Pergamon

Cellular and Molecular Biology Research, Vol. 41, No. 5, pp. 347–359, 1995

0968-8773/95 $9.50 + .00

0968-8773(95)02028-4

Original Contribution

MAGNESIUM AND CARDIOVASCULAR BIOLOGY: AN IMPORTANT LINK BETWEEN CARDIOVASCULAR RISK FACTORS AND ATHEROGENESIS

BURTON M. ALTURA*†‡§ and BELLA T. ALTURA*‡

Departments of *Physiology and †Medicine and ‡The Center for Cardiovascular and Muscle Research, State University of New York, Health Science Center at Brooklyn, NY 11203, USA

(*Received* 26 *May* 1995; *Accepted* 15 *September* 1995)

Abstract—In this review, a rationale is presented for how hypercholesterolemia, hypertension, diabetes mellitus, end-stage renal disease, renal dialysis, and prolonged stress can all lead to atherosclerosis, ischemic heart disease, and stroke. The data indicate that Mg deficiency caused either by poor diet and/or errors in Mg metabolism may be a missing link between diverse cardiovascular risk factors and atherosclerosis. Data from our laboratories and others indicate that reduction in extracellular and intracellular free Mg ions (Mg^{2+}) can induce an entire array of pathophysiological phenomena known to be important in atherogenesis, that is, vasospasm, increased vascular reactivity, elevation in $[Ca^{2+}]_i$, formation of proinflammatory agents, oxygen radicals, platelet aggegation, reduction in cardiac bioenergetics, cardiac failure, oxidation of lipoproteins, gender-related modulation of endothelial-derived relaxing factor/NO, changes in membrane fatty acid saturation, changes in membrane plasmalogens and N-phospholipids (suggesting changes in intracellular phospholipid signals), and probably transcription factors.

Keywords—Magnesium, Atherosclerosis, Cardiovascular risk factors, Cell bioenergetics, Cytosolic free calcium, Ion selective electrodes

INTRODUCTION

Hypercholesterolemia, hypertension, diabetes mellitus, immune injury, end-stage renal disease (ESRD), renal dialysis, and prolonged stress all are widely accepted as risk factors for atherosclerosis. No common link has been identified that forms a rational underlying basis to these disorders and atherogenesis. Moreover, it is not clear how lipoproteins, Ca^{2+}, and macrophages gain access to the normally impermeable arterial walls or what allows plyable physiologic vascular smooth muscle (VSM) cells to change their state (phenotype) from a contractile cell to a noncontractile synthetic (or modulatory) cell (Ross, 1993; Schwartz et al, 1995), nor is it clear how these diverse risk factors lead to ischemic heart disease (IHD) and stroke.

During the past two decades, information and data has accumulated to suggest that magnesium deficiency caused either by poor diet and/or errors in body Mg metabolism may be a missing link between the diverse cardiovascular risk factors and atherosclerosis (Altura and Altura, 1990, 1994, 1995a). New evidence from our laboratories points to potential intracellular signals linking Mg^{2+} deficiency to etiology of atherogenesis and vascular disease.

PROBLEMS IN DAILY INTAKE OF MAGNESIUM, WATER HARDNESS, AND CARDIOVASCULAR-IHD DEATH RATES

At a typical daily total fluid intake (approximately 2 l), Mg intake from water can be as low as 2 mg (in some parts of the United States, South Africa, Europe, and Canada) or as high as 350 mg (in parts of Texas). The extremes of Mg content in drinking water are <6 mg/l in Newfoundland and >400 mg/l in certain regions of Italy, France, and the former Yugoslavia. This wide range has confounded correlations between epidemiologic studies done in hard vs. soft water areas (Altura and Altura, 1990).

§To whom correspondence should be addressed.

However, it is now clear from several studies in which Mg levels were carefully ascertained that there is an inverse relationship between Mg intake and IHD, sudden cardiac death, and hypertensive vascular disease (Altura and Altura, 1990). Support comes from a recent report by Bloom and Peric-Golia (1989) that the frequency of myocardial calcification at autopsy after cardiac death correlated inversely to Mg levels in the drinking water.

Moreover, the death rate from cardiovascular diseases in the eastern United States has long been known to be significantly higher than in western states: 429 ± 39 vs. 366 ± 32 per 100,000 population/y ($p < 0.01$) (Masironi, 1979). On the average, the hardness of drinking water in the eastern United States is approximately one-half that in the western states. Similar phenomena have been observed in Canada, Finland, South Africa, and some parts of Europe (Altura and Altura, 1990).

Patients who have lived in areas of soft water and Mg-poor soil and who die of IHD or sudden cardiac death demonstrate on average a 20% loss of intracardiac Mg at autopsy. Mg is the only metal decreased to this extent consistently in such cardiac deaths (Chipperfield and Chipperfield, 1973, 1978). In addition, the coronary arteries of such patients often show deficits of 30–40% in total Mg content. A concomitant elevation in Ca is seen in both tissues (Altura and Altura, 1984; Crawford and Crawford, 1967; Chipperfield and Chipperfield, 1973, 1978).

Average dietary intake of Mg in the United States was about 450–485 mg/d at the turn of the century. It has been steadily decreasing (Table 1); the most recent figures indicate a typical daily intake of about 185–260 mg/d for men and about 172–235 mg/d for women. The Recommended Daily Allowance is 350 mg/d, so there is a typical dietary shortfall of 90–178 mg/d in this country. Surveys in Europe and Canada reveal similar shortfalls (Altura and Altura, 1995b). Thus, the typical daily Mg intake in the United States is somewhere between 35% and 75% of the desired amount. Such pronounced deficits raise the possibility of important health effects because so many biochemical and physiologic functions could be compromised (Table 2).

Table 1. Progressive Decline in Dietary Intake of Magnesium Over Past 90 Years

Years	Mg Intake/d
1900–1908	475–500
1909–1913	415–435
1915–1929	385–398
1935–1939	360–375
1947–1949	358–370
1957–1959	340–360
1965–1976	300–340
1978–1985	225–318
1987–1992	175–248

According to recent epidemologic studies, in which dietary variables were assessed in volunteers by the 24-h recall method, Mg intake was strongly and inversely associated with blood pressure (Altura and Altura, 1995a; Joffres et al., 1987). Strict vegetarians exhibit significantly decreased incidences of ischemic heart disease, sudden cardiac death, and hypertension, and they generally have above-average dietary Mg intakes (Marier, 1986; Miller et al., 1992; Rouse et al., 1983). Legumes, beans, nuts, soybeans, green leafy vegetables, and unprocessed cereals are rich in Mg and form the basis of vegetarian diets.

In addition to a probable dietary deficiency of Mg, in the average human subject's diet in North America and Europe, it should be stressed that we tend to forget that when foods (containing Mg) are cooked and processed (or refined), most food staples lose more than 65% of the Mg content (Altura and Altura, 1995b). Such a situation perforce results in further reductions in dietary intake of Mg.

MAGNESIUM DEPLETION FROM BODY IS COMMON

A variety of commonly used drugs, such as alcohol, diuretics, chemotherapeutic agents (e.g., cisplatin, bleomycin, cyclosporine), certain antibiotics (amphotericin B and other fungicides), and cardiac glycosides, among others (Altura and Altura, 1984, 1995b), produce a loss of body Mg, with alcohol being the most notorious Mg waster. Diarrhea, malnutrition, vomiting, dehydration, high salt diets, malabsorption syndromes, and certain renal disorders also result in considerable body loss of Mg.

Six to 50% of all hospitalized patients exhibit low serum Mg; patients with numerous different cardiovascular disorders (hypertension, diabetes mellitus, congestive heart failure, Prinzmetal angina, cardiac arrhythmias, and so on) associated with atherogenesis demonstrate the most profound deficits in body Mg (Altura et al., 1994b; Gottlieb et al., 1990; Keller and Aronson, 1990; Miyagi et al., 1989; Millane et al., 1992; Sheehan, 1992). Did the disease(s) produce the Mg deficiency or was an existing Mg deficiency responsible, in part, for the etiology of the disease state?

MAGNESIUM EXISTS IN THREE STATES IN BLOOD

Blood normally contains magnesium ions (Mg^{2+}) in three states: bound to proteins; complexed to small anion ligands such as bicarbonate, phosphate, citrate,

Table 2. Physiologic Functions of Magnesium

Enzyme Functions	Structural Functions	Membrane Functions	Calcium Antagonist
7 Glycolytic enzymes	Proteins	Hormone-receptor binding	Muscle contraction/relaxation
4 TCA cycle enzymes	Polyribosomes	Gating of Ca^{2+} channels	Neurotransmitter release
Membrane-bound ATPases	Nucleic acids	Transmembrane flux of ions	Action potential conduction
Kinases, e.g., creatine kinase	Mitochondria	Adenylate cyclase system	In nodal tissue
Alkaline phosphatase	Multienzyme complexes, e.g., G-proteins, NMDA	Ca^{2+}-Ca^{2+} release	
12 Photosynthetic enzymes			

TCA, tricarboxylic acid; NMDA, *N*-methyl-D-aspartic acid.

lactate, acetate, and so on; and free. In tissues, most of the Mg^{2+} is bound to ATP. Most clinical laboratories measure total Mg (TMg) levels by colorimetry or atomic absorption spectroscopy. However, it is the free ionized form of Mg that is physiologically active in blood and body fluids. Usual estimates of free Mg^{2+} concentration have relied on TMg measurements in protein-free ultrafiltrates that, of course, exclude the protein-bound Mg (D'Costa and Cheng, 1983).

Because the levels of anions can vary significantly in pathologic states and in view of the role apparently played by Mg in cellular homeostasis (Table 2) (Aikawa, 1981), it is desirable to directly measure free Mg^{2+} in blood and other body fluids (Altura, 1994). With this in mind, Mg^{2+}-sensitive ion-selective electrodes (ISEs) have been designed to obtain these measurements in the presence of potential cationic interferences and other interferents (Altura and Lewenstam, 1994).

MAGNESIUM MODULATION OF HYPERTENSIVE EVENTS

Animal studies provide evidence for Mg modulation of hypertensive events. With respect to experimental and genetically induced hypertension, several reports support the concept that Mg deficiency or derangements in Mg metabolism can result in high blood pressure (Altura and Altura, 1990, 1994, 1995b; Altura et al., 1993a).

Experiments from our laboratory indicate that in rats, dietary deficiency of Mg results in elevation of arterial blood pressure; decreased arteriolar, venular, and precapillary lumen sizes; and decreased numbers of microvessels (rarefaction) concomitant with decreased flow in the capillaries (Altura et al., 1984, 1992). An elevated ratio of Ca^{2+} to Mg^{2+} is found in the vascular walls, which would be expected to result in enhanced vascular tone (decreased lumen size), enhanced reactivity to endogenous vasoconstrictors, and diminished reactivity to endogenous vasodilators. Stress-induced hypertension and alcohol-induced hypertension are also associated with Mg deficiency (Altura and Altura, 1994; Altura et al., 1992).

In addition to the inverse relationship between precapillary lumen size and serum Mg concentration in these dietary studies (Altura et al., 1992), we have found that ultrafilterable Mg^{2+} is significantly attenuated and that there is an elevated Ca/Mg ratio in spontaneously hypertensive rats (Altura and Altura, 1983, 1995c). It has been reported recently that intracellular free Mg^{2+} ($[Mg^{2+}]_i$) is lowered in striated muscle and aortic smooth muscle of these rats (Ng et al., 1992). Overall, these findings suggest that the level of free ionized Mg^{2+} in the extracellular fluid and at the level of the vascular smooth muscle cell membrane plays an important role in controlling vascular tone, contractility of blood vessels, and eventual prevention of hypertensive vascular disease.

At least 10 independent clinical studies show that patients with hypertension of diverse etiologies exhibit hypomagnesemia either in serum or in tissues, or both (Altura and Altura, 1990, 1995a). On the average, patients with long-term hypertension have at least a 15% deficit in total serum Mg. Reports from a number of groups around the globe clearly demonstrate inverse correlations between total Mg concentrations in serum or tissue and arterial blood pressure. Nadler and associates (1987) showed that controlled short-term (4 weeks) dietary deprivation of Mg in humans significantly elevates arterial blood pressure, similar to our rat studies (Altura et al., 1984). Other studies provide evidence that certain hypertensive patients exhibit reduced urinary excretion of Mg, which is inversely correlated with diastolic blood pressure levels (Altura and Altura, 1990).

We have noted that even borderline hypertensives (140/90–150/95 mm Hg) often have depressed serum ionized (IMg^{2+}), but not total, Mg levels (Table 3). Essential hypertensive subjects, diagnosed on the basis of elevated blood pressure readings found repeatedly to be in excess of 150/95 mmHg, in the absence of secondary hypertension, exhibited significant lowered serum IMg^{2+} and elevated serum ionized Ca^{2+} (ICa^{2+})/ionized Mg (IMg^{2+}) levels (Table 4). We

Table 3. Serum Ionized Magnesium, Total Magnesium, and Ionized Calcium to Ionized Magnesium Ratios in Normotensive vs. Hypertensive Subjects

		Mg (mM/l)		
Group	n	IMg^{2+}	TMg	ICa^{2+}/IMg^{2+}
Normotensive controls	61	0.620 ± 0.007	0.88 ± 0.01	1.96 ± 0.03
Hypertensives	23	0.584 ± 0.011*	0.82 ± 0.02	2.13 ± 0.04*

Values are means ± SEM. Adapted from Resnick et al., 1995.
* Significantly different from normotensive controls ($p < 0.01$).

had noted previously that elevated ICa^{2+}/IMg^{2+} levels were associated with enhanced vasomotor tone, spasm, and atherogenesis (Altura and Altura, 1974, 1990; Altura et al., 1990).

MAGNESIUM MODULATES BLOOD LIPIDS AND ATHEROGENESIS

Evidence from both animals and humans suggests that dietary and blood levels of Mg^{2+} may modulate serum levels of lipids and lipoproteins (Altura, 1982, 1988; Altura and Altura, 1990, 1995c; Altura et al., 1990; Altura et al., 1993; Davis et al., 1984; Rasmussen et al., 1989; Rayssiguier, 1986). Using rabbits, our laboratory has reported that the dietary level of Mg modulates the serum level of cholesterol and triglycerides in normal animals; the lower the Mg intake, the higher the serum lipid levels (Altura et al., 1990; Altura and Altura, 1995c).

In rabbits fed a high-cholesterol diet, Mg deficiency, comparable with the reduced dietary intake seen today (i.e., 30–40% normal) in the adult population, exacerbates atherogenesis and lipid deposition in arterial muscle (Fig. 1) and stimulates macrophages. Pretreatment with orally administered Mg aspartate attenuated the atherosclerotic state (Fig. 1) and lowered serum cholesterol and triglycerides (Altura et al., 1990; Altura and Altura, 1995c). In these studies, the extent of atherogenic lesions was poorly correlated with the level of serum cholesterol and highly dependent on the level of dietary Mg and on the Ca/Mg ratio.

High blood levels of lipids and lipoproteins can mask a "true" Mg-deficient state (Altura et al., 1990). A similar hidden state of Mg deficiency has been observed by our group in renal transplant patients (Markell et al., 1993a) and in ESRD (Markell et al., 1993b). These people are characterized by extensive unexplained atherogenesis. In all such patients, we found a marked lowering of free but not total Mg, decreased urinary output of Mg, hyperinsulinemia, and elevated total serum cholesterol or triglycerides, or both.

ICa^{2+}/IMg^{2+} ratios, signs of elevated vascular tone, increased vascular reactivity, and risk of atherogenesis were also significantly elevated in our stable renal transplant recipients and subjects with ESRD. TMg in these patients is usually normal or elevated, respectively, suggesting that in advanced atherogenesis and states of hyperlipidemia, a normal TMg level would often be seen, despite the fact that the body and, specifically, the vascular walls would be deficient in free Mg^{2+}.

EXTRACELLULAR AND INTRACELLULAR MAGNESIUM DEFICIENCY IS COMMONLY SEEN IN DIABETES

In the hypertensive-diabetic patient population, hyperinsulinemia, insulin resistance, and hypertensive vascular disease are often associated with decreased serum high-density lipoprotein (HDL) cholesterol, increased low-density lipoprotein (LDL) cholesterol, and elevated triglyceride levels (Moller, 1993). These lipid, LDL, and HDL changes are the subject of much speculation.

Table 4. Serum Ionized Magnesium, Total Mg, and Serum Ionized Calcium to Magnesium Ratios in Normotensive vs. Subjects with Untreated Essential Hypertension

		Mg (mM/l)		
Group	n	IMg^{2+}	TMg	ICa^{2+}/IMg^{2+}
Normotensive	40	0.622 ± 0.008	0.84 ± 0.02	1.98 ± 0.04
Essential hypertensive	44	0.522 ± 0.011*	0.83 ± 0.04	2.36 ± 0.05*

Values are means ± SEM.
* Significantly different from normotensive controls ($p < 0.01$).

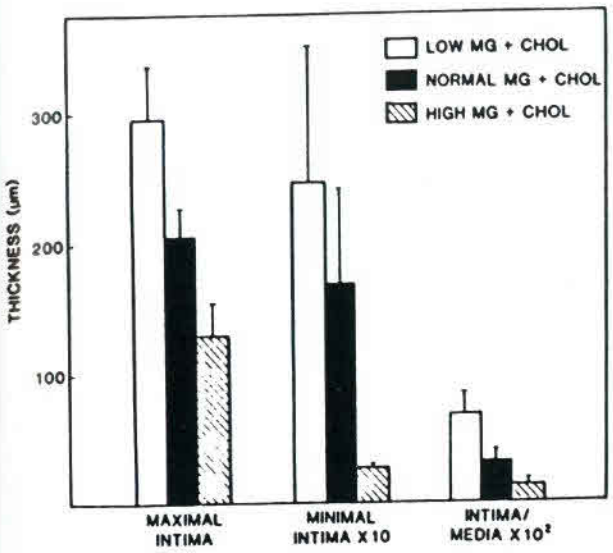

Fig. 1. Influence of varying Mg intake on thickness and intima/media ratio of intimal atheroma in rabbits fed 2% cholesterol. Values are means ± SD (n = 4 or 5 each). All high Mg and low Mg values are significantly different from their respective normal Mg values ($p < 0.02$, Student's t test). See Altura et al (1990) for details.

With respect to experimental diabetes and Mg, a number of studies point to a firm relationship between the disease state and Mg deficiency (Altura and Altura, 1990, 1995a,b; Altura et al., 1993). Both streptozotocin-induced and alloxan-induced diabetes in rats are associated with an intense magnesuria, glycosuria, and polyuria. Studying alloxan-induced diabetes in rats, we found that serum TMg is often reduced after 8 weeks in many animals, but ultrafilterable Mg^{2+} is reduced relatively more consistently (Table 5) (Altura and Altura, 1995c). Most importantly, the few animals that develop high blood pressure show marked deficits in ultrafilterable Mg^{2+}. These animals also demonstrate considerable elevation in serum total cholesterol and triglycerides, suggesting a rational basis for diabetic (atherosclerotic) vascular disease linked to Mg deficiency. Concomitant with these substantive vascular risk factors, the arterial walls of alloxan-treated diabetic rats show increases in exchangeable, membrane-bound, and intracellular ^{45}Ca with an accompanying reduction in cellular Mg content (Altura and Altura, 1983, 1995c). Such data are consistent with the idea of increased vessel wall tone, increased vascular reactivity, and angiopathy known to be present in diabetes mellitus.

Evidence has accumulated during the past decade to suggest a strong association between clinical diabetes, hypertension, dyslipidemias, and abnormal glucose tolerance. Data scattered in the literature collectively suggest that control of diabetes is inversely related to Mg deficiency (Altura et al., 1993; Altura and Altura, 1995a). Diabetic retinopathy is clearly associated with a state of Mg deficiency (Hatwal et al., 1989; McNair et al 1978; Wada et al., 1983). Both insulin-dependent and noninsulin-dependent (type II) diabetes mellitus (NIDDM) are associated with reduced serum TMg and intracellular Mg concentration and increased urinary loss of Mg (Altura et al., 1979; Bachem et al., 1980; Johansson et al., 1981; Paolisso et al., 1989; Sheehan, 1992; Sjogren et al., 1988). Even though not all diabetic patients studied exhibit a simultaneous reduction in serum TMg and intracellular free Mg^{2+}, oral treatment with Mg salts improves control of both types of diabetes, in the few studies done so far (Paolisso et al., 1989, 1992).

Using ISEs to measure free IMg^{2+} in fasting subjects with and without type II diabetes and ^{31}P-nuclear magnetic resonance (NMR) spectroscopy to measure intracellular free Mg^{2+} in red blood cells, we found that both Mg^{2+} levels were significantly reduced in diabetics compared with nondiabetic subjects (Table 6) (Resnick et al., 1993). A close relationship (r = 0.725, $p < 0.001$) was noted between serum IMg^{2+} and intracellular free Mg^{2+}. Studying women who develop diabetes during pregnancy (gestational diabetes), we recently reported similar data (Bardicef et al., 1995). Very recently, working with euglycemic-clamped NIDDM insulin-resistant vs. insulin-sensitive subjects, we noted inverse correlations of IMg^{2+} levels with arterial blood pressure and serum lipids in many of the insulin-resistant subjects only (Barron, Altura, Leibovitz, and Altura, unpublished data, 1995).

We thus propose that Mg deficiency, both extracel-

Table 5. Total and Ultrafilterable Magnesium in Alloxan-Diabetic Rats*

Group	n	TMg (mM)	Ultrafilterable Mg (mM)	% Ultrafilterable Mg
Saline controls	36	0.92 ± 0.02	0.60 ± 0.03	65.2 ± 1.72
Alloxan	18	0.73 ± 0.05†	0.42 ± 0.06†	57.5 ± 1.03†

* Experimental animals received 150 mg/kg alloxan i.p.; serum and ultrafiltration obtained at 8 wk. Values are means ± SE.

† Significantly different from saline controls ($p < 0.01$).

Table 6. Extracellular Ionized and Intracellular Free Mg Levels in Normal and NIDDM Subjects

Group	n	Serum (mM/l) TMg	Serum (mM/l) IMg^{2+}	RBC $[Mg^{2+}]_i$ (μM)
Normal controls	30	0.86 ± 0.01	0.630 ± 0.008	223.3 ± 8.3
NIDDM	22	0.81 ± 0.05	0.552 ± 0.008*	184.1 ± 13.7*

Values are means ± SEM. Adapted from Resnick et al. (1993).
* $p < 0.001$ vs. normal controls.

lular and intracellular, is a characteristic of chronic stable mild NIDDM and may predispose patients to the excess cardiovascular mortality of the diabetic state. By adequately reflecting cellular Mg metabolism, free Mg^{2+} measurements should provide a more useful tool than has heretofore been available to analyze Mg metabolism in diabetes.

MAGNESIUM METABOLISM IN IHD, ACUTE MYOCARDIAL INFARCTION, AND STROKE

If deficiency in cardiac and vascular tissue Mg^{2+} does play a role in etiology of atherogenesis and vascular disease, as the above suggests, then one would anticipate that a large number of patients with IHD, acute myocardial infarction (AMI), and stroke should demonstrate blood, cardiac, and vascular tissue deficiency of Mg. Although some evidence for decreased content of blood TMg, cardiac and coronary VSM TMg has been seen in some patients with IHD, AMI, and stroke, the data are not consistent (Altura, 1979; Altura and Altura, 1982, 1984; Chipperfield and Chipperfield, 1973; Seelig, 1980); most of the consistent data has been noted in cardiac tissue (i.e., mean 20% loss of TMg in hearts) of patients who died of sudden death IHD (Altura, 1979, 1988; Altura and Altura, 1984, 1990, 1995a; Crawford and Crawford, 1967; Chipperfield and Chipperfield, 1973, 1978; Seelig, 1980). We believe a great deal of these inconsistencies, until recently, have been due to the inability to measure serum IMg^{2+} and $[Mg^{2+}]_i$.

Data obtained recently by our group clearly shows, for the first time, consistent deficits in serum IMg^{2+} in many IHD patients (New York Heart Association classes 2–4) (Table 7), patients early after AMI (Table 8), and patients early after stroke (e.g., cerebral infarction, subarachnoid hemorrhage, cerebral hemorrhage) (Table 9). Measurements on $[Mg^{2+}]_i$ in cardiac and vascular tissue are not as yet available. Because we recently demonstrated a close correspondence between extracellular serum IMg^{2+} measurements (but not TMg) and red blood cell $[Mg^{2+}]_i$ measurements in untreated hypertension (Altura and Altura, 1995c; Altura et al., 1994b), NIDDM (Resnick et al., 1993), gestational diabetes in pregnant women (Bardicef et al., 1995), and ESRD patients (see below), we believe that the deficits in IMg^{2+} noted in the IHD, AMI, and stroke patients are most likely reflecting cardiovascular cell and tissue loss of $[Mg^{2+}]_i$.

EXTRACELLULAR AND INTRACELLULAR DEFICITS OF Mg^{2+} ARE OBSERVED IN ESRD

If deficits in blood and tissue Mg^{2+} are causative in the etiology of the widespread atherogenesis and hypertensive disease noted in ESRD, then we should expect to observe significant reductions in both extra- and intracellular Mg^{2+} in ESRD patients. Studying 26 hemodialysis patients (HEMO) and 10 continuous ambulatory peritoneal dialysis (CAPD) patients on dialysis for approximately 12 and 48 months, respectively, we found a 10–15% reduction in serum IMg^{2+} in the HEMO patients and a 15–25% reduction in IMg^{2+} in the CAPD patients (Markell et al., 1993b); ICa^{2+}/IMg^{2+} ratios were significantly elevated in both groups, suggesting the presence of peripheral vasoconstriction, increased vascular reactivity, and atherosclerosis. Because almost one-half of these subjects had underlying diabetic vascular disease and most were hypertensive, the IMg^{2+} and ICa^{2+}/IMg^{2+} data support our hypothesis.

Table 7. Plasma Ionized Magnesium vs. Total Magnesium in Ischemic Heart Disease Patients Scheduled for Coronary Bypass Surgery

Group	n	Mg (mM/l) IMg^{2+}	Mg (mM/l) TMg
Controls	42	0.60 ± 0.005	0.84 ± 0.008
IHD	35	0.50 ± 0.008*	0.82 ± 0.006

IMg^{2+} obtained with NOVA ISE on Stat Profile 8 Analyzer; TMg obtained with AAS. Values are means ± SEM.
* Significantly different from controls ($p < 0.001$).

Table 8. Serum Ionized Mg, Total Mg and Percent Ionized Mg Levels in Patients Early After Acute Myocardial Infarction

		Serum Mg (mM/l)		
Group	n	IMg^{2+}	TMg	$\%IMg^{2+}$
Controls	60	0.59 ± 0.003	0.82 ± 0.02	71.9 ± 0.57
AMI	6	0.52 ± 0.018*	0.833 ± 0.024	62.2 ± 1.58*

Values are means ± SEM.
* $p < 0.001$ vs. controls.

HOW DOES A PROLONGED STATE OF MAGNESIUM DEFICIENCY CAUSE ATHEROGENESIS AND VASCULAR DISEASE?

Low $[Mg^{2+}]_o$ *produces vasospasm, increased vascular tone, and elevation in* $[Ca^{2+}]_i$

Approximately 25 years ago, we demonstrated, for the first time, that reduction in $[Mg^{2+}]_o$ resulted in sustained vasospasm of most types of small peripheral and cerebral blood vessels; the lower the $[Mg^{2+}]_o$, the greater the developed tension and decreased arteriolar lumen sizes (Altura and Altura, 1974, 1978, 1980). Reduction of $[Mg^{2+}]_o$ also resulted in potentiation of most vasoconstrictor and pressor agents and decreased responses to dilator agents (Turlapaty and Altura, 1980; Altura and Altura, 1981a). The end result of lowered serum IMg^{2+} and $[Mg^{2+}]_i$ would be vasospasm, increased vascular tone, increased vascular reactivity, reduction in peripheral blood flow (hypoxia), and thus local vascular injury, a potent stimulus for hypertension and atherogenesis (Ross, 1993).

Using a combination of radiolabeled ^{45}Ca, digital image analysis with fluorescent probes and confocal laser scanning microscopy with fluorescent probes, we found that Mg^{2+} gates special membrane Ca^{2+} channels in both VSM and endothelial cells and controls the release of intracellular free cytosolic Ca^{2+} ($[Ca^{2+}]_i$) from these cells (Altura and Altura, 1981a,b, 1995a,c; Altura et al., 1982, 1987; Turlapaty and Altura, 1978; Zhang et al., 1991b, 1992a, 1993). The entry and release of $[Ca^{2+}]_i$ and loss of membrane-Mg^{2+} most likely initiates a number of intracellular signals (i.e., growth factors, adhesion factors, cytokines, and phospholipids; see below) that could result in VSM cell profiferation (and cell transformation), alteration in endothelial cell wall permeability, macrophage attraction, adhesion and cell entry. Low $[Mg^{2+}]_o$-induced vasospasm could accelerate uptake of oxidized lipids and/or stimulate generation of oxygen radicals (see below).

Low $[Mg^{2+}]_o$ *results in formation of proinflammatory agents*

Feeding rats and hamsters diets deficient in Mg, for periods of 14–21 days, results in cardiomyopathic lesions, cytokine formation and release (e.g., interleukins 1, 2, and 6; tumor necrosis factor-α), and endothelin formation (Freedman et al., 1990, 1991; Weglicki et al., 1992). Although other growth factors (e.g., transforming growth factor β; fibroblast growth factor) have not as yet been identified in Mg deficient states, it is likely that Mg^{2+}-deficiency will be found to cause production of many of the well-known growth factors implicated in atherogenesis (Ross, 1993; Schwartz et al., 1995).

Low $[Mg^{2+}]_o$ *results in lipid peroxidation and free radicals*

Until recently, it was not known how Mg deficiency could promote cardiovascular damage. During the past 5 years, reports from several laboratories, including ours, have suggested that Mg deficiency can lead to formation of ferrylmyoglobin and lipid peroxidation products.

We suggest that the link between Ca^{2+} overload induced by Mg deficiency and cardiac myocyte membrane damage is formation and action of the ferrylmyoglobin radical (Wu et al., 1994). Using rat perfused working hearts, we have prevented such injury by using one-electron reductants such as ascorbate (Wu et al., 1994).

Other lipid peroxidation products appear 7–21 days after dietary Mg deficiency is initiated in rats and ham-

Table 9. Serum Ionized Mg^{2+}, Ca^{2+}, and pH and Total Mg Assessed by Ion Selective Electrodes Early After Stroke in Men and Women

Group	n	IMg^{2+} (mM/l)	TMg (mM/l)	ICa^{2+}/IMg^{2+}	pH
Controls	40	0.58 ± 0.008	0.85 ± 0.009	2.04 ± 0.03	7.43 ± 0.02
Stroke	65	0.51 ± 0.005*	0.83 ± 0.007	2.29 ± 0.04*	7.42 ± 0.04

Values are means ± SEM. Stroke = subarachnoid hemorrhage, cerebral infarction, intracerebral hemorrhage.
* Significantly different from healthy control subjects ($p < 0.01$).

sters (Dickens et al., 1992; Weglicki et al., 1992; Weglicki, personal communication,1995). Several different antioxidants have been reported to afford complete protection against myocardial necrosis in these animal models (Atrakchi et al., 1992; Freedman et al., 1990, 1991) and against cardiac failure in our perfused hearts (Wu et al., 1994), strengthening the idea that free radicals are important in vascular injury induced by Mg deprivation. It is also possible that these antioxidants prevent loss of cellular Mg^{2+} or promote enhanced transport of Mg^{2+} into the vascular, endothelial, and cardiac cells.

Low $[Mg^{2+}]_o$ *enhances platelet aggregation and thrombotic tendencies*

Several studies dating back to the late 1950s indicate that low $[Mg^{2+}]_o$ promotes blood coagulation (Seelig, 1980; Weaver, 1980). More recently, several investigators have demonstrated that platelet agregation is enhanced in low $[Mg^{2+}]_o$ environments (Nadler et al., 1993) and that megakaryocyte numbers and white cell counts are increased in Mg-deficient hamsters (Rishi et al., 1990). Such pathways would obviously tend to produce thrombotic tendencies in atherogenesis and might play important roles in the "cracking and bleeding" of atherosclerotic plaques known to be seen in IHD and AMI patients.

Low $[Mg^{2+}]_o$ *results in deficits in coronary flow, reduced cardiac bioenergetic reserves, and cardiac failure*

Although Prinzmetal angina, sudden death ischemic heart disease, and AMI all can lead to arrhythmias and cardiac failure, until recently it has not been possible to explain these events as due to a Mg deficient state. Using intact perfused working rat hearts exposed to low, normal, or high $[Mg^{2+}]_o$, we have been able to establish a rational basis for how Mg^{2+} deficiency might provoke cardiac failure.

In vitro ^{31}P-NMR spectroscopy studies performed in our laboratories, on the intact perfused rat heart, indicates that acute elevation of extracellular free Mg^{2+} concentrations can increase phosphocreatine levels, intracellular pH, and intracellular free Mg^{2+} levels (Barbour et al., 1992) (Table 10). Intracellular inorganic phosphate levels decreased and the cytosolic phosphorylation potential and free energy of ATP hydrolysis increased accordingly (Barbour et al., 1992). Elevated levels of $[Mg^{2+}]_o$ also resulted in increases in coronary flow, cardiac output, and stroke volume, and there was a concomitant fall in oxygen consumption (Barbour et al., 1992; Wu et al., 1992). Many of these beneficial hemodynamic effects were also observed in intact dog hearts in vivo (Friedman et al., 1987).

Acute reductions in $[Mg^{2+}]_o$ result in opposite effects on cardiac performance, high-energy phosphates, intracellular pH, $[Mg^{2+}]_i$, cytosolic phosphorylation potential, and free energy of ATP hydrolysis (Table 10) (Altura et al., 1993b). Low $[Mg^{2+}]_o$ also resulted in decreased coronary flow, stroke volume, and cardiac output, leading to cardiac failure. These observations suggest that Mg^{2+} regulates cardiac performance, oxygenation, and substrate delivery in the myocardium. Elevated intracellular free Mg^{2+} and pH could be expected to enhance creatine kinase activity as well as that of other myocardial cellular enzymes, increasing cardiac efficiency, stroke volume, and coronary flow, whereas reductions in $[Mg^{2+}]_i$ and pH (cytosolic acidosis) would do the opposite.

Low $[Mg^{2+}]_o$ *results in oxidation of LDL*

Oxidized modification of lipoproteins is a well-recognized step in atherogenesis (Morel and Chisolm, 1989; Ross, 1993), and Mg deficiency is known to result in alterations in plasma lipoproteins and triglycerides (see above) (Altura et al., 1990; Rasmussen, 1993; Raysigguier, 1986). A recent study from France has now clearly demonstrated that Mg levels affect the susceptibility of lipoproteins to peroxidation (Raysigguier et al., 1993). Oxidized lipoproteins (e.g., oxidized very-low density lipoproteins [vlDL] and LDL) are clearly cytotoxic to cells in culture (Morel and Chisolm, 1989; Ross, 1993). This Mg^{2+}-mediated pathway may be an important step whereby oxidized LDL stimulates monocyte adhesion as has been shown recently for LDL treatment of endothelial cells (Berliner et al., 1990).

Gender-related hemodynamic differences appear to involve Mg^{2+}*: Roles of EDRF and* NO

Gender-related differences in hemodynamic characteristics have received attention because premenopausal women are known to be less susceptible than men to numerous cardiovascular disorders, including atherogenesis.

In the early 1980s, a variety of endogenous and exogenous vasodilators were demonstrated to exert their effects by acting on endothelial cells (Furchgott and Zawadzki, 1980). These investigators demonstrated that endothelial cells released a substance, or substances, termed EDRF, in response to vascular smooth muscle relaxants. It is now believed most EDRF activity is attributed to nitric oxide.

Table 10. Effects of Different Concentrations of Extracellular Mg^{2+} on Cytoplasmic Phosphates, Phosphorylation Potential, Intracellular Free Mg^{2+}, and Intracellular pH in Working Rat Perfused Hearts

$[Mg^{2+}]_o$ (mM)	P_i (mM)	ATP (mM)	PCr (mM)	CPP (mM^{-1})	$[Mg^{2+}]_i$ (μM)	pH_i
4.8	2.2 ± 0.6*	6.2 ± 0.4*	11.3 ± 0.5*	112 ± 24*	1210 ± 120*	7.21 ± 0.004*
1.2	4.7 ± 1.2	7.5 ± 0.1	9.2 ± 0.5	38.4 ± 10.2	651 ± 55	7.14 ± 0.044
0.3	13.9 ± 3.5*	4.7 ± 0.1*	6.3 ± 0.7*	7.6 ± 2.0*	426 ± 54*	7.00 ± 0.050†

Values are means ± SEM.
Adapted from Altura et al. (1993b) and Barbour et al. (1992).
* $p < 0.01$; † $p < 0.05$.
P_i, intracellular phosphorous; PCr, phosphocreatine; CPP, cytosolic phosphorylation potential; pH_i, intracellular pH.

Our laboratory has demonstrated gender-related differences in endothelial-cell dependent vascular responsiveness (Zhang et al., 1990, 1991a,b, 1992b). In aortas isolated from male rats, withdrawal of $[Mg^{2+}]_o$ and concomitant reduction in extracellular sodium ($[Na^+]_o$) induced significant increases of basal tone. Surprisingly, this did not occur in intact aortas removed from female rats, although it was observed in endothelium-denuded preparations from both sexes (Zhang et al., 1992b).

The observed gender-related differences were not dependent on animal strain or type of tissue preparation. No tension development was observed in aortas from castrated males treated with estradiol. Aortic tissues of sexually immature male and female rats, however, exhibited marked tension development when exposed to zero $[Mg^{2+}]_o$ and low $[Na^+]_o$. These studies suggest that sex steroid hormones, probably 17-β estradiol, can influence contractile responsiveness of VSM, and Mg^{2+} may regulate internal Na^+-dependent Ca^{2+} influx in endothelial cells. These data may help to explain why Mg-deficient women, unlike men, are protected against IHD, hypertensive vascular disease, and cerebrovascular disease until menopause.

MAGNESIUM-DEPENDENT CHANGES IN FATTY ACID SATURATION AND PLASMALOGEN CONTENT OF VSM: POSSIBLE RELATION TO INTRACELLULAR SIGNALING PATHWAYS

In atherogenesis and vascular disease, it has been demonstrated that membrane phospholipids are altered. The failure to focus on the latter may be one of the chief reasons that neither the frequency of restenosis after percutaneous transluminal coronary angioplasty has improved nor have the varied recommended treatments met with success. Approximately 8 years ago, our laboratory reported that dietary Mg deficiency in rats results in alterations of membrane phospholipids and fatty acid saturation in intact cardiac tissue (Brautbar and Altura, 1987). This early finding may be particularly important in light of recent evidence that indicates that phosphoinositide-derived second messengers are important in regulation of Ca^{2+} in VSM (Nahorski et al., 1994).

With such a background, we recently investigated whether Mg^{2+} modulated fatty acid saturation and plasmalogen (α,β-unsaturated ether) content in rat aortic and canine cerebral VSM membranes (Kostellow et al., 1995a). In our preliminary observations, using ion exchange chromatography to separate lipids and ^{1}H-NMR spectroscopy, we noted that exposure of primary cultured aortic VSM cells to low $[Mg^{2+}]_o$ for 18 h increased the fatty acid content of aortic VSM about 3-fold (Fig. 2). Fatty acid saturation clearly was increased 2-fold when aortic VSM cells were exposed to a medium containing 0.17 mM $[Mg^{2+}]_o$ vs. one containing 1.2 mM $[Mg^{2+}]_o$. These data show that the level of fatty acid unsaturation is quite sensitive to $[Mg^{2+}]_o$ concentrations over the human pathophysiologic range (0.3–0.6 mM) found by our ISEs in patients with hypertension, ESRD, IHD, AMI, NIDDM, and stroke (see above). Although not shown here, a similar Mg^{2+}-dependent increase in fatty acid saturation was observed in segments of canine cerebrovascular smooth muscle (Kostellow et al., 1995a).

We also found changes in the α,β-unsaturated ether protons of extracts of canine cerebrovascular smooth muscle cells incubated for 18 h in different $[Mg^{2+}]_o$ (Fig. 3) (Kostellow et al., 1995a). In contrast to the classic double bonds, discussed above, the α,β-unsaturated ether content of the N-containing phospholipid fraction increases in VSM cells as the $[Mg^{2+}]_o$ decreases. Collectively, such data, when viewed in light of our earlier data on cardiac muscle (Brautbar and Altura, 1987), suggest that Mg^{2+} can modulate phospholipid moieties and content in VSM cell membranes. Such attributes of Mg^{2+} could be pivotal in generating and controlling intracellular signal pathways.

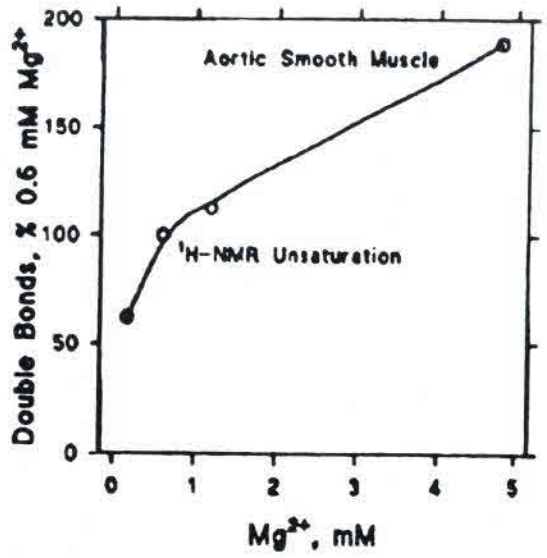

Fig. 2. Effect of $[^{2+}]_o$ on the saturated fatty acid content of rat aortic smooth muscle cells in culture. Primary cell cultures were incubated for 18 h in media containing the $[^{2+}]_o$ concentrations indicated (Kostellow et al., 1995a).

DOES MAGNESIUM MODULATE INTRACELLULAR FREE CALCIUM COMPARTMENTATION AND TRANSCRIPTION FACTOR NUCLEAR FACTOR-$\kappa\beta$?

Two years ago, it was suggested by Collins (1993) that endothelial nuclear factor-$\kappa\beta$ (NF-$\kappa\beta$) might be a pivotal factor in the initiation of the atherosclerotic lesion. He reviewed data suggesting that genes for a number of the endothelial-leukocyte adhesion molecules implicated in the etiology of atherogenesis contain functional NF-$\kappa\beta$ binding sites that are needed for cytokine induction in endothelial cells. Because the above indicates that Mg^{2+}-deficient states can generate cytokines, activation of this pleiotropic family of transcription factors may be important in the diversity of growth factor and cytokine gene expression associated with the dysfunctional endothelial and VSM cells seen in atherosclerosis. The fact that reduced $[Mg^{2+}]_o$ can result in oxygen radical generation and oxidation of LDL and VLDL (reviewed above) would support the ideas of oxidant stress-activation of the NF-$\kappa\beta$ family of transcription factors, linking "the atherosclerotic lesion into a final common pathway for induced endothelial gene activation," as suggested for NF-$\kappa\beta$ by Collins (1993). But, the missing link in this hypothesis may be $[Mg^{2+}]$.

Using aortic and cerebral VSM primary cell cultures, Kostellow, Morrill, Altura, Altura, and Gupta (unpublished data, 1995) have found presumptive preliminary evidence for the idea that low $[Mg^{2+}]_o$ can result in activation of the NF-$\kappa\beta$ family of transcription factors. This, and the proposed phospholipid intracellular signals, may be a consequence of a translocation of $[Ca^{2+}]_i$ by reduction in $[Mg^{2+}]_o$. Using primary rat aortic and canine cerebral VSM cell cultures, fura-2 (and fluo-3), digital image microscopy, and laser scanning confocal microscopy, we found that a reduction of $[Mg^{2+}]_o$ by 35–40% can result in an approximate 35-fold rise in nuclear $[Ca^{2+}]_i$ but only a 6- and 10-fold rise, respectively, in perinuclear and cytosolic cell $[Ca^{2+}]_i$ (Altura et al., 1993a; Altura and Altura, 1995a). Because large pre-RNA and tRNA transcription could be activated by such rises in nuclear $[Ca^{2+}]_i$, it is possible that this rapid elevation in $[Ca^{2+}]_i$ is pivotal in setting all other subcellular signaling and molecular events into motion.

CONCLUSIONS

This short review does not cover all the interactions between Mg^{2+} and the cardiovascular system. We have not touched on basic Mg^{2+} biochemistry, electrophysiologic aspects of Mg^{2+}, or membrane transport processes. Instead, we attempted to put together a working hypothesis that has evolved over our 30-plus years of research in the Mg^{2+} field, that is, to show the importance of measuring Mg^{2+} in patients with cardiovascular-renal disorders and how such measurements with ion-selective electrodes and ^{31}P-NMR spectroscopy can be useful in diagnosing and potentially preventing some of these diseases. By demonstrating that reduc-

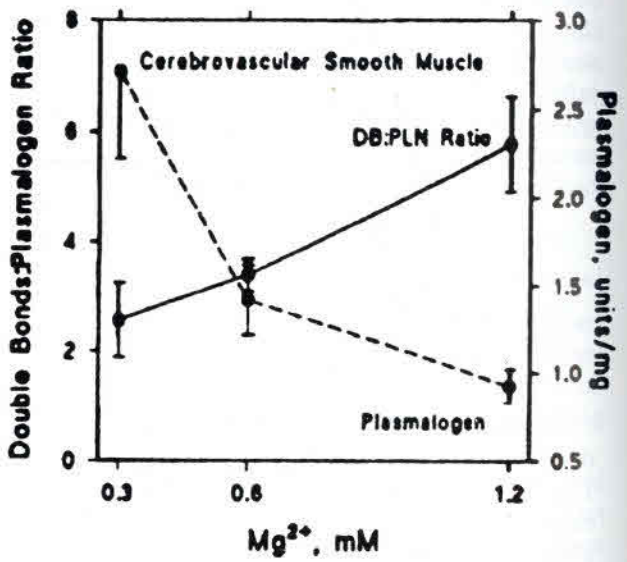

Fig. 3. ^{1}H-NMR measurements of the α,β-unsaturated ether of plasmalogen and double bond protons in total lipid extracts of canine cerebrovascular smooth muscle segments. Segments were incubated for 18 h in the $[^{2+}]_o$ concentrations shown. Values are means ± SD for three dogs (Kostellow et al., 1995a).

tion in $[Mg^{2+}]_o$ can induce an entire array of pathophysiologic phenomena (e.g., vasospasm, increased vascular reactivity, elevation in $[Ca^{2+}]_i$, formation of proinflammatory agents, oxygen radicals, platelet aggregation, reduction in cardiac bioenergetics, cardiac failure, oxidation of lipoproteins, gender-related modulation of EDRF-NO, changes in membrane fatty acid saturation, plasmalogen content and N-phospholipids [suggesting changes in intracellular signals], and probably the transcription factor nuclear factor-$\kappa\beta$), we provide a rationale for how Mg deficiency could result in hypertensive vascular disease, atherogenesis, IHD, AMI and stroke. Inadequate dietary intake of Mg or errors in Mg metabolism could result in dyslipidemias, insulin resistance, atherosclerosis, vascular thrombosis, stroke, and sudden cardiac death.

Acknowledgements — We are grateful for all discussions and collaboration extended to us over the years by Aimin Zhang, Randall Barbour, Adele Kostellow, Gene Morrill, Raj K. Gupta, Asefa Gebrewold, Roger Q. Cracco, Zaitoon Memon, Alexander Mauskop, Eli A. Friedman, Theo Günther, Hartmut Ising, Lawrence M. Resnick, Mariana S. Merkell, Terry L. Shirey, C. C. Young, Fann Wu, Karen Dell'Orfano, Toni P.-O. Cheng, Terry L. Dowd, Sara M. Handwerker, and Joshua Burack. We are also grateful to the National Institutes of Health (Research Grant AA-08674) for the support they have extended to us.

REFERENCES

Aikawa, J. K. Magnesium: its biologic significance. Boca Raton: CRC Press; 1981.

Altura, B. M. Sudden-death ischemic heart disease and dietary magnesium intake: is the target site coronary vascular smooth muscle? Med. Hypoth. 5:843–849; 1979.

Altura, B. M. Magnesium and regulation of contractility of vascular smooth muscle. Adv. in Microcirculation 11:77–113; 1982.

Altura, B. M. Ischemic heart disease and magnesium. Magnesium 7:57–67, 1988.

Altura, B. M. Importance of Mg in physiology and medicine and the need for ion selective electrodes. Scand. J. Clin. Lab. Invest. 54(Suppl. 217):5–10; 1994.

Altura, B. M.; Altura, B. T. Magnesium and contraction of arterial smooth muscle. Microvasc. Res. 7:145–155; 1974.

Altura, B. M.; Altura, B. T. Magnesium and vascular tone and reactivity. Blood Vessels 15:5–16; 1978.

Altura, B. M.; Altura, B. T. Magnesium ions and contraction of vascular smooth muscles: relationship to some vascular diseases. Federation Proc. 40:2672–2679, 1981a.

Altura, B. M.; Altura, B. T. Magnesium modulates calcium entry and contractility in vascular smooth muscle. In: Ohnishi, T.; Endo, M., eds. The mechanism of gated calcium transport across biological membranes. New York: Academic Press; 1981b:137–145.

Altura, B. M.; Altura, B. T. Influence of magnesium on vascular smooth muscle and serum biochemical parameters from diabetic and hypertensive rats. Magnesium 2:253–266; 1983.

Altura, B. M.; Altura, B. T. Magnesium, electrolyte transport and coronary vascular tone. Drugs 28(Suppl. 1):120–142; 1984.

Altura, B. M.; Altura, B. T. Role of magnesium in pathogenesis of high blood pressure: relationship to its actions on cardiac and vascular smooth muscle. In: Laragh, J. H.; Brenner, B. M., eds. Hypertension: pathophysiology, diagnosis, and management. New York: Raven Press; 1990:1003–1025.

Altura, B. M.; Altura, B. T. Role of magnesium and calcium in alcohol-induced hypertension and strokes as probed by in-vivo television microscopy, digital image microscropy, optical spectroscopy ^{31}P-NMR, spectroscopy and a unique magnesium ion-sensitive electrode. Alcoholism: Clin. Exp. Res. 18:1057–1068; 1994.

Altura, B. M.; Altura, B. T. Role of magnesium in the pathogenesis of hypertension updated: relationship to its actions on cardiac, vascular smooth muscle, and endothelial cells. In: Laragh, J. H.; Brenner, B. M., eds. Hypertension: pathophysiology, diagnosis, and management. New York: Raven Press, 1995a:1213–1242.

Altura, B. M.; Altura, B. T. Magnesium and cardiovascular diseases. In: Berthon, G., ed. Handbook of metal-ligand interactions in biological fluids, vol. 2. New York: Marcel Dekker, Inc., 1995b:822–842.

Altura, B. M.; Altura, B. T. Magnesium metabolism and cardiovascular pathobiology. In: Vecchiet, L., ed. Magnesium and physical activity. Lancaster, UK: Parthenon Press; 1995c:37–70.

Altura, B. M.; Turlapaty, P. D. M. V.; Halevy, S. Vascular smooth muscle in diabetes and its influence on reactivity of blood vessels. In: Davis, E., ed. The microcirculation in diabetes mellitus. Basel: Karger; 1979:118–150.

Altura, B. M.; Altura, B. T.; Carella, A.; Turlapaty, P. D. M. V. Ca^{2+} coupling in vascular smooth muscle: Mg^{2+} and buffer effects on contractility and membrane Ca^{2+} movements. Can. J. Physiol. Pharmacol. 60:459–482, 1982.

Altura, B. M.; Altura, B.T; Carella, A. Magnesium deficiency-induced spasms of umbilical vessels: relation to preeclampsia, hypertension, growth retardation. Science 221:376–378; 1983.

Altura, B. M.; Altura, B. T.; Gebrewold, A.; Ising, H.; Gunther, T. Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes in situ. Science 223:1315–1317, 1984.

Altura, B. M.; Altura, B. T.; Carella, A.; Gebrewold, A.; Murakawa, T.; Nishio, A. Mg^{2+}-Ca^{2+} interaction in contractility of vascular smooth muscle: Mg^{2+} versus organic calcium channel blockers on myogenic tone and agonist-induced responsiveness of blood vessels. Can. J. Physiol. Pharmacol. 65:729–745; 1987.

Altura, B. M.; Altura, B. T.; Gebrewold, A.; Gunther, T.; Ising, H. Noise-induced hypertension and magnesium: Relationship to microcirculation and Ca. J. Appl. Physiol. 72:194–202; 1992.

Altura, B. M.; Zhang, A.; Altura, B. T. Magnesium, hypertensive vascular diseases, atherogenesis, subcellular compartmentation of Ca^{2+} and Mg^{2+} and vascular contractility. Mineral Electrolyte Metab. 19:323–336, 1993a.

Altura, B. M., Barbour, R. L.; Dowd, T. L.; Wu, F.; Altura, B. T.; Gupta, R. K. Low extracellular magnesium induces intracellular free Mg deficits, ischemia, depletion of high-energy phosphates and cardiac failure in intact working rat hearts: A ^{31}P-NMR study. Biochim. Biophys. Acta 1181:328–332, 1993b.

Altura, B. M.; Lewenstam, A. Unique magnesium ion selective electrodes. Scand. J. Clin. Lab Invest. 54(Suppl. 217):1–100, 1994.

Altura, B. T.; Altura, B. M. Withdrawal of magnesium causes vasospasm while elevated magnesium produces relaxation of tone in cerebral arteries. Neurosci. Lett. 20:323–327, 1980.

Altura, B. T.; Altura, B. M. The role of magnesium in etiology of strokes and cerebrovasospasm. Magnesium 1:277–291, 1982.

Altura, B. T.; Brust, M.; Barbour, R. L.; Bloom, S.; Stempak, J.; Altura, B. M. Magnesium dietary intake modulates blood lipid levels and atherogenesis. Proc. Natl. Acad. Sci. USA 87:1840–1844; 1990.

Altura, B. T.; Shirey,T. L.; Young, C. C.; Dell'orfano, K.; Hiti, J.; Welch, R.; Yen, Q.; Barbour, R. L.; Altura, B. M. Characterization of a new ion selective electrode for ionized magnesium in whole blood, plasma, serum and aqueous samples. Scand. J. Clin. Lab. Invest. 54(Suppl. 217):21–36, 1994a.

Altura, B. T.; Burack, J.; Cracco, R. Q.; Galland, L.; Handwerker, S. M.; Markell, M. S.; Mauskop, A.; Memon, Z. S.; Resnick, L. M.; Zisbrod, Z.; Altura, B. M. Clinical studies with the NOVA ISE for Mg^{2+}. Scand. J. Clin. Lab. Invest. 54(Suppl. 217):53–68, 1994b.

Atrakchi, A. H.; Bloom, S.; Dickens, B. F.; Mak, I. T.; Weglicki,

W. B. Hypomagnesemia and isoproterenol cardiomyopathies: protection by probucol. Cardiovasc. Pathol. 1:155–160; 1992.
Bachem, V.; Strobel, B.; Jastram, U.; Jannsen, E. G.; Paschen, K. Magnesium and diabetes. Magn. Bull. 1:35–39; 1980.
Bardicef, M.; Bardicef, O.; Sorokin, Y.; Altura, B. M.; Altura, B. T.; Cotton, D. B.; Resnick, L. M. Extracellular and intracellular magnesium depletion in pregnancy and gestational diabetes. Am. J. Obstet. Gynecol. 172:1009–1013; 1995.
Barbour, R. L.; Altura, B. M.; Reiner, S. D.; Dowd, J. L.; Wu, F.; Altura, B. T.; Gupta, R. K. Influence of Mg^{2+} on cardiac performance, intracellular free Mg^{2+} and pH in perfused hearts as assessed with ^{31}P-nuclear magnetic resonance spectroscopy. Magnes. Trace Elem. 10:99–116, 1992.
Bloom, S.; Peric-Golia, L. Geographic variations in the incidence of myocardial calcification associated with acute myocardial infarction. Hum. Pathol. 20:726–731; 1989.
Brautbar, N.; Altura, B. M. Hypophosphatemia and hypomagnesemia result in cardiovascular dysfunction: theoretical basis for alcohol-induced cellular injury. Alcoholism. Clin. Exp. Res. 11:118–126, 1987.
Chipperfield, B.; Chipperfield, J. R. Heart muscle magnesium, potassium, and zinc concentrations after sudden death from heart disease. Lancet 2:293–296; 1973.
Chipperfield, B.; Chipperfield, J. R. Differences in metal content of the heart muscle in death from ischemic heart disease. Am. Heart J. 95:732–737; 1978.
Collins, T. Biology of disease. Endothelial nuclear factor-κB and the initiation of the atherosclerotic lesion. Lab. Invest. 68:499–508; 1993.
Crawford, T.; Crawford, M. D. Prevalence and pathological changes of ischaemic heart disease in a hard-water and in a soft-water area. Lancet 1:229–232; 1967.
Davis, W. H.; Leary, W. P.; Reyes, A. J.; Olhaberry, J. V. Monotherapy with magnesium increases abnormally low high density lipoprotein cholesterol: a clinical assay. Curr. Ther. Res. 36:341–346; 1984.
Dickens, B. F.; Weglicki, W. B.; Li, S.; Mak, I. T. Magnesium deficiency in vitro enhances free-radical intracellular oxidation and cytotoxicity in endothelial cells. FEBS Lett. 311:187–191;1992.
Eisenberg, M. J. Magnesium deficiency and sudden death. Am. Heart J. 10:269–280; 1991.
Freedman, A. M.; Atrakchi, A. H.; Cassidy, M. M.; Weglicki, W. B. Magnesium deficiency-induced cardiomyopathy: protection by vitamin E. Biochem. Biophys. Res. Commun. 170:1102–1106; 1990.
Freedman, A. M.; Cassidy, M. M.; Weglicki, W. B. Captopril protects against myocardial injury by magnesium deficiency. Hypertension 18:142–147; 1991.
Friedman, H. S.; Nguyen, T. N.; Mokroaui, A. M.; Barbour, R. L.; Murakawa, T.; Altura, B. M. Effects of magnesium chloride on cardiovascular dynamics in the neurally intact dog. J. Pharmacol. Exp. Ther. 243:126–130; 1987.
Furchgott, R. F.; Zawadzki, J. V. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376; 1980.
Gottlieb, S. S.; Baruch, L.; Kukin, M. L.; Berstein, J. L.; Fisher, M. L.; Packer, M. Prognostic importance of the serum magnesium concentration in patients with congestive heart failure. J. Am. Coll. Cardiol. 16:827–831; 1990.
Hatwal, A.; Gujral, A. S.; Bhatia, R. P. S.; Agrawal, J. K.; Bajpai, H. S. Association of hypomagnesemia with diabetic retinopathy. Acta Ophthamol. 67:714–716; 1989.
Joffres, M. R.; Reed, D. M.; Yano, K. Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu Heart Study. Am. J. Clin. Nutr. 45:469–475; 1987.
Johansson, G.; Danielson, B. G.; Ljunghall, J.; Wibell, L. Evidence for a disturbed magnesium metabolism in diabetes mellitus. Magnes. Bull. 2:178–180; 1981.
Keller, P. K.; Aronson, R. S. The role of magnesium in cardiac arrhythmias. Prog. Cardiovasc. Dis. 32:433–448; 1990.
Kostellow, A. B.; Morrill, G. A.; Ma, G.-Y.; Zhang, A.; Altura, B. T.; Altura, B. M.; Gupta, R. K. Mg^{2+}-dependent changes in fatty acid unsaturation and plasmalogen content of vascular smooth muscle cells: A ^{1}H-NMR study (abstract). Proc. Intl. NMR Meeting, Nice, August 1995.
Markell, M. S.; Altura, B. T.; Barbour, R. L.; Altura, B. M. Ionized and total magnesium levels in cyclosporin-treated renal transplant recipients: relationship with cholesterol and cyclosporin levels. Clin. Sci. 85:315–318; 1993a.
Markell, M. S.; Altura, B. T.; Sarn, Y.; Delano, B. G.; Ifudo, O.; Friedman, E. A.; Altura, B. M. Deficiency of serum ionized magnesium in patients receiving hemodialysis or peritoneal dialysis. ASAIO J. 39:M801-M804; 1993b.
Marier, J. R. Magnesium content of the food supply in the modern-day world. Magnesium 5:1–8; 1986.
Masironi, R. Geochemistry and cardiovascular diseases. Philos. Trans. R. Soc. Lond. 288:193–203, 1979.
McNair, P.; Christiansen, C.; Madsbad, S.; Lauritzen, E.; Faber, O.; Bindr, C.; Transbol, I. Hypomagnesemia, a risk factor in diabetic retinopathy. Diabetes 27:1075–1078, 1978.
Millane, T. A.; Jennison, S. H.; Mann, J. M.; Holt, D. W.; McKenna, W. J.; Camm, A. J. Myocardial magnesium depletion associated with prolonged hypomagnesemia: a longitudinal study in heart transplant recipients. J. Am. Coll. Cardiol. 20:806–812, 1992.
Miller, W. L.; Crabtree, B. F.; Evans, D. K. Exploratory study of the relationship between hypertension and diet diversity among Saba islanders. Public Health Rep. 107:426–432; 1992.
Miyagi, H.; Yasue, H.; Okumura, K.; Ogawa, H.; Goto, K.; Oshimia, S. Effect of magnesium on anginal attack induced by hyperventillation in patients with variant angina. Circulation 79:597–602; 1989.
Moller, D. E. Insulin resistance. New York: Wiley; 1993.
Morel, D. W.; Chisolm, G. M. Antioxidant treatment of diabetic rat inhibits lipoprotein oxidation and toxicity. J. Lipid Res. 30:1827–1834; 1989.
Nadler, J. L.; Goodson, S.; Rude, P. Evidence that prostacyclin mediates the vascular action of magnesium in humans. Hypertension 9:379–383; 1987.
Nadler, J. L.; Buchanan, T.; Natarajan, R.; Antonipillai, I.; Bergman, R; Rude, R. Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. Hypertension 21(part 2):1024–1029; 1993.
Nahorski, S. R.; Wilcox, R. A.; Mackrill, J. J.; Challiss, R. A. J. Phosphoinositide-derived second messengers and the regulation of Ca^{2+} in vascular smooth muscle. J. Hypertens. 12(Suppl. 10):S133-S143; 1994.
Ng, L. L.; Davies, J. E.; Ameen, M. Intracellular free-magnesium levels in vascular smooth muscle and striated muscle cells of the spontaneously hypertensive rats. Metabolism 41:722–727; 1992.
Paolisso, G.; Sgambato, S.; Pizza, G.; Passariello, N.; Varicchio, M.; D'Onofrio, F. Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. Diabetes Care 12:265–269; 1989.
Paolisso, G.; DiMaro, G.; Cozzolino, T.; Salvator, T.; D'Amore, A.; Lama, D.; Varicchio, M.; O'Onofrio, F. Chronic magnesium administraiton enhances oxidative glucose metabolism in thiazide treated hypertensive patients. Am. J. Hypertens. 5:681–686; 1992.
Rasmussen, H. S. Justification for magnesium therapy in acute ischaemic heart disease. Clinical and experimental studies. Danish Med. Bull. 40:84–99; 1989.
Rasmussen, H. S.; Autrup, P.; Golstein, K.; Mcnair, P.; Mortensen, P. B.; Larsen, O. G.; Lawaetz, H. Influence of magnesium substitution therapy on blood lipid composition in patients with ischemic heart diseases. Arch. Intern. Med. 149:1050–1053; 1989.
Rayssiguier, Y. Magnesium, lipids and vascular disease. Magnesium 5:182–190; 1986.
Rayssiguier, Y.; Gueux, E.; Bussiere, L.; Durlach, J.; Mazur, A. Dietary magnesium affects susceptibility of lipoproteins and tissues to peroxidation in rats. J. Am. Coll. Nutr. 12:133–137; 1993.
Resnick, L. M.; Altura, B. T.; Gupta, R. K.; Alderman, M. H.; Altura, B. M. Intracellular and extracellular magnesium depletion in type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 36:767–770; 1993.

Resnick, L. M.; Bardicef, O.; Altura, B. T.; Alderman, M. H.; Altura, B. M. Serum ionized magnesium. Relation to blood pressure and racial factors. Submitted, 1995.

Rishi, M.; Ahmad, A.; Makheja, A.; Karcher, D.; Bloom, S. Effects of reduced dietary magnesium on platelet production and function in hamsters. Lab. Invest. 63:717–721; 1990.

Ross, R. Atherosclerosis: a defense mechanism gone awry. Am. J. Pathol. 143:987–1001, 1993.

Rouse, I. L.; Beillin, L. J.; Armstrong, B. K.; Vandongen, R. Blood pressure-lowering effect of a vegetarian diet. Lancet 1:5–10; 1983.

Schwartz, S. M.; deBois, D.; O'Brien, E. R. M. The intima. Soil for atherosclerosis and restenosis. Circ. Res. 77:445–465; 1995.

Seelig, M. S. Magnesium deficiency in the pathogenesis of disease. New York: Plenum Press; 1980.

Sheehan, J. P. Magnesium deficiency and diabetes mellitus. Magnes. Trace Elements 10:215–219, 1992.

Sjogren, A.; Floren, E.; Nilsson, A. Magnesium, potassium and zinc deficiency in subjects with Type II diabetes mellitus. Acta Med. Scand. 224:461–465, 1988.

Turlapaty, P. D. M. V.; Altura, B. M. Extracellular magnesium ions control calcium exchange and content of vascular smooth muscle. Eur. J. Pharmacol. 52:421–423; 1978.

Turlapaty, P. D. M. V.; Altura, B. M. Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. Science 208:198–200; 1980.

Wada, M.; Jujii, S.; Takemura, T.; Seki, J.; Akai, T. Magnesium levels and diabetic retinopathy. Magnes. Bull 1:12–14, 1983.

Weaver, K. A possible anticoagulant effect of magnesium in preeclampsia. In: Cantin, M.; Seelig, M. S., eds. Magnesium in health and disease. Jamaica: Spectrum Publishers Inc., 1980:833–838.

Weglicki, W. B.; Phillips, T. M.; Freedman, A. M.; Cassidy, M. M.; Dickens, B. F. Magnesium-deficiency elevates circulating levels of inflammatory cytokines and endothelin. Mol. Cell. Biochem. 118:105–111, 1992.

Wu, F.; Zou, L. Y.; Altura, B. T.; Barbour, R. L.; Altura, B. M. Low extracellular magnesium results in cardiac failure in isolated perfused hearts. Magnes. Trace Elements 10:364–373; 1992.

Wu, F.; Altura, B. T.; Gao, J.; Barbour, R. L.; Altura, B. M. Ferrylmyoglobin formation induced by magnesium deficiency in perfused rat heart causes cardiac failure. Biochim. Biophys. Acta Mol. Dis. 1225:158–164, 1994.

Zhang, A.; Altura, B. T.; Altura, B. M. Endothelium-dependent differences in responsiveness of rat aortic smooth muscle to reduction in extracellular magnesium and sodium ions. Magnes. Trace Elements 9:186–190; 1990.

Zhang, A., Altura, B. T.; Altura, B. M. Sexual dimorphism of vascular smooth muscle responsiveness is dependent on anions and estrogen. Steroids 56:524–526,; 1991a.

Zhang, A.; Carella, A.; Altura, B. T.; Altura, B. M. Interactions of magnesium and chloride ions on tone and contractility of vascular muscle. Eur. J. Pharmacol. 203:223–235; 1991b.

Zhang, A.; Cheng, T. P.-O.; Altura, B. M. Magnesium regulates intracellular free ionized calcium concentration and cell geometry in vascular smooth muscle cells. Biochim. Biophys. Acta Mol. Cell Res. 1134:25–29, 1992a.

Zhang, A.; Altura, B. T.; Altura, B. M. Endothelial-dependent sexual dimorphism in vascular smooth muscle: role of Mg^{2+} and Na^{+}. Br. J. Pharmacol. 105:305–310; 1992b.

Zhang, A.; Cheng, T. P.-O.; Altura, B. T.; Altura, B. M. Mg^{2+} and caffeine-induced intracellular Ca^{2+} release in human vascular endothelial cells. Br.J. Pharmacol. 109:291–292; 1993.