BLOOD PRESSURE AND RED BLOOD CELL MAGNESIUM, POTASSIUM, AND CALCIUM

RESPONSES TO DIETARY FATS

A Thesis

by

NOREEN ELAINE WEHRMAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

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August 1993

Major Subject: Nutrition

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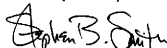A Thesis

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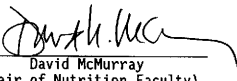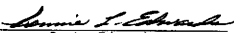for the degree of

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**ABSTRACT**

Blood Pressure and Red Blood Cell Magnesium, Potassium, and Calcium
Responses to Dietary Fats.  (August 1993)

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Hypertension and cardiovascular disease are leading causes of
death in the United States.  Many nutritional factors, including
minerals and dietary fats, have been implicated in the etiology of
hypertension.  The role of dietary fats in blood pressure regulation
is being studied more closely, with careful consideration to their
actions on prostaglandin synthesis, membrane fluidity and
permeability, and electrolyte balance.  This study examined the
changes in blood pressure and red blood cell magnesium, calcium, and
potassium concentrations in response to diets enriched in six
different test fats in normotensive men.  This study also examined
the relationship between dietary calcium, magnesium, and potassium,
and erythrocyte mineral concentrations and blood pressure.

No significant reduction in either systolic or diastolic blood
pressure was observed in subjects after following any of the diets.
There was no significant change in red blood cell mineral
concentrations in response to dietary treatment.

Erythrocyte calcium, potassium, and magnesium concentrations
were found to be significantly correlated with each other.  However,

no relationship was apparent between red blood cell mineral concentrations and blood pressure.  In addition, no evidence of a relationship between dietary minerals and red blood cell minerals was observed.

These results suggest that the fatty acid composition of the diet and mineral intake do not have a significant impact on blood pressure and erythrocyte mineral concentrations.

## DEDICATION

This thesis is dedicated to my husband, Michael Wehrman, whom I was very lucky to meet during my short stay in Texas. Michael always had faith in my work and never doubted my abilities, even when I did. I'll never be able to express my most heartfelt gratitude to him for his support and encouragement. His love is greathearted, his kindness unsparing, and his achievements and passion for his work are inspirational.

## ACKNOWLEDGEMENT

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## INTRODUCTION

Hypertension and cardiovascular disease (CVD) are two leading causes of death in the United States today. Cardiovascular disease alone accounted for nearly one million deaths and hypertension for over 20,000 deaths in 1987 (1). Hypertension is known to accelerate and increase the severity of atherosclerosis and CVD (2-7). Control of blood pressure has been shown to have a beneficial effect upon cardiovascular morbidity and mortality (8-11).

Many nutritional factors have been associated with hypertension, and researchers have focused chiefly on the role of sodium, potassium, calcium, and magnesium, (12-18). More recently, however, the role of dietary fats in blood pressure control has been studied. Many researchers believe that certain dietary fats may control blood pressure or play a part in blood pressure regulation through their actions on prostaglandin (PG) synthesis, membrane fluidity and permeability, cell membrane transport function, and electrolyte balance (19-34). There is very little evidence in the literature today, however, about the way in which blood pressure and tissue mineral levels respond to specific dietary fats.

This thesis is written in the style of the American Journal of Clinical Nutrition

## LITERATURE REVIEW

### PROBLEM IDENTIFICATION

Hypertension and atherosclerosis are the two diseases in the United States that account for nearly 50% of all deaths (1). Hypertension (HTN) is known to play an important role in the development of CVD. Hypertension is one of the major risk factors for coronary heart disease (CHD) and may lead to myocardial ischemia, left ventricular hypertrophy and failure due to a greater load on the heart, hypertensive vascular disease of the coronary arteries, acceleration of atherosclerosis extending to the small branches of the coronary arteries, cerebral atherosclerosis and cerebral ischemia. Hypertension both predisposes to heart attack and is more likely to cause heart attacks to be fatal once they do occur. According to Gubner, left ventricular hypertrophy is the "key lesion" in HTN, and it greatly increases the tendency toward ventricular arrhythmias and sudden death (35).

Atherosclerosis has been shown to be accelerated by HTN with an increased extent of fatty streaks in the aorta and coronary arteries, a greater extent of raised atherosclerotic lesions, more frequent fibrous plaques, complicated lesions, and calcified lesions, and higher prevalence of stenosis in the coronary arteries (2). Koletsky and coworkers demonstrated a more rapid and severe development of atherosclerosis in the intra-abdominal arteries, leading to thrombosis and aneurysm (3). Hypertension in experimental monkeys and rabbits has led to more severe aortic and coronary lesions when

coupled with an atherogenic diet (4-6). In addition, Bronte-Stewart and Heptinstall showed that only small rises in pressure were necessary to increase atheroma, even when not sustained (5). Hypertension increased the severity of aortic atherosclerosis in dogs as well (7). According to Dzau, HTN may cause "shear-related injury" to blood vessels, and injury to the endothelium and vascular cell proliferation amplify the development of atherosclerosis (36).

Some believe that the danger of HTN on the cardiovascular system is derived mainly from the diastolic blood pressure. However, researchers have found that both systolic and diastolic blood pressures were statistically significant indices of risk of CHD, and either a high systolic or diastolic pressure is a good predictor of the risk of CHD (37-38).

Many nutritional factors have been implicated in the etiology of HTN (12-18). Specifically, much of the attention is focused on dietary sodium, calcium, potassium, magnesium, and fats.

There is evidence that control of blood pressure may have a beneficial effect upon cardiovascular morbidity and mortality. Control of blood pressure has been associated with a decrease in the incidence of stroke, fatal heart attacks, cardiovascular deaths, fatal myocardial infarctions, cerebrovascular events, and total cardiovascular mortality (8-11). Subjects who had recently stopped using beta-blockers for treatment of HTN had a transient four-fold increase in relative risk of CHD (39).

**ELECTROLYTES**

Considerable attention has been given to the role of

electrolyte imbalances in the etiology and pathogenesis of disease, particularly CVD. Several epidemiological studies demonstrated a decrease in the incidence of CHD, CVD, and death from ischemic heart disease (IHD) in areas where water and soil are rich in minerals (40-41).

There is accumulating evidence from both animal and human studies which indicates that disturbances of electrolytes may induce arrhythmias, high blood pressure, CVD and CHD, and even cardiac lesions. Numerous clinical abnormalities in both animals and humans have been associated with imbalances of magnesium, potassium, and calcium, including ventricular tachycardia, fibrillation, arrhythmias, premature ventricular beats, acute myocardial infarction (AMI), extrasystoles, congestive heart failure, cardiac lesions, necrosis, and HTN. Electrolyte disturbances are believed to play a role in arrhythmias, blood pressure, and vascular smooth muscle control (42-44). Lower bioavailability of minerals or abnormal mineral metabolism have also been recently implicated in the etiology of HTN (45).

Erythrocytes were chosen as the experimental system to study electrolytes because of their ease in collection and availability. Although erythrocytes may not be the ideal system because they may not accurately reflect mineral status at the time of analysis or total body stores (46-47), they should be an adequate system for the study of changes in cations over time.

## Magnesium

Magnesium deficiency has been shown to produce coronary

arterial spasm in dogs (48). Experimental magnesium deficiency in rats resulted in gross morphological lesions of the myocardium, large areas of necrosis, calcification extending through the entire ventricular wall, congestion of the subepicardial vessels, and marked dilation of the cardiac chambers. In addition, many of the lesions found in the rats were characterized by proliferation of fibroblasts and deposition of collagen (49-50). In spontaneously hypertensive rats, magnesium deficiency caused widespread tissue calcification and aggravated high blood pressure (51). In other rat studies, magnesium deficiency produced marked hyperlipidemia (52).

In humans, hypomagnesemia has been found to be common among patients entering intensive care units (53-54). Serum and erythrocyte magnesium levels have been negatively correlated with the size of myocardial infarction (MI) (55). Decreased cellular magnesium, even in the presence of normal serum magnesium, has been found to predispose or contribute to digitalis-toxic arrhythmias (56-57). The incidence of serious ventricular ectopic beats, tachycardia and fibrillation was significantly higher upon admission among hypomagnesemic patients with AMI, and the incidence of atrial fibrillation and supraventricular tachycardia was also higher among hypomagnesemic patients (58). Exchangable magnesium was found to be significantly lower in patients with CHD than in a group with normal coronary arteries (59). Experimental human magnesium deficiency has produced changes in electrocardiograms, including broadening and decreased amplitude of the T waves, and slight prolongation of the QT interval, which reflects the duration of ventricular fibrillation

(60). Iseri and coworkers described two cases of ventricular arrhythmias associated with magnesium depletion (61).

Studies even have shown that persons with "Type A" behavior are more likely to experience an acute loss of intracellular magnesium than their calmer counterparts when stressed which, at least in part, may ultimately lead to the increase in development of CVD associated with "Type A" behavior (62).

It has been suggested that magnesium deficiency decreases $Na^+$-$K^+$-ATPase pump activity, leading to an increase in intracellular sodium. This, in turn, causes a change in membrane potential which may contribute to the arrhythmias so commonly associated with magnesium deficiency (63).

Magnesium is believed to have anti-ischemic properties. According to Shattock and coworkers, magnesium may limit cellular calcium overload under conditions where efflux of calcium via sodium-calcium exchange is reduced (64). (Increased cellular calcium has been implicated in the etiology of HTN.) Epidemiological studies have indicated that low intake of dietary magnesium appears to be related to an increased incidence of IHD (65). Patients with IHD retained more magnesium than controls when presented with an intravenous magnesium infusion, suggesting that patients with IHD may be magnesium deficient (66).

Magnesium has been shown to have therapeutic value in some cases. Magnesium therapy has been reported to completely and immediately abolish arrhythmias and restore normal sinus rhythm (61). Magnesium sulfate has been proven to be an effective therapy for

Torsades de Pointes and for toxic arrhythmias associated with hypomagnesemia (61,67). After intravenous bolus injections of magnesium sulfate, patients with resistant ventricular fibrillation have been defibrillated (68). Multifocal atrial tachycardia associated with hypomagnesemia and hypokalemia in the absence of other predisposing factors has been resolved after normalization of both serum potassium and magnesium (69). Magnesium infusion has led to an increase in cellular potassium and a decrease in the frequency of ventricular ectopic beats (70). Magnesium replacement has restored normal intracellular levels of both potassium and magnesium (71). Magnesium may even help protect against the blood pressure response to stress and high salt intake (72).

There is conflicting evidence in the literature about the role of dietary magnesium on blood pressure. Although, as previously mentioned, epidemiological studies point to the fact that dietary magnesium appears to play a role in HTN, supplementing the diet with magnesium oxide was found to have no significant effect on systolic blood pressure among spontaneously hypertensive rats fed low, normal, and high magnesium diets (73). In another study however, spontaneously hypertensive rats fed a magnesium-free diet for two months had a greater mean arterial pressure, total peripheral resistance, and renal vascular resistance (51).

Serum and erythrocyte magnesium has been related to blood pressure as well. Serum magnesium has been inversely correlated with systolic blood pressure and mean blood pressure (74). On the other hand, plasma and erythrocyte magnesium did not differ significantly

between normal pregnant and pregnancy-induced hypertensives (75).

**The Role of Magnesium in Ion Transport**

Magnesium appears to have an important role in ion transport in red blood cells (76). Intracellular magnesium influences the activities of several membrane transport systems, including the sodium pump which exchanges three sodium ions for two potassium ions, the $Na^+$-$K^+$-$Cl^-$ cotransport system which moves these three ions across the cell membrane, and the $K^+$-$Cl^-$ cotransport system which moves one potassium ion with one chloride ion across the cell membrane. Intracellular ionized magnesium itself can be altered by the rate of magnesium transport across the cell membrane.

Alterations in magnesium status have been associated with subsequent alterations in the status of calcium and potassium. Hypokalemia and hypocalcemia occurred in the majority of patients with experimental hypomagnesemia (60). In the magnesium-deficient rat, lymphocyte potassium was decreased while lymphocyte calcium was increased (77).

**The Relationship Between Magnesium and Potassium**

Cellular concentrations of magnesium and potassium are very closely associated with each other. A highly significant correlation has been found between potassium and magnesium concentrations within the lymphocytes and erythrocytes from normotensive subjects (78). A significant correlation has also been found between muscle magnesium and potassium content in muscles with normal calcium (79).

Because magnesium is a necessary coenzyme for ATPases, one of which supplies the energy to transport sodium out of the cell and

potassium into the cell, a deficiency of magnesium may lead to an inability of the cell to accumulate potassium. The resting membrane potential is largely dependent on the ratio of intracellular and extracellular potassium. A decrease in the intracellular potassium concentration causes the cell to be more easily excited, and this has been implicated in the etiology of high blood pressure. Chronic potassium depletion, on the other hand, lowers vascular resistance and blood pressure (80), although increasing dietary intake of potassium alone in normotensives has been shown to have no effect on blood pressure (81).

To demonstrate the relationship between magnesium and potassium, several studies presented evidence that potassium deficiency cannot be corrected without the subsequent infusion of magnesium. One study showed that the cellular potassium content of subjects on long-term diuretic treatment, who were suffering from congestive heart failure and were being treated for arterial HTN, and who presented with symptoms of ventricular ectopic beats, did not respond to potassium infusion alone. Rather, magnesium infusion led to an increase in cellular potassium and a decrease in the frequency of ventricular ectopic beats (70).

Ventricular fibrillation has been related to low serum potassium levels in patients in coronary care units (82). Cumulative potassium deficit, myocardial tissue potassium, and plasma potassium levels have been correlated to the likelihood of spontaneous ventricular fibrillation during AMI (83).

## The Relationship Between Magnesium and Calcium

A deficiency of magnesium seems to have a similar effect on the balance between intracellular and extracellular calcium. The resting membrane is only slightly permeable to calcium. Cellular calcium balance is maintained by the activities of pumps in addition to voltage-dependent and receptor-operated channels (84). Indirectly, the calcium balance of the cell is partially maintained by the magnesium-dependent $Na^+$-$K^+$-ATPase pump because the inwardly moving sodium is used by the $Na^+$-$Ca^{2+}$-ATPase pump to extrude calcium from the cell. Hannaert and coworkers have shown that an increased sodium content in vascular smooth muscle cells decreases net calcium extrusion through sodium and calcium exchange, thus increasing cytosolic free calcium (85).

Decreases in intracellular magnesium reduce the activity of the magnesium-dependent $Ca^{2+}$-ATPase pump, which normally pumps calcium to the outside of the cell and incorporates calcium into the endoplasmic reticulum by activity on the endoplasmic reticular membrane. Normally, calcium is sequestered in the endoplasmic reticulum, which helps maintain cytosolic calcium balance. However, with a decreased $Ca^{2+}$-ATPase activity, cytosolic calcium is increased. In addition, with magnesium deficiency, there is an increased interchange across the mitochondrial membrane between sodium and calcium, which produces an even greater increase in cytosolic calcium. Thus, an impairment of the pump systems or the ionic gradient may cause an increase in cytosolic calcium. An impaired intracellular calcium content has been confirmed in patients suffering from essential HTN (86).

### The Relationship Between Calcium and Blood Pressure

An increased intracellular level of calcium has been related to HTN. When the concentration of intracellular calcium reaches a certain threshold, calcium binds to the protein calmodulin, which initiates a series of biochemical events that eventually result in actin-myosin interaction and smooth muscle contraction. This reaction is dependent upon the concentration of calcium available in the cytosol (87). If the concentration of cytosolic calcium reaches an abnormally high level, the calcium will enter the mitochondria, the major storage site of intracellular calcium. The efflux pathway will become saturated and the net accumulation of calcium will exceed mitochondrial capacity, eventually causing cell dysfunction and cell death (84). Magnesium, in this respect, actually may act as a calcium-blocking agent. In addition, experimentally increasing intracellular calcium caused a decrease in intracellular potassium and an increase in sodium (88).

Calcium overload and HTN may play a vital role in the development of AMI. Cellular calcium overload is known to be highly pathogenic, and leads to necrotization and calcinosis of the arterial media in rats. High intracellular calcium enhances the contractile response to vasoconstrictive stimulus in systemic HTN, leading to much more severe coronary spasm than in normotensives, possibly ending in AMI. In addition, Gasser noted that calcium overload has been shown to be the basic physiologic principle in the development of myocardial cell necrosis (89).

Calcium supplementation has been shown to decrease blood

pressure in rats (90-92) and humans (93-95). Weanling spontaneously hypertensive rats placed on a low calcium diet developed higher blood pressure than those on an intermediate or high calcium diet (96).

A low plasma ionized calcium concentration is inversely related to blood pressure (97-98). An epidemiological study found a positive relationship between serum calcium and blood pressure (99). Hvarfner and coworkers found an impaired renal tubular reabsorption of calcium among essential hypertensives, resulting in a decreased level of plasma ionized calcium (97).

## POLYUNSATURATED FATS

Dietary fats may be related to blood pressure (100). An increase in the intake of polyunsaturated fats in the diet has been associated with a subsequent decrease in blood pressure. Both normotensive and hypertensive subjects significantly decreased their blood pressures when fed a diet low in total fat (25%) with high P:S ratio (1:0), and high in vegetables. The decrease was greater among the hypertensives, especially for diastolic pressure (101). In another study in which the subjects were fed a vegetarian diet, both systolic and diastolic blood pressures fell during the vegetarian diet and rose to the level observed before the vegetarian diet after resumption of an omnivorous diet. The P:S ratio increased from 0.4 during the omnivorous diet to 0.8 during the vegetarian diet (102). The dietary changes in these two studies, however, were not confined to a single nutrient, so it is difficult to exclude the effects that other nutrients may have on blood pressure.

Beilin and coworkers reviewed studies of vegetarians and the

effect of vegetarian diets on blood pressure. The authors agreed that there is strong evidence for an antihypertensive effect of a vegetarian diet, independent of sodium intake and other lifestyle changes (103). In addition, Margetts and coworkers concluded that the effect on blood pressure of a vegetarian diet cannot be contributed solely to the effect of a change in P:S ratio or dietary fiber. Rather, a combination of these two factors, or perhaps another aspect of diet may be responsible (104).

Even small changes in the amount of polyunsaturated fats in the diet may cause a significant decrease in blood pressure. Comberg and coworkers demonstrated that a significant decrease in diastolic blood pressure and a small, although non-significant, decrease in systolic blood pressure followed an increase in polyunsaturated fatty acids (PUFAs) from only 2% to 4% of total calories (19).

Hetzel and coworkers reviewed epidemiological studies which indicated that the fall in CHD mortality in the USA and Australia since 1967, characterized by a fall in sudden deaths, was associated with an increase in polyunsaturated fat consumption since 1960. These researchers supported these studies with their own laboratory experiments which also indicated a protective effect of polyunsaturated fats against sudden cardiac death (105).

Supplementing the diet with safflower seed oil (containing 72% linoleic oil) has also caused a decrease in blood pressure (20). Treating subjects with both groundnut oil (20-28% PUFA) and safflower oil (70-75% PUFA) resulted in a significant decrease in diastolic blood pressure, and significant decreases in both systolic and

diastolic blood pressures occurred in subjects treated with these oils in addition to traditional antihypertensive medication (106). Additionally, rat studies have shown that feeding sunflower oil lowers systolic blood pressure, and when chain length is similar, the degree of unsaturation is a vital factor in controlling blood pressure (21).

Little has been tested about the effect of specific dietary fats on blood pressure. Some researchers claim that it is the particular fatty acid profile rather than the P:S ratio which affects blood pressure. In a recent study by Karanja and coworkers, fish oil consumption (36% kcals as fat, P:S = 0.84) resulted in lower blood pressure compared to butterfat (P:S = 0.07) and corn oil (P:S = 4.54) in spontaneously hypertensive rats (107). In another study, polyunsaturated fatty acids had a more positive effect on the utilization of calcium than monounsaturated fatty acids. Poor bioavailablity of calcium occurred when feeding a diet high in canola oil, and the bioavailability of magnesium was improved by the addition of fish oil to soybean oil in the diet (22).

PROSTAGLANDINS

The supposed effect that changing the P:S ratio has on blood pressure has been attributed, at least in part, to the influence of increased linoleic acid on prostaglandin (PG) synthesis. Linoleic acid (18:2), the most abundant polyunsaturated fatty acid in the diet, can be desaturated and elongated to form arachidonic acid (20:4), which is the precursor of prostanoids of the diene series. Prostaglandins, 20-carbon unsaturated fatty acids, are formed from

arachidonic acid by oxygenation and cyclization (108). Certain PGs are known to exert a lowering effect on blood pressure.

Researchers believe that physiological levels of PGs may serve to regulate blood pressure through renal diuretic and saliuretic effects and renal vasodilation (109). In fact, Comberg and coworkers had speculated that renin and aldosterone activity may also be involved in the effect that polyunsaturated fats, the PG precursors, have on blood pressure (19).

Berl and coworkers suggested a direct or indirect role for renal PGs in the action of vasopressin (110). Altsheler and coworkers suggested that PGs play a role in natriuresis (111). In addition, Lee and coworkers demonstrated that intravenous PG infusion in hypertensive patients induced normotension associated with normal renal blood flow and normal sodium excretion (112). Stokes proposed that PGs enhance water excretion by affecting three main physiological functions. These include reducing vasopressin-dependent osmotic water permeability of the collecting tubules, enhancing medullary blood flow, and inhibiting NaCl absorption. In this regard, renal PGs antagonize the action of vasopressin (a hormone which has antidiuretic effects and elevates blood pressure) (113).

There is accumulating evidence that PG production can be modified through diet. Grataroli and coworkers suggested that the decrease in gastric $PGE_2$ demonstrated in rats fed fish oil may be the result of substitution of arachidonic acid by n-3 fatty acids or by activation of $PGE_2$ catabolism (23). Watanabe and coworkers found

that dietary fats significantly altered fatty acid composition of the brain, adrenal gland, renal medulla, and cortex phospholipids in spontaneously hypertensive rats, although they did not affect blood pressure (114).

The precursors of PGs, linoleic and arachidonic acids, are released from plasma membrane phospholipids by the action of phospholipase $A_2$. Because the composition of the plasma membrane is constantly changing and is affected by diet, it may be possible that increasing the amount of unsaturated fatty acids in the diet, thus increasing the concentration of linoleic and arachidonic acids in the plasma membrane, will have an effect on substrate concentration for PG synthesis.

Significant changes in plasma fatty acids and the composition of phospholipids have been brought about by dietary fat supplements between five and 40% of dietary calories (24). Supplementing the diet with linoleic acid has been shown to elevate peripheral leukocyte membrane linoleic acid significantly (20). Epstein and coworkers demonstrated that the infusion of Liposyn (a fat emulsion derived from safflower oil) increased the rate of excretion of PGs, supporting the fact that providing linoleic acid can increase PG synthesis in man (25).

An increase in linoleic acid in the diet has been shown to stimulate PGE biosynthesis in man, leading to effects in systems which control renal function and sodium and potassium balance (26). Supplementing the diet with linoleic acid increased gastric PGE and $PGE_2$ production in humans (27). Mahoney and coworkers, on the other

hand, showed that linoleic acid supplementation (40% energy as sunflower seed oil) did not protect against experimentally-induced renal HTN, in spite of a small increase in renal linoleic acid content and an increase in PG systhesis (28).

Tobian and coworkers demonstrated that Dahl rats, which were genetically susceptible to salt HTN, had much lower $PGE_2$ levels in renal papillary tissue than a similar strain which was resistant to salt HTN. A high linoleic diet increased the concentration of $PGE_2$ in the renal papillae of both susceptible and resistant rats. The susceptible rats had a decreased capacity for natriuresis, and the low papillary $PGE_2$ was believed to contribute to their intrinsic inability to excrete sodium rapidly. A high linoleic diet eliminated part of the large rise in blood pressure brought on in the susceptible rats by a diet high in NaCl, delayed the start of the blood pressure rise and prevented it from ever reaching the level that would have been reached on a diet lower in linoleic acid (29).

Dietary linoleic deprivation significantly increased systolic blood pressure while suppressing vascular $PGI_2$, a vasodilator PG (30). In rats fed a diet deficient in linoleic acid, blood pressure rose significantly when fed a high salt diet, and less sodium, potassium, and chloride were excreted. In addition, renal cyclooxygenase metabolism was significantly depressed in linoleic acid deprived rats (31).

Watanabe and coworkers (114) demonstrated that spontaneously hypertensive rats fed differing levels of n-6 and n-3 fatty acids had relatively higher levels of the 2-series PG precursor (20:4 n-6) and

a relatively low level of 1- and 3-series PG precursors (20:3 n-6 and 20:5 n-3). The 2-series PGs act both as vasodilators and as vasoconstrictors, and the 1- and 3-series PGs act as vasodilators. The researchers proposed that unbalanced PG precursor levels may be partially responsible for the development of HTN in spontaneously hypertensive rats (114).

There was a three-fold decrease in plasma and dermal PGE2 levels in mice fed menhadin oil (rich in n-3 fatty acids) versus corn oil (rich in n-6 fatty acids). The difference may be attributed to metabolic competition for cyclooxygenase activity (32).

Houwelingen and coworkers, in a review of studies on the effect of dietary fat on PGE production, stated that the type of dietary fat is important in stimulating prostanoid production in vitro (33). Dietary oils did not cause significant differences in renal $PGE_2$ production in vivo. They did, however, modulate the fatty acid composition and phospholipid composition of renal medulla, believed to be the major site of renal $PGE_2$ production, in vivo (33).

Margetts and coworkers showed that omnivorous normotensive subjects significantly increased the relative concentrations of linoleic acid in plasma phospholipids when on a high P:S ratio diet, even though this produced no consistent effect on mean blood pressure (115). Sachs and coworkers found that although linoleic and oleic acids were enriched significantly in the plasma cholesteryl esters, phospholipids and triglycerides of normotensive persons supplemented with dietary linoleic or oleic acids, there was no increase in arachidonic acid in any of these fractions (34).

However, it has been previously shown that the phospholipid and fatty acid composition of human red blood cells changes very little, even with extreme variations in dietary fat (116).

## LIPID BILAYER MEMBRANE

Another proposed way in which an increase in polyunsaturated fats in the diet may decrease blood pressure is through an increase in the fluidity and permeability of cell membranes. The cell is surrounded by a lipid bilayer membrane which is selectively permeable to certain ions, including magnesium, potassium, calcium, and sodium. The ionic gradient of the cell in its environment is maintained by the cell membrane through several processes, including the energy-requiring, magnesium-dependent $Na^+$-$K^+$-ATPase pump. When these processes are impaired, the ionic gradient is impaired as well, and certain electrolyte imbalances may occur. Cooper has reviewed the role of cell membrane fluidity abnormalities in the pathogenesis of disease (117).

Gobel and coworkers demonstrated that cation transport systems which were modified in alcoholics were associated with increased blood pressure (118). $Na^+$-Li countertransport has been shown to be higher among hypertensives than normotensives (119). $Na^+$-$K^+$-$Cl^-$ cotransport in the vascular smooth muscle cells, the putative target tissue for essential HTN of spontaneously hypertensive rats, was found to be significantly lower than that of normotensive rats (120). On the other hand, pregnant women who developed pre-eclampsia (pregnancy associated HTN) showed no difference in sodium-potassium cotransport in erythrocytes, compared to normotensive pregnant women

(121).

The dry weight of the red blood cell membrane contains approximately 48% protein and 44% lipid (122). The process of maintaining cellular electrolyte balance has been shown to be related to the composition of the lipid fraction of the cell membrane.

The composition of the cell membrane is not fixed, and changes in its composition occur readily. Red blood cells have a deficiency of acetyl CoA carboxylase, an enzyme needed for de novo synthesis of fatty acids. Therefore, these cells are incapable of synthesizing fatty acids on their own (123). The cells, however, are involved in mechanisms for turnover of lipids and lipid renewal (124).

Plasma unesterified fatty acids have been shown to interact strongly with human erythrocytes. Unesterified fatty acids in plasma are transferred from serum albumin to receptor binding sites on cell surfaces (125). The fatty acids are transferred initially to a "superficial" red cell pool, subsequently transferred to a "deeper" pool in the membrane, then incorporated into membrane phospholipids (126). In addition, the major mechanisms of lipid renewal are passive exchange with preformed serum phosphatides, and active assembly of phosphatides from lysophosphatides and free fatty acids (127).

Of the four major erythrocyte phospholipids, phosphatidylserine, phosphatidyethanolamine, phosphatidylcholine, and sphingomyelin, only the latter two have been known to be actively exchangable with plasma. Reed concluded from his research that "...alterations in the fatty acid composition of the plasma compounds

can affect, in a very significant manner, the corresponding erythrocyte membrane lipids during the life span of a given red blood cell" (128). It is because of this exchange between plasma lipids and those in red blood cell membranes that the phospholipids and fatty acids in red blood cells tend to resemble those in plasma (117).

Plasma fatty acids can be altered through diet. Results from one study demonstrated that an olive oil-rich diet, containing a large amount of oleic acid, influenced the lipid composition of the cell membrane and the cation transport systems which are altered in essential HTN, specifically the $Na^+$-$K^+$-ATPase system (129). In another study in which the erythrocytes of normotensives and hypertensives were compared, the researchers found that the lipid composition of the erythrocytes membranes influences the Li-$Na^+$ countertransport system of the cells (130). Dietary linoleic acid supplementation may affect the flux of ions across the cell membrane, thus causing changes in blood pressure (131). Plasma triglycerides and cholesterol have also been associated with ion transport systems (132).

In one study, membrane fluidity in platelets of hypertensive subjects was shown to be less than that of normotensive subjects, and the platelet membranes of normotensive subjects were shown to contain a significantly greater fraction of linoleic acid than the platelet membranes of hypertensive subjects. In addition, erythrocytes from hypertensives were shown to be less deformable that those of normotensives. The researchers proposed that the abnormality of the

membrane may be global, affecting membranes of all cells (133).

An in vitro study of mouse neuroblastoma cells showed that short-term supplementation of the growth medium with oleic or linoleic acid increased the permeability of the neuroblastoma membranes to $Na^+$ while causing a subsequent decrease in the permeability to $K^+$. These effects were not seen when the growth medium was supplemented with stearic acid (18:0). The researchers concluded that these changes were not caused by changes in the $Na^+$-$K^+$-ATPase-mediated $K^+$ influx or in the $K^+$ gradient (134). Another study showed that dietary linoleic and oleic acids are unlikely to have major effects on membrane-dependent functions of red blood cells related to HTN (135).

Papahadjopoulos and coworkers stated that the fluidity of the fatty acyl chains of membrane phospholipids is essential for several transport systems, including the $Na^+$-$K^+$-ATPase system (136). The fluidity probably provides the motional freedom needed to allow proteins within the membranes to undergo conformational changes and rotational and translational movements associated with their activity (136). Cheng and Chen noted that membrane fluidity is significantly influenced by the composition of the membrane lipids, and a reduction in the fluidity of membranes is due to the deceleration of lipid movement (137).

The cholesterol content of the plasma membrane is also believed to affect the permeability and fluidity of the membrane by affecting the degree of "tightness" of the lipid components. Kroes and Ostwald demonstrated a decrease in the permeability of cholesterol-loaded

erythrocytes to electrolytes both in vitro and in vivo (138). Both the active and the passive components of the $Na^+$ efflux decreased in the cholesterol-loaded cells with a subsequent decrease in the number of Na+ pump sites (138). Papahadjopoulos and coworkers demonstrated that the presence of cholesterol partially or totally inhibited the activity of the phospholipid-activated $Na^+$-$K^+$-ATPase, depending on the degree of unsaturation of the phospholipid component. An increase in plasma cholesterol induced a decrease in membrane fluidity and an increase in $Na^+$-$K^+$-ATPase in plasma red cells (139). In addition, an increase in the level of cholesterol in cell membranes could interfere with some functions of the membrane, probably by controlling the fluidity of the acyl chains of the phospholipids, with injurious effects to cell metabolism and viability (136). Changes in the fluidity of membranes due to lipid accumulation may cause changes in the functioning of receptors or carriers within the membrane, thus contributing to metabolic disturbances (140).

It has been shown that plasma cholesterol, which can be increased by an increase in dietary cholesterol and/or saturated fat, may modify cellular calcium since a high membrane cholesterol content induces a greater influx of calcium and decreases the activity of the calcium pumps, which may play a role in the development of arterial HTN (141).

The purpose of this study was to determine blood pressure and red blood cell magnesium, potassium, and calcium responses associated with intake of various dietary fats.

## MATERIALS AND METHODS

### BACKGROUND INFORMATION ON THE CLINICAL TRIAL

Thirty male volunteers between the ages of 30 and 60 (recruited as subjects in an investigation sponsored by the Palm Oil Research Institute of Malaysia) were subjects in this investigation. The volunteers qualified for the study on the basis of the information obtained from a complete medical examination, including an electrocardiogram (EKG) and an analysis of blood chemistry. The subjects had a blood cholesterol level between 130-220 mg/dl, a blood pressure of not more than 90 mm Hg diastolic and not more than 150 mm Hg systolic, and were within 80-120% of their ideal body weight. The subjects were not on any medication except that which was approved by the physician working with this study. The subjects showed no evidence of metabolic disorders, psychiatric disorders, or alcoholism. The subjects were expected to provide medical and diet histories, to adhere to specific diet modifications, to submit to venipuncture following a 12-hour fast, to keep diet records, to maintain body weight within five pounds (2.27 kilograms), to maintain pretrial exercise level and alcohol consumption, and to refrain from taking diet supplements (vitamins), steroid injections, or illicit drugs. In return, the subjects were provided with nutrition information, access to all data resulting from analyses pertaining to them, provision of food products containing the test fats, and financial reimbursement contingent upon completion of the study.

The diets consisted of 40% energy as fat. Test fats were

incorporated into cookies, ice cream, milk, and spreads and oils and were consumed by the subjects as 60% of the total fat. The test fats include crude palm oil (CPO), refined palm oil (RPO), butter (BUT), sunflower oil (SUN), Parkay margarine (PAR), and a sunflower/refined palm oil blend (SPO). The percentages of types of fatty acids for each test fat were determined by the laboratory of Dr. Randall Wood, Texas A&M University (Table 1). The diets were designed to fit the usual diets of the participants through the use of exchange lists. The design of the study was that of a Latin Square in which all subjects followed all diets.

The experiment consisted of six diet periods, each of six weeks duration and corresponding to one specific test fat at each time, in which the subjects were expected to follow a prescribed diet plan. Each diet period was followed by a six week "washout period" in which the subjects were expected to eat their normal diet. During each diet period, blood was drawn weekly at the same time (approximately 8:00 a.m.) following a 12-hour fast, by venipuncture by a registered medical technician. The blood was collected in Venoject AutoSep serum separation evacuated blood collection tubes (Terumo Medical Corporation, Elkton, Maryland) containing an inert barrier-forming material (specific gravity between serum and coagulum after clotting and centrifugation) and a clot activator (disk carrying silica particles). Body weights were also measured during the visit when blood was sampled.

**TABLE 1**
Percentages of types of fatty acids in test fats*+

|     | SAT (<15:0) | SAT (>15:0) | TOTAL SAT | CIS MONO | TRANS MONO | TOTAL POLY |
|-----|-------------|-------------|-----------|----------|------------|------------|
|     | %           | %           | %         | %        | %          | %          |
| BUT | 16.0        | 46.8        | 62.8      | 25.2     | 4.0        | 3.9        |
| PAR | 0.5         | 20.4        | 20.9      | 33.6     | 26.2       | 14.6       |
| SUN | 0.0         | 12.8        | 12.8      | 17.5     | 0.0        | 65.6       |
| CPO | 0.0         | 40.7        | 40.7      | 46.0     | 0.0        | 10.9       |
| RPO | 0.0         | 46.5        | 46.5      | 40.8     | 0.0        | 9.3        |
| SPO | 0.0         | 34.1        | 34.1      | 33.2     | 0.0        | 25.3       |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend; Sat, saturated fatty acids; Mono, monounsaturated fatty acids; Poly, polyunsaturatd fatty acids.
+ Only major fatty acids of even chain lengths >10:0 and <20:0 have been included.

PRESENT STUDY

Number of Subjects by Treatment Group

One of the 30 subjects was not present for blood drawings and blood pressure readings for two of the weeks (weeks 3 and 6), and subsequently was dropped from this study. There were between three and seven subjects per treatment group, and the breakdown of the 29 subjects by dietary group is listed in Table 2.

Electrolytes and Blood Pressures

The present study involved analysis of red blood cell calcium, magnesium, and potassium from the second baseline week (week 0), week 3 and week 6 of Diet Period 4. In addition, both systolic and diastolic blood pressures were measured during these same weeks with a sphygmomanometer (W.A. Baum and Company, Incorporated, Copiague, N.Y.) and stethoscope (Premier) by the author who was trained by the American Red Cross. The blood pressures were taken from both the right and left arms of the subjects while in a sitting position before the blood sample was drawn. Left and right blood pressures were combined to obtain an average systolic and average diastolic blood pressure for each subject.

Dietary Data

Three three-day food intake records were kept by the subjects during week 0, week 3, and week 6 of Diet Period 4. In cases where food intake records were missing, the food intake records available were used to calculate average intakes of nutrients. A nutritional anaylsis was performed on each of the food intake records (Nutrapractor 6000, Practocare, Inc., San Diego, CA).

**TABLE 2**
Number of subjects by dietary group and total number*

| Dietary Group | n |
|---|---|
| BUT | 4 |
| PAR | 5 |
| SUN | 6 |
| CPO | 7 |
| RPO | 4 |
| SPO | 3 |
| Total | 29 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend;

## SAMPLE PREPARATION

All stainless steel instruments used for this analysis were soaked in a 5% EDTA solution (Appendix, J.T. Baker Incorporated, Phillipsburg, New Jersey) for at least one hour and rinsed with distilled deionized water (Milli-Q Pyrogen-free Water System, Millipore Corporation). All glassware was soaked in a 10% nitric acid solution (Appendix, Mallinckrodt Incorporated, Paris, Kentucky) for at least 24 hours, rinsed in distilled deionized water, and allowed to air dry.

The blood samples were frozen and transferred on ice to the laboratory where they remained frozen at -10 C until time of analysis. The samples were then thawed at room temperature.

Any remaining serum was removed by inversion of the tube onto a piece of absorbent paper. The inert gel separator was removed from the blood collection tube with a stainless steel spatula, using a scooping motion, and discarded. The coagulum was transferred to a beaker. The sample in the beaker was placed in a 100-140 C oven (Precision Scientific Group, GCA Corporation, Chicago, IL) and dried for a minimum of 48 hours. The samples were removed from the oven and transferred to a dessicator to cool.

After the samples had cooled, the samples were broken up and an aliquot of approximately 0.5 gram of the dried coagulum was weighed, in duplicate, using an electronic analytical balance (Series 1600, Sartorious Balances and Scales, Brinkmann Instruments Company, Division of Sybron Corporation, Cantiague, New York). The weights were recorded, and the samples were transferred to a beaker. The

samples were digested with aproximately 10 ml concentrated nitric
acid using a remote control hot plate (Model RC2240, Series 411,
Barnstead/Thermolyne Corporation, Dubuque, Iowa). More nitric acid
was added as needed throughout the digestion. When the digest was
clear (not cloudy), the nitric acid was allowed to evaporate until
approximately 0.5-1.0 ml digested sample remained in the beaker. The
digest was transferred to a 50-ml volumetric flask with a 1%
lanthanum chloride solution (Appendix, Fischer Scientific, Fair Lawn,
New Jersey), rinsing the sides of the beaker to ensure complete
transfer, and adjusted to volume with the lanthanum chloride
solution. The digest was filtered to remove any silica particles,
and the digest was transferred into culture tubes for mineral
analysis.

**RED BLOOD CELL MINERAL ANALYSIS**

The digests were analyzed for magnesium, calcium, and potassium
using an atomic absorption spectrophotometer (Model AA-6, Varian
Techtron, Palo Alto, California). Magnesium samples were diluted
500:1, potassium samples 397:1, and calcium was not diluted for
anaylsis. Wavelengths used for calcium, magnesium, and potassium
were 422.7, 285.2, and 766.5 nm respectively.

The results from the atomic absorption spectrophotometry were
reported in parts per million (PPM) (Appendix).

**STATISTICAL ANALYSIS**

All statistical tests were performed using the Statistical
Analysis System (SAS). Blood pressure and red blood cell data were
analyzed using the general linear model with repeated measures.

Correlation coefficients were also calculated for blood pressure and
red blood cell data and dietary intake of minerals and
blood pressures.

## RESULTS

### DIETARY DATA

#### Modified Foods

Modified fats were incorporated into the diets of the subjects in the form of spreads, ice cream, milk, and cookies. The fatty acid profiles of the modified foods varied according to which test fat was incorporated into the foods and are listed in Tables 3-7.

#### Intake of Total Fat, Modified Fat, Magnesium, Calcium, Potassium, Linoleic Acid, and P:S Ratios

All six treatment groups consumed approximately 35 to 42% of their total daily calories from fat, and 14 to 22% calories from modified fat. These percentages were not significantly different among treatment groups. Average percentages of calories as fat and percentages of calories as modified fat are listed in Table 8 by treatment group. Average percentages of modified fat as total fat are listed in Table 9 by treatment group, and averaged between approximately 36 and 62%. These too were not significantly different.

There were no significant differences among treatment groups in the amount of dietary magnesium, calcium, and potassium consumed during Diet Period 4. These figures are listed in Table 10 and are compared to their Recommended Dietary Allowances (RDAs) or Estimated Minimum Requirements (EMRs) and reference values found in the literature (144,145). The SUN diet contained the largest percentage of calories from linoleic acid and the highest P:S ratio. Average percentages of calories as linoleic acid and P:S ratios differed by

**TABLE 3**
Amounts of fatty acids per tablespoon of modified spreads and oils*

|              | BUT   | PAR   | SUN   | CPO   | RPO   | SPO   |
|--------------|-------|-------|-------|-------|-------|-------|
|              |       |       | g     |       |       |       |
| total fatty acids | 11.47 | 11.58 | 13.81 | 13.81 | 13.81 | 13.97 |
| 10:0         | 0.58  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| 12:0         | 0.59  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| 14:0         | 1.54  | 0.06  | 0.00  | 0.00  | 0.00  | 0.00  |
| 16:0         | 3.58  | 1.45  | 1.09  | 5.15  | 5.80  | 4.12  |
| 16:1         | 0.16  | 0.00  | 0.00  | 0.04  | 0.03  | 0.03  |
| 18:0         | 1.26  | 0.91  | 0.68  | 0.47  | 0.62  | 0.64  |
| 18:1         | 2.44  | 6.94  | 2.42  | 6.31  | 5.61  | 4.61  |
| 18:2         | 0.41  | 1.63  | 9.00  | 1.46  | 1.23  | 3.48  |
| 18:3         | 0.05  | 0.06  | 0.06  | 0.04  | 0.06  | 0.06  |
| P:S          | 0.06  | 1.25  | 6.36  | 0.19  | 0.19  | 0.37  |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend; and P:S, polyunsaturated to saturated fatty acid ratio.

**TABLE 4**
Amounts of fatty acids per cup of modified milk*

|  | BUT | PAR | SUN | CPO | RPO | SPO |
|---|---|---|---|---|---|---|
|  |  |  | g |  |  |  |
| total fatty acids | 8.38 | 8.47 | 8.11 | 8.11 | 8.11 | 8.19 |
| 10:0 | 0.43 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12:0 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:0 | 1.12 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 2.61 | 1.06 | 2.42 | 3.03 | 3.41 | 2.42 |
| 16:1 | 0.12 | 0.00 | 0.00 | 0.02 | 0.02 | 0.02 |
| 18:0 | 0.92 | 0.67 | 0.40 | 0.28 | 0.36 | 0.38 |
| 18:1 | 1.78 | 5.07 | 1.42 | 3.71 | 3.29 | 2.70 |
| 18:2 | 0.30 | 1.19 | 5.29 | 0.86 | 0.72 | 2.04 |
| 18:3 | 0.03 | 0.04 | 0.03 | 0.02 | 0.03 | 0.03 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 5**
Amounts of fatty acids per cup of modified ice cream*

|  | BUT | PAR | SUN | CPO | RPO | SPO |
|---|---|---|---|---|---|---|
|  |  |  | g |  |  |  |
| total fatty acids | 19.47 | 19.66 | 18.78 | 18.78 | 18.78 | 19.00 |
| 10:0 | 0.99 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12:0 | 1.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:0 | 2.61 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 6.07 | 2.46 | 1.48 | 7.00 | 7.89 | 5.61 |
| 16:1 | 0.27 | 0.00 | 0.00 | 0.06 | 0.04 | 0.04 |
| 18:0 | 2.14 | 1.55 | 0.92 | 0.64 | 0.85 | 0.87 |
| 18:1 | 4.15 | 11.78 | 3.29 | 8.58 | 7.62 | 6.27 |
| 18:2 | 0.70 | 2.77 | 12.24 | 1.99 | 7.62 | 4.73 |
| 18:3 | 0.08 | 0.10 | 0.08 | 0.06 | 0.08 | 0.08 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 6**
Amounts of fatty acids per serving of modified molasses cookie*

|                    | BUT  | PAR  | SUN  | CPO  | RPO  | SPO  |
|--------------------|------|------|------|------|------|------|
|                    |      |      | g    |      |      |      |
| total fatty acids  | 2.26 | 2.25 | 2.33 | 2.33 | 2.33 | 2.35 |
| 10:0               | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12:0               | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:0               | 0.30 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0               | 0.71 | 0.28 | 0.18 | 0.87 | 0.98 | 0.69 |
| 16:1               | 0.03 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 18:0               | 0.25 | 0.18 | 0.11 | 0.08 | 0.18 | 0.11 |
| 18:1               | 0.48 | 1.35 | 0.41 | 1.06 | 0.95 | 0.78 |
| 18:2               | 0.08 | 0.32 | 1.52 | 0.25 | 0.21 | 0.59 |
| 18:3               | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 7**
Amounts of fatty acids per serving of modified sugar cookie*

|                    | BUT  | PAR  | SUN  | CPO  | RPO  | SPO  |
|--------------------|------|------|------|------|------|------|
|                    |      |      | g    |      |      |      |
| total fatty acids  | 2.22 | 2.21 | 2.27 | 2.27 | 2.27 | 2.29 |
| 10:0               | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 12:0               | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:0               | 0.30 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0               | 0.67 | 0.28 | 0.18 | 0.85 | 0.95 | 0.68 |
| 16:1               | 0.03 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| 18:0               | 0.24 | 0.17 | 0.11 | 0.08 | 0.10 | 0.11 |
| 18:1               | 0.47 | 1.32 | 0.40 | 1.04 | 0.92 | 0.76 |
| 18:2               | 0.08 | 0.31 | 1.48 | 0.24 | 0.20 | 0.57 |
| 18:3               | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 8**
Dietary intake of kcals as fat and kcals as modified fat*

|  | BUT | PAR | SUN | CPO | RPO | SPO | Goal |
|---|---|---|---|---|---|---|---|
|  | % | % | % | % | % | % | % |
| Kcals as fat | 36.71 | 39.03 | 35.72 | 38.68 | 34.86 | 42.06 | 40.00 |
| Kcals as modified fat | 21.85 | 14.04 | 18.44 | 19.14 | 15.92 | 16.47 | 24.00 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 9**
Dietary intake of modified fat of total fat*

| Dietary Group | Average Intake Modified Fat of Total Fat | Goal |
|---|---|---|
|  | % | % |
| BUT | 61.85 | 60.00 |
| PAR | 35.81 | 60.00 |
| SUN | 50.68 | 60.00 |
| CPO | 45.02 | 60.00 |
| RPO | 45.41 | 60.00 |
| SPO | 39.01 | 60.00 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 10**
Dietary intake of magnesium, calcium, and potassium*

|  | BUT | PAR | SUN | CPO | RPO | SPO | RDA or EMR | Mean | Reference Value |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | mg |  |  |  |  |  |
| Magnesium | 317 | 309 | 254 | 261 | 327 | 259 | 350 | 289 | 298 |
| % RDA |  |  |  |  |  |  |  | 83 | 85 |
| Calcium | 906 | 1007 | 887 | 1160 | 1312 | 1201 | 800 | 1078 | 868 |
| % RDA |  |  |  |  |  |  |  | 135 | 109 |
| Potassium | 3318 | 3422 | 2949 | 3287 | 3613 | 2918 | 2000 | 3251 | 2930 |
| % EMR |  |  |  |  |  |  |  | 163 | 147 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend; RDA, Recommended Dietary Allowance; and EMR, Estimated Minimum Requirement.

treatment group and are listed in **Table 11**.

**CLINICAL DATA**

**Anthropometric Data**

Anthropometric data collected before the beginning of the study, including height, weight, age, and body mass index (BMI), is listed in **Table 12**. Body mass index is weight (kilograms) divided by height (meters squared).

Individual variations in weight throughout Diet Period 4 ranged from -4.32 to +2.5 kilograms from week 0 to week 6. Only two of the subjects' weights varied more than five pounds (2.27 kilograms) from their original weight at week 0. **Table 13** provides the average weight variations by dietary group. None of these variations in weight among treatment groups were significantly different.

**Diastolic Blood Pressure**

**Table 14** lists the mean diastolic blood pressures (DBPs) by week within treatment. DBPs during week 0, week 3, and week 6 were not significantly different. Treatment had no significant effect on DBP. Week also had no significant effect on DBP, and there was no significant week by treatment interaction.

DBP was highly correlated ($p < 0.001$) with systolic blood pressure (SBP), but not with any other variable. Correlation coefficients between DBP and other variables can be found in **Table 15**.

**Systolic Blood Pressure**

Mean systolic blood pressures (SBPs) during week 0 were significantly different ($p < 0.05$, $r = 0.60$), but they were not

**TABLE 11**
Dietary intakes of linoleic acid and P:S ratios*

|  | BUT | PAR | SUN | CPO | RPO | SPO | Reference Value |
|---|---|---|---|---|---|---|---|
| Linoleic Acid (% kcals) | 2.48 | 5.24 | 14.45 | 3.87 | 3.46 | 10.32 | 5.07 |
| P:S Ratio | 0.13 | 0.55 | 1.39 | 0.27 | 0.27 | 0.98 | |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  and P:S ratio, polyunsaturated to saturated fatty acid ratio.

**TABLE 12**
Weight (kg), height (cm), age, and body mass index (BMI) of subjects
by treatment group*
(Mean ± standard deviation)

| Treatment | Weight | | Height | | Age | | BMI | |
|---|---|---|---|---|---|---|---|---|
| | kg | | cm | | years | | | |
| BUT | 92.07 | 4.27 | 180.17 | 2.07 | 37.33 | 0.85 | 28.35 | 1.13 |
| PAR | 76.25 | 3.67 | 172.88 | 2.98 | 38.25 | 3.44 | 25.26 | 0.66 |
| SUN | 78.90 | 2.27 | 177.75 | 2.28 | 42.00 | 3.46 | 24.97 | 0.57 |
| CPO | 84.85 | 3.81 | 179.88 | 3.37 | 46.50 | 3.65 | 26.12 | 0.40 |
| RPO | 82.22 | 4.69 | 180.63 | 4.08 | 37.50 | 1.15 | 25.13 | 0.65 |
| SPO | 85.00 | 3.72 | 181.17 | 1.91 | 42.00 | 6.55 | 25.98 | 1.63 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  Kg, kilogram; Cm, centimeter; and BMI, body mass index.

**TABLE 13**
Average variation in weight (kg) from week 0 to week 6 by dietary
group*

| Dietary Group | Weight Variation |
|---------------|------------------|
|               | kg               |
| BUT           | 0.80             |
| PAR           | 0.64             |
| SUN           | 0.59             |
| CPO           | 0.62             |
| RPO           | 0.28             |
| SPO           | 1.74             |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  and kg, kilogram.

**TABLE 14**
Mean diastolic blood pressures by week within treatment*
(Mean $\pm$ SEM)

| Week | 0 | 3 | 6 |
|------|-----|-----|-----|
|      | mm Hg | | |
| BUT  | $67.0 \pm 3.46$ | $69.5 \pm 3.94$ | $69.5 \pm 3.65$ |
| PAR  | $77.0 \pm 3.99$ | $75.3 \pm 4.55$ | $78.7 \pm 4.21$ |
| SUN  | $65.4 \pm 3.09$ | $66.0 \pm 3.53$ | $67.6 \pm 3.26$ |
| CPO  | $79.0 \pm 3.46$ | $75.0 \pm 3.94$ | $76.0 \pm 3.65$ |
| RPO  | $68.0 \pm 6.91$ | $74.0 \pm 7.88$ | $71.0 \pm 7.30$ |
| SPO  | $69.0 \pm 3.99$ | $71.3 \pm 4.55$ | $67.7 \pm 4.21$ |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

TABLE 15
Correlation matrix between variables*

|       | Mg   | Ca    | K    | SBP   | DBP   | MgI   | CaI   | KI    |
|-------|------|-------|------|-------|-------|-------|-------|-------|
|       |      |       |      | /r/   |       |       |       |       |
| Mg    | 1.00 | 0.32  | 0.13 | 0.07  | 0.09  | 0.21  | 0.26  | 0.29  |
| Ca    | 0.32 | 1.00  | 0.33 | 0.31  | 0.31  | 0.33  | 0.37  | 0.47+ |
| K     | 0.13 | 0.33  | 1.00 | 0.26  | 0.19  | 0.15  | 0.32  | 0.20  |
| SBP   | 0.07 | 0.31  | 0.26 | 1.00  | 0.60| | 0.31  | 0.23  | 0.18  |
| DBP   | 0.09 | 0.31  | 0.19 | 0.60| | 1.00  | 0.31  | 0.32  | 0.30  |
| MgI   | 0.21 | 0.33  | 0.15 | 0.31  | 0.31  | 1.00  | 0.43+ | 0.85‡ |
| CaI   | 0.26 | 0.37  | 0.32 | 0.23  | 0.32  | 0.43+ | 1.00  | 0.58§ |
| KI    | 0.29 | 0.47+ | 0.20 | 0.18  | 0.30  | 0.85‡ | 0.58§ | 1.00  |

* Mg, erythrocyte magnesium concentration; Ca, erythrocyte calcium
  concentration; K, erythrocyte potassium concentration; SBP,
  systolic blood pressure; DBP, diastolic blood pressure; MgI,
  magnesium intake; CaI, calcium intake; and KI, potassium intake.
+ Significant, p <0.05.
‡ Significant, p <0.0001.
§ Significant, p <0.005.
| Significant, p <0.001.

significantly different during week 3 and week 6.  Treatment had an effect on SBP (p <0.05) but there was no week effect or week by treatment interaction.  Table 16 lists the mean SBPs by treatment group.

SBP was significantly correlated (p <0.001) with diastolic blood pressure, but not with any other variable.  Table 15 lists correlation coefficients between SBP and other variables.

## Erythrocyte Mineral Concentrations

Table 17 lists the mean magnesium concentration of red blood cells by week within treatment group.  There were no significant differences found in magnesium concentration during week 0 or week 6.  However, there were significant differences among magnesium concentrations during week 3 (p <0.05).  Neither treatment nor week had a significant effect on magnesium concentration.  There was no significant week by treatment interaction.

Table 15 provides the correlation coefficients of magnesium concentration with other variables.  Magnesium concentration was not significantly correlated with any other variable.

Red blood cell calcium concentration was not significantly different during week 0, week 3, or week 6.  Table 18 lists the mean calcium concentration of red blood cells by week within treatment group.

Treatment did not have a significant effect on calcium concentration.  Week, however, did have a significant effect on calcium concentration in red blood cells (p <0.05), but there was no significant week by treatment interaction.

**TABLE 16**
Mean systolic blood pressures (SBP) by week within treatment*
(Mean ± SEM)

| Week | 0 | 3 | 6 |
|------|---|---|---|
| | | mm Hg | |
| BUT | 106.0 ± 2.52 | 113.0 ± 3.39 | 111.5 ± 4.25 |
| PAR | 118.3 ± 2.91 | 119.0 ± 3.91 | 120.0 ± 4.91 |
| SUN | 110.4 ± 2.25 | 111.4 ± 3.03 | 104.2 ± 3.80 |
| CPO | 112.8 ± 2.52 | 120.0 ± 3.39 | 117.0 ± 4.25 |
| RPO | 123.0 ± 5.03 | 120.0 ± 6.77 | 124.0 ± 8.50 |
| SPO | 107.8 ± 2.91 | 113.7 ± 3.91 | 112.0 ± 4.91 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude palm oil; RPO, refined palm oil; and SPO, sunflower/palm oil blend.

**TABLE 17**
Mean erythrocyte magnesium concentration by week within treatment*
(Mean ± SEM)

| Week | 0 | 3 | 6 |
|------|-----|-----|-----|
| | | PPM | |
| BUT | 638.44 ± 224.43 | 898.47 ± 99.64 | 996.47 ± 133.88 |
| PAR | 657.45 ± 173.84 | 567.45 ± 77.18 | 894.97 ± 103.70 |
| SUN | 716.15 ± 173.84 | 768.28 ± 77.18 | 768.46 ± 103.70 |
| CPO | 822.84 ± 173.84 | 880.98 ± 77.18 | 802.13 ± 103.70 |
| RPO | 854.20 ± 224.43 | 610.24 ± 99.64 | 844.60 ± 133.88 |
| SPO | 365.77 ± 224.43 | 555.60 ± 99.64 | 867.73 ± 133.88 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  and PPM, parts per million.

**TABLE 18**
Mean erythrocyte calcium concentration by week within treatment*
(Mean $\pm$ SEM)

| Week | 0 | 3 | 6 |
|------|-----|-----|-----|
| | | PPM | |
| BUT | 12.05 $\pm$ 0.80 | 14.46 $\pm$ 1.68 | 13.21 $\pm$ 1.07 |
| PAR | 12.07 $\pm$ 0.62 | 13.49 $\pm$ 1.30 | 12.33 $\pm$ 0.83 |
| SUN | 13.11 $\pm$ 0.62 | 16.24 $\pm$ 1.30 | 10.48 $\pm$ 0.83 |
| CPO | 14.72 $\pm$ 0.62 | 13.39 $\pm$ 1.30 | 12.59 $\pm$ 0.83 |
| RPO | 13.88 $\pm$ 0.80 | 12.99 $\pm$ 1.68 | 12.03 $\pm$ 1.07 |
| SPO | 13.76 $\pm$ 0.80 | 13.81 $\pm$ 1.68 | 12.32 $\pm$ 1.07 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  and PPM, parts per million.

Calcium concentration was significantly correlated with potassium intake (p< 0.05), but not with any other variable. Table 15 lists the correlation coefficients of calcium concentration with other variables.

There were no significant differences among potassium concentrations during week 0, week 3, or week 6. Table 19 lists the mean potassium concentration of red blood cells by week within treatment group.

Treatment had no significant effect on potassium concentration. Week, however, had a significant effect on potassium concentration (p <0.01), but there was no significant week by treatment interaction.

Red blood cell potassium concentration was not correlated with other variables. Table 15 lists the correlation coefficients of potassium concentration with other variables.

Correlations with Dietary Intake of Minerals

Magnesium intake was significantly correlated with intake of calcium (p <0.05) and potassium (p <0.0001). Similarly, dietary calcium was found to be significantly correlated with potassium intake (p <0.005) and magnesium (p <0.05). Correlation coefficients are listed in Table 15. There were no significant correlations among dietary minerals and erythrocyte mineral concentrations (Table 15).

TABLE 19
Mean erythrocyte potassium concentration by week within treatment*
(Mean ± SEM)

| Week | 0 | 3 | 6 |
|------|---|---|---|
| | | PPM | |
| BUT | 2626.04 ± 316.26 | 2298.94 ± 275.53 | 2996.29 ± 353.24 |
| PAR | 2565.65 ± 244.97 | 2148.86 ± 213.42 | 1845.44 ± 273.62 |
| SUN | 2804.94 ± 244.97 | 2492.56 ± 213.42 | 2308.43 ± 273.62 |
| CPO | 2576.88 ± 244.97 | 1960.87 ± 213.42 | 2460.33 ± 273.62 |
| RPO | 2994.24 ± 316.26 | 1830.50 ± 275.53 | 2363.70 ± 353.24 |
| SPO | 2622.55 ± 316.26 | 1754.98 ± 275.53 | 1902.16 ± 353.24 |

* BUT, butter; PAR, Parkay margarine; SUN, sunflower oil; CPO, crude
  palm oil; RPO, refined palm oil; SPO, sunflower/palm oil blend;
  and PPM, parts per million.

# DISCUSSION

Six different types of test fats were incorporated into the diets of 29 men. The subjects' blood pressures and red blood cell magnesium, calcium, and potassium were measured at intervals throughout one of six diet periods, although the original plan of this study was to analyze blood pressures and red blood cell minerals from two or three diet periods.

An average of seven days of food intake records was used to compute the dietary data in this study. Several days of food intake records have been shown to be more accurate than 24-hour recalls in determining usual and current intake of nutrients (144).

Subjects were asked to keep three days of food intake records at three different times throughout diet period four, during week 0, week 3, and week 6, rather than for nine consecutive days. Record keeping for longer than seven consecutive days may become tedious and result in greater error (144).

The majority of the subjects in this study consumed less than recommended (goal) levels of calories from fat and calories from modified fat (Tables 8-9). This may have been due in part to the less than favorable response to the taste, smell, and appearance of some of the modified foods, and to the difficulty that some of the subjects had modifying their usual intake of fat, particularly if the subjects typically ate a diet low in fat relative to the study's goals.

Average fat intake among study participants was similar to reference values found in the literature (145). Although fat intake

was higher than the level which is recommended for Americans by the American Heart Association, National Research Council, and National Cholesterol Education Program (146), fat intake was comparable to typical fat intake among Americans, particularly among American men aged 19-50 years (147).

Linoleic acid intake, expressed as percentage of calories, varied according to treatment group and test fat (Table 11). Linoleic acid intake for subjects in the Parkay group, however, was similar to values found in the literature (145).

The crude palm oil products were the least acceptable, and several of the subjects from the crude palm oil treatment group complained of a "motor oil"-like taste and smell, particularly when cooking with the products. Ironically, the average percentage of calories from modified fat within the crude palm oil treatment group was the second highest among all treatment groups, contrary to what the author had expected (Table 8). The average percentage of calories from modified fat within the butter group, on the other hand, was the highest among all treatment groups, as the products were generally well accepted (Table 8).

The average intakes of calcium and potassium in all treatment groups met and exceeded the Recommended Dietary Allowance (RDA) of 800 mg of calcium and the Estimated Minimum Requirement of 2000 mg of potassium. The average intake of magnesium, however, did not meet the RDA of 350 mg in any of the treatment groups (142). Although the average intake of magnesium did not meet the RDA, the average intake of magnesium, expressed as a percentage of RDA, did compare favorably

to the values found in the literature (Table 10) (143).

Average calcium intake among study participants was higher than reference values found in the literature (Table 10) (145). High levels of dietary calcium among study participants may have been attributed to the fact that the participants were encouraged to drink milk and eat ice cream provided in the study in order to meet the study's goals for intake of test fats.

Average potassium intake among study participants was similar to values found in the literature (145), although slightly higher than reference values (Table 10). Differences in intake may have been due to factors such as geographic or regional differences in availability of food and food preferences, seasonal differences, and socioeconomic status.

At the beginning of this study, one of the hypotheses had been that blood pressure would decrease in subjects following diets high in polyunsaturated fatty acids (linoleic acid). No evidence was presented from this study to support this hypothesis.

There has been evidence presented in the literature which indicates that tissue prostaglandin synthesis can be altered by increasing the amount of linoleic acid in the diet. Because linoleic acid acts as a precursor for prostaglandin synthesis, an increase in linoleic acid would essentially increase the substrate concentration for prostaglandin synthesis.

Polyunsaturated fats and oils have also been shown to increase the permeability of the RBC membrane to ions. An increase in the degree of unsaturation makes a membrane more permeable, and this may

affect efflux of electrolytes from red blood cells. However, in this study, there was no evidence of this as there were no significant changes in RBC minerals. Previous studies have shown an increased efflux of electrolytes, including potassium, from erythrocytes in subjects after following an oleic acid-enriched diet for three weeks. In one study, the oleic acid content of erythrocyte membranes was increased while the saturated fatty acid composition was decreased. This decrease in the saturated fatty acid composition was associated with an increase in potassium efflux (130). Hunt and colleagues noted that one way that membrane composition may be affected by serum lipids is by changing the pore size of the membrane, thereby affecting the passive leak of ions (133).

Both systolic and diastolic blood pressures among study participants were lower than reference values found in the literture for adult men (148). This may be attributed to the fact that only normotensive men were involved in this study. In other words, the values found in the literature would reflect blood pressures from a representative sample of the total population, not just from normotensives. Had hypertensive subjects been included in this study, it is possible that greater differences in blood pressure would have been seen as a result of treatment. However, other studies have shown decreases in blood pressure even among normotensive subjects in response to certain dietary factors (20,94,95).

Significant changes in blood pressure in response to dietary linoleic acid supplementation have been seen in human studies of

relatively short duration (19,20). Comberg and colleagues demonstrated a 10 mmHg decrease in diastolic blood pressure in hypertensive men after just three weeks of supplementation with linoleic acid (19). Similarly, Heagery and coworkers showed a decrease in blood pressure in normotensive men after four weeks of supplementing the diet with 3 grams of safflower seed oil capsules (20). Therefore, it can be concluded that a diet period of six weeks duration should have been of sufficient length to detect changes in blood pressure in response to dietary fats.

There is conflict in the literature about which component of blood pressure is the more reliable predictor of risk of CHD. Researchers have shown that both pressures were statistically significant indices of risk of CHD, and an elevation of either pressure is a good predictor of the risk of CHD (37-38). Therefore, statistical tests were performed on both the systolic and diastolic blood pressures, which only acted to increase the blood pressure data pool.

Cellular mineral levels in this study were measured using atomic absorption spectrophotometry, a method used to determine mineral levels in biological materials. In this study, the biological materials (red blood cells) were first ashed, and after simple dilution, were aspirated into a flame. The light absorbed at a particular wavelength, corresponding to the mineral being measure, is compared with the absorption of a standard mineral solution. The absorptions of light at the specific wavelengths are proportional to the concentrations of minerals in the sample.

The red blood cell calcium concentrations calculated in this study compared quite favorably to concentrations in other studies involving erythrocyte calcium. Calcium concentrations in this study ranged from 10.48 to 16.24 PPM (0.524 to 0.736 mEq/1). Normal levels found in the literature ranged from 12.0 to 28.0 PPM (0.6-1.4 mEq/1) (149).

Potassium levels in this study were significantly lower than values found in the literature, probably due to overdilution of the samples prior to analysis on the spectrophotometer. The atomic absorption reading did not fall within the working range and therefore cannot be compared to values in the literature. Potassium concentration in this study ranged from 1754.98 to 2996.29 PPM (175.49 to 299.63 mg/100 ml). Concentrations found in the literature range from 3710 to 6050 PPM (371 to 605 mg/100 ml) (149).

Magnesium concentrations ranged from 365.77 to 996.47 PPM (36.58 to 99.65 mg/100 ml) in this study, a much wider range than that found in the literature. Ranges in the literature from studies involving direct measurement of erythrocyte magnesium are from 454.0 to 655.0 PPM (45.4 to 65.5 mg/100 ml) and 400 to 612 PPM (40.0-61.2 mg/100 ml) (150). Values involving indirect measurement ranged from 44.0 to 80 PPM (4.4 to 8.0 mg/100 ml) (150). Other erythrocyte magnesium concentrations found in the literature include 26 to 131 PPM (2.6 to 13.1 mg/100 ml) and 34 to 56 PPM (3.4 to 5.6 mg/100 ml) (149), and 45.71 to 63.0 PPM (4.57 to 6.30 mg/100 ml) (151).

The results of this study suggest no relationship between blood pressures and red blood cell mineral concentrations. At the

beginning of the study, one hypothesis was that there would be such a relationship. Specifically, a positive correlation was expected to be found between blood pressure and red blood cell calcium. The fact that no evidence to support this hypothesis was observed is contrary to evidence presented in other studies which indicated that intracellular calcium initiates smooth muscle contraction, and a high intracellular calcium enhances the contractile response to vasoconstrictive stimuli in systemic HTN (89). An impaired intracellular calcium content has been confirmed in patients suffering from essential HTN (86). This finding however, does agree with the results of Cooper and colleagues, who reported that intracellular calcium was not correlated with blood pressure in either hypertensives or normotensive subjects (152). Once again, it must be noted that all subjects in this study were normotensive, which may account for the results.

An inverse relationship between blood pressure and red blood cell magnesium and potassium also was expected. However, results from this study did not indicate such a relationship. This is contrary to evidence presented in the literature in which magnesium deficiency aggravated high blood pressure in spontaneously hypertensive rats (51), or with evidence that even small reductions in erythrocyte magnesium led to increased blood pressure (153). This does agree with evidence, however, that plasma and erythrocyte magnesium did not differ significantly between normal pregnant and pregnancy-induced hypertensives (75). In addition, this does not agree with the concept that a decrease in intracellular potassium

causes the cell to be more easily excited and may play a role in the development of HTN.

No significant changes in red blood cell magnesium or calcium concentration were found in this clinical trial as a result of a diet high in any particular test fat. This is contrary to evidence presented in the literature which indicates that dietary linoleic acid supplementation may effect the flux of ions across the cell membrane (131). This is consistent with results from previous studies which indicate that dietary linoleic and oleic acids are unlikely to have major effects on membrane-dependent functions of red blood cells (135).

Although the lifespan of the red blood cell is approximately 120 days, the diet period of this clinical trial was of six weeks duration. It is probable that greater differences among erythrocyte mineral concentrations may have been seen as a result of treatment if the length of the treatment period had corresponded more closely to the lifespan of the red blood cell. Heagerty and colleagues, however, reported that after just four weeks of supplementing the diet with linoleic acid, significant changes were seen in sodium transport across the plasma membrane (20). Other studies also have shown significant changes in the fatty acid composition and function of the red blood cell membrane after just a few weeks (130).

In this study, dietary calcium was not correlated with blood pressure. This disagrees with results from other studies which showed a change in either diastolic blood pressure alone (95), a decrease in systolic blood pressure with calcium supplementation (90-

92), or in both systolic and diastolic blood pressures (93-95.) Even normotensive men showed decreases in systolic and diastolic blood pressures in response to calcium supplementation (93,94).

There was no evidence presented in this study of a relationship between dietary minerals and red blood cell mineral concentrations. Moser and colleagues reported similar findings that dietary magnesium was not significantly correlated with the level of magnesium in either the plasma or erythrocytes (154).

A significant positive correlation was found among all red blood cell mineral levels. Magnesium was positively correlated with both calcium and potassium. Calcium was positively correlated with magnesium and potassium, and potassium with magnesium and calcium. The fact that magnesium and calcium were positively correlated is contrary to evidence which indicates that low levels of intracellular magnesium may cause an increase in cytosolic calcium (86). The subjects in the present study, however, were not magnesium deficient, and therefore probably did not have abnormally elevated intracellular calcium levels as a result.

The positive correlation between intracellular concentrations of magnesium and potassium found in this study have been similarly demonstrated in previous studies (76,78). Magnesium deficiency has also been related to decreased concentrations of potassium in lymphocytes (77).

# CONCLUSION

This study demonstrates that neither systolic nor diatolic blood pressure changed significantly in subjects who followed diets rich in crude or refined palm oil, butter or Parkay margarine, sunflower oil, or a sunflower/palm oil blend.

This particular study found no evidence of a relationship between blood pressures and red blood cell mineral concentrations.  A positive correlation was expected to be found between intracellular calcium concentrations and blood pressure, and an inverse relationship was hypothesized between intracellular magnesium and potassium.  However, all subjects in this study were normotensive, therefore it is difficult to conclude from this study alone that there is no relationship between blood pressure and erythrocyte minerals.

This study did not find any change in blood cell mineral concentrations in subjects after following a diet rich in any particular test fat.

Red blood cell calcium concentration was found to be correlated with both dietary calcium and potassium.  No other correlations were found between mineral intake and mineral concentration in red blood cells.

All three erythrocyte minerals were found to be positively correlated with each other.  The relationship between magnesium and potassium concentrations is supported by findings from previous studies, but the relationship between intracellular magnesium and calcium contradicts previous research.

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# APPENDIX

1% Lanthanum Chloride Solution:
Formula Weight $LaCl_3.7H_2O$ = 371.38
15.142 grams $LaCl_3$/1000 ml $H_2O$


5% EDTA Solution:
Formula Weight $Na_2C_{10}H_{14}O_8N_2.2H_2O$ = 372.24
52.54 grams/1000 ml


10% Nitric Acid Solution:
100 ml nitric acid/1000 ml distilled deionized $H_2O$



(50 ml/weight of sample 1) X spectrophotometer reading X Dilution
Factor = PPM 1

(50 ml/weight of sample 2) X spectrophotometer reading X Dilution
Factor = PPM 2

(PPM 1 + PPM 2) / 2 = Average PPM

Dilution Factors:
|           |     |
|-----------|-----|
| Magnesium | 500 |
| Potassium | 397 |
| Calcium   | 1   |

## VITA

Noreen Elaine Wehrman was born to Laura Therese (Siegrist) Cichosz and Kenneth John Cichosz of Lewiston, New York. She graduated in 1988 with a Bachelors Degree in Nutrition from Penn State University, University Park, Pennsylvania. Noreen is married to Michael Eugene Wehrman of Billings, Montana and is a nutrition consultant in Lincoln, Nebraska. Her permanent mailing address is 2320 North 68th Street, Lincoln, Nebraska, 68507.