Blood pressure regulation and micronutrients

Krishnamurti Dakshinamurti*[1] and Shyamala Dakshinamurti[2]

[1]*Department of Biochemistry and Medical Genetics and* [2]*Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3*

This review attempts to delineate the underlying mechanisms leading to the development of hypertension as well as the function of vitamins and minerals in the regulation of blood pressure. Physiological processes that regulate cardiac output and systemic vascular resistance impact on the control of blood pressure. Metabolic abnormalities associated with the tetrad of hypertension, dyslipidaemia, glucose intolerance and obesity share insulin resistance, which might be organ or cell specific, as an underlying feature representing different tissue manifestation of a common cellular ionic defect. As Ca is at the centre of ionic regulation of cellular functions, vitamins involved in Ca regulation have a significant role in the control of blood pressure. The endothelium-dependent vasodilator, NO, is susceptible to oxidative damage. Hence, antioxidant vitamins and related factors regulate blood pressure through protection of NO. Robust evidence for the involvement of vitamin B_6 (pyridoxine), vitamin C, vitamin D and vitamin E in the regulation of blood pressure have been reported. The well-known roles of Na, K, Ca, Mg and Cl have been explored further. The action of various vitamins on blood pressure regulation cannot always be explained on the basis of their conventionally recognised 'vitamin function'. The non-traditional functions of vitamins and their derivatives can

---

be exploited as an adjunct to available pharmacological modalities in the treatment of hypertension.

Hypertension mechanisms: Micronutrients: Vitamins: Ion channels: Calcium: Pyridoxal phosphate

Introduction

Hypertension is the sustained elevation of systemic arterial pressure. Under resting conditions the mean arterial pressure (MAP) is about 110 mmHg (with the systolic blood pressure (SBP) in the range 135–140 mmHg and the diastolic blood pressure not greater than 90 mmHg). MAP is normally tightly regulated such that tissue perfusion is maintained with minimum vascular trauma. A subject with a MAP elevated above the value of 110 mmHg on at least two measurements is considered to be hypertensive. In industrialized Western societies, about 20–30 % of the adult population have some degree of elevation of blood pressure. Hypertension is not a single disease entity with a single identifiable aetiology; it is a clinical condition brought on by a number of factors. It is generally recognized that stress, poor diet, lack of exercise and related features of our civilization contribute significantly to the development of hypertension. Age, heredity, racial proclivity, obesity and dietary intake of salt are considered to be among the main factors in the development of hypertension. There are, as yet, unknown contributors. The use of anti-hypertensive drugs in the last 40 years has resulted in a significant control of hypertension in about half of the patients so that mortality from stroke and cardiovascular disease in hypertensive subjects has dropped considerably. The Systolic Hypertension in the Elderly Program (SHEP) Cooperative Research Group (1991), the reports of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (1993, 1997), the MRC/BHF Heart Protection Study (1999) and the Canadian Recommendations for the Management of Hypertension (1999) have indicated the effectiveness of various treatment programmes. However, as Reaven & Hoffman (1988) have pointed out, therapy has been based primarily on pharmacological approaches to lowering blood pressure, with little attention being paid to the underlying metabolic abnormalities. Recent research on the underlying pathophysiology of hypertension and on the roles played by environment and nutrients in the development or amelioration of hypertension will promote more effective treatment modalities.

This review delineates the underlying mechanisms leading to the development of hypertension as well as the function of micronutrients (vitamins and minerals) in blood pressure regulation. With regard to the vitamins, robust evidence for the involvement of vitamin B_6 (pyridoxine), vitamin C, vitamin D and vitamin E has been reported. In hypertensive patients, plasma homocysteine levels are strongly and independently correlated to arterial stiffness. Plasma levels of homocysteine are influenced by levels of folate, vitamin B_{12} and vitamin B_6. The well-known roles of Na, K, Ca, Mg and Cl have been extended further. Recent work has indicated the interrelationship among micronutrients in the control of blood pressure. With regard to the non-nutrient minerals, the toxic effects of As and Pb (which include the development of hypertension) are of particular public health significance.

Mechanisms that regulate cardiac output and systemic vascular resistance affect the control of blood pressure. Apart from the baroreceptor reflex, mechanisms involved in the renin–angiotensin–aldosterone (RAS) system and in the alteration of extracellular fluid volume

are the main players. Activation of the sympathetic nervous system, as well as the abnormal function of the vascular endothelium, have been recognized to contribute to the development of hypertension. The vast majority of hypertensive subjects who do not have a single identifiable cause for their hypertension are referred to as having 'essential hypertension'; multiple factors contribute to their elevated blood pressure. The deleterious consequences of hypertension arise out of increased workload on the heart and damage to the arteries themselves due to the increased pressure; thus, it is a major cause of cardiovascular disease, stroke and renal failure. In this review, hypertension refers primarily to 'essential hypertension'.

Hypertension and 'syndrome X'

It has been known for quite some time that the abnormal plasma lipoprotein concentrations in untreated hypertensive subjects increase their risk of coronary artery disease (MacMahon *et al*. 1985). Epidemiological data have documented the association between obesity and hypertension (Widgren *et al*. 1992). Increases in plasma of both glucose and insulin following a load of glucose in hypertensive subjects indicate an insulin-resistant condition (Ferranni *et al*. 1987). It has been reported that, in hypercholesterolaemia, increased levels of VLDL and decreased levels of HDL are associated with hyperinsulinaemia (Stadler *et al*. 1981); this may be secondary to insulin resistance (IR). The term 'syndrome X' was used by Reaven & Hoffman (1988) to describe the association of what has been referred to as the 'deadly quartet' of obesity, hyperlipidaemia, hypertension and hyperinsulinaemia/IR. The term 'insulin resistance syndrome' was suggested (Haffner *et al*. 1992*a*) for this as the underlying pathology for this cluster of metabolic disorders (hypertension, dyslipidaemia, obesity and glucose intolerance) is IR. The San Antonio Heart Study indicates that the existence of hyperinsulinaemia is a predictor of the development of hypertension, hypertriacylglycerolaemia, type 2 diabetes (NIDDM) and abnormal lipoproteinaemia (Morales *et al*. 1993).

Hypertension and associated metabolic abnormalities

Metabolic abnormalities associated with the tetrad of hypertension, dyslipidaemia, glucose intolerance and obesity can be understood by comparing the metabolic connections between hypertension, obesity and dyslipidaemia, on the one hand, with those between hypertension and glucose intolerance, on the other. IR is the common thread connecting these abnormalities. Alterations in intracellular Ca and its sequelae, vascular endothelial function and sympathetic nervous system activation, are other levels at which the tetrad of 'syndrome X' are connected.

Cross-sectional surveys provide much evidence relating obesity to hypertension. This evidence is further strengthened by longitudinal studies which show that obesity can be a predictor of hypertension (Haffner *et al*. 1992*b*; Widgren *et al*. 1992). Subjects in the San Antonio and Finnish studies, before the onset of hypertension had increased obesity, an adverse body-fat distribution, increased triacylglycerol levels and increased insulin concentration compared with those in subjects who remained normotensive at follow-up (Morales *et al*. 1993; Mykkanen *et al*. 1994; Haffner *et al*. 1996). There is a correlation between the degree of obesity and the level of circulating insulin. The incidence of hyperinsulinaemia is further increased in central obesity where the waist:hip ratio is high (Spangler *et al*. 1998). This relationship appears quite early in life, as prepubescent, obese, non-diabetic children show a positive correlation between hyperinsulinaemia and blood pressure (Kanai *et al*. 1990).

Independently, both obesity and hypertension, even when they do not coexist, are characterized by hyperinsulinaemia and insulin insensitivity. There is a positive correlation between hyperinsulinaemia and blood pressure in non-obese hypertensive subjects. Total insulin-mediated glucose uptake was suppressed in hypertensive subjects compared with normal controls: Ferrannini *et al.* (1987) showed that IR correlated with systolic and mean blood pressure. Hypertension is an insulin-resistant state with relative IR even in non-obese, non-glucose-intolerant hypertensive subjects compared with matched controls. There is selective IR primarily in skeletal and vascular smooth muscle while renal and sympathetic nervous system responses to insulin are maintained (Zemel, 1998). In obesity, on the other hand, IR is widespread with a decreased number of cell surface receptors as well as post-receptor defects (Ciaraldy *et al.* 1981). When both obesity and hypertension coexist there is greater IR than in normotensive obese subjects (Manicardi *et al.* 1986; Istfan *et al.* 1992). The relationship between IR and hypertension has been described in a multicentre study (Ferrannini *et al.* 1987). There was correlation between IR and blood pressure even after adjusting for BMI. Increased fasting insulin predicted the development of hypertension across all strata of BMI (Haffner *et al.* 1992*b*; Shetterly *et al.* 1994).

Type 2 diabetes (non-insulin-dependent diabetes; NIDDM) is more common in incidence than type 1 (insulin-dependent diabetes). Patients with NIDDM are generally overweight; in fact, obesity is a risk factor for NIDDM. Subjects with NIDDM secrete decreased amounts of insulin following an oral load of glucose; in addition, they are resistant to the action of insulin. IR is due to defective binding to its receptor on the plasma membrane as well as to post-receptor defects in insulin action. IR is not unique to NIDDM; it is also observed in hypertension, microalbuminuria, dyslipidaemia and abnormal obesity (Groop *et al.* 1993, 1996, 1997; Nosadini *et al.* 1994).

Targets of insulin action

Insulin administration increases sympathetic nervous system activity (Rowe *et al.* 1981). In obese individuals, insulin infusion increases plasma noradrenaline (O'Hare *et al.* 1989). The obese retain normal insulin sensitivity to stimulation of the sympathetic nervous system while having a reduced sensitivity to glucose disposal. Selective IR leads to hyperinsulinaemia which, in turn, stimulates sympathetic nervous system activity and increases renal Na retention, thereby increasing peripheral resistance. Insulin exerts an anti-natriuretic effect; it also has a direct vasodilatory effect on vascular smooth muscle of several vascular beds in normal subjects but not in the obese (Anderson *et al.* 1991). The vasoconstrictor action of insulin through sympathetic nervous system activation is opposed by the direct effect of insulin as a vasodilator. In the obese, the vasodilatory effect of insulin is attenuated, tipping the balance in favour of vasoconstriction.

Insulin regulates vascular tone by regulating $[Ca^{2+}]_i$ of the vascular smooth muscle (Zemel, 1995). Insulin attenuates both voltage- and receptor-mediated Ca^{2+} influx in cultured vascular smooth muscle (VSM) cells (Standley *et al.* 1991). Physiological concentrations of insulin attenuate the $[Ca^{2+}]_i$ response of arginine vasopressin, angiotensin II and noradrenaline (Touyz *et al.* 1994). In addition, the attenuation of vasoconstriction in canine femoral artery cells has been shown to be dependent on the inhibition of Ca influx. This direct vasodilatory action of insulin is due to the stimulation of VSM Ca^{2+}-ATPase. In the insulin-resistant state, Ca efflux from VSM is attenuated, leading to vasoconstriction (Zemel *et al.* 1992*a*).

Activation of the sympathetic nervous system

A significant number of even borderline hypertensive individuals have increased autonomic drive, resulting in increase in heart rate and cardiac output (Julius, 1996). This was shown in young borderline hypertensive subjects; in such subjects, plasma noradrenaline (an index of sympathetic tone) was also increased. Plasma noradrenaline concentration increases with age, perhaps reflecting the increase in blood pressure with age. In hypertensive subjects, micro-neurography has shown enhanced muscle sympathetic nerve activity (Flora & Hara, 1993; Grassi *et al*. 1997; Mancia, 1997). There was progressive increase in sympathetic tone with increasing severity of essential hypertension. Patients with secondary hypertension did not show any increase in muscle sympathetic nerve activity compared with normotensive subjects. Muscle sympathetic nerve activity was also increased in obese subjects, whether hypertensive or normotensive. Sympathetic activation has been shown to be elevated in subjects with IR (Ferrannini *et al*. 1987). Sympathetic activation stimulates renin secretion and the resulting angiotension II further activates the sympathetic nervous system, both centrally and peripherally. The cause of sympathetic activation in essential hypertension is not yet known, although the phenomenon has been demonstrated repeatedly (Mancia, 1997; Rahn *et al*. 1999).

Impaired vascular endothelial function

The vascular endothelium is important for determining vascular tone through its production of vasoconstrictor and vasodilator factors which control the activity of the underlying smooth muscle layer (Vane *et al*. 1990). The term 'endothelial function' is used to refer to stimulated endothelium-dependent vasodilatation. Impaired endothelial function *in vivo* in human arterial hypertension and hypercholesterolaemia has been reviewed (Vanhoutte, 1999; John & Schmieder, 2000). NO, the most important vasodilator produced by the endothelium, is formed from arginine through the action of NO synthase. This enzyme is stimulated by shear stress caused by blood flow across the endothelium, as well as by chemical mediators such as acetylcholine which act on its receptors on the endothelial cell membrane. NO is produced and released both tonically and under stimulation by endothelial agonists. NO diffuses to the underlying smooth muscle cells and activates soluble guanylate cyclase to generate cGMP, which causes smooth muscle relaxation and, thence, endothelium-dependent vasodilatation (Vallance *et al*. 1989).

The plasma concentrations of the oxidative metabolites of NO such as nitrite and nitrate are reduced in patients with essential hypertension, indicating that both resting and stimulated production of NO are impaired in this condition (Forte *et al*. 1997). In hypercholesterolaemia, on the other hand, stimulated release of NO is impaired whereas the tonic release is unaffected. In patients with essential hypertension the release of NO is augmented by a reduction in blood pressure brought about by angiotension-converting enzyme (ACE) inhibitor or by Ca-channel antagonists (Lyons *et al*. 1994). Ca-channel antagonists are effective in reversing endothelial dysfunction in vessels in several vascular beds. Angiotension II increases the production of superoxide which is associated with impaired VSM relaxation stimulated by acetylcholine. Angiotension receptor (AT_1) antagonists are also effective in reversing the impairment of endothelial function in hypertensive subjects (Dijkhorst-Oei *et al*. 1999). In both hypertension and hypercholesterolaemia, oxidative stress is a major mechanism leading to impairment of endothelial function.

Impairment of vascular endothelial function due to decreased NO release not only affects vascular tone but also accelerates the pathogenetic processes in dyslipidaemic subjects leading

to atherosclerosis, such as platelet aggregation, VSM cell proliferation and migration, mono-cyte adhesion as well as expression of adhesion molecules (John & Schmieder, 2000). Lipid-lowering drugs are as effective in reversing the impairment of endothelial function as Ca-channel antagonists are in essential hypertensives.

Natriuretic peptides

Atrial natriuretic peptide (ANP), a member of the family of natriuretic peptides, regulates a variety of physiological processes including blood pressure, progesterone secretion, renin release and vasopressin release through its effect on the generation of second messengers cAMP or cGMP and through its effect on ion channels (Anand-Srivastava & Trachte, 1993). Functionally, ANP is antagonistic to the roles of vasopressin, endothelins and the RAS system. There is a decrease in ANP receptor binding in hypertensive subjects and animals. Sympathetic nervous system activation in the kidney results in the activation of the RAS system, leading to increased production of angiotension II (AII). AII increases aldosterone secretion; it causes vasoconstriction through an increase in Ca influx. Noradrenaline, through α-adrenoreceptor agonism, and AII, through activation of Na^+K^+-ATPase, profoundly decrease renal Na excretion. These actions are opposed by dopamine (DA) and ANP. ANP recruits silent DA D_1 receptors from the cell interior to the plasma membrane and DA acting through these receptors inhibits Na^+K^+-ATPase. These actions are mimicked by cGMP, the second messenger for ANP, and require DA binding to D_1 receptors (Holtback *et al*. 2000). ANP, through ANP–C-receptor binding, inhibits AII-mediated increase in adenyl cyclase. It also inhibits endothelin (vasoconstrictor) secretion and MAP kinase activity required for mitogenesis. DA itself is produced intrarenally from the precursor amino acid L-3,4-dihydroxyphenylalanine through the action of 3,4-dihydroxyphenylalanine decarboxylase, for which pyridoxal phosphate (PLP; the active form of vitamin B_6) is a cofactor. Thus, ANP antagonizes the anti-natriuretic actions of nor-adrenaline and the RAS System. The inhibition of adenylate cyclase by ANP is general. Heart and aorta from genetic models of hypertension, as well as from other animal models such as the deoxycorticosterone acetate (DOCA)–NaCl and the 'one kidney–one clip' hypertensive rats, all show this inhibition (Anand-Srivastava *et al*. 1993; Anand-Srivastava, 1997). There are significant gender-specific differences in the incidence of hypertension, indicating the protective action of oestrogen. In addition to its effect on lipoproteins, oestrogen appears to modulate the renin–angiotension system. Oestrogen directly affects natriuretic peptide gene expression and also affects the mediator mechanisms (de Bold, 1999). Oestrogen affects the RAS through modulating angiotensinogen gene expression, reduces ACE activity and also decreases gene expression and density of AT_1, the G-protein-coupled AII receptor. Oestrogen also diminishes vascular tone by increasing the production of endothelium-derived vasodilators.

Ion channels in the control of blood pressure

Resnick (1999*a*) has proposed a unifying 'ionic' hypothesis taking into consideration cytosolic free Ca and Mg concentrations in a variety of cells and tissues and in a variety of clinical conditions in relation to the concentrations of these ions in normal subjects. These ions contribute to the final common pathways mediating not only blood pressure but also the major metabolic processes in all cells. Resnick provides a cellular explanation for the heterogeneity of hypertension as well as the coexistence of several clinical conditions such as hypertension, obesity and

NIDDM. Steady-state elevation of cytosolic free Ca is associated with a suppression of cytosolic free Mg in many tissues. These concomitant changes alter many cellular functions. In blood vessels, this causes vasoconstriction, arterial stiffness and hypertension. These ionic changes result in hypertrophy in the heart, aggregation and thrombosis in platelets, IR in skeletal muscle and fat tissue, hyperinsulinaemia in the pancreatic β-cells and activation of the central nervous system. The alterations in cellular ionic steady state become critical in defining the pathophysiology shared by hypertension, cardiac hypertrophy, dyslipidaemia including obesity, NIDDM and vascular disease. This would, as detailed later, also explain the heterogeneity of hypertension.

Vascular plasma membrane depolarization through decreased K^+ channel activation or stretch produces opening of the voltage-dependent T and L Ca channels or 'leak' channels, with subsequent Ca current representing influx into the cytosol of extracellular Ca. This increase in intracellular Ca induces activation of specific ryanodine-sensitive Ca-release channels in the sarcoplasmic reticulum and magnifies the initial localized increase in $[Ca^{2+}]_i$ more generally throughout the cytosol. At the contractile site, the increase in Ca initiates the cascade of molecular rearrangements of calmodulin and myosin light chain kinase (MLCK), resulting in myofilament shortening and vasoconstriction. Muscle relaxation is a reversal of the ionic balance through the sarcoplasmic reticulum-mediated reuptake of Ca into intracellular storage as well as the plasma membrane Ca-ATPase and Na/Ca exchange-mediated Ca egress from the cell. These would result in reducing $[Ca^{2+}]_i$.

In electrically non-excitable tissues, intracellular Ca is augmented primarily through stimulation of the phospholipase c-mediated inositol-1,4,5-triphosphate (IP_3) pathway. Some tissues have both voltage/receptor-mediated as well as intracellular mobilization pathways to augment intracellular Ca. The end result of this would include processes such as hormone secretion and renal ion secretion.

The role of Ca in regulating cellular processes, including vascular tone, is dependent upon (and is modified by) cellular Mg. Mg is a cofactor for all phosphotransferase reactions, in all ATP-dependent reactions where Mg-ATP is the substrate and in all thiamine triphosphate-dependent reactions. As such, it participates in intermediate metabolism of lipids, carbohydrates and amino acids. Mg is required for (and modifies) Ca entry and egress and other processes that use plasma membrane or endoplasmic reticulum Ca-ATPase, Na^+K^+-ATPase, L-channel Ca current, Ca binding to calmodulin, Ca-activated K^+ current as well as IP_3-mediated Ca release from cellular stores. The steady-state concentrations of Ca and Mg are reciprocally related. The net result is to maintain 'steady state cell metabolism' (Resnick, 1999a) and buffer cell response to Ca-dependent stimuli. A cellular Mg deficiency, by decreasing the function of a wide range of enzymes, membrane pumps and ion pumps, would exaggerate Ca-induced cell stimulation in all cells and tissues. In the vasculature this would result in vasoconstriction. Elevation of $[Ca^{2+}]_i$ with a reciprocal decrease in $[Mg^{2+}]_i$ is consistently seen in subjects with hypertension, obesity and NIDDM. These same changes are also observed in cells from healthy elderly persons.

There is a strong correlation between cellular ionic steady state in individual subjects and the pathophysiological changes in the tissues of these individuals (Resnick *et al*. 1997). Procedures which exaggerate or minimize these ionic abnormalities have corresponding effects on the clinical condition. Aortic distensibility of hypertensive or normal subjects, as determined by magnetic resonance imaging (MRI), is closely related to concomitantly measured Mg levels in brain or skeletal muscle using ^{32}P-NMR spectroscopy (MRS): the more depressed the Mg, the less distensible the aorta (Resnick *et al.* 1997).

The fasting steady-state $[Ca^{2+}]_i$ values are higher in hypertension, obesity and NIDDM. Along with lower $[Mg^{2+}]_i$ they predict fasting insulin and insulinaemia after glucose load, as well as peripheral IR. The MRI-determined abdominal visceral fat mass, a major indicator of IR,

is inversely related to MRS-determined values for $[Mg^{2+}]_i$ (Resnick *et al.* 1997). The higher the $[Ca^{2+}]_i$ and, reciprocally, the lower the $[Mg^{2+}]_i$, the more severe was the hypertension. Thus, the various clinical abnormalities associated with syndrome X represent different tissue manifestations of a common cellular ionic defect (Julius *et al.* 1992; Rockstroh *et al.* 1997; Maier *et al.* 1998). The hypothesis of Resnick (1999*a*), although not specifying the origin of the ionic defect, unifies the cellular defects in a variety of related clinical abnormalities. This would also indicate that, for any given extracellular concentration of calcium, states characterized by a high $[Ca^{2+}]_i$ (e.g. hypertension/obesity/NIDDM) will exhibit a blunted cellular and tissue response (e.g. IR).

Calcium and renin in hypertension

Subjects with essential hypertension are classified as having low or high levels of plasma renin activity. Those with low renin activity have lower levels of serum Ca than normotensive subjects; this is biologically relevant, as these subjects have high levels of serum parathyroid hormone (PTH) and 1,25-dihydroxycholecalciferol (1,25-(OH)$_2$ D$_3$) and low levels of calcitonin compared with normotensive individuals. The hormone distribution is consistent with a perceived extracellular deficit of Ca (Resnick *et al.* 1993); they are salt sensitive and their MAP is inversely proportional to the concentration of extracellular free Ca. The increased $[Ca^{2+}]_i$ of these subjects presumably comes from an extracellular source and hence the extracellular concentration of free Ca is low.

Subjects with essential hypertension and a high plasma concentration of renin belong to the category with a high extracellular free Ca concentration compared with normotensive subjects. The source of their $[Ca^{2+}]_i$ is intracellular stores. The serum distribution of the Ca-regulatory hormones in these subjects (low levels of PTH and 1,25-(OH)$_2$ D$_3$ and high levels of calcitonin) is opposite to that seen in those with hypertension and low renin, and reflects the high concentration of extracellular free Ca. The RAS system mobilizes calcium from the IP$_3$-dependent endoplasmic reticulum sources. The high extracellular Ca results from the plasma membrane compensating for increased $[Ca^{2+}]_i$ by Ca extrusion from the cell interior and by inhibiting Ca influx into the cell. In hypertensive subjects with high renin, the MAP is directly proportional to the concentration of free Ca in serum. The lack of response of this hypertensive subset to Ca-channel blockers reflects only the source of their increased $[Ca^{2+}]_i$ as through the IP$_3$-dependent mobilization from the endoplasmic reticulum and not from Ca influx from extracellular sources.

The ability of dietary salt (NaCl) to increase blood pressure in hypertensive subjects is proportional to the ability of NaCl to increase $[Ca^{2+}]_i$ and decrease $[Mg^{2+}]_i$. Ca-channel blockers are able to ameliorate hypertension in these low-renin hypertensive subjects. NaCl-induced rise in $[Ca^{2+}]_i$ is due to increased influx of Ca from extracellular space and exceeds the compensatory ability of the plasma membrane to extrude Ca. NaCl-induced increases in Ca-regulatory hormones and the parathyroid hypertensive factor (Resnick *et al.* 1993), NaCl-induced increase in peripheral noradrenaline (Campese *et al.* 1982) or arginine vasopressin level (Huang & Leenen, 1996), or increased sympathetic flow (Chen *et al.* 1988) contribute to the augmentation of extracellular Ca entry into the cell in this hypertensive subset.

Sodium overload

The relationship between dietary NaCl and hypertension has been studied extensively. About half the population with essential hypertension is NaCl sensitive (Weinberger, 1996). The

African-American population with a high incidence of hypertension belongs to the low-renin NaCl-sensitive group. Na intake has also been linked to the rise in blood pressure in older individuals. Exclusive Na intake leads to extracellular volume expansion and consequent increase in cardiac output. Chronic increase in NaCl intake has been related to increase in vascular resistance. A genetic predisposition toward impaired renal handling of a Na load has been suggested (Weinberger, 1996). Activation of the sympathetic nervous system as well as abnormalities in ion transport have been linked to Na overload-induced hypertension. Chronic Na overload has been reported to increase the levels of an endogenous ouabain-like compound, a Na^+K^+-ATPase inhibitor, which inhibits the cell membrane Na pump resulting in an increase in intracellular Na. As a result of decreased activity of Na/Ca exchanger, there is an increase in $[Ca^{2+}]_i$ with an increase in vascular tone.

Toxic minerals: lead and arsenic

Exposure to high levels of Pb is known to cause nephropathy. Prolonged exposure to low levels of Pb has been shown to cause hypertension in man and animals (Nowack *et al*. 1993; Khalil-Manesh *et al*. 1994; Gonic *et al*. 1997). A significant increase in plasma malondialdehyde, a lipid peroxidation product, has been reported. Vaziri *et al*. (1999) postulate that the increase in reactive oxygen species in Pb-treated animals, through inactivating endothelially produced vasodilator NO, might lead to hypertension. Treatment of Pb-exposed rats with des-methyltirilazad resulted in amelioration of the hypertensive condition and reduction to near normal of plasma malondialdehyde levels without any reduction of the Pb concentration in blood.

The consumption of As-contaminated artesian-well water has been reported to cause hypertension in a study from Taiwan (Chen *et al*. 1995). As is leached from naturally occurring minerals into drilled wells. Rahman *et al*. (1999) have reported an unfolding devastating health crisis in Bangladesh in populations consuming As-contaminated well water. Pump sets were installed extensively in rural areas of this country to provide people with a safe water supply. The prevalence of hypertension in people consuming As-contaminated water confirms the earlier Taiwan experience. As is known to cause black foot disease, a peripheral vascular disease. The recent epidemiological studies point to hypertension as an additional hazard of As contamination of drinking water. The toxicity of As is probably through its direct effect on the atherosclerotic process involving endothelial cells, smooth muscle cells, platelets and macrophages (Ross, 1986). Renal insufficiency, impairment of the endothelial barrier in the vascular system and initiation of atherosclerotic plaque formation have been reported following chronic As exposure (Taylor, 1996). These mechanisms could also contribute to the development of hypertension in the exposed population.

Micronutrients in the amelioration of hypertension

The goal of treatment of hypertension is to reduce blood pressure to less than 140/90 or even lower levels. The *(JNC VI) US National Recommendations for the Pharmacological Treatment of High Blood Pressure* (1997) have been reviewed (Moser, 1999). A variety of drugs with diverse modes of action have been added over the years to the armamentarium for the pharmacological treatment of hypertension. These include diuretics, β-blockers, ACE inhibitors, Ca-channel blockers (both dihydropyridine and non-dihydropyridine compounds) and AII receptor blockers. More recently, long-acting dihydropyridine Ca-channel blockers have been

added to this list. Combination therapy, usually starting with a diuretic and a β-blocker, is generally the first choice and, depending on the clinical condition, specific additions or deletions are recommended. As suggested by the JNC VI, the inclusion of a diuretic is necessary in whatever combination regimen is required to achieve the desired blood-pressure values in hypertensive patients. Despite the clear benefits of pharmacological treatment, adequate blood pressure control is achieved in only half of the treated patients (Nurminen *et al*. 1998). Data from various epidemiological observations, intervention trials and observations on experimental animal models of hypertension have provided a large body of evidence establishing the beneficial effects of various dietary components in lowering the elevated blood pressure. These interventions could be very useful as definitive or adjunct treatment modalities. Apart from regulating the intake of macronutrients to produce weight reduction in overweight subjects, and the avoidance of excessive alcohol consumption, various normal dietary components (at recommended daily amount levels or at increased levels of intake) have been shown to reduce blood pressure. Four mineral micronutrients (Na, K, Ca and Mg) have been shown to have a direct effect on blood pressure based on epidemiological, laboratory and clinical investigations (Reusser & McCarron, 1994).

The relationships among hypercholesterolaemia, hypertension and CHD are well known. The development of atherosclerosis is related to the generation of reactive oxygen species, lipid peroxidation and the oxidation of LDL. The production of free radicals is a normal cellular process. However, the cell normally possesses very efficient protective antioxidants and their generating mechanisms to prevent the accumulation of free radicals and consequent injury. These include α-tocopherol, ascorbic acid, β-carotene, glutathione, metal-binding proteins such as transferrin and ceruloplasmin and enzymes such as manganese superoxide dismutase, Cu-Zn superoxide dismutase (SOD), the selenoenzyme glutathione peroxidase and the Fe-containing catalase. In spite of this, the cellular protective mechanisms may be overwhelmed and severe free radical-mediated injury might ensue. *In vitro* experiments indicate that LDL oxidation is accentuated when antioxidants are depleted. For instance, under conditions of Cu deficiency there is increased LDL peroxidation due to a reduction in Cu, Zn SOD and an increase in body Fe stores due to the antagonistic relation between Cu and Fe. Fe is a strong oxidant and catalyses LDL oxidation *in vitro* (Fields, 1999). Rats fed a high-Fe diet deficient in antioxidants such as α-tocopherol and β-carotene exhibited increased lipid peroxidation and hypercholesterolaemia (Fields *et al*. 1993). The median intake of Cu in food is lower than the estimated safe and adequate dietary intake values for most population groups (Third Report on Nutrition Monitoring in the United States, 1995). An analysis of the National Diet and Nutrition Survey in the UK (Bates *et al*. 1999) indicates that, for people aged 65 years and over, intakes of vitamin D, Mg, K and Cu are low; there is biochemical evidence of vitamin D deficiency in this group. The deterioration of micronutrient status with age is an additional risk factor for chronic diseases in this age group. It has been reiterated that specific modifications of human diet can exert positive effects on several chronic conditions including hypertension (Trials of Hypertension Prevention Collaborative Research Group, 1992; Geleijnse *et al*. 1994; Sacks *et al*., 1995 (DASH trial); McCarron *et al*. 1997). McCarron *et al*. (1998) have reported that improving the overall diet in persons with mild to moderate hypertension yielded benefits far beyond improved arterial pressure control. Most of the subjects in this study were on prescribed anti-hypertensive medication and their hypertension was considered well controlled at the beginning of this study. However, the dietary intervention produced further clinically significant reductions in blood pressure, suggesting that some component of blood pressure dysregulation in the hypertensive subjects was beyond the control of the anti-hypertensive drugs used but responded to nutritional factors.

The anti-hypertensive effectiveness of non-pharmacological manipulation is a hotly debated area. Resnick (1999*b*) has pointed out the problems that distort scientific inquiry. He states that the exclusivity given to the process of epidemiological analysis in a uniform manner to hypertension which is an inherently non-uniform phenomenon in identifying 'evidence-based medicine' is a major hurdle. Meta-analysis is the primary tool of this process. As long as hypertension is treated as a single disease entity in primary clinical trials, meta-analysis of clinical trials can be carried out *ad infinitum* with the same inconclusive and inconsistent conclusions (Drueke, 1999; Griffith *et al.* 1999). Resnick (1999*a*) has analysed the cellular ionic basis of hypertension and pointed out the heterogeneity of this disease process in terms of its aetiology. In view of this, all human essential hypertension cannot be treated as a single entity: available clinical and laboratory parameters must be employed to categorize this disease process into identifiable clinical subgroups; only then will clinical trials make any scientific sense.

In succeeding sections the effects of selected single nutrients on essential hypertension in man or in appropriate animal models are described to provide a biochemical basis for the nutrient action. This list includes the minerals Ca, Mg and K as well as vitamins and antioxidants such as vitamins D, B_6, C and E and β-carotene.

Calcium

The relationship between pregnancy-induced hypertension (PIH) and Ca was reported over 20 years ago. Pre-eclampsia appears in late gestation in nulliparous pregnant women as hypertension, oedema and proteinuria. Belizan & Villar (1980) attributed the low incidence of PIH in women in Central American countries (compared with Western Europe and North America), in spite of poor prenatal care and nutrition, to their high Ca intake, which resulted from their practice of soaking maize in lime water. Belizan *et al.* (1991) hypothesized that the lowering of blood pressure through changes in Ca metabolism prevented PIH. Pregnancy is characterized by enhanced Ca absorption, physiological hypercalciuria and an enhanced demand for Ca deposition in the fetal skeleton. There is a progressive decrease in the concentration of serum Ca up to the middle of the third trimester during pregnancy. In subjects with PIH the level of serum Ca is much lower. There are corresponding changes in the Ca-regulatory hormones with high levels of PTH. Ca supplementation has been reported to lower blood pressure in pregnant as well as non-pregnant women: the largest reduction in blood pressure occurred in those with low pretreatment serum Ca levels (Repke *et al.* 1989). Ca supplementation during gestation may lower PTH levels which, in turn, may reduce intracellular Ca concentration in vascular smooth muscle and decrease its responsiveness to pressor stimuli. Changes in renal Na handling could also be affected by Ca (Belizan *et al.* 1991).

Other than in women at risk for PIH, Ca supplementation has also been shown to decrease blood pressure in hypertensive patients (McCarron, 1985; Saito *et al.* 1989) and in animal models of hypertension (Hatton & McCarron, 1994; Sallinen *et al.* 1996). Hypertensive subjects manifest a number of disturbances of Ca metabolism that are consistent with increased bone resorption and bone loss, such as elevated PTH and increased urinary Ca excretion. Rat models of hypertension are also associated with increased bone loss and reduced levels of bone mineral mass compared with normotensive controls (Wang *et al.* 1993). Metz *et al.* (1999) have reported findings in human subjects that support the concept that elevated blood pressure varies inversely with bone mass and density, known predictors of osteoporotic fractures. McCarron & Reusser (1999) have collected a large body of evidence based on clinical studies and analyses which seem unequivocally to point to a beneficial influence of dietary Ca on blood pressure.

They have noted that the most impact of Ca supplementation on blood pressure was on persons who had consumed low levels of dietary Ca. Of significance is the analysis by Griffith *et al.* (1999), which included a meta-analysis of sixty-six randomized trials which showed a distinct beneficial effect of adequate dietary Ca intake. There were significant reductions in systolic blood pressure of 1·3 and 4·3 mmHg in the general population and in hypertensive subjects respectively. The corresponding Ca supplement-induced reductions in diastolic blood pressure were 0·2 and 1·5 mmHg respectively. They have also pointed out that groups at high risk for hypertension, such as African-Americans, NaCl-sensitive persons and pregnant women, were particularly sensitive to the effect of increased Ca intake. McCarron (1998) has pointed out the striking agreement between the blood pressure findings in the DASH study (Appel *et al.* 1997) and his own prediction of the relationship between dietary Ca and systolic blood pressure (McCarron *et al.* 1984). It is to be noted that, in the DASH study, very significant reductions in systolic and diastolic blood pressures (of 5·5 and 3·0 mmHg respectively) were achieved on the DASH diet. Although this diet had a low level of fat, the considerable increase in dietary Ca should be kept in mind (Appel *et al.* 1997). The physiological relevance of the decrease in urinary Ca on this diet is supported by a decrease in circulating PTH levels, suggesting that blood pressure reduction was mediated through a Ca-sparing mechanism.

Although experimental animal work and results of clinical trials point to the role of a deficit of extracellular ionized Ca in the aetiology of hypertension in some subjects, this hypothesis has met with strong resistance for several reasons. The very well recognized increased incidences of hypertension among persons with hypercalcaemia of primary hyperparathyroidism, and of hypotension associated with hypocalcaemia (Kesteloot & Geboers, 1982), have left a strong impression that hypertension is a disease associated with excess Ca. The other major reason was the well-entrenched concept supported by numerous observations that excess NaCl intake is directly responsible for the development of essential hypertension in human subjects. Although these observations are quite valid, what has not been recognized is what circumscribes these observations. Much of the credit for focusing attention on the 'Ca deficiency' and 'the heterogeneity of Ca relationship to hypertension' theories is shared by Resnick and McCarron for their perseverance.

Resnick (1999*b*) has provided an integrated view of Ca metabolism in hypertension and how Ca deficiency and Ca excess both contribute to the development of hypertension. The steady-state cell activity has a central role in determining the influence of environmental stimuli on tissue and organ function through the respective hormonal systems. The RAS system determines the homoeostasis of Na and K. Ca-regulating hormones PTH, the metabolically active vitamin D derivative and parathyroid hypertensive factor, regulate Ca homoeostasis. Insulin regulates carbohydrate and fat metabolism. Growth hormone, glucocorticoids and catecholamines mediate responses to environmental stress. Excess cytosolic free Ca and reciprocal decrease in intracellular free Mg have roles in the final common pathways which regulate metabolic, cardiovascular, neural, renal and thrombotic activities and, as such, in the development of disease processes such as hypertension, left ventricular hypertrophy, atherosclerosis and IR. Resnick *et al.* (1983) recognized that, for subjects with the same level of urinary Na excretion, serum ionized Ca levels were suppressed in low-renin hypertensive subjects and were correspondingly increased in those hypertensive subjects with high plasma renin activity. Serum ionized Ca levels were even more decreased in patients with primary aldosteronism than in low-renin hypertensive patients. These subjects had a further decrease in their plasma renin activity (Resnick & Laragh, 1985). Low-renin essential hypertensive subjects had a significant increase in serum PTH and $1,25-(OH)_2 D_3$ levels compared with normotensive subjects, and patients with primary aldosteronism had a further increase in circulating PTH levels. The Ca

and Ca-regulating hormone profile of high-renin hypertensive subjects was exactly opposite to that seen in those with low renin.

NaCl overload stimulates Ca-regulating hormones, whereas Ca supplementation has the opposite effect on these hormones (Zemel *et al.* 1986). The ability of NaCl loading to elevate blood pressure and of Ca supplements to decrease blood pressure was found to be proportional to the NaCl- or Ca-induced changes in Ca metabolism (Resnick, 1999*b,c*). As pointed out earlier (p. 8), cytosolic free Ca has a role in stimulus–contraction coupling in the muscle and stimulus–secretion coupling in neurohormone secretion. The action of catecholamines to increase vascular tone, of AII to constrict peripheral and renal vasculature and stimulate aldosterone secretion, and of other pressor factors are all mediated by their ability to increase cytosolic free Ca levels. This discussion emphasizes the heterogeneity of human essential hypertension based on the physiological differences among the subjects in regulating Ca homoeostasis.

Magnesium

A negative correlation between Mg content (hardness) of drinking water and hypertension and stroke as well as IHD was reported quite early (Schroeder, 1960). Resnick *et al.* (1984) described a strong inverse relationship between erythrocyte free Mg and diastolic blood pressure. Using ion-selective probes and NMR spectroscopy, others have confirmed the low levels of Mg in VSM and in erythrocytes of subjects with essential hypertension compared with normotensive controls. More recent prospective studies of the relationship of nutrient intake to subsequent changes in blood pressure are the Nurses' Health Study (Witteman *et al.* 1989) and the Health Professionals Study (Ascherio *et al.* 1992, 1998). Both these studies have reported a significant inverse relationship between Mg intake and the development of hypertension. A significant protective effect of Mg and Ca intake on the risk of cerebrovascular disease was reported (Yang, 1998). Magnesium sulfate is widely used as a routine therapy to prevent eclamptic seizures. The collaborative eclampsia trial (The Eclampsia Trial Collaborative Group, 1995) has provided incontrovertible evidence for the use of magnesium sulfate in preference to diazepam or phenytoin in the treatment of eclampsia (Lucas *et al.* 1995). Other epidemiological studies have also reported an inverse relationship between dietary Mg intake and blood pressure (Kawano *et al.* 1998; Mizushima *et al.* 1998). The inverse relationship between Mg status and the related disease processes (hypertension, diabetes and dyslipidaemia) have been reported (Altura & Altura, 1995; Paolisso & Barbagallo, 1997). Cytosolic free Mg has been reported to be low in hypertensive and diabetic subjects. Mg deficiency exacerbates insulin resistance and predisposes diabetics to early onset of cardiovascular diseases. Mg deficiency in experimental animals leads to dyslipidaemia, including atherosclerosis and vascular damage. Mg supplementation improves the control of diabetes in human subjects (Paolisso *et al.* 1992*a*) and lowers serum cholesterol and triacylglycerols and also attenuates the development of atherosclerotic lesions in experimental animals (Altura & Altura, 1995; Nasir *et al.* 1995). The infusion of Mg at pharmacological concentrations produces vasodilatation of systemic vasculature and coronary arteries. It also protects the myocardium against ischaemia-reperfusion injury in experimental animals. The second Leicester Intravenous Magnesium Intervention trial has demonstrated that intravenous Mg has a protective effect during the treatment of acute myocardial infarction (Woods & Fletcher, 1994).

Laboratory investigations have underlined the relationship between Mg deficiency and hypertension (Altura *et al.* 1984, 1992). The elevation of blood pressure was directly related to

the severity of Mg deficiency in animals. In the spontaneously hypertensive rat (SHR), Mg deficiency accelerated the development of hypertension (Berthelot & Esposito, 1983) although, once hypertension was established, a deficiency of Mg did not increase the blood pressure further (Overback *et al*. 1987). However, there are reports that pharmacological Mg supplementation of SHR results in attenuation of the hypertension. In a reinvestigation of the effects of Mg deficiency in Wistar rats, Laurant *et al*. (1999) have reported that Mg deficiency induced a transitory hypotension about 2 weeks after the rats were on the Mg-deficient diet. However, this was followed by sustained hypertension in rats after about 15 weeks on the deficient diet. During the early transient hypotensive phase of Mg deficiency, hyperaemia was observed. This corresponded to the increased production and release of circulatory inflammatory agents such as cytokines, prostacyclin and histamine, which are all vasodilators and reduce blood pressure. The sustained hypertensive second phase of Mg deficiency is associated with activation of the sympathetic nervous system.

As pointed out earlier (p. 8), there is a reciprocal relationship between intracellular Ca and Mg concentrations. The role of Ca in regulating vascular tone is dependent on (and is also modified by) Mg. An intracellular Mg deficiency, by decreasing the activities of a variety of cellular processes (including phosphotransferases, membrane pumps and ion channels), exaggerates Ca-induced stimulation. Such cellular ionic abnormalities are characteristic of hypertension, obesity, NIDDM and hyperlipidaemia. Mg deficiency increases the susceptibility of lipoproteins to peroxidation (Rayssiguier *et al*. 1993*a*,*b*). A low intracellular concentration of Mg results in a high rate of free-radical formation (Weglicki *et al*. 1996). Free radicals inactivate the endothelium-derived relaxation factor, NO. Increased degradation of NO by superoxide anions could contribute to the enhancement of the arterial contractile response (Yang, 1998), resulting in the development and maintenance of sustained hypertension during chronic Mg deficiency.

Observations from distinct lines of investigation including experimental animal work, epidemiological studies and therapeutic studies, as well as clinical intervention trials, all point to the contribution of Mg deficiency in the development of hypertension. However, an intracellular deficiency of Mg is not an isolated event but is related to cellular ionic homoeostasis of Ca, as pointed out by Resnick (1999*b*). In a recent critical review, Suter (1998) has pointed out that K, Mg and fibre have been identified as modulators of the risk of vascular diseases including stroke. The protective effects of these nutrients were particularly pronounced in hypertensive subjects. However, in a consensus statement to provide an evidence-based recommendation, Burgess *et al*. (1999) on the basis of a MEDLINE search of reports of trials, meta-analyses and review articles, have concluded that Mg supplementation is not recommended as a treatment for hypertension.

Potassium

A protective effect of K on the risk for stroke based on epidemiological studies was reported (Tobian *et al*. 1984; Khaw & Barrett-Connor, 1987). Ascherio *et al*. (1998) examined the association of K and related nutrients with the risk of stroke and concluded that the results were consistent with the hypothesis that diets rich in K, Mg and cereal fibre reduced the risk of stroke and that supplements of K alone may also be beneficial. They found that the protective effect was particularly pronounced in hypertensive subjects. A similar inverse relationship between K intake and blood pressure, as well as a direct association between urinary Na:K and blood pressure, has been reported (Krishna, 1990, 1994; Linas, 1991). A negative correlation

between blood pressure and K intake has also been reported in the Intersalt Study (The Intersalt Cooperative Research Group, 1988) and in the Health Professionals' Follow-up Study (Ascherio *et al.* 1992). These studies represent an association rather than a cause–effect relationship, but they do indicate a strong correlation. Although many studies indicate a beneficial effect of K on hypertension, some reports do not support this, or find the correlation weak (Grimm *et al.* 1990). However, still other studies indicate that K intake is inversely related to a risk of fatal thromboembolic stroke (Lee *et al.* 1988) or that high K intake causes a modest reduction in blood pressure of hypertensive subjects (Sacks *et al.* 1998).

In experimental animal studies, Dahl *et al.* (1972) showed the hypotensive potential of K in NaCl-sensitive hypertension. A similar beneficial effect of K in hypertensive rats was reported by Tobian *et al.* (1984) and Tobian (1986). K has been shown to ameliorate hypertension in other animal models of hypertension, both NaCl-sensitive and NaCl-insensitive (Linas, 1991).

The protective effect of K against the development of vascular diseases might primarily be due to its effect on blood pressure regulation, although other mechanisms have been suggested (Suter, 1998). The direct effect of K on blood pressure regulation would be through its effect on natriuresis, baroreceptor sensitivity, the RAS system, vasodilatation, and sympathetic nervous system activation. Alternate mechanisms include the role of K in inhibition of free-radical formation (McCabe *et al.* 1994; Young *et al.* 1995), VSM proliferation (McCabe & Young, 1994) and arterial thrombosis (Lin & Young, 1994).

Vitamins and blood pressure control

Some vitamins have been identified as having a role in blood pressure regulation. As Ca is at the centre of ionic regulation of cellular activities (Resnick, 1999*a*), it is conceivable that vitamins involved in intracellular Ca regulation would have a significant role. Thus, vitamin D seems to be involved indirectly through its effect on cellular Ca homoeostasis and also through direct mechanisms. The role of stress and cellular oxidative damage through the formation of free radicals is well recognized as contributing to several chronic conditions. Free radicals have an effect on LDL oxidation and hence in atherogenesis, which is associated with hypertension. Also, the most important endothelium-dependent vasodilator, NO, is susceptible to oxidative damage and is protected by mechanisms inhibiting the formation of free radicals. Vitamin C (ascorbic acid) and vitamin E are the antioxidant vitamins in the aqueous and membrane regions of the cell respectively and, hence, indirectly affect blood pressure regulation. Other mechanisms have also been suggested in this protective action. The reported beneficial role of β-carotene is not through its being a provitamin for vitamin A but through its effect as an antioxidant.

Apart from these vitamins, the most consistent and significant effect of a vitamin on blood pressure regulation is that of vitamin B_6. Investigations in the past have established that even a moderate deficiency of vitamin B_6 could lead to hypertension. The hypertensive effect is through both central and peripheral mechanisms. Centrally, the stimulation of the central nervous system is under control of monoamine neurotransmitters, which are products of PLP (active form of vitamin B_6)-dependent decarboxylation of amino acid derivatives. The key role of Ca in maintaining vascular tone is well known. PLP has a role in intracellular Ca transport: it is an antagonist of both the voltage-mediated L-type Ca-channel receptor and the ATP-mediated purinergic receptor-mediated influx of Ca intracellularly. Thus, vitamin B_6 plays a significant role in Ca transport and blood pressure regulation. Another aspect of vitamin B_6 function to be noted is that it is not just vitamin B_6 deficiency that is implicated in generation of hypertension:

non-physiological doses of vitamin B_6 appear to decrease the high blood pressure associated with both genetic and non-genetic animal models of hypertension. The following sections describe various reports based on experimental and epidemiological observations.

Vitamin D

There is a close association between Ca metabolism and vitamin D. It is well known that Ca absorption and whole-body Ca metabolism are regulated by vitamin D, through much clinical evidence based on studies of rickets, osteomalacia and osteoporosis. The active form of vitamin D is 1,25-$(OH)_2 D_3$. This is considered to be a hormone as it is formed in the kidney and has its sites of action in various organs and cell types. A decrease in serum Ca triggers a series of events which result in the restoration of Ca homoeostasis. This starts with the parathyroids: the decrease in serum ionized Ca is a trigger for the release of PTH from the parathyroids by the activation of an extracellular Ca receptor. PTH stimulates osteoclasts and osteocytes and also enhances renal tubular Ca reabsorption; thus, it increases serum Ca. PTH is also a stimulator of a hydroxylase which converts 25-hydroxycholecalciferol (25-$(OH)D_3$) to 1,25-$(OH)_2 D_3$. 25-$(OH)D_3$ itself is formed from calciferol through a hydroxylase in the liver. In renal and intestinal epithelial cells, 1,25-$(OH)_2 D_3$ increases the synthesis of vitamin D-dependent Ca-binding proteins, which are active in the intestinal absorption and renal reuptake of Ca. In addition to PTH and 1,25-$(OH)_2 D_3$ there is another Ca-regulating hormone, calcitonin, which is synthesized and secreted by the C cells of the thyroid. Calcitonin acts as a Ca-lowering agent by decreasing Ca mobilization through inhibition of osteoclast activity. The manner of regulation of calcitonin is opposite to that of PTH. The net effect of the orchestrated responses of these three calcitropic hormones is the homoeostasis of Ca^{2+}. In view of its intimate relationship to Ca homoeostasis through the hormonal form (1,25-$(OH)_2 D_3$) and the knowledge of the central role played by cellular ionized Ca in the process of blood pressure regulation, a role for vitamin D in blood pressure regulation is a natural extension.

On the basis of much clinical evidence, PTH has been related to hypertension. In primary and secondary hyperparathyroidism there are elevated serum PTH and Ca levels. These patients are hypertensive; hence, it was assumed that the increased blood pressure seen in essential hypertensive subjects and in animal models of hypertension such as the SHR is also associated with increased PTH secretion. This was confirmed by the beneficial effects of parathyroidectomy in hyperparathyroid subjects, as well as in the animal models (Schleiffer *et al*. 1986; Bukoski *et al*. 1995). Essential hypertension was considered to be a clinical condition related to Ca excess. There was considerable resistance to acceptance of evidence from experimental and epidemiological sources that suggested that hypertension is not a single disease entity and that some hypertensive situations are associated with a decrease in serum ionized Ca. Resnick and McCarron have been persistent in emphasizing these observations and Resnick (1999*b*) has provided an ionic basis for the hormonal roles of 1,25-$(OH)_2 D_3$ and PTH in intracellular Ca regulation. The distinction between low-renin and NaCl-sensitive and other types of hypertension has been emphasized.

The prevalence of hypertension in a population increases with the distance north or south of the equator (Rostand, 1997). Vitamin D status has been shown to relate inversely to the severity of hypertension in Caucasian populations both with and without vitamin deficiency (Lind *et al*. 1989; Barger-Lux & Heaney, 1994; Boucher, 1998). Serum 25-(OH) D_3 is used as an indicator of vitamin D status and has been shown to be inversely related to the severity of hypertension (Krause *et al*. 1998). A direct relationship between serum 25-(OH) D_3 and HDL-

cholesterol has also been reported (Auwerx *et al.* 1992). An increased prevalence of myocardial infarction has been reported in northern compared with southern European communities in populations with vitamin D deficiency (Balarajan *et al.* 1987). The seasonal variations of blood pressure and the finding that the incidence of IHD increases with increasing altitude where u.v. radiation is increased are suggestive that the vitamin D status of the population may be important in determining the long-term circulatory risk in these populations (Boucher, 1998).

Clinical evidence would indicate that PTH is a prime candidate for development of hypertension and that it would have a pressor action on vasculature. However, PTH, when infused or added to vascular preparations, is a vasodilator (Pang *et al.* 1987; Bukoski & Kremer, 1991). An explanation had to be found for the antihypertensive effects of the ablation of the parathyroids.

Studies of the vascular effects of the hormone form of vitamin D (1,25-$(OH)_2 D_3$) strongly indicate a modulator role in Ca metabolism, contractile activity and growth of VSM. There are 1,25-$(OH)_2 D_3$-specific receptors on VSM cell membrane (Kawashima, 1987). Both acute and chronic treatment with 1,25-$(OH)_2 D_3$ results in vasoconstriction. It stimulates ^{45}Ca uptake by primary cultures of mesenteric artery myocytes from SHR and Wistar–Kyoto (WKY) rats through a process dependent on protein synthesis. Treatment of SHR or WKY rats for 3 d with 1,25-$(OH)_2 D_3$ leads to generation of contractile force in subsequently isolated resistance arteries, possibly through the same protein-synthetic events that are responsible for the increased Ca uptake (Bukoski *et al.* 1990). Short-term infusion of 1,25-$(OH)_2 D_3$ potentiated the *in vivo* pressor response to noradrenaline in SHR but not in WKY rats. It also potentiated the contractile response to serotonin and noradrenaline in isolated resistance arteries (Bukoski *et al.* 1989) and increased constriction in renal circulation in normotensive rats. The vitamin D hormone also stimulated the growth of cultured mesenteric myocytes (Bukoski *et al.* 1989). Xue *et al.* (1991) reported that resistance artery segments in culture lose stress-generating capacity in reponse to noradrenaline; this was completely protected by 1,25-$(OH)_2 D_3$. As the loss of contractile ability and the protection by 1,25-$(OH)_2 D_3$ were also observed in de-endothelialized preparations, the effects are exerted directly on the VSM cell.

In view of the vasodilatory effect of PTH, the question about the attenuation of blood pressure by parathyroidectomy was sought to be answered from other observations. PTH stimulates the production of 1,25-$(OH)_2 D_3$; its hypertensive action in the whole animal might, therefore, be related to the increased production of this vitamin D hormone which has a pressor function. Another interesting finding was that special cell types in the parathyroid produced other factors with hypertensive action (Pang & Lewanczuk, 1989). The parathyroid hypertensive factor (PHF) administered to normotensive rats induced a developing rise in blood pressure. PHF is responsive to Ca intake and is found in the serum of hypertensive subjects and animal models of hypertension but not in normotensive subjects. It has been speculated that the tropic action of 1,25-$(OH)_2 D_3$ (Minghetti & Norman, 1988) might cause enhanced growth of specific cell types in the parathyroid which produce PHF. PHF, in turn, suppresses 1,25-$(OH)_2 D_3$ production which might account for the fall in the concentration of 1,25-$(OH)_2 D_3$ in established hypertensive subjects.

The linkage between calcitropic hormones, NaCl sensitivity, renin status and hypertension has been clarified by Resnick (1999*a*). As stated earlier (p. 9), he has categorized essential hypertensive subjects on the basis of their renin response. The low-renin type shows NaCl sensitivity and the high-renin type is NaCl resistant. Increased circulating levels of PTH and 1,25-$(OH)_2 D_3$, which facilitate cellular Ca uptake from extracellular space, are seen in this type. NaCl-induced increases in intracellular accumulation of Ca ($[Ca^{2+}]_i$) and the concomitant decrease in $[Mg^{2+}]_i$ are paralleled by the increase in PTH and 1,25-$(OH)_2 D_3$; the greater the NaCl-induced increase in serum 1,25-$(OH)_2 D_3$, the greater the blood pressure in this group of

low-renin hypertensive subjects. Oral Ca supplementation of these subjects, who were on the NaCl load, had the opposite effect on blood pressure and on vitamin D hormone. Resnick proposed that NaCl and Ca both operate through modulation of $1,25\text{-(OH)}_2 D_3$. This is significant, because normotensive black subjects, who are prone to NaCl overload hypertension, have higher circulating levels of $1,25\text{-(OH)}_2 D_3$ and PHF. Among essential hypertensive subjects, those with the highest levels of PHF were of the low-renin type. In experimental animal models, the NaCl-sensitive, low-renin DOCA–NaCl rat had high levels of PHF. Also, among those with essential hypertension, the pressor response to NaCl loading was directly proportional to the basal level of PHF. Dietary Ca suppresses PHF secretion whereas PHF stimulates $1,25\text{-(OH)}_2 D_3$ synthesis. Thus, PHF has taken the place of PTH in the original Ca-regulation scheme.

As stated earlier, vitamin D (cholecalciferol) is 25-hydroxylated in the liver and this is the substrate for the subsequent 1-hydroxylation in the kidney to form the vitamin D hormone $1,25\text{-(OH)}_2 D_3$. The kidney enzyme $25\text{-(OH)}D_3$ 1 hydroxylase is under stringent control. When concentrations of vitamin D, phosphate and Ca are low (consistent with high concentrations of PTH and also PHF), the synthesis of $1,25\text{-(OH)}_2 D_3$ is stimulated. Plasma concentrations of $1,25\text{-(OH)}_2 D_3$ are higher for salt-sensitive than for salt-resistant rats. Inverse associations between plasma $25\text{-(OH)} D_3$ concentration and blood pressure of salt-sensitive rats and also between plasma $25\text{-(OH)} D_3$ and the time the rats were on salt loading have been reported (Thierry-Palmer *et al*. 1998).

The acute direct effects of $1,25\text{-(OH)}_2 D_3$ on blood pressure, cardiac output and renal haemodynamics were investigated (Jespersen *et al*. 1998). The rapid effects of vitamin D hormone were assessed over 120 min following a bolus injection in subjects with essential hypertension and compared with controls. Ionized Ca was kept constant during the study with a clamping technique. Acute $1,25\text{-(OH)}_2 D_3$ caused a fast and non-genomic-mediated decrease in cardiac output together with a transient increase in blood pressure; such changes were not seen in normal control subjects. The changes in hypertensive subjects could not be associated with any change in related hormone systems (including the RAS system) as these interactions require a longer time owing to genomic or secondary effects (Burgess *et al*. 1990). The results indicate a role for $1,25\text{-(OH)}_2 D_3$ in the regulation of vascular contractility in essential hypertension.

To provide a direct cellular basis for the pressor effect of vitamin D hormone, Shan *et al*. (1993) studied the effect of $1,25\text{-(OH)}_2 D_3$ on the voltage-dependent Ca-channel activity as well as on cytosolic free Ca accumulation using the whole-cell version of the patch clamp method and fluorescence spectroscopic technique: $1,25\text{-(OH)}_2 D_3$ applied for 20 min produced a significant increase in the Ca current over the entire range of test pulses; this effect was time and dose dependent. Correspondingly, $1,25\text{-(OH)}_2 D_3$ significantly increased free Ca levels. These results provide a cellular basis for the vascular function of the vitamin D hormone.

Vitamin D is a prohormone for more polar metabolites of which $1,25\text{-(OH)}_2 D_3$ is the hormone. The other polar metabolite is the 24,25-dihydroxy $(24,25\text{-(OH)}_2 D_3)$ derivative, which was considered to be an inactive metabolite of vitamin D although, quite early, it was suggested that this metabolite, too, might have a function in endochondral bone formation (Malluche *et al*. 1980). Specific receptors for $24,25\text{-(OH)}_2 D_3$ were found in the parathyroids (Merke & Norman, 1981), chondrocytes (Corvol *et al*. 1980), epiphyseal growth plate (Somjen *et al*. 1982*a*) and limb bud mesenchymal cells (Somjen *et al*. 1982*b*). Shan *et al*. (1996) have investigated the vascular effects of $24,25\text{-(OH)}_2 D_3$: they found that it caused a dose-dependent relaxation of the tonic or phasic tension induced by K^+ or by noradrenaline. These were direct effects on the VSM as the artery strips used were de-endothelialized. $24,25\text{-(OH)}_2 D_3$ inhibited vasoconstriction dependent on the release of intracellular Ca stores released by noradrenaline. Tension dependent on extracellular entry of Ca induced by noradrenaline was also attenuated.

Tension dependent on entry of external Ca induced by K^+ or arginine vasopressin was also inhibited. In patch clamp experiments, 24,25-$(OH)_2 D_3$ reduced the amplitude of inward Ca currents in VSM cells.

1,25-$(OH)_2 D_3$ has been shown to open VSM cell-membrane Ca channels and to increase intracellular free Ca concentration (Shan *et al*. 1993). 24,25-$(OH)_2 D_3$ also acts on the voltage-dependent Ca channel with effects in the opposite direction, acting as an inhibitor of Ca entry, similar in action to oestrogen, progesterone (Shan *et al*. 1994) and PLP (described in a later section; p. 26). As 1,25-$(OH)_2 D_3$ and 24,25-$(OH)_2 D_3$ are immediate metabolites of 24-hydroxy-cholecalciferol and have opposite vascular effects, is it possible that the metabolic switch has a role in blood pressure regulation or even in the development of hypertension? In most of the investigations cited, the concentrations of 1,25-$(OH)_2 D_3$ or 24,25-$(OH)_2 D_3$ used have been high compared with their circulating concentrations, which are 30 pg/ml for 1,25-$(OH)_2 D_3$ and 2 ng/ml for 24,25-$(OH)_2 D_3$. One can implicate local high concentrations to answer the quibble of unphysiological concentrations. Still, the possibility of using these polar metabolites of 24-hydroxycholecalciferol metabolically or pharmacologically to control blood pressure is a worthy prospect.

Vitamin B₆

Vitamin B_6

Early reports have indicated a relationship between vitamin B_6 status and hypertension in pregnant women. The toxaemic placenta is markedly deficient in PLP and the lack of demonstrable effect of vitamin B_6 in toxaemia was ascribed to a low pyridoxal kinase activity. Seizures associated with pregnancy-induced hypertension were prevented by Mg alone or in association with vitamin B_6 (pyridoxine). The enzyme pyridoxal kinase requires Mg for its activity. Various clinical reports indicate a correlation between alcoholism, hypothyroidism, diabetes and hypertension. However, many factors confound the underlying vitamin B_6 status of these individuals (Dakshinamurti & Lal, 1992).

We have investigated the effect of moderate vitamin B_6 deficiency on blood pressure regulation (Paulose *et al*. 1986, 1988). The blood pressure changes in the vitamin B_6-deficient rat can be classified into three phases: (1) pre-hypertensive (1–4 weeks); (2) hypertensive (5–11 weeks); (3) post-hypertensive (starting from week 12). Vitamin B_6-deficient rats during the hypertensive phase were only moderately pyridoxine deficient; they did not have any clinical signs of deficiency. This moderately vitamin B_6-deficient hypertensive rat has been biochemically characterized (Dakshinamurti *et al*. 1990*a*) in terms of tissue vitamin B_6 levels and as functionally deficient in neurotransmitters serotonin and γ-aminobutyric acid (GABA). Brain regional PLP levels were significantly ($P < 0.05$) reduced after 8 weeks of vitamin B_6 depletion. PLP levels in cerebral cortex, hippocampus and thalamus were 5·5 (SD 0·39), 7·45 (SD 0·45) and 8·70 (SD 0·36) nmol/g, respectively, in controls (pair-fed a vitamin B_6-supplemented diet) and 3·36 (SD 0·40), 5·20 (SD 0·32) and 6·3 (SD 0·34) nmol/g in vitamin B_6-deficient rats, respectively. We refer to this as the 'moderately deficient' condition. After 11 weeks of vitamin B_6 depletion, the PLP levels were further reduced to 2·78 (SD 0·28), 4·42 (SD 0·35) and 5·10 (SD 0·32) nmol/g in cerebral cortex, hippocampus and thalamus respectively. At this stage of vitamin B_6 deficiency the rats were no longer hypertensive: they were normotensive or even hypotensive. We refer to this as the advanced vitamin B_6-depleted state. It is the 'moderately vitamin B_6-deficient rat' which we have used as an animal model of moderate hypertension.

The nature of the hypertension that developed in the pyridoxine-deficient animal needed to be characterized in an effort to identify the causative factor(s). Using drugs such as phenytoin,

valproic acid and diazepam, it was shown that the hypertension was not the result of a hyperexcitable state in these animals. Although pyridoxine treatment reversed both the hypothyroidism and hypertension, there was no indication that the hypothyroid condition initiated hypertension. An association between hypertension and sympathetic stimulation has been observed in both hypertensive animals and human subjects; therefore, the possibility that the reversible hypertension seen in pyridoxine-deficient rats was related to sympathetic stimulation was considered. The concentration of noradrenaline in plasma is a valid reflection of sympathetic activity. However, blood samples have to be withdrawn from the conscious animal without trauma. We developed such a system by implanting a vascular-access port with catheterization to the jugular vein (Paulose & Dakshinamurti, 1987). We showed (Viswanathan *et al*. 1990) that both adrenaline and noradrenaline levels in the plasma of pyridoxine-deficient rats were nearly threefold higher than those of controls. Treatment of the rats with pyridoxine restored the blood pressure and catecholamine levels to normal within 24 h, whereas pyridoxine administration to control rats had no significant effect on either of these parameters. The complete reversibility of hypertension in such a short time would preclude a permanent structural damage to the vessel wall of the deficient rat; the lesion might possibly be at the level of neurotransmitter regulation. We also determined noradrenaline turnover in the hearts of deficient and control rats, but found no difference in myocardial noradrenaline content between the two groups; however, noradrenaline turnover was significantly increased in deficient rats compared with controls, thus supporting the contention that peripheral sympathetic activity is increased in the pyridoxine-deficient hypertensive animal.

Cardiovascular effects of serotonin.　　Serotonin (5-HT) is involved in a wide variety of functions of the central nervous system. Serotonergic cell bodies occur mainly in the raphe nuclei of the brain stem; however, the nerve axons project into virtually all parts of the brain and spinal cord and thus control a variety of functions such as blood pressure, emotional behaviour, endocrine function, perception of pain and sleep. In addition, there are effects on the peripheral neurons and non-neural tissues. 5-HT, when administered into the brain, elicits complex cardiovascular responses. The receptors that mediate these effects are different and have been categorized into major families. Each 'family' consists of multiple receptor subtypes that share similarities in their molecular biological, pharmacological, biochemical and physiological properties. These receptors are present throughout the central and peripheral nervous systems. The development of centrally acting 5-HT agonists such as 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), ipsapirone and flesinoxan with specificity to 5-HT_{1A} subtype receptors has led to the recognition that 5-HT_{1A} receptors are involved in the central control of autonomic flow (Schoeffler & Hoyer, 1988). It is possible that the decrease in neuronal 5-HT and the consequent changes in its receptors, particularly 5-HT_{1A}, may cause hypertension in these animals. Hence, we investigated the effect of serotonergic 5-HT_{1A} receptor agonists such as 8-OH-DPAT, flesinoxan, urapidil or methyl urapidil on the SBP of conscious vitamin B_6-deficient hypertensive rats. After hypertension developed and had reached its peak (8–10 weeks on the deficient diet), the rats were used for assessment of the effect of the drugs under investigation. Intraperitoneal injection of 8-OH-DPAT, flesinoxan, urapidil, or 5-methyl urapidil (0·1–10 µmol/kg) caused a significant fall in systolic blood pressure of vitamin B_6-deficient hypertensive rats (Lal & Dakshinamurti, 1993*a*). The effect clearly depended on the dose used and was seen within 30 min of drug administration. When doses that caused a fall in SBP of 20 mmHg were compared, the following rank order was established: 8-OH-DPAT >flesinoxan > 5-methyl urapidil > urapidil; none of these drugs, however, had a consistent effect on heart rate. The

affinity of the agonists for the 5-HT$_{1A}$ receptor site (Groz *et al.* 1987) correlates with the order of their anti-hypertensive activity, indicating that this effect is mediated through the 5-HT$_{1A}$ receptor site. The selective 5-HT$_{1A}$ receptor antagonist spiroxatrine (Nelson & Taylor, 1986) dose-dependently antagonized the hypotensive activity of 5-HT$_{1A}$ receptor agonists. The moderately pyridoxine-deficient hypertensive rats have a low concentration of 5-HT in various brain areas, reflected in the increased 5-HT$_{1A}$ receptor number in membrane preparations. Lesioning of central serotonergic tracts with 5,7-dihydroxytryptamine results in a similar increase in the 5-HT$_{1A}$ receptor numbers, indicating that central serotonergic depletion is one of the contributors to the development of hypertension. What is the mechanism of the hypertensive action of the 5-HT$_{1A}$ agonist? The hypertension of the moderately pyridoxine-deficient rat is characterized by central sympathetic stimulation, as in other hypertensive animal models. When α_2 adrenoreceptors in the nucleus tractus solitarii are stimulated, inhibitory neurons of the vasomotor centre are activated. Sympathetic outflow, which originates from the vasomotor centre and innervates the peripheral vasculature, heart and kidney, is reduced. As a result, peripheral vascular tone, heart rate and renin release are decreased, leading to a decrease in total peripheral resistance and cardiovascular output. Drugs such as clonidine, an α_2 agonist, exert their cardiovascular effect through stimulation of the α_2 adrenoreceptors in the brain stem. Activation of central α_2 adrenoreceptors in the nucleus tractus solitarii required a serotonergic input through 5-HT$_{1A}$ receptors (Rappaport *et al.* 1985); hence, the hypotensive action of 5-HT$_{1A}$ receptor agonists.

Calcium channels. The end result of centrally mediated sympathetic stimulation is an increase in peripheral resistance. This is reflected in elevation of both resting and stimulated vascular tone in the resistance arteries of the moderately pyridoxine-deficient hypertensive rats. Elevated peripheral resistance is the hallmark of hypertension, as seen in other models of hypertension (Postnov & Orlov, 1985). The increase in tone of caudal artery segments from the hypertensive deficient rats is Ca dependent, as seen by the decrease in unstimulated isometric tension observed in response to the presence of the specific Ca chelator ethylene glycol tetra-acetic acid in the medium and also the response to the addition of Ca to the medium (Viswanathan *et al*. 1991). The decrease in tone following the addition to the medium of the Ca-channel antagonist nifedipine indicates that increased peripheral resistance, resulting from increased permeability of smooth muscle plasma membrane to Ca^{2+}, might be central to the development of hypertension (Rapp *et al*. 1986).

The initiation of smooth muscle contraction is principally dependent on the short-term increase in cytosolic free Ca. Ca moves in and out of the cell and intracellular storage sites in response to chemical, electrical, pressure and other physical stimuli. Ca influx essentially occurs through plasma membrane Ca^{2+} channels which are receptor operated or voltage operated (Glossman & Striessnig, 1988). Voltage-sensitive Ca channels participate in action potential generation by mediating voltage-activated inward movement of Ca ions which depolarize the cell (Catterall *et al*. 1998). These channels couple cell-surface electrical signals to the physiological response by mediating a voltage-dependent increase in the cytosolic concentration of Ca, which is a key intracellular second messenger. The slow channel (L-type) is the major pathway by which Ca ions enter the cell during excitation for initiation and regulation of the force of contraction of cardiac and skeletal muscle. VSM also contains the (L-type) slow channel. We evaluated the possibility that, in the pyridoxine-deficient hypertensive rat, a higher concentration of cytosolic free Ca^{2+} might be responsible for the higher tension in the VSM. The higher cytosolic free Ca^{2+} could be caused by an abnormal increase in the permeability of dihydropyri-

dine-sensitive Ca^{2+} channel of the VSM plasma membrane. We determined the intracellular Ca uptake by caudal artery segments of normal control and pyridoxine-deficient rats during progressive depletion using La-resistant ^{45}Ca uptake as an index (Lal & Dakshinamurti, 1993b). In the prehypertensive vitamin B_6-deficient phase (weeks 3–4 on the deficient diet) the $^{45}Ca^{2+}$ influx into VSM did not differ significantly from control values. In the hypertensive rat (SBP 145 (SD 2) mmHg), the $^{45}Ca^{2+}$ influx into the VSM was significantly increased to twice that of the control. As the degree of vitamin B_6 deficiency increased (post-hypertensive phase, after 12 weeks on the deficient diet) there was a further large increase in $^{45}Ca^{2+}$ uptake. The mechanisms leading to a decrease in SBP during this advanced phase of deficiency are not yet understood. The possibility of altered sensitivity of the contractile apparatus to increased intracellular Ca and calmodulin cannot be discounted (Ngheim & Rapp, 1983). It is significant that changing the diet of vitamin B_6-deficient rats in the post-hypertensive phase (after 12–14 weeks on the deficient diet) to that of a vitamin B_6-containing normal diet led to a significant increase in their SBP within 2 weeks.

In a further study, we explored the defects in the Ca channel of the vitamin B_6-deficient hypertensive rat by studying the effect of Ca-channel antagonists on the SBP of conscious hypertensive rats (Lal & Dakshinamurti, 1993b). They were on the deficient diet for 7–10 weeks and had an SBP of 145–150 mmHg. The intraperitoneal injection of nifedipine produced a dose-dependent decline in SBP. All of the other Ca-channel antagonists used were also effective in lowering the SBP of the vitamin B_6-deficient hypertensive rats. The rank order of potency was: nifedipine > (-) 202–791 > verapamil > diltiazem. The specificity of the effect was seen in the effect of the dihydropyridine agonist, BAY K 8644, on the SBP of the vitamin B_6-deficient hypertensive rats: injection of BAY K 8644 did not alter the SBP of the deficient rat; however, when it was co-administered with the dihydropyridine Ca-channel antagonist nifedipine at equimolar doses, BAY K 8644 significantly antagonized the hypotensive effect of nifedipine. BAY K 8644 is known to prolong the open state of Ca channels during activation (Kokubun & Reuter, 1984), thereby promoting Ca entry into the cell. The failure of BAY K 8644 to do this in the deficient hypertensive rats suggests that the dihydropyridine-sensitive Ca channel is probably maximally open, implying that the vitamin B_6 status might be an important contributor to the variation in Ca-channel function.

Dietary-induced hypertension and vitamin B_6. In further experiments we investigated the relationship between the vitamin B_6 levels in the diet and some diet-induced hypertensive conditions. Dietary manipulations such as decrease in the Ca content of the diet or increase in the sucrose or fructose content of the diet led to a consistent, although modest, increase in SBP. The effect of altering the level of Ca in the diet at different phases (prehypertensive, hypertensive and post-hypertensive) of vitamin B_6 deficiency was studied (Lal & Dakshinamurti, 1995). Lowering dietary Ca from 1·0 to 0·1 % caused a significant increase in the SBP in rats on a vitamin B_6-sufficient diet. This occurred during weeks 3–4 on the low-Ca diet. Similar effects of a low-Ca diet on blood pressure have been reported by others (Baksi *et al.* 1989). Low levels of Ca in the diet potentiated the hypertension induced by the vitamin B_6-deficient diet when both deficiencies were present from the beginning of the experiment. Feeding a low-Ca diet during the hypertensive or post-hypertensive phase failed to raise the SBP in these rats, whereas normalizing the vitamin B_6 status of post-hypertensive vitamin B_6-deficient rats restored the ability of low dietary Ca to increase SBP.

It has been suggested that reduced dietary Ca depletes Ca from membrane storage sites, causing a less stable membrane of the VSM (Brickman *et al.* 1990). This results in enhanced Ca

influx and increased tone and reactivity. Peripheral resistance is elevated, leading to hypertension. Stabilizing the membrane abnormality in VSM by using high dietary Ca has been demonstrated. We have shown (Dakshinamurti & Lal, 1992) that high dietary Ca also reduces hypertension in rats with vitamin B_6 deficiency-induced hypertension, as has been shown in other models of hypertension such as the one-kidney deoxycorticosterone–NaCl hypertensive Wistar rat (Arvola et al. 1993). A low-Ca diet decreases ionic serum Ca; vitamin B_6 deficiency appears to cause a similar abnormality. Ca uptake by enterocytes is reduced in vitamin B_6 deficiency. An interesting finding was that an increased concentration of vitamin B_6 in the diet attenuated the blood-pressure-increasing effect of low dietary Ca. Vitamin B_6 might correct the membrane abnormality by a mechanism similar to that of the Ca-channel antagonists (Dominiczak & Bohr, 1990; Lal et al. 1993).

Acute or chronic ingestion of simple carbohydrates such as sucrose or fructose has been shown to cause an increase in SBP of varying degrees in several strains of rats (Zein et al. 1990). The ingestion of sucrose by male Sprague–Dawley rats resulted in a modest elevation of SBP. This was attenuated by the inclusion of a vitamin B_6 supplement (five times the normal intake) in their diet (Lal et al. 1996). The results show that hypertension induced by dietary means such as low Ca or an increase in simple carbohydrates in the diet of rats receiving normal amounts of vitamin B_6 in their diet responds to a further dietary supplement of vitamin B_6 (five times the normal intake).

Genetic hypertension and vitamin B_6. In further work we investigated whether a dietary supplement of vitamin B_6 could attenuate the elevation of SBP in genetically hypertensive animal models such as the Zucker obese rat or SHR. The Zucker obese (*fa/fa*) rat was originally studied as a model of obesity and atherosclerosis and has found extensive use in the study of diabetes mellitus. Various reports have shown that the Zucker obese rat also develops hypertension, which is specifically associated with the obese genotype (*fa/fa*); in contrast, Zucker lean rats are normal in all parameters. Metabolic alterations associated with obesity are believed to be the pathogenetic determinants of hypertension. Energy restriction of Zucker obese rats reduced the weight gain but did not attenuate the hypertension. SHR have been used extensively as an experimental model for the study of essential hypertension in human subjects.

Zucker obese (fa/fa), SHR and corresponding control rats were tested for the effects of vitamin B_6 ingestion in different ways:

(1) vitamin B_6 was included as a supplement (five times the normal intake) from the start of the experiment until development of hypertension;
(2) vitamin B_6 supplement was removed from the diet of Zucker obese and Zucker lean control groups after 16 weeks on the dietary supplements;
(3) a diet deficient in vitamin B_6 was instituted in SHR and control WKY;
(4) the SBP of rats in all groups was monitored in the conscious animal by tail-cuff plethysmography;
(5) the effects of the various treatments on the uptake of Ca by caudal artery segments were also examined.

Male Zucker obese rats (*fa/fa*) of age 6 weeks fed a commercial rat chow developed hypertension in 3–4 weeks, whereas their lean controls (*Fa/Fa*) did not. Similar increases in the SBP of the Zucker obese rat have been reported using direct (Zemel et al. 1992*b*) and indirect (Yoshioka et al. 1993) measurements. The inclusion of a vitamin B_6 supplement (five times the normal intake) resulted in complete attenuation of the hypertension in the obese strain; in

fact, the SBP of the obese rats after 16 weeks of supplementation was lower than their initial level. Heart rate was also lowered as a result of feeding the high vitamin B_6 diet. The age-associated increase in the blood pressure of the Zucker lean rat was also attenuated by the high vitamin B_6 diet. When the high vitamin B_6 diet of the Zucker obese rats was changed to a normal vitamin B_6 diet, the SBP of the rats increased by about 30 mmHg in 12 d. Similar switch of the diet in the Zucker lean rat also resulted in a rise in the SBP within 2 d to the same level as shown by Zucker lean rats fed the normal vitamin diet for the entire experimental period. In contrast to the effects seen in the Zucker obese rats, there was no response to the inclusion or removal of dietary vitamin B_6 supplement in the SHR. However, the WKY responded to both these conditions in a manner similar to that seen in the Sprague–Dawley strain. Thus, the SBP of SHR, unlike that of WKY, was insensitive to the vitamin B_6 concentration in the diet. The changes in SBP in the Zucker as well as in the sucrose-fed rats correlated with changes in the uptake of Ca by caudal artery segments in all these groups. This is the first observation that animal models of hypertension can be classified on the basis of their response to a vitamin B_6 supplement. On this basis, the aetiology of hypertension in SHR is quite distinct from that in Zucker obese rats.

Effect of high dietary vitamin B_6 on blood glucose of Zucker rats and Sprague–Dawley rats ingesting sucrose. Zucker obese rats on the high-vitamin B_6 diet for 16 weeks had a lower blood glucose level than rats on the normal vitamin B_6 diet. This blood-glucose-lowering effect was seen in the both the fasting and non-fasting state. The hyperglycaemia seen in Sprague–Dawley rats ingesting sucrose was also attenuated. Zucker lean rats and Sprague–Dawley rats on commercial rat chow did not respond to the ingestion of high levels of vitamin B_6 as these rats were normoglycaemic (Lal *et al*. 1996).

Effect of pyridoxal phosphate on ^{45}Ca uptake by rat caudal artery segments. We investigated the possibility that pyridoxine or, more particularly, PLP, could directly modulate the cellular Ca uptake process. Cold La-resistant $^{45}Ca^{2+}$ uptake by segments of caudal artery was determined. The effect of PLP on the BAY K 8644-induced $^{45}Ca^{2+}$ influx was examined in artery segments from control (normal) rats. The DHP-sensitive Ca-channel agonist was ineffective in increasing further the basal Ca uptake by caudal artery segments from vitamin B_6-deficient hypertensive rats. However, BAY K 8644 stimulated $^{45}Ca^{2+}$ entry into artery segments from control (normal) rats. PLP dose-dependently ($0.1–10$ μM) reduced the BAY K 8644-stimulated Ca uptake by control artery segments. As seen earlier (Viswanathan *et al*. 1991), the basal uptake of $^{45}Ca^{2+}$ by caudal artery segments from vitamin B_6-deficient hypertensive rats was at least twice the uptake by artery segments from control (normal) rats. PLP or nifedipine added to the incubation medium reduced significantly the $^{45}Ca^{2+}$ uptake by artery segments from the deficient hypertensive rats. However, in the presence of BAY K 8644 (which, by itself, had no effect) in the incubation medium, both PLP and nifedipine were much less effective in attenuating the $^{45}Ca^{2+}$ uptake by artery segments from the deficient hypertensive rats. These *in vitro* direct antagonisms indicate the possibility that the Ca-channel agonist BAY K 8644, the Ca-channel antagonist nifedipine and PLP might all act at the same site on the Ca channel.

Effect of pyridoxal phosphate on [^3H]nitrendipine binding by artery membrane preparations. We have also examined the effect of PLP on the binding of tritiated [^3H]nitrendipine, a dihy-

dropyridine Ca-channel antagonist, to membrane preparations from caudal artery of normal rats and have analysed the Scatchard plots of the binding assay. The equilibrium dissociation constant value for [3H]nitrendipine binding was 0.57 ± 0.03 nM and the total number of binding sites was 150 ± 7 fmol/mg protein in control membranes. [3H]Nitrendipine showed corresponding values of 0.69 ± 0.04 nM and 98 ± 6 fmol/mg protein in PLP-treated membranes. The results indicate that PLP treatment of membranes caused a decrease in the number of [3H]nitrendipine binding sites.

Effect of pyridoxal phosphate on ATP-induced positive inotropic action in the isolated heart. In other experiments (Dakshinamurti *et al.* 1998, 2000) we investigated the effect of PLP on the ATP-induced positive inotropic effect in isolated perfused normal rat heart. The infusion of ATP caused an immediate increase (within a few seconds) in left ventricular developed pressure, $+dP/dt$ and $-dP/dt$. This effect was completely blocked in hearts pretreated with PLP for 10 min (Wang *et al.* 1999). The antagonistic effect of PLP was concentration dependent: the median effective dose (ED_{50}) for PLP was in the range of 10–15 μM. The marked increase in contractile activity upon infusing 1 μM-isoproterenol was not affected by perfusing the heart with 50 μM-PLP, which prevented the positive inotropic action of ATP. Likewise, 10 μM-propranolol, which prevented the positive ionotropic action of 1 μM-isoproterenol, showed no effect on the positive inotropic action of 50 μM-ATP.

Effect of pyridoxal phosphate on ATP-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes. The incubation of cardiomyocytes with 15 or 50 μM-PLP for 10 min did not alter the basal $[Ca^{2+}]_i$. However, the ATP-induced increase in $[Ca^{2+}]_i$ was significantly decreased in PLP-treated cardiomyocytes. The effect of PLP (1–50 μM) on the ATP (50 μM)-induced increase in $[Ca^{2+}]_i$ was dose dependent. The effective concentration of PLP was as low as 1 μM. ED_{50} for PLP was in the range of 10–20 μM.

Effect of pyridoxal phosphate on ATP binding in cardiac sarcolemma. Since cardiac sarcolemmal membrane has been reported to contain both high- and low-affinity ATP-binding sites, we studied the effects of PLP on the high-affinity and low-affinity binding sites by employing 1–10 nM- and 1–10 μM-[35S]ATPγS, respectively. The maximal ATP binding at both high- and low-affinity sites was inhibited by both 50 μM-PLP and 100 μM-suramin without any change in their respective equilibrium dissociation constant values. PLP almost completely blocked the low-affinity binding. Binding at the high-affinity sites was depressed by about 60 % of the control value. Suramin (100 μM) has effects comparable to 50 μM-PLP on both high- and low-affinity sites. Cibacron blue and 4,4′-diisothiocyanatostilbene-2,2′-disulfonate at 50 μM concentrations inhibited ATP binding at high-affinity and low-affinity sites by 40–45 % and 60–75 % respectively. The ED_{50} values for inhibitory effects of PLP on high- and low-affinity ATP binding sites were about 10 μM and 15 μM, respectively. Agents such as propranolol (β-adrenoceptor blocker), prazosin (α adrenoceptor blocker), verapamil (L-type Ca^{2+}-channel blocker) did not show any significant effect on the high- or low-affinity ATP-binding sites.

PLP *in vitro* attenuates the influx of extracellular Ca. This effect is achieved through modulation of ligand binding. This is analogous to the effect of PLP on steroid hormone activity (Litwack, 1988). Voltage-sensitive Ca channels undergo long-term modulation by neurotrans-

mitters and a variety of second messengers. Activation of the channels is enhanced by cAMP and cAMP-dependent protein kinase (Schmid *et al*. 1985). In common with the pharmacological receptors, Ca channels are regulated by homologous and heterologous factors. Chronic channel activation, chronic drug exposure, hormonal influence and specific diseases are all associated with altered expression of Ca channel function and numbers (Ferrante & Triggle, 1990). The action of drugs at the Ca channels would indicate that endogenous factors or ligands might serve as physiological regulators, a function which is mimicked by Ca channel agonists or antagonists. The KCl-induced increase in $[Ca^{2+}]_i$ was augmented in cardiomyocytes from the vitamin B_6-deficient rats. The administration of vitamin B_6 to the vitamin B_6-deficient animals was found to abolish this augmentation. It is possible that the observed augmentations of KCl-induced increase in $[Ca^{2+}]_i$ is related to an increase in Ca^{2+} influx through the sarcolemmal Ca^{2+} channels. An increase in Ca^{2+} influx in smooth muscle cells caused an increase in smooth muscle tone and hypertension in vitamin B_6 deficiency (Paulose *et al*. 1988; Lal *et al*. 1993).

The increase in $[Ca^{2+}]_i$ in cardiomyocytes from vitamin B_6-deficient animals may contribute towards heart dysfunction and increased susceptibility to myocardial infarction (Ellis & McCully, 1995). These observations can explain the high incidence of hypertension and coronary artery disease in vitamin B_6-deficient patients (Vermaak *et al*. 1987), as well as the beneficial effects of vitamin B_6 in patients with hypertension (Ayback *et al*. 1995) and myocardial infarction (Ellis & McCully, 1995). The antagonistic effect of PLP may not be of a generalized depressant nature. Since ATP is considered to influence cellular functions by acting on purinoceptors (Dubyak & El-Moatassium, 1993), it is possible that PLP may affect the cardiac action of ATP by blocking these receptors in the myocardium. This view is consistent with pharmacological studies showing the antagonistic effect of PLP on ATP-induced changes in rat vagus and vas deferens (Trezise *et al*. 1994). Furthermore, PLP decreased the specific binding of ATP to both high- and low-affinity binding sites similar to purinoceptor antagonists such as suramin, Cibacron blue and 4,4′-diisothiocyanatostilbene-2,2′-disulfonate. PLP may be a selective antagonist of purinoceptors because other receptor channel blocking agents (such as propranolol, prazosin, verapamil and ryanodine) did not show any effect on the specific binding of ATP to sarcolemma. It is generally accepted that ATP acts as a sympathetic cotransmitter with noradrenaline, causing vasoconstriction acting through purinergic receptors of the $P2X_1$ subtype (Boarder & Hourani, 1998). Electrophysiological responses similar to those of cloned $P2X_1$ receptor are reported in VSM cells (Evans & Kennedy, 1994). Immunocytochemical evidence has been presented for the presence of $P2X_1$ receptor on the smooth muscle cells of submucosal arteries (Vulchanova *et al*. 1996). In addition α, β methylene L-ATP has been shown to contract blood vessels (Hourani *et al*. 1986).

Hypertension is associated with even moderate deficiency of vitamin B_6. Physiological levels of vitamin supplementation reverses this hypertension. The development of hypertension in vitamin B_6 deficiency is due to a variety of mechanisms which are not mutually exclusive. The central effects on blood pressure regulation are due to vitamin B_6 deficiency mediated decreases in brain GABA and 5-HT. The stimulation of sympathetic nervous system activity is due to decreased 5-HT at specific serotonergic receptor ($5-HT_{1A}$) sites. The increased catecholamine secretion in the autonomic nervous system results in increased muscle tone. PLP appears to have a role in cellular Ca transport, acting through both the significant Ca channels (the voltage-mediated L-type channel as well as the ATP-mediated purinergic $P2X_1$ channel). Physiological concentrations of vitamin B_6 would reverse the abnormalities seen in deficiency. Hypertension is also produced in a variety of experimental animal models with replete vitamin B_6 status. Genetic and dietary influences are responsible for development of these hypertensive conditions. High levels of vitamin B_6 attenuate many (although not all) of the hypertensive con-

ditions of genetic or dietary origin. It has been shown (Dakshinamurti *et al.* 1990*b*) that there is a continuum in many of the PLP-mediated enzymic activities from vitamin deficiency to pharmacological levels of the vitamin in the organism, leading to increased synthesis and secretion of various monoamines with very beneficial effects to the organism as a whole (Bender & Totoe, 1984). This would include effects on sympathetic nervous system activity as well as on the Ca channels. Other suggested mechanisms might also include enhanced sensitivity and end-organ responsiveness to glucocorticoids, mineralocorticoids and aldosterone (Bender, 1999), all of which can result in hypertension. PLP acts to terminate the action of steroid and other nuclear-acting hormones (Tully *et al.* 1994).

We have shown that the hyperglycaemia associated with hypertensive Zucker obese and sucrose-fed rats were normalized by the high vitamin B_6 supplement. Reaven & Hoffman (1988) have suggested that abnormalities in carbohydrate metabolism may underlie the aetiology of hypertension in this condition. However, as pointed out by Resnick (1999*a*), the alterations in $[Ca^{2+}]_i$ might be the central abnormality which expresses itself differently in different organ systems. Correction of this basic defect ameliorates the related clinical condition, be it hypertension or hyperglycaemia.

Vitamin B_6 has been referred to as a 'vitamin without a deficiency' (Van den Berg, 1999). However, low plasma levels of PLP have been reported in many studies, particularly in the elderly (Van der Wielen *et al.* 1996; Brussard *et al.* 1997; Bates *et al.* 1999). The prevalence of hypertension in the elderly population might be related to this deficiency and/or to the chronic use of anti-inflammatory drugs, many of which have a hypertensive side effect. Ribaya Mercado *et al.* (1991) showed that more vitamin B_6 was required to normalize plasma PLP levels and tryptophan load test results in the elderly than in younger adult populations. There are age-related changes in vitamin B_6 metabolism. In the elderly group, plasma homocysteine is inversely correlated with plasma PLP. Homocysteine is an independent risk factor for cardiovascular disease. Van den Berg (1999) has commented that this 'vitamin without a deficiency' has 'indeed protective effects beyond known functions.' Many of these functions are being unravelled.

Vitamin C

Various associations that might suggest that a deficiency of vitamin C (ascorbic acid) may lead to hypertension in human subjects have been listed (Bulpitt, 1990). Mortality from stroke is highest in regions with lowest intake of vitamin C. Blood pressure and diabetes are inversely related to intake of vitamin C; so, too, is hypercholesterolaemia, and all three are associated with old age. Stress leads to a depletion of vitamin C and stress is related to hypertension. Although these associations may be explained by other factors, increasingly the antioxidant function of various nutrients as protective against hypertension is being considered. High blood pressure, an important risk factor for cardiovascular disease, has been inversely associated with both intake and status indices of vitamin C (Jacques, 1992; Moran *et al.* 1993; Ness *et al.* 1996; Bates *et al.* 1999). If the relationship between vitamin C and blood pressure is causal, it has implications for dietary intervention; however, dietary intervention trials have been inconclusive. The effect of vitamin C supplementation, even when decreasing blood pressure, was not quite unequivocal (Feldman *et al.* 1992; Lovat *et al.* 1993; Ghosh *et al.* 1994). In the Finnish study with smokers, where a controlled trial of mixed antioxidants including ascorbic acid, organic Se, vitamin E and β-carotene was undertaken, there was a significant reduction in systolic blood pressure (Salonen *et al.* 1994). In a randomized double-blind placebo-controlled trial, Duffy *et al.* (1999) have shown that long-term (up to 4 weeks) treatment with ascorbic

acid (500 mg daily) of hypertensive patients reduced their blood pressure. In experimental animal studies, Yoshioka *et al*. (1985) and Ziemalanski *et al*. (1991) have shown a very significant effect of vitamin C in reducing the blood pressure in SHR. Increasing evidence from animal and human interventional studies indicates that the beneficial effect of vitamin C may be due to its antioxidant function.

Cardiovascular pathology in insulin-dependent diabetes mellitus, particularly the nephropathy subset and hypertension, is mainly due to atherosclerosis or large vessel disease, although microangiopathy is also a significant contributor. Reactive oxygen free radicals are implicated in this vascular injury. Free radical scavenging systems including ascorbic acid and α-tocopherol provide a natural defence against accumulating reactive oxygen species. A valuable clue to the association between low ascorbic acid status and conditions such as diabetic nephropathy and hypertension has been provided by Ng *et al*. (1998), who found that the uptake mechanisms for ascorbic acid and dehydroascorbic acid were impaired in lymphoblasts from subjects with diabetic nephropathy or hypertension. The depletion of ascorbic acid may be due to the defective cycling of dihydroascorbic acid. This would impair the ability to recycle oxidized ascorbic acid and hence would deplete antioxidant defences.

As detailed in an earlier section (p. 6), the vascular endothelium has a very significant role in regulating vascular tone. The vascular endothelium-derived relaxing factor NO has an important function in this. NO is released following stimulation of the muscarinic receptor on endothelial cells by acetylcholine or by stretch. There is strong evidence to indicate that NO is inactivated by increased vascular superoxide or other free radicals, leading to endothelial dysfunction in subjects with diabetes and coronary artery disease (Levine *et al*. 1996; Ting *et al*. 1996). Solzbach *et al*. (1997) have extended experimental animal studies and studies with the human brachial artery to human coronary arteries. They investigated acetylcholine-induced vascular responses and papavarine-induced flow-dependent vasodilatation in the coronary circulation of hypertensive patients both before and after administration of vitamin C; thus the acute effects of vitamin C were monitored. They have shown constriction of epicardial coronary arteries during intracoronary infusion of acetylcholine in hypertensive patients. This was dramatically improved after vitamin C infusion. Flow-dependent vasodilatation is another tool to assess endothelial function and this was increased following treatment with vitamin C. Taddei *et al*. (1998) have reported similar results.

In other work, Kugiyama *et al*. (1998) studied patients with coronary spastic angina. They infused acetylcholine into the left coronary arteries and measured epicardial artery diameters by quantitative coronary angiography both before and during combined intracoronary infusion with vitamin C (10 mg/min) or saline. They found that vitamin C attenuated the constrictor response of the epicardial arteries to acetylcholine in coronary arteries but had no effect on control coronary arteries, and suggested that vitamin C suppressed vasomotor dysfunction in coronary arteries of patients with coronary spastic angina.

Jeserich *et al*. (1999) investigated whether the abnormal constriction of epicardial coronary arteries due to sympathetic stimulation induced by the cold pressor test in patients with essential hypertension or hypercholesterolaemia could be reversed by administration of vitamin C. The cold pressor test has been shown to dilate normal coronary arteries but to constrict epicardial arteries in patients with hypertension or hypercholesterolaemia. Jeserich *et al*. (1999) found that impaired coronary vasomotor activity following sympathetic nervous system stimulation in essential hypertensive or hypercholesterolaemic subjects was improved by vitamin C. Conti (1999), in an editorial in the *European Heart Journal*, has commented on the observations of Kugiyama *et al*. (1998) and Jeserich *et al*. (1999) to suggest the need for vitamin C therapy to be tested in a large population of patients with coronary artery disease.

In a more revealing investigation, Sherman *et al.* (2000) studied the dose-dependent effects of ascorbic acid on endothelial vasomotor function in patients with hypertension to seek kinetic evidence that superoxide anion contributes to endothelial dysfunction. They demonstrated that the forearm blood flow response to methacholine infusion was impaired in patients with hypertension, whereas response to sodium nitroprusside was not affected. Concomitant infusion of ascorbic acid at 24 mg/min, which produced a high physiological concentration of about 3·2 mmol/l in forearm circulation, returned the methacholine response to normal. A tenfold lower concentration of ascorbic acid had no effect on the response to methancholine. On the basis of this study, they state that, although there was a lower baseline plasma ascorbic acid concentration in hypertensive patients, there was no correlation between this and endothelium-dependent dilatation at the baseline or during ascorbic acid infusion. They contend that the improvement in endothelial vasomotor function due to ascorbic acid cannot be explained by correction of an ascorbic acid-deficient state and that alternative mechanisms for the effects of pharmacological doses of ascorbic acid should be considered. This recalls the comment of Van den Berg (1999) regarding 'the protective action of vitamin B_6 beyond its known functions'. These recent observations are very significant in the context of our currently accepted function of vitamins. We have to re-evaluate our concepts regarding all aspects of vitamin biology, including their biological effects at high physiological or pharmacological concentrations in the body.

Vitamin E and other antioxidants

Free radical production is increased in patients with NIDDM and those with essential hypertension (Collier *et al.* 1990; Sagar *et al.* 1992; Moran *et al.* 1993). Free radical-mediated peroxidation of membrane polyunsaturated fatty acids results in impairment of membrane integrity and its function (Slater, 1984). Vitamin E is an endogenous antioxidant: it breaks the chain of lipid peroxidation in cell membranes, prevents the formation of lipid hydroperoxides (Machlin & Bendich, 1987) and also stabilizes the membrane (Erin *et al.* 1984). Thus, vitamin E improves cellular free radical defence potential and seems to have a beneficial effect on glucose transport and insulin sensitivity (Paolisso *et al.* 1992*b*; Faure *et al.* 1997).

Pregnancy-induced hypertension complicates a small but significant percentage of all pregnancies. The hypertension is categorized as pre-eclampsia or gestational hypertension. Lipid peroxides in serum and placental tissue were significantly increased and serum vitamin E levels significantly reduced in women with severe gestational hypertension and pre-eclampsia compared with normal subjects. In mild gestational hypertensive as well as in chronic hypertensive subjects who were pregnant, there were no changes either in serum vitamin E levels or in serum lipid peroxides (Gratacos *et al.* 1998).

Wen *et al.* (1996) investigated lipid peroxidation, plasma vitamin C, and plasma and erythrocyte levels of α-tocopherol in patients with essential hypertension and healthy controls. Hypertensive subjects had a significantly higher concentration of plasma malondialdehyde and significantly lower levels of plasma ascorbic acid. Erythrocyte α-tocopherol levels, which reflect the content of vitamin E in membranes, were also significantly lower in hypertensive than in healthy normotensive subjects; however, plasma levels of vitamin E were comparable in both hypertensive and normotensive subjects. These results suggest that hypertensive patients might have reduced tissue levels of the antioxidant vitamins and, hence, increased lipid peroxidation.

In experimental animal work, antioxidant levels were reported to be low in SHR compared with WKY controls (Janero & Burghardt, 1988). In view of the known function of α-

tocopherol as a scavenger of free radicals, Newaz & Nawal (1998) have examined lipid peroxides and antioxidant status of SHR and their WKY controls. They also evaluated the effect of vitamin E supplementation on blood pressure of rats in their experimental groups. Treatment of SHR with α-tocopherol prevented the age-related increase in the blood pressure of SHR. SHR had significantly higher levels of lipid peroxides in blood vessels and plasma and reduced total antioxidant status and SOD activity. After 12 weeks of α-tocopherol supplementation of SHR, there was a significant decrease in lipid peroxidation in blood vessels. The levels of total antioxidant status and SOD increased significantly in rats supplemented with α-tocopherol. They concluded that α-tocopherol prevented an increase in blood pressure, reduced lipid peroxides in blood vessels and in plasma, and also increased the total antioxidant status.

In further work, Newaz *et al*. (1999) compared the NO synthase activity in blood vessels of SHR and of SHR treated with α-tocopherol. NO synthase produces NO from L-arginine through a Ca- and calmodulin-dependent process. NO is responsible for the acetylcholine-mediated vascular relaxation. Reduced activity of NO synthase or accelerated degradation of NO may lead to impaired vasodilatation and, hence, increased blood pressure. The blood pressure of α-tocopherol-treated SHR is significantly lower than that of untreated SHR. Compared with the WKY controls, the SHR had a lower NO synthase activity in blood vessels. Treatment with α-tocopherol increased the NO synthase activity and concomitantly reduced the blood pressure of SHR.

Stroke-prone SHR are regarded as an animal model for ischaemic stroke in human subjects. Cerebral ischaemia for 20 min in stroke-prone SHR induced massive efflux of glutamate which caused delayed neuronal death in the hippocampal CA1 region; no such phenomenon was found in WKY (Gemba *et al*. 1992). Cortical neurons isolated from stroke-prone SHR were more vulnerable than those from WKY and resulted in apoptosis when exposed to NO- and NMDA-mediated neurotoxic agents (Tagami *et al*. 1997*b*). Vitamin E inhibited the reoxygenation injury in cultured cortical neurons. Reperfusion after carotid artery occlusion resulted in an increase in apoptotic neurons in the stroke-prone SHR (Tagami *et al*. 1997*a*). In further work, Tagami *et al*. (1999) examined the protective effect on the reperfusion injury of feeding the stroke-prone SHR with a high-vitamin E diet for 3 weeks. Cerebral ischaemia followed by 6 or 9 d of reperfusion very significantly increased the number of apoptotic neurons in the hippocampal CA1 region of the stroke-prone SHR fed the normal diet. However, if the rats were fed the high-vitamin E diet for 3 weeks before ischaemia and reperfusion, there was a dramatic decrease in the number of apoptotic neurons. Tagami *et al*. (1999) have demonstrated that oxygen radical generation occurs after reperfusion and the free radicals heavily damage the neurons in stroke-prone SHR. Vitamin E pretreatment of these rats increases its concentration in all tissues, including the brain. Vitamin E reacts with the free radicals and thus prevents neuronal apoptosis caused by reperfusion.

GSH has intracellular antioxidant properties: it inhibits free radical formation and functions generally as a redox buffer. GSH may also have an important role in glucose homoeostasis (Paolisso *et al*. 1992*b*) and in lowering blood pressure in diabetic and hypertensive subjects (Ceriello *et al*. 1991). As stated in an earlier section (pp. 8–9), the decrease in intracellular free Mg has been recognized as a feature common to all these clinical conditions (Resnick, 1999*a*). Mg deficiency has been associated with increased membrane susceptibility to oxidative stress (Freedman *et al*. 1992; Rayssiguier *et al*. 1993*b*). Mg dose-dependently reduced blood pressure (Widman *et al*. 1993). It has been suggested that the beneficial effect of glutathione on glucose metabolism may be mediated by Mg (Barbagallo *et al*. 1999). These authors have also examined whether vitamin E had similar effects in subjects with essential hypertension. In

hypertensive subjects, vitamin E (600 mg/d) administration significantly increased whole-body glucose disposal, GSH:GSSG and $[Mg^{2+}]_i$. In the basal condition, whole-body glucose disposal correlated directly with GSH:GSSG and $[Mg^{2+}]_i$. Their results show a clinical relationship between vitamin E administration, intracellular Mg levels, GSH:GSSG and tissue glucose metabolism.

δ-Tocopherol is a minor component of dietary vitamin E, which is predominantly α-tocopherol. Murray *et al*. (1997) have isolated and characterized a natriuretic δ-tocopherol metabolite, LLU-α, which inhibits the intermediate conductance (70 ps) ATP-sensitive K^+ channel in the thick ascending limb. Unlike ANP, LLU-α exhibits a prolonged natriuresis. This factor could be part of the system controlling extracellular volume; if so, δ-tocopherol might be analogous to vitamin D being a precursor to a hormone.

The role of vitamin E as an antioxidant is well recognized. A supplement of vitamin E appears to decrease blood pressure in a variety of hypertensive conditions. Several studies indicate an association between dietary intake or plasma concentrations of the antioxidants vitamin E, vitamin C and β-carotene, and hypertension (Gey *et al*. 1993). Suboptimal levels of vitamin C, vitamin E and β-carotene have been reported as additive risk factors for cardiovascular diseases. Galley *et al*. (1997) have investigated the effect of a short-term high dose of antioxidant supplement consisting of zinc sulfate (200 mg), ascorbic acid (500 mg), α-tocopherol (600 mg) and β-carotene (30 mg). They found that this combination therapy reduced blood pressure in both hypertensive and normal subjects, although the effects were more marked in hypertensive subjects. The results confirm the well-known synergism between vitamin E and vitamin C, β-carotene and vitamin E, and Zn and vitamin E respectively (Goody *et al*. 1991; Palozza & Krinsky, 1992).

Conclusions

Hypertension is not a single disease entity with a single identifiable aetiology; a variety of factors contribute to the initiation and maintenance of this clinical condition. Metabolic abnormalities associated with the tetrad of hypertension, dyslipidaemia, glucose intolerance and obesity, share IR that might be organ, tissue or cell specific as an underlying feature. Oxidative stress is a major mechanism leading to impaired endothelial function and is present in hypertension and dyslipidaemia. In addition to the coexistence of several metabolic abnormalities with hypertension, the heterogeneity of hypertension itself is recognized. These metabolic abnormalities represent different tissue manifestations of a common cellular ionic defect. The intracellular concentrations of Ca and Mg, which are reciprocally related, regulate cellular metabolic processes and so define the pathophysiology shared by the tetrad of 'syndrome X'. The heterogeneity of hypertension is defined in terms of plasma renin activity, which seems to be related to the source of the increase in intracellular free Ca. In the high-renin type this is intracellular; in the low-renin type it is extracellular. This is reflected in the inverse concentrations of extracellular ionized Ca in these two conditions. The calcitrophic hormones, particularly the derivatives of vitamin D, are involved in this regulation.

Hypertension is associated with the deficiency of some micronutrients. Various normal dietary components in recommended daily amounts or, more significantly, at much higher levels of intake, have been shown to decrease further the blood pressure of hypertensive subjects who are receiving pharmacological treatment, indicating that some aspects of blood pressure regulation not amenable to manipulation by antihypertensive drugs do respond to these 'micronutrients' which include minerals and vitamins. As Ca is at the centre of ionic regulation

of cellular function, vitamins involved in Ca regulation have a significant role. As the endothelium-dependent vasodilator, NO is susceptible to oxidative damage, antioxidant vitamins also have a role.

The function of vitamin D in Ca homoeostasis is well known. More interesting are the direct effects of vitamin D metabolites. $1,25\text{-}(OH)_2\,D_3$ is a vasoconstrictor, whereas $24,25\text{-}(OH)_2\,D_3$ is a vasodilator, both acting directly and reciprocally at the level of the VSM. Vitamin B_6 has a unique role in blood pressure regulation. The active form of this vitamin, PLP, seems to have a direct role in the regulation of Ca channels; this includes both the voltage-mediated L-type Ca channel and the ATP-mediated purinergic P2X-type Ca channel. Vitamin B_6 functions in blood pressure control through other mechanisms as well, particularly via regulation of sympathetic nervous system activation. This central function is mediated by the synthesis and secretion of the neurotransmitter serotonin. These subsume the 'protective effects of vitamin B_6 beyond its known function'.

Vitamin C and vitamin E, because of their antioxidant nature, inhibit the oxidation of NO, the most important vasodilator of the vascular endothelium; they thus maintain the vasodilator status of blood vessels. Vitamin C also seems to have a direct acute effect on inhibition of the constrictor response of resistance arteries to a variety of stimuli. This improvement in endothelial vasomotor function may be explained, not as due to the correction of an ascorbic acid deficiency but as due to a direct effect on the VSM. A minor constituent of natural vitamin E, δ-tocopherol, is a precursor of LLUα, which exhibits prolonged natriuresis and may control extracellular fluid volume. Synergism between the actions of vitamin E and vitamin C and between vitamin E and β-carotene have been reported.

The actions of these vitamins on blood pressure regulation cannot always be understood on the basis of their conventionally recognized 'vitamin function'. In addition, there is a continuum of vitamin effects from the deficient state to pharmacological levels of the vitamin in the organism. The non-traditional functions of the vitamins and their derivatives can be exploited as an adjunct to the available modalities in the treatment of hypertension.

References

Altura BM & Altura BT (1995) Magnesium and cardiovascular biology: an important link between cardiovascular risk factors and atherosclerosis. *Cellular and Molecular Biology Research* **41**, 347–359.

Altura BM, Altura BT, Gebrewold A, Ising H & Gunther T (1984) Magnesium deficiency and hypertension: correlation between magnesium deficient diets and microcirculatory changes in situ. *Science* **223**, 1315–1317.

Altura BM, Altura BT, Gebrewold A, Ising H & Gunther T (1992) Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium. *Journal of Applied Physiology* **72**, 194–202.

Anand-Srivastava MB (1997) Atrial natriuretic peptide-C receptor and membrane signaling in hypertension. *Journal of Hypertension* **15**, 815–826.

Anand-Srivastava MB, de Champlain J & Thibault C (1993) DOCA-salt hypertensive rat hearts exhibit altered expression of G-proteins. *American Journal of Hypertension* **6**, 72–75.

Anand-Srivastava MB & Trachte GJ (1993) Atrial natriuretic factor receptors and signal transduction mechanisms. *Pharmacological Reviews* **45**, 455–497.

Anderson EA, Hoffman RP & Balon TW (1991) Hyperinsulinemia produced both sympathetic neural activation and vasodilation in normal humans. *Journal of Clinical Investigation* **87**, 2246–2252.

Appel LJ, Moore TJ, Obrzanek E, Vollmer WM, Svetskey LP, Sacks FM, Bray GA, Vogt TM, Cutlet JA, Windhauser MM, Lin P-H & Karanja N (1997) DASH Collaborative Research Group: a clinical trial of the effects of dietary patterns on blood pressure. *New England Journal of Medicine* **336**, 1117–1124.

Arvola P, Ruskoaho H & Portsi I (1993) Effects of high calcium diet on arterial smooth muscle functions and electrolyte balance in mineralo-corticoid-salt hypertensive rat. *British Journal of Pharmacology* **106**, 948–958.

Ascherio A, Rimm EB & Giovannuchi EL (1992) A prospective study of nutritional factors and hypertension among US men. *Circulation* **86**, 1475–1484.

Ascherio A, Rimm EB, Hernan MA, Giovannuchi EL, Kawachi I, Stampfer MJ & Willett WC (1998) Intake of potassium, magnesium, calcium and fibre and risk of stroke among US men. *Circulation* **98**, 1198–1204.

Auwerx J, Bouillon R & Kesteloot H (1992) Relation between 25,hydroxyvitamin D$_3$, apolipoprotein cholesterol. *Arteriosclerosis and Thrombosis* **12**, 671-674.

Ayback M, Sermet A, Ayyilidiz MO & Karakilcik AZ (1995) Effect of oral pyridoxine hydrochloride supplementation on arterial blood pressure in patients with essential hypertension. *Drug Research* **45**, 1271–1273.

Baksi SN, Abhold RH & Speth RC (1989) Low calcium diet increases blood pressure and alters peripheral but not central angiotensin II binding sites in rats. *Journal of Hypertension* **7**, 423–427.

Balarajan R, Yuen P & Machin D (1987) Inequalities in health: changes in RHA's in the past decade. *British Medical Journal* **294**, 1561–1564.

Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM & Paolisso G (1999) Effects of vitamin E and glutathione on glucose metabolism. Role of magnesium. *Hypertension* **34**, 1002–1006.

Barger-Lux MJ & Heaney RP (1994) The role of calcium intake in preventing bone fragility, hypertension and certain cancers. *Journal of Nutrition* **124**, 1406S-1411S.

Bates CJ, Prentice A, Cole TJ, Van der Pols JC, Doyle W, Finch S, Smithers G & Clarke PC (1999) Micronutrients: highlights and research challenges from the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. *British Journal of Nutrition* **82**, 7–15.

Belizan JM & Villar J (1980) The relationship between calcium intake and edema, proteinuria and hypertension-gestosis: an hypothesis. *American Journal of Clinical Nutrition* **33**, 2202–2210.

Belizan JM, Villar J, Gonzalez L, Campodonico L & Bergal E (1991) Calcium supplementation to prevent hypertensive disorders of pregnancy. *New England Journal of Medicine* **325**, 1399–1405.

Bender DA (1999) Non-nutritional uses of vitamin B$_6$. *British Journal of Nutrition* **81**, 7–20.

Bender DA & Totoe L (1984) High doses of vitamin B$_6$ are associated with inhibition of hepatic tryptophan metabolism and increased uptake of tryptophan into the brain. *Journal of Neurochemistry* **43**, 269–275.

Berthelot A & Esposito J (1983) Effects of dietary magnesium on the development of hypertension in the spontaneously hypertensive rat. *Journal of the American College of Nutrition* **4**, 345–353.

Boarder MR & Hourani SMO (1998) The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Trends in Pharmacological Science* **19**, 99–107.

Boucher BJ (1998) Inadequate vitamin D status: does it contribute to the disorders comprising syndrome 'x'? *British Journal of Nutrition* **79**, 315–327.

Brickman AS, Nyby MD, von Hungen K, Eggena P & Tuck ML (1990) Calcitropic hormones, platelet calcium and blood pressure in essential hypertension. *Hypertension* **16**, 515–522.

Brussard JH, Lowik MRH, Van den Berg H, Brants HAM & Kistemaker C (1997) Micronutrient status, with special reference to vitamin B$_6$. *European Journal of Clinical Nutrition* **51**, Suppl. 3, S32–S38.

Bukoski RD, DeWan P & McCarron DA (1989) 1,25 (OH)$_2$ vitamin D$_3$ modifies growth and contractile function of vascular smooth muscle of spontaneously hypertensive rats. *American Journal of Hypertension* **2**, 533–556.

Bukoski RD, Ishibashi K & Bian K (1995) Vascular actions of the calcium-regulating hormones. *Seminars in Nephrology* **15**, 536–549.

Bukoski RD & Kremer D (1991) Calcium-regulating hormones in hypertension: vascular actions. *American Journal of Clinical Nutrition* **54**, 220S–226S.

Bukoski RD, Wagman DW & Wang D (1990) Injection of 1,25 vitamin D enhances resistance artery contractile properties. *Hypertension* **16**, 523–531.

Bulpitt CJ (1990) Vitamin C and blood pressure. *Journal of Hypertension* **8**, 1071–1075.

Burgess ED, Hawkins RG & Watanabe M (1990) Interaction of 1,25-dihydroxy vitamin D and plasma renin activity in high renin essential hypertension. *American Journal of Hypertension* **3**, 903–905.

Burgess E, Lewanczuk R, Bolli P, Chockalingam A, Cutler H, Taylot G & Hamet P (1999) Recommendations on potassium, magnesium and calcium. *Canadian Medical Association Journal* **160**, Suppl. 9, S35–S45.

Campese VM, Romoff MS & Levitan D (1982) Abnormal relationship between sodium intake and sympathetic nervous system activity in salt-sensitive patients with essential hypertension. *Kidney International* **21**, 371–378.

Canadian Recommendations for the Management of Hypertension (1999) *Canadian Medical Association Journal* **161**, S1–S22.

Catterall WA, Seage MJ & Takahashi M. (1998) Molecular properties of dihydropyridine-sensitive calcium channels in skeletal muscle. *Journal of Biological Chemistry* **263**, 3535–3538.

Ceriello A, Giugliano D, Quatraro A & Lefebvre PJ (1991) Antioxidants show an antihypertensive effect in diabetics and hypertensive subjects. *Clinical Science* **81**, 739–742.

Chen CJ, Hsuek Ym, Lai MS, Shyu MP, Chen SY, Wu MM, Kuo TL & Tai TY (1995) Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* **25**, 53–60.

Chen YF, Meng Q & Wyss JM (1988) High NaCl diet reduces hypothalamic norepinephrine turnover in hypertensive rats. *Hypertension* **11**, 55–62.

Ciaraldi TP, Kolterman OG & Olefsky JM (1981) Mechanism of the post-receptor defect in insulin action in human obesity. Decrease in glucose transport system activity. *Journal of Clinical Investigation* **68**, 875–880.

Collier A, Wilson R, Bradley H, Thomas JA & Small M (1990) Free radical activity in type II diabetes. *Diabetic Medicine* **7**, 27–30.

Conti CR (1999) Vitamin C and coronary vasoactivity. *European Heart Journal* **20**, 1615–1617.

Corvol MM, Ulman A & Garabedian M (1980) Specific nuclear uptake of 24,25-dihydroxycholecalciferol, a vitamin D$_3$ metabolite biologically active in cartilage. *FEBS Letters* **116**, 273–276.

Dahl LK, Leitl G & Heine M (1972) Influence of dietary potassium and sodium/potassium molar ratios on the development of salt hypertension. *Journal of Experimental Medicine* **136**, 318–330.

Dakshinamurti K & Lal KJ (1992) Vitamins and hypertension. *World Review of Nutrition and Dietetics* **69**, 40–73.

Dakshinamurti K, Lal KJ, Dhalla NS, Musat S & Wang Xi (2000) Pyridoxal 5-phosphate and calcium channels. In *Biochemistry and Molecular Biology of Vitamin B_6 and PQQ-dependent proteins* pp. 307–314 [A Iriarte, HM Kagan and M Martinez-Carrion editors]. Basel: Birkhauser Verlag.

Dakshinamurti K, Lal KJ & Ganguly PK (1998) Hypertension, calcium channels and pyridoxine (vitamin B_6). *Molecular and Cellular Biochemistry* **188**, 137–148.

Dakshinamurti K, Paulose CS, Viswanathan M, Siow YL, Sharma SK & Bolster B (1990a) Neurobiology of pyridoxine. *Annals of the New York Academy of Sciences* **585**, 129–144.

Dakshinamurti K, Sharma SK & Bonke D (1990b) Influence of B vitamins on binding properties of serotonin receptors in the CNS of rats. *Klinische Wochenschrift* **68**, 142–148.

de Bold, MLK (1999) Estrogen, natriuretic peptides and the renin–angiotensin system. *Cardiovascular Research* **41**, 524–531.

Dijkhorst-Oei LT, Stross ES, Koomans HA & Rabelink TJ (1999) Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. *Journal of Cardiovascular Pharmacology* **33**, 420–424.

Dominiczak AF & Bohr DF (1990) Cell membrane abnormalities and the regulation of intracellular calcium concentration in hypertension. *Clinical Science* **79**, 415–423.

Drueke TB (1999) Dietary calcium and blood pressure. *American Journal of Hypertension* **12**, 96–98.

Dubyak GR & El-Moatassium C (1993) Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. *American Journal of Physiology* **265**, C577-C606.

Duffy SJ, Gokce N, Holbrook M, Huang A, Frei B, Kearney Jr JF & Vita JA (1999) Treatment of hypertension with ascorbic acid. *Lancet* **354**, 2048–2049.

Ellis JM & McCully KS (1995) Prevention of myocardial infarction by vitamin B_6. *Research Communications in Chemical Pathology and Pharmacology* **89**, 208–220.

Erin AN, Spirin MM & Tabidze LV (1984) Formation of α-tocopherol complex with fatty acids: a hypothetical mechanism of stabilization of biomembranes by vitamin E. *Biochimica et Biophysica Acta* **774**, 96–102.

Evans RJ & Kennedy C (1994) Characterization of P$_2$-purinoceptors in the smooth muscle of the rat tail artery: a comparison between contractile and electrophysiological responses. *British Journal of Pharmacology* **113**, 853–860.

Faure P, Rossini E, Lafond JL, Richard MJ, Favier A & Halimi S (1997) Vitamin E improves the free radical defence system potential and insulin sensitivity of rats fed high fructose diets. *Journal of Nutrition* **127**, 103–107.

Feldman EB, Gold S, Green J, Moran J, Xu G & Shultz GG (1992) Ascorbic acid supplements and blood pressure. A four week pilot study. *Annals of the New York Academy of Sciences* **669**, 342–344.

Ferrannini E, Buzzigoli G & Bonadonna R (1987) Insulin resistance in essential hypertension. *New England Journal of Medicine* **317**, 350–356.

Ferrante J & Triggle DJ (1990) Drug and disease-induced regulation of voltage-dependent calcium channels. *Pharmacological Reviews* **42**, 29–44.

Fields M (1999) Role of trace elements in coronary heart disease. *British Journal of Nutrition* **81**, 85–86.

Fields M, Lewis CG, Lure MD, Burns WA & Antholine WE (1993) Low dietary iron prevents free radical formation and heart pathology of copper-deficient rats fed fructose. *Proceedings of the Society for Experimental Biology and Medicine* **202**, 225–231.

Flora JS & Hara K (1993) Sympathoneural and hemodynamic characteristics of young subjects with mild essential hypertension. *Journal of Hypertension* **11**, 647–655.

Forte P, Copland M, Smith LM, Milne E, Sutherland J & Benjamin N (1997) Basal nitric oxide synthesis in essential hypertension. *Lancet* **349**, 837–842.

Freedman AM, Mark IT, Stafford RE, Dickens BF, Cassidy MM, Muesing RA & Weglicki WB (1992) Erythrocytes from magnesium-deficient hamsters display an enhanced susceptibility to oxidative stress. *American Journal of Physiology* **262**, C1371–C1375.

Galley HF, Thornton J, Howdle PD, Walker BE & Webster NR (1997) Combination oral antioxidant supplementation reduces blood pressure. *Clinical Science* **9**, 361–365.

Geleijnse JM, Wittman JCM, Back AAA, den Breejen JH & Grobbe DE (1994) Reduction in blood pressure with a low sodium, high magnesium salt in older subjects with mild to moderate hypertension. *British Medical Journal* **309**, 436–440.

Gemba T, Ninomiya M, Matsunaga K & Veda M (1992) Changes in extracellular concentration of amino acids in the hippocampus during cerebral ischemia in stroke-prone SHR, stroke-resistant SHR and normotensive rats. *Neuroscience Letters* **135**, 184–188.

Gey KF, Stahelin HB & Eicholzer M (1993) Poor plasma status of carotene and vitamin C is associated with a higher mortality from ischemic heart disease and stroke. Basal prospective study. *Clinical Investigation* **71**, 3–6.

Ghosh SK, Ekpo EB, Shah IU, Gerling AJ, Jankins C & Sinclair AJ (1994) A double blind, placebo-controlled parallel trial of vitamin C treatment in elderly patients with hypertension. *Gerontology* **40**, 268–272.

Glossman H & Striessnig J (1988) Calcium channels. *Vitamins and Hormones* **44**, 155–328.

Gonic HC, Ding V, Bondy SC & Vaziri ND (1997) Lead-induced hypertension: interplay of nitric oxide and reactive oxygen species. *Hypertension* **30**, 1487–1492.

Goody HF, Purkins L, Heatley RV & Kelleher J (1991) The effect of vitamin E deficiency on plasma zinc and copper concentrations. *Clinical Nutrition* **10**, 233–235.

Grassi G, Catteneo BM, Seravalle G, Lanfranchie A & Mancia G (1997) Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension* **31**, 68–72.

Gratacos E, Casals E, Deulofen R, Carrach V, Alonso PL & Fortuny A (1998) Lipid peroxide and vitamin E patterns in pregnant women with different types of hypertension in pregnancy. *American Journal of Obstetrics and Gynecology* **178**, 1072–1076.

Griffith LE, Guyatt GH, Cook RJ, Bucher HC & Cook DJ (1999) The influence of dietary and non dietary calcium supplementation on blood pressure. *American Journal of Hypertension* **12**, 84–92.

Grimm RH, Neaton JD & Elmer PJ (1990) The influence of oral potassium chloride on blood pressure in hypertensive men on a low sodium diet. *New England Journal of Medicine* **322**, 569–574.

Groop L, Ekstrand A & Forsblom C (1993) Insulin resistance, hypertension and microalbuminuria in patients with type 2 diabetes mellitus. *Diabetologia* **36**, 642–647.

Groop L, Forsblom C & Lehtovirta M (1996) Metabolic consequences of a family history of NIDDM (the Botnia Study): evidence of sex-specific parental effects. *Diabetes* **45**, 1585–1593.

Groop L, Forsblom C & Lehtovirta M (1997) Characterization of the prediabetic state. *American Journal of Hypertension* **10**, 172S–180S.

Groz G, Hantt G & Kolassa N (1987) Urapidil and some analogs with hypotensive properties show high affinities for 5-HT binding sites of the 5 HT_{1A} subtype and for α_1-adrenoreceptor binding sites. *Naunyn-Schmiedeberg's Archives of Pharmacology* **336**, 597–601.

Haffner SM, Ferrannini E, Hazuda HP & Stern MP (1992*a*) Clustering of cardiovascular risk factors in confirmed prehypertensive individuals. *Hypertension* **20**, 38–45.

Haffner SM, Miettinen H, Gaskill SP & Stern MP (1992*b*) Metabolic precursors of hypertension. *Archives of Internal Medicine* **156**, 1994–2001.

Haffner SM, Valdez RA, Hazuda HP, Mitchel BD, Morales PA & Stern MP (1996) Prospective analyses of the insulin resistance syndrome (syndrome X). *Diabetes* **41**, 715–722.

Hatton IC & McCarron DA (1994) Dietary calcium and blood pressure in experimental models of hypertension. *Hypertension* **23**, 513–530.

Holtback U, Kruse MS, Brismar H & Aperia A (2000) Intrarenal dopamine co-ordinates the effects of antinatriuretic and natriuretic factors. *Acta Physiologica Scandinavica* **168**, 215–218.

Hourani SMO, Loizon GD & Cusak NJ (1986) Pharmacological effects of L-AMP-PCP on ATP receptors in smooth muscle. *European Journal of Pharmacology* **131**, 99–103.

Huang BS & Leenen FHH (1996) Sympathoexcitatory and pressor responses to increased brain sodium and oubain are mediated via brain ANG II. *American Journal of Physiology* **270**, H275–H280.

Istfan NW, Plaisted CS & Bistrain BR (1992) Insulin resistance versus insulin secretion in the hypertension of obesity. *Hypertension* **19**, 385–392.

Jacques PF (1992) Relationship of vitamin C status to cholesterol and blood pressure. *Annals of the New York Academy of Sciences* **669**, 205–214.

Janero DR & Burghardt B (1988) Myocardial vitamin E (α-tocopherol) contents of SHR & WKY normal rats. *International Journal of Vitamin and Nutrition Research* **58**, 292–294.

Jeserich M, Schindler T, Olscherwski M, Unmussig M, Just H & Solzback U (1999) Vitamin C improves endothelial function of epicardial coronary arteries in patients with hypercholesterolemia or essential hypertension – assessed by cold pressor testing. *European Heart Journal* **20**, 1676–1680.

Jespersen B, Randlov A, Abrahamsen J, Fogh-Anderson N, Olsen NV & Kanstrup I-L (1998) Acute cardiovascular effects of 1,25-dihydroxycholecalciferol in essential hypertension. *American Journal of Hypertension* **11**, 659–666.

John S & Schmieder RE (2000) Impaired endothelial function in arterial hypertension and hypercholesterolemia: potential mechanisms and differences. *Journal of Hypertension* **18**, 363–374.

Julius S (1996) The evidence for a pathophysiologic significance of the sympathetic overactivity in hypertension. *Clinical and Experimental Hypertension* **18**, 305–321.

Julius S, Gundbrandsson T, Jamerson K & Andersson O (1992) The interconnection between sympathetics, microcirculation and insulin resistance in hypertension. *Blood Pressure* **1**, 9–19.

Kanai H, Matsuzawa Y & Togunaga K (1990) Hypertension in obese children: fasting serum insulin levels are closely correlated with blood pressure. *International Journal of Obstetrics* **14**, 1047–1056.

Kawano Y, Matsuoka H, Takshita S & Omae T (1998) Effect of magnesium supplementation in hypertensive patients. Assessment by office, home and ambulatory blood pressure. *Hypertension* **32**, 260–265.

Kawashima H (1987) Receptor for 1,25 dihydroxy-vitamin D in a vascular cell line derived from rat aorta. *Biochemical Biophysical Research Communications* **146**, 1–6.

Kesteloot H and Geboers J (1982) Calcium and blood pressure. *Lancet* **1**, 813–815.

Khalil-Manesh F, Gonic HC, Weiler FW, Prin B, Weber MA, Purdy R & Reno Q (1994) Effect of chelation treatment with dimercapto succinic acid on lead-related blood pressure changes. *Environmental Research* **65**, 86–99.

Khaw KT & Barrett-Connor E (1987) Dietary potassium and stroke-associated mortality: a 12-year prospective population study. *New England Journal of Medicine* **316**, 235–240.

Kokubun S & Reuter H (1984) Dihydropyridine derivatives prolong the open state of calcium channels in cultured cardiac cells. *Proceedings of the National Academy of Sciences USA* **81**, 4824–4827.

Krause S, Buhring M, Hopfen Muller W, Holick MG & Sharma AM (1998) Ultraviolet B and blood pressure. *Lancet* **352**, 709–710.

Krishna GG (1990) Effect of potassium intake on blood pressure. *Journal of the American Society of Nephrology* **39**, 771–776.

Krishna GG (1994) Role of potassium in the pathogenesis and treatment of hypertension. *American Journal of Medical Sciences* **307**, S21-S25.

Kugiyama K, Motoyama T & Hiroshima O (1998) Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteris in patients with coronary spastic angina. *Journal of the American College of Cardiology* **32**, 103–109.

Lal KJ & Dakshinamurti K (1993*a*) Hypotensive action of 5-HT receptor agonists in the vitamin B_6 deficient hypotensive rat. *European Journal of Pharmacology* **234**, 183–189.

Lal KJ & Dakshinamurti K (1993*b*) Calcium channels in vitamin B_6 deficiency-induced hypertension. *Hypertension* **11**, 1357–1362.

Lal KJ & Dakshinamurti K (1995) Relationship between low calcium induced hypertension and vitamin B_6 status. *Journal of Hypertension* **13**, 327–332.

Lal KJ, Dakshinamurti K & Thliveris J (1996) The effect of vitamin B_6 on the systolic blood pressure of rats in various animal models of hypertension. *Journal of Hypertension* **14**, 355–363.

Lal KJ, Sharma SK & Dakshinamurti K (1993) Regulation of calcium influx into vascular smooth muscle by vitamin B_6. *Clinical and Experimental Hypertension* **15**, 489–500.

Laurant P, Dalle M, Berthelot A & Rayssiguier Y (1999) Time-course of the change in blood pressure level in magnesium-deficient Wistar rats. *British Journal of Nutrition* **82**, 243–251.

Lee CN, Reed DM, MacLean CJ, Yano K & Chin D (1988) Dietary potassium and stroke. *New England Journal of Medicine* **318**, 995–996.

Levine GN, Frei B, Koulouris SN, Gerhard MD, Kearney JF Jr & Vita JA (1996) Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* **93**, 1107–1113.

Lin H &Young DB (1994) Interaction between plasma potassium and epinephrine in coronary thrombosis in dogs. *Circulation* **89**, 331–338.

Linas SL (1991) The role of potassium in the pathogenesis and treatment of hypertension. *Kidney International* **39**, 771–789.

Lind L, Wengle B, Wide L & Ljunghall S (1989) Reduction of blood pressure during long-term treatment with active vitamin D (alphacalcidol) is dependent on plasma renin activity and calcium status: a double-blind, placebo-controlled study. *American Journal of Hypertension* **2**, 20–25.

Litwack G (1988) The glucocorticoid receptor at the protein level. *Cancer Research* **48**, 2636–2640.

Lovat LB, Lu Y, Palmer AJ, Edwards R, Fletcher AE & Bulpit CJ (1993) Double blind trial of vitamin C in elderly hypertensives. *Journal of Human Hypertension* **7**, 403–405.

Lucas MJ, Leveno KJ & Cunningham FG (1995) A comparison of magnesium sulfate with phenytoin for the prevention of eclampsia. *New England Journal of Medicine* **333**, 201–205.

Lyons D, Webster J & Benjamin N (1994) The effect of antihypertensive therapy on responsiveness to local intra-arterial NG-monomethyl-L-arginine in patients with essential hypertension. *Journal of Hypertension* **12**, 1047–1052.

McCabe RD, Backarich MA, Srivastava K & Young DB (1994) Potassium inhibits free radical formation. *Hypertension* **24**, 77–82.

McCabe RD & Young DB (1994) Potassium inhibits cultured vascular smooth muscle proliferation. *American Journal of Hypertension* **7**, 346–350.

McCarron DA (1985) Is calcium more important than sodium in the pathogenesis of essential hypertension? *Hypertension* **7**, 607–627.

McCarron DA (1998) Diet and blood pressure – the paradigm shift. *Science* **281**, 933–934.

McCarron DA, Morris CD, Henry HJ & Stanton JL (1984) Blood pressure and nutrient intake in the United States. *Science* **224**, 1392–1398.

McCarron DA, Oparil S, Chait Ä, Haynes RB, Kris-Etherton P, Stern JS, Resnick LM, Clark S, Morris CD, Hatton DC, Metz JA, McMohan M, Holcomb S, Snyder GW & Pi-Sunyer FX (1997) Nutritional management of cardiovascular risk factors. A randomized clinical trial. *Archives of Internal Medicine* **153**, 154–183.

McCarron DA, Oparil S, Resnick LM, Chait A, Haynes B, Kris-Etherton P, Pi-Sunyer FX, Stern JS, Morris CD, Clark S, Hatton DC, Metz JA, McMohan M, Holcomb S & Snyder GW (1998) Comprehensive nutrition plan improved cardiovascular risk factors in essential hypertension. *American Journal of Hypertension* **11**, 31–40.

McCarron DA & Reusser ME (1999) Finding consensus in the dietary calcium–blood pressure debate. *Journal of the American College of Nutrition* **18**, 398S-405S.

Machlin IJ & Bendich A (1987) Free radical tissue damage: protective role of antioxidant nutrients. *FASEB Journal* **1**, 441–445.

MacMahon SW, MacDonald GJ & Blacket RB (1985) Plasma lipoprotein levels in treated and untreated hypertensive men and women. The National Heart Foundation of Australia Risk Factor Prevalence Study. *Arteriosclerosis* **67**, 49–55.

Maier LS, Brandes R, Pieske B & Bers DM (1998) Effects of left ventricular hypertrophy on force and Ca^{2+} handling in isolated rat myocardium. *American Journal of Physiology* **274**, H1361–H1370.

Malluche HH, Henry H, Meyer-Saballek W, Sherman S, Massry SG & Norman AN (1980) Effects and interactions of 24,25 $(OH)_2 D_3$ and 1,25 $(OH)_2 D_3$ on bone. *American Journal of Physiology* **238**, E494–E496.

Manicardi V, Camellini L & Bellodi G (1986) Evidence for an association of high blood pressure and hyperinsulinemia in obese man. *Journal of Clinical Endocrinology and Metabolism* **62**, 1302–1304.

Mancia G (1997) The sympathetic nervous system in hypertension. *Journal of Hypertension* **15**, 1553–1565.

Massry SG, Iseki K & Campese VM (1986) Serum calcium, parathyroid hormone and blood pressure. *American Journal of Nephrology* **6**, 1–28.

Merke J & Norman AW (1981) Evidence of 24 (R) $25(OH)_2$ D_3 receptor on the parathyroid gland of rachitic chicken. *Biochemical and Biophysical Research Communications* **100**, 551–558.

Metz JA, Morris CD, Roberts LA, McClung MR & McCarron DA (1999) Blood pressure and calcium intake are related to bone density in adult males. *British Journal of Nutrition* **81**, 383–388.

Minghetti PP & Norman AW (1988) $1,25(OH)_2$-vitamin D3 receptors: gene regulation and genetic circuitry. *FASEB Journal* **2**, 3043–3053.

Mizushima S, Cappuccio FP, Nichols R & Elliot P (1998) Dietary magnesium intake and blood pressure: a qualitative overview of observational studies. *Journal of Human Hypertension* **12**, 447–453.

Morales PA, Mitchell BD, Valdez RA, Hazuda HP, Stern MP & Haffner SM (1993) Incidence of non-insulin dependent diabetes mellitus and impaired glucose tolerance in hypertensive subjects: the San Antonio Heart Study. *Diabetes* **42**, 154–163.

Moran JP, Cohen L, Green JM, Xu G, Feldman EB & Hames CG (1993) Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. *American Journal of Clinical Nutrition* **57**, 213–217.

Moser M (1999) National recommendations for the pharmacological treatment of hypertension. *Archives of Internal Medicine* **159**, 1403–1406.

MRC/BHF (1999) Heart protection study of cholesterol-lowering therapy and of antioxidant vitamin supplementation in a wide range of patients at increased risk of coronary heart disease death: early safety and efficacy experience. *European Heart Journal* **20**, 725–741.

Murray ED Jr, Wechter WJ, Kantochi D, Wang W-H, Pham T, Quiggle DD, Gibson KM, Leipold D & Anner BM (1997) Endogenous natriuretic factors 7: Biospecificity of a natriuretic δ-tocopherol metabolite LLU-α. *Journal of Pharmacology and Experimental Therapeutics* **282**, 657–662.

Mykkanen L, Kuusisto J, Pyorala K, Laasko M & Haffner SM (1994) Increased risk of non-insulin dependent diabetes mellitus in elderly hypertensive subjects. *Journal of Hypertension* **12**, 1425–1432.

Nasir F, Mazure A, Gionnoni F, Gueux E, Davidson O & Rayssiguer Y (1995) Magnesium deficiency modulates hepatic lipogenesis and apolipoprotein gene expression in the rat. *Biochimica et Biophysica Acta* **1257**, 125–132.

Nelson D & Taylor WE (1986) A selective serotonin$_{1A}$ receptor agonist. *European Journal of Pharmacology* **124**, 207–208.

Ness AR, Khaw KT, Bingham S & Day NE (1996) Vitamin C status and blood pressure. *Journal of Hypertension* **14**, 503–508.

Newaz MA & Nawal NNA (1998) Effect of α-tocopherol on lipid peroxidation and total antioxidant status in spontaneously hypertensive rats. *American Journal of Hypertension* **11**, 1480–1485.

Newaz MA, Nawal NNA, Rohaizan CH, Muslim N & Gapor A (1999) α-Tocopherol increased nitric oxide synthase activity in blood vessels of spontaneously hypertensive rats. *American Journal of Hypertension* **12,** 839–844.

Ng LL, Ngkeekwong FC, Quinn PA & Davies JE (1998) Uptake mechanisms for ascorbate and dehydroascorbate in lymphoblasts from diabetic nephropathy and hypertensive patients. *Diabetologia* **41**, 435–442.

Ngheim CX & Rapp JP (1983) Responses to calcium of chemically skinned vascular smooth muscle from spontaneously hypertensive rats. *Clinical and Experimental Hypertension* **4**, 849–856.

Nosadini R, Solini A, Velussi M, Muollo B, Frigato F, Sambataro M, Cipollina MR, DeRiva F, Brocco I & Crepaldi G (1994) Impaired insulin-induced glucose uptake by extra hepatic tissue is hallmark of NIDDM patients who have or will develop hypertension and microalbuminuria. *Diabetes* **43**, 491–499.

Nowack R, Wiecer A, Exner B, Gretz N & Ritz E (1993) Chronic lead exposure in rats: effects on blood pressure. *European Journal of Clinical Investigation* **23**, 433–448.

Nurminen M-L, Korpela R & Vapaatalo H (1998) Dietary factors in the pathogenesis and treatment of hypertension. *Annals of Medicine* **30**, 143–150.

O'Hare JA, Minaker KL & Meneilly GS (1989) Effect of insulin on plasma norepinephrine and 3,4 dihydroxyphenylalanine in obese men. *Metabolism* **38**, 322–329.

Overback A, Zenzen JG, Ressel C, Muller HM & Stumpe KO (1987) Influence of magnesium on blood pressure and the effect of nifedipine in rats. *Hypertension* **9**, 139–143.

Palozza P & Krinsky NI (1992) Beta carotene and alpha tocopherol are synergistic antioxidants. *Archives of Biochemistry and Biophysics* **297**, 184–187.

Pang PKT & Lewanczuk RZ (1989) Parathyroid origin of a new circulating hypertensive factor in spontaneously hypertensive rats. *American Journal of Hypertension* **2**, 898–902.

Pang PKT, Narvey S & Doris PA (1987) Plasma parathyroid hormone during the development of spontaneous hypertension in rats. *Canadian Journal of Physiology and Pharmacology* **65**, 2386–2389.

Paolisso G & Barbagallo M (1997) Hypertension, diabetes mellitus and insulin resistance. The role of intracellular magnesium. *American Journal of Hypertension* **10**, 346–355.

Paolisso G, DiMaro G & Cozzolino B (1992a) Chronic magnesium administration enhances oxidative glucose metabolism in thiazid treated hyperensive patients. *American Journal of Hypertension* **5**, 681–686.

Paolisso G, DiMaro G, Pizza G, D'Amore A, Scambato S, Tesauro P, Varricchio M & d'Onofrio F (1992b) Plasma

GHS/GSSG affects glucose homeostasis in healthy subjects and non-insulin dependent diabetics. *American Journal of Physiology* **263**, E435–E440.

Paulose CS & Dakshinamurti K (1987) Chronic catheterization using vascular access port in rats: blood sampling with minimal stress for plasma catecholamine determination. *Journal of Neuroscience Methods* **22**, 141–146.

Paulose CS, Dakshinamurti K, Packer S & Stephens NL (1986) Hypertension in pyridoxine deficiency. *Journal of Hypertension* **4**, Suppl. 5, S174–S175.

Paulose CS, Dakshinamurti K, Packer S & Stephens NL (1988) Sympathetic stimulation and hypertension in the pyridoxine-deficient adult rat. *Hypertension* **11**, 387–391.

Postnor YP & Orlov SN (1985) Ion transport across phasma in primary hypertension. *Physiological Reviews* **65**, 904–905.

Rahman M, Tondel M, Ahmed SA, Chowdhury IA, Faruquee MH & Axelson O (1999) Hypertension and arsenic exposure in Bangladesh. *Hypertension* **33**, 74–78.

Rahn KH, Barenbrock M & Hausberg M (1999) The sympathetic nervous system in the pathogenesis of hypertension. *Journal of Hypertension* **17**, Suppl. 3, S11–S14.

Rappaport A, Sturtz F & Guicheney P (1985) Regulation of central α-adrenoreceptor by serotonergic denervation. *Brain Research* **344**, 158–161.

Rapp JP, Nghiem CX & Oniwochi MO (1986) Aortic calcium uptake and efflux in spontaneously hypertensive and inbred Dahl rats. *Journal of Hypertension* **4**, 493–499.

Rayssiguier Y, Durlach J, Gueux E, Rock E & Mazur A (1993*a*) Magnesium and aging: experimental data—importance of oxidative damage. *Magnesium Research* **3**, 369–378.

Rayssiguier Y, Gueux E, Bussiere L, Durlach J & Mazur A (1993*b*) Dietary magnesium affects susceptibility of lipoproteins and tissue to peroxidation in rats. *Journal of the American College of Nutrition* **12**, 133–137.

Reaven GR & Hoffman BB (1988) Abnormalities of carbohydrate metabolism may play a role in the etiology and clinical course of hypertension. *Trends in Pharmacological Science* **9**, 78–79.

Repke JT, Villar J, Anderson C, Pareja G, Dubin N & Belizan JM (1989) Biochemical changes associated with blood pressure reduction induced by calcium supplementation during pregnancy. *American Journal of Obstetrics and Gynecology* **160**, 684–690.

Resnick LM (1999*a*) The cellular ionic basis of hypertension and allied clinical conditions. *Progress in Cardiovascular Diseases* **42**, 1–22.

Resnick LM (1999*b*) Therapeutic implications of calcium intake in hypertension. *American Journal of Hypertension* **12**, 82–83.

Resnick LM (1999*c*) The role of dietary calcium in hypertension. *American Journal of Hypertension* **12**, 99–112.

Resnick LM, Gupta RK & Laragh JH (1984) Intracellular free magnesium in erythrocytes of essential hypertension: relation to blood pressure and serum divalent cations. *Proceedings of the National Academy of Sciences USA* **81**, 6511–6515.

Resnick LM & Laragh J (1985) Calcium metabolism and parathyroid function in primary aldosteronism. *American Journal of Medicine* **78**, 385–390.

Resnick LM, Laragh J, Sealey J & Alderman M (1983) Divalent cations in essential hypertension: relations between serum ionized calcium, magnesium and plasma renin activity. *New England Journal of Medicine* **309**, 888–891.

Resnick LM, Lewanczuk RZ, Laragh JH & Pang PKT (1993) Parathyroid hypertensive factor-like activity in human essential hypertension. Relationship to plasma renin activity and dietary salt sensitivity. *Journal of Hypertension* **11**, 1235–1241.

Resnick LM, Militianu DM & Cunnings AJ (1997) Direct magnetic resonance determination of aortic distensibility in essential hypertension: relation to age, abdominal visceral fat, and in situ intracellular free magnesium. *Hypertension* **30**, 654–659.

Reusser ME & McCarron DA (1994) Micronutrient effects on blood pressure regulation. *Nutrition Reviews* **52**, 367–375.

Ribaya Mercado JD, Russel RM, Sayhoun N, Morrow FD & Gershoff SN (1991) Vitamin B_6 requirements of elderly men and women. *Journal of Nutrition* **121**, 1062–1074.

Rockstroh JK, Schobel HP & Vogt-Ladner G (1997) Blood pressure independent effects of nitrendipine on cardiac structure in patients after renal transplantation. *Nephrology, Dialysis and Transplantation* **12**, 1441–1447.

Ross R (1986) The pathogenesis of atherosclerosis: an update. *New England Journal of Medicine* **314**, 488–500.

Rostand SG (1997) Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension* **30**, 150–156.

Rowe JW, Young JB & Minaker KL (1981) Effect of insulin and glucose infusion on sympathetic nervous system activity in normal man. *Diabetes* **30**, 219–225.

Sacks FM, Willet WC, Smith AM, Brown LE, Rosner B & Moore TJ (1998) Effect on blood pressure of potassium, calcium and magnesium in women with low habitual intake. *Hypertension* **31**, 131–138.

Sacks FM, Obarzanek E & Windhauser MM (1995) Rationale and design of the dietary approaches to stop hypertension trial (DASH): a multicentered controlled-feeding study of dietary patterns to lower blood pressure. *Annals of Epidemiology* **5**, 108–118.

Sagar S, Kallo U, Kaul N, Ganguly NK & Sharma BK (1992) Oxygen free radicals in essential hypertension. *Molecular and Cellular Biochemistry* **111**, 103–108.

Saito K, Sano H, Furuta Y & Fukuzaki H (1989) Effect of oral calcium on blood pressure response in salt loaded borderline hypertensive patients. *Hypertension* **13**, 219–226.

Sallinen K, Arvola P, Wuorela H, Ruskoaho H, Vapaatalo H & Porsti I (1996) High calcium diet reduces blood pressure in exercised and nonexercised hypertensive rats. *American Journal of Hypertension* **9**, 144–156.

Salonen R, Korpela H, Nyyssonen K, Porkkala E & Salonen JT (1994) Reduction of blood pressure by antioxidant supplementation: a randomized double-blind clinical trial. *Life Chemistry Reports* **12**, 65–68.

Schleiffer R, Pernot F & Gairard A (1986) Parathyroidectomy, cardiovascular reactivity and calcium distribution in aorta and heart of spontaneously hypertensive rats. *Clinical Science* **71**, 505–511.

Schmid A, Renaud J & Luxdunski M (1985) Short term and long term effects of β adrenergic effectors and cyclic AMP on nitrendipine-sensitive voltage-dependent Ca^{2+} channels of skeletal muscle. *Journal of Biological Chemistry* **260**, 13041–13046.

Schoeffler P & Hoyer D (1988) Centrally acting hypotensive agents with affinity for $5HT_{1A}$ binding sites inhibit forskolin-stimulated adenyl cyclase activity in calf hippocampus. *British Journal of Pharmacology* **95**, 975–985.

Schroeder HA (1960) Relations between mortality from cardiovascular disease and treated water supplies. Variations in sites and 163 largest municipalities of the United States. *Journal of the American Medical Association* **172**, 1902–1908.

Shan J, Bing L, Taniguchi N & Pang PKT (1996) Inhibition of membrane L-type calcium channel activity and intracellular calcium concentration by 24 R, 25-dihydroxyvitamin D_3 in vascular smooth muscle. *Steroids* **61**, 657–663.

Shan J, Resnick LM, Lewanczuk RZ, Karpinski E, Li B & Pang PKT (1993) 1,25-Dihydroxyvitamin D as a cardiovascular hormone. *American Journal of Hypertension* **6**, 983–988.

Shan J, Resnick LM, Liu Q, Wu X, Barbagallo M & Pang PKT (1994) Vascular effects of 17β-estradiol in male Sprague-Dawley rats. *American Journal of Physiology* **266**, H967–H973.

SHEP Cooperative Research Group, Clinical Trials Branch (1991) Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension: final results of the Systolic Hypertension in Elderly Program (SHEP). *Journal of the American Medical Association* **265**, 3255–3264.

Sherman DL, Keanney JF, Biegelsen ES, Duffy SJ, Coffman JD & Vita JA (2000) Pharmacological concentrations of ascorbic acid are required for the beneficial effect on endothelial vasomotor function in hypertension. *Hypertension* **35**, 936–941.

Shetterly SM, Rewars M, Hamman R & Marshall JA (1994) Patterns and predictors of hypertension incidence among hispanics and nonhispanic whites: the San Luis Valley Diabetes Study. *Journal of Hypertension* **12**, 1095–1102.

Slater TF (1984) Free radical mechanisms in tissue injury. *Biochemical Journal* **222**, 1–15.

Solzbach U, Hornig B, Jeserich M & Just H (1997) Vitamin C improves endothelial dysfunction of epicardial coronary arteries in hypertensive patients. *Circulation* **96**, 1513–1519.

Somjen D, Somjen GJ, Harell A, Mechanic GL & Binderman I (1982a) Partial characterization of a specific high affinity binding macromolecule for 24 R, 25-dihydroxyvitamin D_3 in differentiating skeletal mesenchyme. *Biochemical and Biophysical Research Communications* **106**, 664–651.

Somjen D, Somjen GJ, Weisman Y & Binderman I (1982b) Evidence for 24,25-dihydroxycholecalciferol receptors in long bones of new born rats. *Biochemical Journal* **204**, 31–36.

Spangler JG, Bell R & Summerson JH (1998) Hyperinsulinemia in hypertension. Association with race, abdominal obesity and hyperlipidemia. *Archives of Family Medicine* **7**, 53–56.

Stadler M, Pometta D & Suenram A (1981) Relationship between plasma insulin levels and high density lipoprotein cholesterol levels in healthy men. *Diabetologia* **21**, 544–548.

Standley PR, Zhang F, Ram JL, Zemel MB & Sowers JR (1991) Insulin attenuates vasopressin-induced calcium transient and voltage-dependent calcium current in rat vascular smooth muscle cells. *Journal of Clinical Investigation* **88**, 1230–1236.

Suter PM (1998) Potassium and hypertension. *Nutrition Reviews* **56**, 151–153.

Taddei S, Virdis A, Ghiadoni L, Magagna A & Salvetti A (1998) Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* **97**, 2222–2229.

Tagami M, Ikeda K, Nara Y, Kubota A, Numano F & Yamori Y (1997a) Insulin-like growth factor-1 attenuates apotosis in hippocampal neurons caused by cerebral ischemia and reperfusion in stroke-prone spontaneously hypertensive rats. *Laboratory Investigation* **74**, 613–617.

Tagami M, Ikeda K, Yamagata K, Nara Y, Fujino H, Kubota A, Numano F & Yamori Y (1999) Vitamin E prevents apoptosis in hippocampal neurons caused by cerebral ischemia and reperfusion in stroke-prone spontaneously hypertensive rats. *Laboratory Investigation* **79**, 609–615.

Tagami M, Yamagata K, Mara Y, Kubota A, Numano F & Yamori Y (1997b) Insulin-like growth factors prevent apoptosis in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. *Laboratory Investigation* **74**, 603–612.

Taylor AE (1996) Cardiovascular effects of environmental chemicals. *Otolaryngology and Head and Neck Surgery* **114**, 209–211.

The Eclampsia Trial Collaborative Group (1995) Which anticonvulsant for women with eclampsia? Evidence from the Collaborative Eclampsia Trial. *Lancet* **345**, 1455–1463.

The Fifth Report of the Joint National Committee on detection, evaluation and treatment of high blood pressure (1993) *Archives of Internal Medicine* **153**, 154–183.

The Intersalt Cooperative Research Group (1988) Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24-hour urinary sodium and potassium. *British Medical Journal* **297**, 319–328.

The Sixth Report of the Joint National Committee on detection, evaluation and treatment of high blood pressure (1997) *Archives of Internal Medicine* **157**, 2413–2446.

Thierry-Palmer M, Carlyl KS, Williams MD, Tewolde T, Caines-Mckenzie S, Bayrog MA, Emmett NL, Harris-Hooker SA, Sanford GL & Williams EF (1998) Plasma 25-hydroxyvitamin D concentrations are inversely associated with blood pressure of Dahl salt-sensitive rats. *Journal of Steroid Biochemistry and Molecular Biology* **66**, 255–261.

Third Report on Nutrition Monitoring in the United States (1995) Volume 1. Washington DC: US Government Printing Office.

Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P & Creageter MA (1996) Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of Clinical Investigation* **97**, 22–28.

Tobian L (1986) High potassium diets markedly protect against stroke deaths and kidney disease in hypertensive rats, a possible legacy from prehistoric times. *Canadian Journal of Physiology and Pharmacology* **64**, 840–848.

Tobian L, Lange JM, Ulm KM, Wold IJ & Iwai J (1984) Potassium prevents death from strokes in hypertensive rats without lowering blood pressure. *Journal of Hypertension* **2**, Suppl., 363–366.

Touyz RM, Tolloczko B & Schiffrin EL (1994) Insulin attenuates agonist-evoked calcium transients in vascular smooth muscle cells. *Hypertension* **23**, Suppl., 125–128.

Trezise DJ, Bell NJ, Khakh BS, Michael AD & Humphrey PA (1994) P_2 purinoceptor antagonist properties of pyridoxal 5-phosphate. *European Journal of Pharmacology* **259**, 295–300.

Trials of Hypertension Prevention Collaborative Research Group (1992) The effects of nonpharmacologic interventions on blood pressure of persons with high normal levels: results of the Trials of Hypertension Prevention, phase 1. *Journal of the American Medical Association* **267**, 1213–1220.

Tully DB, Allgood VE & Cidlowski JA (1994) Modulation of steroid receptor-mediated gene expression by vitamin B_6. *FASEB Journal* **8**, 343–349.

Vallance P, Collier J & Moncada S (1989) Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **ii**, 997–1000.

Van den Berg H (1999) Vitamin B_6 status and requirements in older adults. *British Journal of Nutrition* **81**, 175–176.

Van der Wielen RPJ, Lowik MRH, Haller J, Van den Berg H, Ferry M & Van Staveren WA (1996) Vitamin B_6 malnutrition among elderly Europeans: the SENECA study. *Journal of Gerontology* **51**A, B417–B424.

Vane JR, Aggard EE & Botting RM (1990) Regulatory functions of the vascular endothelium. *New England Journal of Medicine* **323**, 27–36.

Vanhoutte PM (1999) How to assess endothelial function in human blood vessels. *Journal of Hypertension* **17**, 1047–1058.

Vaziri ND, Liang K & Ding Y (1999) Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. *Kidney International* **56**, 1492–1498.

Vermaak WH, Barnard H, Potgieter GM & Theron H (1987) Vitamin B_6 and coronary artery disease. Epidemiological observations and case studies. *Atherosclerosis* **63**, 235–238.

Viswanathan M, Bose R & Dakshinamurti K (1991) Increased calcium influx in caudal artery of rats made hypertensive with pyridoxine deficiency. *American Journal of Hypertension* **4**, 252–255.

Viswanathan M, Paulose KJ, Lal KJ & Dakshinamurti K (1990) Alterations in brain stem α adrenoreceptor activity in pyridoxine-deficient rat model of hypertension. *Neuroscience Letters* **111**, 201–205.

Vulchanova L, Arvidsson U, Riedi M, Wang J, Bull G, Suprenant A & North RA (1996) Differential distribution of two ATP-gated ion channels (P2X receptors) determined by immunocytochemistry. *Proceedings of the National Academy of Sciences USA* **93**, 8063–8067.

Wang TM, Hsu JF, Lee WSS & Mathews JL (1993) Evidence for reduced cancellous bone mass in the spontaneously hypertensive rat. *Bone and Mineral* **20**, 251–264.

Wang X, Dakshinamurti K, Musat S & Dhalla NS (1999) Pyridoxal 5-phosphate is an ATP-receptor antagonist in freshly isolated rat cardiomyocytes. *Journal of Molecular and Cellular Cardiology* **31**, 1063–1072.

Weglicki WB, Mak IT, Kramer JH, Dickens BF, Cassidy MM, Stafford RE & Phillips TM (1996) Role of free radicals and substance P in magnesium deficiency. *Cardiovascular Research* **31**, 677–682.

Weinberger MH (1996) Salt sensitivity of blood pressure in humans. *Hypertension* **27**, 481–490.

Wen Y, Killalea S, McGettigan P & Feely J (1996) Lipid peroxidation and antioxidant vitamin C and E in hypertensive patients. *Irish Journal of Medical Sciences* **165**, 210–212.

Widgren BR, Herlitz H & Wikstrand J (1992) Increased waist/hip ratio, metabolic disturbances, and family history of hypertension. *Hypertension* **20**, 563–568.

Widman L, Wester PO, Stegmayr BG & Wirell MP (1993) The dose dependent reduction in blood pressure through administration of magnesium: a double blind placebo controlled cross-over trial. *American Journal of Hypertension* **6**, 41–45.

Witteman JCM, Willett WC & Stampfer MJ (1989) A prospective study of nutritional factors and hypertension among US women. *Circulation* **80**, 1320–1327.

Woods KL & Fletcher S (1994) Long-term outcome after intravenous magnesium sulfate in suspected acute myocardial infarction: the second Leicester Intravenous Magnesium Intervention Trial. *Lancet* **343**, 816–819.

Xue H, McCarron DA & Bukoski RD (1991) 1,25 $(OH)_2$ vitamin D_3 attenuates the loss of resistance artery contractile function associated with incubation in cultured media. *Biochemical and Biophysical Research Communications* **174**, 11–17.

Yang CY (1998) Calcium and magnesium in drinking water and risk of death from cerebrovascular disease. *Stroke* **29**, 411–414.

Yoshioka M, Aoyama K & Matsushita T (1985) Effects of ascorbic acid on blood pressure and ascorbic acid metabolism in spontaneously hypertensive rats. *International Journal of Vitamin and Nutrition Research* **55**, 301–307.

Yoshioka S, Nishino H, Shiraki T, Ikeda K, Koike H & Okuno A (1993) Antihypertensive effects of Cs-045 treatment in obese Zucker rats. *Metabolism* **42**, 75–80.

Young DB, Lin H & McCabe RD (1995) Potassium's cardiovascular protective mechanisms. *American Journal of Physiology* **268**, R825-R837.

Zein M, Areas JL & Preus HG (1990) Long-term effects of excess sucrose ingestion on three strains of rats. *American Journal of Hypertension* **3**, 560–562.

Zemel MB (1995) Insulin resistance, obesity and hypertension: an overview. *Journal of Nutrition* **125**, 1715S–1717S.

Zemel MB (1998) Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Molecular and Cellular Biochemistry* **188**, 129–136.

Zemel M, Gualdoni S & Walsh M (1986) Effects of sodium and calcium on calcium metabolism and blood pressure regulation in hypertensive black adults. *Journal of Hypertension* **4**, Suppl. 5, S364–S365.

Zemel MB, Johnson BA & Ambrozy SA (1992*a*) Insulin-stimulated vascular relaxation: Role of Ca^{2+}-ATPase. *American Journal of Hypertension* **5**, 637–641.

Zemel MB, Peuler JD, Sowers JR & Simpson L (1992*b*) Hypertension in insulin-resistant Zucker obese rats is independent of sympathetic neural support. *American Journal of Physiology* **262**, E368–E371.

Ziemalanski S, Wartanowicz M, Potribnicka K, Burgos de Moor A, Iwanow K & Ignatowska-Switalska M (1991) Effect of exogenous ascorbic acid on experimental hypertension and mineral balance in rats fed low-and high-fat diets. *Nutrition* **7**, 131–135.

Are you a Registered Nutritionist?

The Nutrition Society

The Society's register is part of a framework of activities that set and promote high standards of education and that support professional development in nutrition. The register helps employers, the media and the general public to identify appropriately qualified individuals. Members on the register are expected to adhere to the Society's Code of Ethics. The Nutrition Society's Register replaced the Register of Accredited Nutritionists, run by the Institute of Biology in conjunction with the Society. Individuals who qualified as Accredited Nutritionists transferred to the Society's register in 1999. The Register is published on the Society's website and, from time to time, in printed form.

Register of Nutritionists
Members of the Nutrition Society have the opportunity to apply for Registration in Nutrition that enables the individual to use the title *Registered Nutritionist* and the letters *R Nutr* after their name. Members of the Society who have been working in the field of nutrition for a minimum of 3 years and who have academic qualifications in nutrition, dietetics or a related subject are eligible to apply. Certain other relevant qualifications may also be acceptable.

Associate registration
Members with appropriate qualifications who have not worked for at least 3 years after graduating may also apply for registration as Associate Nutritionists. This confers the title *Associate Nutritionist* and letters *Assoc Nutr.*

How to Apply
Send a recent CV, photocopied qualification certificate(s) and the names of THREE academic and/ or professional references to the Society's Registrar.

Register of Public Health Nutritionists
The Society has a policy to develop specialist categories of the general Register to cater for members with specialist expertise. In 1997, the first, a Register of Public Health Nutritionists, was launched. Members of the Society who have been working in the field of Public Health Nutrition for a minimum of 3 years in the last 5 and who have appropriate qualifications are eligible to apply. Members who fulfil the criteria for registration are able to use the title *Public Health Nutritionist* and the letters *RPHNutr* after their name.

How to Apply
Send a recent CV, photocopied qualification certificate(s) and the names of TWO academic and/or professional references to the Society's Registrar.

Associate registration
Members who qualify from accredited courses and who have not worked for at least 3 years after graduating are eligible for registration as Associate Public Health Nutritionists. This confers the title *Associate Public Health Nutritionist* and letters *Assoc. PHNutr.*

For more information about the Registers, registration fees and about the Accreditation of Courses in Public Health Nutrition contact:

The Registrar, The Nutrition Society,
10 Cambridge Court, 210 Shepherds Bush Rd, London W6 7NJ
Fax +44 (0) 20 7602 1756; Email: mail@nutsoc.org.uk
Details can be found on the Society's Website: www.nutsoc.org.uk