indirect alterations in renal function (Preuss,1993). In other words, the kidney may possess inborn defects for sodium excretion, or may have a reduced response to ANF (atrial natriuretic factor), which acts to eliminate sodium (Muntzel & Drueke, 1992). So, while all hypertensives do not benefit from a reduced sodium intake, there is no apparent potential for harm from moderate sodium restriction to a level of 75-100mmol/day and it is therefore recommended as part of dietary treatment (Kaplan, 1986).

Many of the reported benefits of a reduced sodium intake may reflect an increased potassium intake (Kaplan, 1986). Generally, whenever sodium is deleted from the diet by substitution of natural foods for processed food, potassium intake increases. Kaplan (1986) further comments that a high K+/Na+ ratio is an important in factor in the prevention of hypertension. Preuss (1993) also reports that many clinical studies show that an increased K consumption lowers BP significantly, and that an inverse relationship between K intake and BP have been shown in several epidemiological studies. Burgess et al. (1999) considers several intervention studies, however, and notes that these failed to demonstrate that potassium supplementation prevents an increase in blood pressure. While it has been suggested that K+ acts to activate the Na+-K+ ATPase pump, thereby reducing intracellular Ca2+ and vascular smooth muscle contraction(Karpannen 1991), Burgess et al. (1999) states that the evidence does not support potassium supplementation for normotensive people as a prevention for hypertension.

Too much calcium in the blood or vascular tissue may be involved in the pathogenesis of hypertension as increased peripheral vascular tone results from elevated intracellular Ca2+ levels (Kaplan, 1986). Nonetheless, the intake of too little, not too much, calcium may be associated with hypertension, and oral supplements may lower, not raise, blood pressure (Kaplan, 1996). In a meta-analysis of 33 Ca supplementation studies, Bucher et al. (1996) concluded that supplementation may lead to a small reduction in SBP, but not DBP. Preuss (1993) also reports that low dietary intakes of Ca have been postulated to increase BP, suggesting that intakes below 400-600mg/day of dietary Ca is associated with a greater risk of hypertension, whereas intakes above this threshold are associated with less risk. One explanation that has been proposed, suggests that the hormones responsible for maintaining Ca2+ homeostasis modulate vascular Ca2+ uptake (Bukoski & Kremer, 1991). For example, a decrease in serum Ca2+ results in the release of parathyroid hormone (PTH) from the parathyroid gland. In turn, this results in the production of 1,25-dihyroxycholecalciferol from the proximal convoluted tubule. This promotes Ca2+ uptake and contraction (Bukoski & Kremer, 1991). Nevertheless, as Burgess et al. (1999) report, intervention studies have failed to demonstrate that calcium supplementation prevents increases in blood pressure in normotensive people or assists in the treatment of hypertensive individuals. Preuss (1993) concludes that there is no good

way to predict how patients may respond to augmented Ca intake. Accordingly, no additional intake above the RDA of 800-1200mg/day is recommended.

As discussed more substantially in the preceding sections, Mg deficiency may contribute to the development of hypertension. Thus, oral replacement could potentially be of benefit (Preuss, 1993). Kaplan (1986) reports that magnesium plays a major role in the control of vascular tone, causing vasodilation when infused into human subjects. Nevertheless, experimental and epidemiological studies have not come to conclusive recommendation for the use of dietary Mg as an anti-hypertensive agent. Burgess et al. (1999) notes that epidemiological studies have not reliably or consistently shown a relation between magnesium intake and blood pressure. It is concluded from this work, that supplementation with magnesium has not yet demonstrated a beneficial effect for the prevention or treatment of hypertension.

2. Caloric & Macronutrient Intakes: Fat, Protein, and Carbohydrate

Calories are very important in battling the development of hypertension. Weight and/or body mass index, in association with age, are the strongest correlates of BP (Preuss, 1993). The relative risk of developing hypertension is 5-6 times higher in obese adults than that of lean individuals (Preuss, 1993). Preuss (1993) reports that obesity, more prevalent in females, is associated with many perturbations that promote elevations in BP. These perturbations include increased sympathetic nervous system activity and the excess intake of nutrients, which influence pressure, elevate cardiac output and increase insulin resistance. Kaplan (1986) also reports that people with a body weight 20% or more above ideal have a frequency of hypertension about two times higher than do the non-obese. Furthermore, Kaplan (1986) states that although few studies have dissociated weight loss from sodium restriction, the evidence is strong supporting dietary restriction. In more recent time, however, Preuss (1993) comments that studies have dissociated the two and have indicated that even modest weight loss, in the absence of sodium restriction, is associated with a significant lowering of BP.

While calories are significant in relationship to BP, so is the manner in which caloric intake is proportioned across fat, carbohydrate, protein, and fibre intakes. Kaplan (1986)

and Preuss (1993) report that BP may fall in response to a decrease in total fat, along with an increase in polyunsaturated fat. The anti-hypertensive effect of polyunsaturated fatty acids, such as linoleic acid, has been assumed to be mediated by way of an increased synthesis of vasodilatory prostaglandins (Preuss, 1993). As for carbohydrate intake, Preuss (1993) reports that current evidence indicates excess refined carbohydrate consumption increases BP, while complex carbohydrate and soluble fibre consumption lowers BP. Ascherio et al. (1991) also supports the observation that an increase in fibre intake is associated with decreased BP, finding an inverse relationship between dietary fibre and DBP in a study of nutrient intakes of normotensive males. Little information is available on the influence of protein intake on BP, although Yamori et al. (1990) reported in their internationally cooperative study on diet and blood pressure, that animal protein was inversely correlated to BP. This supports the recommendation made by Kaplan (1986) that advocates a return to the ancestral diet of man, which our physiology is likely best adapted and evolved to handle. This ancestral diet was higher in protein and potassium content, while lower in fat and sodium content. Kaplan (1986) also reports a study that illustrates a vegetarian diet may lower blood pressure. The ingredients of a vegetarian diet that may lower blood pressure are presumed to be an increased content of polyunsaturated fat, fiber, vegetable protein, potassium, and magnesium.

2.7.4 Recommendations for the Prevention and Treatment of Hypertension

Kaplan (1986) made recommendations for a practical dietary prescription for the prevention and treatment of hypertension. It is likely these recommendations are relevant today. The following is a list of those recommendations:

1. For the overweight, weight reduction should be the primary goal.
2. For all hypertensives, dietary sodium should be restricted to a 2g daily level.
3. Potassium intake need not be specifically increased since it will rise with a lowered sodium intake. Those who are hypokalemic may benefit from potassium supplementation.
4. Supplemental magnesium and calcium is recommended for those who are deficient. Caution is advised in not reducing the dietary sources of calcium when dietary sodium is reduced.
5. More fibre and less saturated fat are beneficial for other reasons and may also help to lower BP.
6. Alcohol should be limited to 2 oz/day.

2.8 SUMMARY

In summary of the literature, it seems that the protective influence of dietary Mg on BP in normotensive individuals has not been reliably or consistently established. It may be that this measure does not accurately reflect the physiologically relevant blood ionic measures of magnesium. Furthermore, Mg supplementation in hypertensive individuals has not been shown to consistently reduce BP. In these instances, it seems important to consider an individual's initial magnesium status (iMg) as hypotensive effects are generally only observed in those subjects that were deficient before treatment.

CHAPTER THREE: METHODS

3.1 SUBJECTS

The sample was comprised of 201 (82 males; 119 females) Lakehead University students enrolled in the Kinesiology program. The average age of the subjects was 21.4 for males and 20.9 for females. The sample was physically active and asymptomatic(normotensive). Ten subjects were smokers.

3.2.PROCEDURES

Subjects were involved in a one-hour testing session and asked to return a three-day dietary record the following week. Testing was comprised of three BP measurements, a blood sample, height and weight measurement, and the completion of a PAQ (physical activity questionnaire).

According to the Canadian Physical Activity Fitness and Lifestyle Assessment (CPAFLA) recommendations on BP assessment, subjects were asked to refrain from strenuous exercise for 24 hours prior to testing and to refrain from caffeine and alcohol consumption 6 hours prior to testing. Additionally, smoking habits were documented and subjects were asked to abstain from smoking at least 2 hours before the test. They were also advised to consume a light carbohydrate meal 2-3 hours prior to reporting to their test. One graduate student took all resting blood pressure measures with a random-zero sphygmomanometer. After 10 minutes of sitting rest, 3 SBP/DBP were taken with 2 minutes of intervening rest between measures. The mode score of these three measures was used in reporting BP and the calculation of MAP. In cases where all three measures differed, the median score was used.

Blood samples were taken following BP measures and obtained by an experienced phlebotomist. Collection was performed by antecubital venipuncture (Pendergraph, 1984) in a 7 mL heparinized Vacutainer tube. The multi-test Stat Profile Ultra Analyzer model 11-3C (Nova Biomedicals Canada Ltd., Mississauga, Ont.) was used for the immediate analysis of iMg, iNa, iK, iCa, and hematocrit of a 25ug sample of whole blood. Measurements were performed by the same technician throughout the study.

Following the blood sample, a PAQ was filled out and height and weight were measured for the calculation of subject body mass index (BMI). Three day dietary records (including one weekend day) were submitted by the subjects the following week. Diets were analyzed using software by NutriQuest (McGraw-Hill, 1998).

3.3 DATA ANALYSIS

Comparisons between the estimates of BP and dietary and blood ionic measures of Mg, Ca, Na, and K were performed to examine significant differences (McNemar chi-square), as well as significant associations (kappa test). In order to conduct these tests, all BP, dietary and blood ionic values were transformed into dichotomous scores relative to the median score for each variable. Those scores less than the median score for that variable were assigned a value of 1, while those scores at or above the median, were assigned a value of 2. For all possible pair-wise comparisons between BP (SBP, DBP, MAP) and dietary and ionic Mg, Ca, Na, and K, as well as BMI and physical activity, a 2X2 frequency table was generated using statistical analysis software (SAS). This was performed across the total group, as well as by sex. For all cases yielding significant differences by McNemar z scores ($z > 1.96$), Relative Risk (RR) and Odds Ratio (OR) were calculated. (See Appendix C,D,E for sample calculations of the McNemar chi-square, kappa test, RR, and OR.)

CHAPTER FOUR: RESULTS

4.1 DESCRIPTION OF SUBJECTS

The demographic characteristics of the subjects are presented in Table 3. Hematologic and dietary characteristics are presented in Tables 4 and 5.

Table 3: General characteristics of subjects.

Parameter	Males(n=82) Mean ±SD	Males(n=82) Range	Females(n=119) Mean ±SD	Females(n=119) Range
Age(years)	21.39 ±1.56	19 - 27	20.89 ±1.60	18 - 33
BMI(kg/m2)	25.40 ±3.88	14.0 - 39.1	23.46 ±3.25	17.2 - 34.6
SBP(mmHg)	122.29 ±7.35	106 - 142	113.41 ±7.80	94 - 140
DBP(mmHg)	76.51 ±7.14	62 - 92	71.9 ±7.85	54 - 94
MAP(mmHg)	91.77 ±6.10	81 - 105	85.74 ±6.75	69 - 103
Activity Score	4.52 ±.61	2 - 5	4.36 ±.65	2 - 5

Table 4: Hematologic characteristics of subjects compared with "normative" measures.

Parameter	"Normal" Values	Males(n=82) Mean ±SD	Males(n=82) Range	Females(n=119) Mean ±SD	Females(n=119) Range
iMg (mmol/L)	0.46-0.60[a]	0.488 ± 0.030	0.420 - 0.550	0.490 ± 0.032	0.410 - 0.580
iCa (mmol/L)	2.1-2.6[b]	1.27 ± 0.037	1.18 - 1.34	1.26 ± 0.039	1.18 - 1.35
iNa (mmol/L)	135-145[b]	145.0 ± 2.16	140 - 150	144.7 ± 2.03	140 - 150
iK (mmol/L)	3.5-5.0[b]	3.993 ± 0.267	3.2 - 4.9	3.913 ± 0.247	3.4 - 5.0
iCa/iMg	/	2.60 ± 0.147	2.27 - 2.95	2.59 ± 0.1582	2.19 - 3.02
iNa/iK	/	36.49 ± 2.56	28.98 - 46.25	37.12 ± 2.25	29.60 - 42.94

a- Taken from Ising et al. (1995)
b- Taken from Van de Graff & Fox (1995)

Table 5: Mineral intakes of subjects as compared with Canadian RNI (recommended nutrient intakes).

Parameter	RNI female / male	Males(n=82) Mean ±SD	Males(n=82) Range	Females(n=119) Mean ±SD	Females(n=119) Range
Magnesium(mg)	280/350	230 ± 124	37.6 - 687.5	205 ±111	8.7 - 707
Calcium(mg)	1200	921 ± 477	166 - 1966	715 ±393	102 - 3144
Sodium(mg)	500	3308 ±1258	923 - 6700	2497 ± 1037	376 - 6871
Potassium(mg)	2000	2632 ±1232	903 - 6461	2074 ± 954	117 - 5256

4.2 STATISTICAL RESULTS

All possible pairwise comparisons between measures of BP (SBP, DBP, MAP) and measures of magnesium, calcium, potassium, and sodium (dietary and blood ionic) were performed by total group and sex. This was also performed for BMI (body mass index) and PAS (physical activity score on standardized CPAFLA questionnaire). RR (risk ratio) and OR (odds ratio) were determined for significant McNemar Chi-square ($z>1.96$) values. The following tables summarize these results. Findings with both significant McNemar z-scores and kappa associations are highlighted for discussion.

4.2.1 Blood Pressure & Blood Ions

Table 6 : 2X2 Comparisons of BP (SBP,DBP, MAP) and Blood Ionic measures (Mg^{2+},Ca^{2+},Na^{+},K^{+},Ca^{2+}/Mg^{2+}, & Na^{+}/K^{+}) by Total Group.

Comparison	A	B	C	D	McNemar (z-score)	Kappa (z-score)	Association	Risk Ratio	Odds Ratio
DBP-Na^{+}	**50**	**49**	**30**	**72**	**2.14***	**3.068***	**0.2117***	**1.59***	**2.45***
DBP-K^{+}	68	31	75	27	4.27	/	/	0.88	1.79
SBP-K^{+}	67	27	76	31	4.83	/	/	1.00	1.01
MAP-K^{+}	62	24	81	34	5.56	/	/	1.03	1.08
MAP-Ca^{2+}/Mg^{2+}	48	38	59	55	2.13	/	/	1.07	1.18
SBP-Na^{+}/K^{+}	52	42	71	36	2.73	/	/	0.80	0.63
DBP-Na^{+}/K^{+}	60	39	63	39	2.38	/	/	0.97	0.95
MAP-Na^{+}/K^{+}	50	36	73	42	3.54	/	/	0.90	0.80

Note: A,B,C,D cells refer to low/low, low/high, high/low, and high/high comparisons of BP with blood ion measures, respectively. Determination of a "low" or "high" classification was based on the position relative to the median split for each variable.

Table 7 : 2X2 Comparisons of BP (SBP,DBP, MAP) and Blood Ionic measures (Mg^{2+},Ca^{2+},Na^{+},K^{+},Ca^{2+}/Mg^{2+}, & Na^{+}/K^{+}) separated by Sex.

Comparison	A	B	C	D	McNemar (z-score)	Kappa (z-score)	Probability	Risk Ratio	Odds Ratio
MALES									
SBP-Mg^{2+}	9	8	35	3	4.12	/	/	0.99	0.96
MAP-Mg^{2+}	12	8	32	30	3.79	/	/	1.08	1.40
MAP-Ca^{2+}	12	8	26	36	3.09	/	/	1.20	2.08
SBP-Na^{+}	7	10	22	43	2.12	/	/	1.07	1.37
SBP-K^{+}	10	7	40	25	4.81	/	/	0.98	0.89
DBP-K^{+}	17	12	33	20	3.13	/	/	1.09	0.86
MAP-K^{+}	13	7	37	25	4.52	/	/	1.06	1.25
SBP-Ca^{2+}/Mg^{2+}	11	6	31	33	4.11	/	/	1.15	1.95
MAP-Ca^{2+}/Mg^{2+}	11	9	31	30	3.48	/	/	1.04	1.18
FEMALES									
SBP-Mg^{2+}	37	40	23	19	2.14	/	/	0.84	0.76
SBP-Ca^{2+}	39	38	21	21	2.21	/	/	1.02	1.01
SBP-Na^{+}	33	44	18	24	3.30	/	/	1.00	1.00
DBP-Na^{+}	**36**	**34**	**15**	**34**	**2.71***	**2.26***	**0.197***	**1.48***	**2.40***
MAP-Na^{+}	**34**	**32**	**17**	**36**	**2.14***	**2.13***	**0.189***	**1.59***	**2.25***
DBP-K^{+}	51	19	42	7	2.94	/	/	0.60	0.44
MAP-K^{+}	49	17	44	9	3.46	/	/	0.73	0.59

4.2.2 Blood Pressure & Dietary Minerals

Table 8 : 2X2 Comparisons of BP (SBP,DBP, MAP) and Mineral Intakes(Mg,Ca,Na,K) by Total Group.

Comparison	A	B	C	D	McNemar (z-score)	Kappa (z-score)	Association	Risk Ratio	Odds Ratio
SBP-K	85	9	91	16	-8.2	/	/	1.24	1.66
DBP-K	84	15	92	10	-7.44	/	/	0.76	0.61
MAP-K	74	12	102	13	-8.43	/	/	0.90	0.79
SBP-Mg	89	5	98	9	-9.16	/	/	1.23	1.82
DBP-Mg	94	5	93	9	-8.89	/	/	1.30	1.78
SBP-Na	**84**	**10**	**83**	**24**	**-7.57**	**2.20***	**0.112***	**1.40***	**2.43***
DBP-Na	82	17	85	17	-6.73	/	/	1.00	0.96
MAP-Na	76	10	91	24	-8.06	/	/	1.30	2.00

Table 9: 2X2 Comparisons of BP (SBP,DBP, MAP) and Mineral Intakes(Mg,Ca,Na,K) separated by Sex.

Comparison	A	B	C	D	McNemar (z-score)	Kappa (z-score)	Association	Risk Ratio	Odds Ratio
MALES									
DBP-K	24	5	45	8	-5.66	/	/	0.94	0.85
DBP-Na	19	10	39	14	-4.14	/	/	0.87	0.68
MAP-Na	15	5	43	19	-5.48	/	/	1.14	1.32
FEMALES									
SBP-K	71	6	36	6	-4.63	/	/	1.49	1.97
MAP-Na	61	5	48	5	-5.91	/	/	1.13	1.27

4.2.3 Blood Pressure, BMI, and Physical Activity

Table 10: 2X2 Comparisons of BP (SBP,DBP, MAP) with BMI and Physical Activity Score by Total Group and Sex

Comparison	A	B	C	D	McNemar (z-score)	Kappa (z-score)	Association	Risk Ratio	Odds Ratio
TOTAL									
SBP-Act	5	89	5	102	8.66	/	/	1.07	1.14
DBP-Act	5	94	5	97	8.94	/	/	1.02	1.03
MAP-Act	5	81	5	110	8.20	/	/	1.15	1.36
SBP-BMI	**86**	**8**	**74**	**33**	**-7.29***	**3.89***	**0.214***	**1.74***	**4.79***
DBP-BMI	**87**	**12**	**73**	**29**	**-6.62***	**2.89***	**0.161***	**1.55***	**2.88***
MAP-BMI	**80**	**6**	**80**	**35**	**-7.98***	**4.07***	**0.212***	**1.71***	**5.83***
MALES									
DBP-BMI	23	6	34	19	-4.43	/	/	1.27	2.14
FEMALES									
SBP-BMI	**72**	**5**	**31**	**11**	**-4.33***	**3.02***	**0.229***	**2.28***	**5.10***
DBP-BMI	64	6	39	10	-4.92	/	/	1.65	2.70

CHAPTER FIVE: DISCUSSION

5.1 BLOOD PRESSURE, BLOOD IONS (Mg2+, Ca2+, K+, & Na+), AND DIETARY MINERALS

The protective influence of magnesium on blood pressure, as suggested by Whelton and Klag (1989), Shirey (1995), and Altura and Altura (1996,) was not demonstrated for the dietary measures in the present study. Although such results are contrary to some studies, these findings are consistent with Burgess et al. (1999). Using a meta-analytic approach, Burgess et al. (1999) showed across eighteen epidemiological and clinical studies, that magnesium intake had no demonstrable effect on BP. From this analysis, it was concluded that the benefit of magnesium supplementation for either the prevention or treatment of hypertension, has not been established.

While Burgess et al. (1999) found no evidence for the benefit of dietary magnesium, the results of Widman et al. (1993) and Lind et al. (1991) suggest that magnesium may be found to be effective as a hypotensive agent in iMg deficient individuals. Widman et al. (1993) and Lind et al. (1991) reported that the treatment of hypertensive individuals with magnesium induced no general effects on blood pressure until corrected for pretreatment iMg status. Both studies concluded that supplementation is not effective at influencing BP in magnesium replete subjects. Findings in the present study are consistent with this conclusion as most subjects were determined to be within a normal iMg range of 0.450-0.60mmol/L (Ising et al., 1995), and no demonstrable relationship with BP was found. The conclusions made by Burgess et al. (1999) may reflect that the iMg status of subjects was not considered, and that dietary measures of Mg may not reflect physiologically relevant iMg. Furthermore, it may also indicate that the subjects involved were not compromised as to their iMg status.

While magnesium may not prove to be a target for hypertension prevention, evidence suggests that dietary sodium is such a target. In the present study, comparisons of dietary and ionic sodium with BP indicated a detrimental association. The chance of having elevated DBP was over one and a half times greater for those with increased iNa as shown by the RR for the total group. The effects of iNa on DBP and MAP for females, and that of dietary sodium on SBP for the total group, were also found to be detrimental

by RR. In all cases, the kappa statistic indicated a significant association between sodium and BP. That is to say that low measures of BP were associated with low measures of sodium, and conversely, high measures of BP were associated with high sodium measures. These findings are consistent with numerous epidemiological studies that link sodium to hypertension (Karpannen, 1991; Muntzel & Drueke, 1992; Preuss, 1993). It is believed that this link is the result of the expansion of the extracellular volume as Na+ and water accumulate (Preuss, 1993) or due to an increase in endogenous Na+-K+ATPase inhibitors (Karpannen, 1991). These inhibitors (digitalis-like) reduce the activity of the Na+-K+ pump, causing a rise in intracellular Na+ and a slowing of Na+-Ca2+ exchange. As a consequence, intracellular vascular smooth muscle Ca2+ rises, producing contraction and vasoconstriction (Karpannen, 1991).

In a manner opposite that of sodium, potassium is suggested to have a protective influence on BP through an activation of the Na+-K+ pump (Karpannen, 1991; Preuss, 1993). In view of this, comparisons were made between BP measures and both the absolute and relative (to Na+) measures of K+. While no significant associations were determined by the kappa statistic, although a protective influence of K+ is suggested with trends showing RR and OR values less than one. Similarly, absolute and relative (to Mg2+) Ca2+ measures were compared with BP. These were performed as it may be the relative ratio of Ca2+/Mg2+ that is of importance with both cations competing for extracellular and intracellular binding sites (Karpannen, 1991; Altura & Altura, 1996). However, no significant associations were demonstrated.

5.2 BLOOD PRESSURE, BMI, AND PHYSICAL ACTIVITY

Comparisons of BMI and BP across the total group and by sex were consistent with reports that BMI is the strongest correlate of BP (Preuss, 1993). RR values greater than one and a half times were determined to exist for all BP measures (DBP, SBP, MAP) with BMI for the total group. These findings were maintained in DBP-BMI comparisons in males, and both SBP-BMI and MAP-BMI comparisons in females. Significant kappa statistics were found in all these cases and indicated that high and low BP measures are associated with high and low BMI measures, respectively. Preuss (1993) proposes that the increased RR associated with a high BMI may be due to perturbations in sympathetic nervous activity and an elevated cardiac output. These findings are consistent with Kaplan (1986) who reported that people with a body weight 20% or more above ideal have a frequency of hypertension about two times greater than do the non-obese.

Contrary to the findings of Tomiak and Gentleman (1993) in the Canada Health Survey, no significant associations were found between PAS (physical activity scores) and BP. It was anticipated that a protective influence of PAS on BP would be demonstrated. This result was not surprising as only 10 subjects scored below the median score for PAS. That is to say that almost all subjects were "highly active" and the few below the median score still achieved an "adequate" PAS. Either the sample contained individuals truly at an adequate level of physical activity or the CPAFLA questionnaire was not sensitive enough to separate individuals across a physical activity continuum for a BP association to be seen.

5.3 CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

This study concluded that a protective relationship between dietary magnesium and BP is not demonstrated in normotensive individuals. As well, there is no relationship between iMg and BP within the normal variation of iMg levels. It may be that there is a threshold effect of iMg on BP such that the contractility of vascular smooth muscle is influenced only at low iMg values. Studies that have shown a hypotensive influence of Mg supplementation may be due to the correction of an underlying magnesium deficiency within hypertensive subjects. In otherwise healthy individuals, it is unlikely that hypomagnesemia will result with the ingestion of a normal diet. As such, it further seems unlikely that supplementation in healthy individuals will prevent the development of hypertension.

Further research is needed in the area of the etiology of hypomagnesemia, as well as the establishment of normal and abnormal iMg levels. A review of clinical studies should be performed in order to determine at what point a threshold influence of iMg on BP is observed. As well, it would be valuable to investigate whether or not magnesium depletion relates to the onset of hypertension. That is to say that hypertension may not initially be accompanied by magnesium deficiency but rather, magnesium deficiency develops transiently through some disease process involved in hypertension. It may be that magnesium supplementation is only a valuable adjunctive therapy for hypertension in such magnesium-depleted individuals.

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Appendix A

CPAFLA PHYSICAL ACTIVITY QUESTIONNAIRE (PAQ)

Please answer the following questions:

1. Over a typical seven day period, how many time do you engage in physical activity that is sufficiently prolonged and intense to cause sweating and a rapid heart beat? (circle).

   At least three times Normally once or twice Rarely or never

2. When you engage in physical activity, do you have the impression that you: (circle)

   Make an intense effort Make a moderate effort Make a light effort

3. Generally, would you say that your current level of physical fitness is: (circle)

   Very good Good Average Poor Very Poor

Appendix B

SCORING FOR THE CPAFLA PAQ

Scoring Template

Item	Male	Female	Male	Female	Male	Female
Question #1 Frequency	*Rarely or Never*		*Once or twice*		*At least three*	
	0	0	2	3	3	5
Question #2 Intensity	*Light*		*Moderate*		*Intense*	
	0	0	1	2	3	3
Question #3 Perceived Fitness	*Very Poor*		*Average*		*Good or Very good*	
	0	0	3	1	5	3

Healthy Physical Activity Participation – Determination of Health Benefit Zones

Health Benefit Zone	Total Score	Rank Score
Excellent	9-11	5
Very Good	6-8	4
Good	4-5	3
Fair	1-3	2
Needs Improvement	0	1

Appendix C

MCNEMAR CHI-SQUARE TEST OF SYMMETRY FOR PAIRED RESPONSE DATA

Computation of McNemar's test

McNemar's test assumes that the response data are binary. In this study, all variables were dichotomously scored relative to their median score such that the binary responses had one the following outcomes:

High factor "a" score & High factor "b" score
High factor "a" score & Low factor "b" score
Low factor "a" score & High factor "b" score
Low factor "a" score & Low factor "b" score

(note: "high" scores are those at or above the median score for that variable; "low scores are those below the median)

A general 2X2 arrangement of these outcomes appears as:

	Low Factor A	*High Factor A*
Low Factor B	*A*	*B*
High Factor B	*C*	*D*

The McNemar equivalence estimate focuses on "discordant pairs", also referred to as "off-diagonal" elements (ie. the paired data in cells "B" and "C"). The null hypothesis (Ho: pB = pC) implies that the proportion of individuals who scored low on factor B and high on factor A will equal the proportion of individuals who scored low on factor A and high on factor B (Suchower and Copenhaver, 1996). The McNemar test is computed using a z formula as shown below:

$$z = \frac{B - C}{\sqrt{B + C}}$$

The null hypothesis is rejected when a significant z score results ($z > 1.96$).

Appendix C continued

Sample Calculation: Comparison of DBP- iNa Responses

	Low iNa	*High iNa*
Low DBP	*50*	*49*
High DBP	*30*	*72*

$$z = \frac{49 - 30}{\sqrt{49 + 30}}$$

$z = 2.14$

Therefore, the null hypothesis is rejected.

Appendix D

AGREEMENT IN PAIRED RESPONSE DATA USING THE KAPPA STATISTIC

Computation of the kappa statistic

The kappa statistic is used to determine the agreement in pairs of responses. Complete agreement between the factors is assumed when kappa equals 1. Conversely, a kappa statistic of 0 indicates that the agreement is coincidental and not different than that which would be expected by chance (Suchower and Copenhaver, 1996).

The kappa statistic focuses on the diagonal elements in cells "A" and "D", as illustrated in the 2X2 table in Appendix C. The formula for the calculation of kappa uses the proportion of events in the 2X2 table according to the following:

$$K = \frac{p_o - p_e}{1 - p_e}$$

Note: p_o refers to the observed proportion and p_e refers to the expected proportion where:

p_o = the sum of the proportion of the total respondents represented by cells A and D
p_e = the sum of the product for each matched row with column proportion

The null hypothesis (Ho: $K = 0$) is tested by z according to:

$$z = \frac{K}{se_o(K)}$$

(Note: $se_o(K)$ refers to the standard error for kappa)

Sample Calculation: Comparison of DBP-iNa Responses:

Using the example in Appendix C and the above formulae, the following is obtained:

kappa = 0.2117
z = 3.068

Therefore, the null hypothesis is rejected and a significant 21% agreement between DBP-iNa paired responses is demonstrated.

Appendix E

RELATIVE RISK

Computation of Relative Risk

Relative Risk (RR) refers to the ratio of the risk of an outcome in individuals with a factor of interest to the risk of an outcome in individuals without the factor of interest. For example, in the 2X2 table shown below, a high BP is the outcome and a high ionic sodium concentration is the factor of interest . In this model, a high BP is also considered to be a "case" and high ionic sodium concentration is considered to constitute "exposure". The RR risk is calculated as the ratio of the incidence of high BP among the ionic sodium group, to the incidence of high BP in the low ionic sodium group.

	Cases: Disease Present High Blood Pressure	***Controls: Disease Absent Low Blood Pressure***
Exposed: High Ionic Sodium	A	B
Non-Exposed: Low Ionic Sodium	C	D

Formulae:

$$RR = \frac{\text{incidence of the disease in the exposed group}}{\text{incidence of the disease in the non-exposed group}}$$

$$RR = \frac{A / (A+B)}{C / (C+D)}$$