

To the Graduate Council:

I am submitting herewith a thesis written by Gloria A. Gaskin entitled "The Influence of Dietary Intake on Hypertension and/or Erythrocyte Na^+, K^+ -ATPase Activity." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science.

Nina L. Marable
Nina L. Marable, Major Professor

We have read this thesis
and recommend its acceptance:

Margaret P. Perfield
Jean D. Perkins

Accepted for the Council:

Vice Provost and
Dean of the Graduate School

THE INFLUENCE OF DIETARY INTAKE ON HYPERTENSION
AND/OR ERYTHROCYTE Na^+, K^+ -ATPase ACTIVITY

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Gloria A. Gaskin

March 1985

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at The University of Tennessee, Knoxville, I agree that the Library shall make it available to borrowers under rules of the Library. Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made.

Permission for extensive quotation from or reproduction of this thesis may be granted by my major professor, or in her absence, by the Head of Interlibrary Services when, in the opinion of either, the proposed use of the material is for scholarly purposes. Any copying or use of the material in this thesis for financial gain shall not be allowed without my written permission.

Signature *Gloria A. Chastain*
Date *Feb 6, 1985*

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Nina L. Marable for her knowledge, guidance, and patience. Her continued support and encouragement, during the research project and throughout the author's graduate studies, are greatly appreciated. The support and suggestions of Dr. Marjorie P. Penfield and Dr. Jean D. Skinner also are appreciated. The author is grateful to the Agricultural Experiment Station for providing the financial assistance necessary to conduct this research project.

Special thanks go to the subjects involved in the study, whose participation made the project possible. Fond mention is made of Lisa Folk for sharing her skill as a phlebotomist. Thanks also is extended to Georgia Bunn for providing her typing services.

The author wishes to acknowledge Noelle Kehrberg for her assistance in perfecting the Na^+, K^+ -ATPase assay. Gratitude is expressed to Walker Stockley, John Brothers, and Gayle White for performing the Na^+, K^+ -ATPase assays. The assistance of Mary Ellen Ralph, Jackie Authur, Gayle White, and Roseann Gray with data collection is greatly appreciated. The author wishes to thank Mary Lou Conley Vann for her work with data collection and computer analysis, and whose efforts and friendship were an inspiration to the author.

Fond mention is made of Julia Pettigrew and Ruthann Swanson for their moral support, and interest in the research project. Kind regard is given to other fellow graduate students, staff, and faculty

members who were instrumental in making the author's graduate work at The University of Tennessee, Knoxville, a pleasant and memorable experience. Lastly, the author wishes to recognize her family, whose love and support contributed to the completion of this task.

ABSTRACT

Normotensive (11) and medicated hypertensive (6) female subjects ages 23 to 58 participated in blood pressure measurements, blood sampling (2 days) following a 10-hour fast, for the determination of maximum ouabain binding, and dietary assessment using 7-day records and/or food frequency forms. Maximum ouabain binding, an index of Na^+, K^+ -ATPase activity, was measured using a tritiated ouabain binding technique in which the number of ouabain binding sites per cell are determined. The number of enzyme units (Na^+, K^+ -ATPase units) was calculated from the number of binding sites per cell because the two are directly proportional.

Stepwise regression procedures in the Statistical Analysis System (SAS) (SAS Institute, 1982b) were used to analyze the relationships among mean systolic blood pressure taken prior to blood sampling (dependent variable, MBP1S), maximum ouabain binding (MBMAX), dietary intake, body mass index (BMI), and age. MBP1S and MBMAX were directly related when data from 15 of the 17 subjects were analyzed. Arthritis medications taken by two of the subjects may have an effect on the blood pressures of the subjects. For this reason, subsequent data analysis excluded these two subjects. Age and BMI were not related to either MBP1S or MBMAX.

Total Inhibition Scores and Total Vanadium Indices, indicators of the consumption of foods containing in vitro Na^+, K^+ -ATPase inhibitors and/or vanadium, were calculated per subject (17) from food frequency data. No associations were found among MBPIS, MBMAX, and the consumption of foods containing in vitro Na^+, K^+ -ATPase inhibitors and/or food containing vanadium (an inhibitor of Na^+, K^+ -ATPase activity). The lack of associations may be due to the scarcity of published data on in vitro Na^+, K^+ -ATPase inhibitors or vanadium contents in foods, and/or the possible inabilities of subjects to determine the quantities and frequencies of consumption. Food frequency forms are not the best method to use when quantitative data are needed.

Energy and nutrient intake means were calculated for the 15 subjects who kept 7-day dietary records. Mean energy intakes ranged from 1002 to 2262 kcal/day, the principal source of calories for most subjects being carbohydrates. Calcium and iron intakes for several subjects were $\leq 70\%$ of the 800-mg RDA. All medicated hypertensive subjects' calcium intakes were low ($\leq 80\%$ of the RDA). None of the subjects consumed $\leq 70\%$ of the RDA for vitamin C, thiamin, riboflavin, and niacin. One subject's mean vitamin A intake was $< 70\%$ of the RDA.

Of the dietary variables analyzed using PROC STEPWISE (SAS Institute, 1982b) (energy, protein, carbohydrate, crude fiber, calcium, iron, vitamin A, vitamin C, thiamin, riboflavin, niacin, and cholesterol), only crude fiber was related to MBPIS. A negative

relationship was found between MBP1S and crude fiber intake only when MBMAX was included in the regression equation. MBMAX and crude fiber intake also were inversely related. Crude fiber may appear as a proxy for other variables that independently do not significantly alter blood pressure: including cholesterol, thiamin, niacin, and carbohydrate. Multiple vitamins taken by several subjects may have obscured the relationship between MBP1S and some of the dietary variables. This should be a consideration in future research studies conducted with a larger number of subjects.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. REVIEW OF LITERATURE.	4
Na ⁺ and K ⁺ Transport in Essential Hypertension.	4
Ion transport in human erythrocytes and leucocytes. . .	4
Na ⁺ ,K ⁺ -ATPase and essential hypertension.	7
Na ⁺ ,K ⁺ -ATPase inhibitors and essential hypertension . .	8
Dietary Intake of Essential Nutrients and Essential	
Hypertension.	10
Sodium and potassium.	10
Calcium	17
Magnesium	20
Other Environmental Factors and Essential Hypertension. .	21
Caloric intake, sucrose intake, fat intake, body	
weight, alcohol consumption, and essential	
hypertension.	21
Diet, Na ⁺ ,K ⁺ -ATPase inhibitors/enhancers and	
essential hypertension.	24
Dietary Survey Techniques	28
Diet history/dietary questionnaire.	29
Dietary record.	31
24-Hour dietary recalls	36
Food frequency.	37
III. METHODS	39
General Plan.	39
Screening Process and Description of Subjects	39
Blood Pressure Measurement.	40
Blood Sampling and Red Blood Cell Counting.	41
Determination of Maximum Ouabain Binding: An Indicator	
of Erythrocyte Na ⁺ ,K ⁺ -ATPase Activity	42
Dietary Analysis.	43
Food frequency form	43
7-Day dietary record.	53
Statistical Analysis.	55
IV. RESULTS AND DISCUSSION.	57
Blood Pressure Measurements	57
Maximum Ouabain Binding and Blood Pressure.	58
Body Mass Index, Age, Blood Pressure, and Maximum	
Ouabain Binding	68

CHAPTER	PAGE
IV. (Continued)	
Total Inhibition Scores, Total Vanadium Indices, Blood Pressure, and Maximum Ouabain Binding	69
Evaluation of Dietary Intakes	71
Energy, protein, fat, and carbohydrate intakes.	71
Calcium and iron intakes.	78
Sodium intake	79
Potassium intake.	80
Vitamin A, vitamin C, thiamin, riboflavin, and niacin intakes.	80
Fiber and cholesterol intakes	81
Effect of Maximum Ouabain Binding and Dietary Intake on Mean Systolic Blood Pressure	83
V. SUMMARY	87
REFERENCES.	90
APPENDICES.	103
A. ACTIVITY TIMETABLE: HYPERTENSION, ERYTHROCYTE Na^+, K^+ - ATPase, AND DIET STUDY.	104
B. MEMORANDUM TO PROSPECTIVE SUBJECTS.	106
C. SUBJECT QUESTIONNAIRE	109
D. CONSENT FORM.	113
E. SUBJECT CHARACTERISTICS	116
F. FOOD FREQUENCY FORM	118
G. DIETARY INTAKE RECORD FORM.	126
H. Na^+, K^+ -ATPase INHIBITION SCORE AND VANADIUM INDEX CALCULATIONS.	128
I. SERVING SIZES FOR FOODS AND BEVERAGES CONTAINING <u>IN VITRO</u> Na^+, K^+ -ATPase INHIBITORS AND/OR VANADIUM.	131
J. SUBJECT RESPONSIBILITIES.	135
K. VITAMIN SUPPLEMENT COMPOSITIONS	137
VITA.	139

LIST OF TABLES

TABLE	PAGE
1. Scaling Factors Representing the Frequency of Food Consumption.	45
2. <u>In Vitro</u> Na ⁺ ,K ⁺ -ATPase Inhibitor Levels in Foods	46
3. <u>In Vitro</u> Na ⁺ ,K ⁺ -ATPase Inhibitor Levels (Units/Ml) in Selected Beverages	48
4. Vanadium Concentrations and Vanadium Content Scores of Selected Foods	49
5. Mean Systolic Blood Pressure (MBPIS), Mean Maximum Ouabain Binding (MBMAX), and Medication Descriptions for Normotensive Control, Medicated and Controlled Hypertensive, and Medicated Moderately Uncontrolled Hypertensive Subjects	59
6. Subjects Ranked According to MBMAX with Corresponding MBPIS.	63
7. Mean Square, Significance of F Ratio, and Partial Regression Coefficients for MBPIS Versus MBMAX with 15 Subjects.	67
8. Total Inhibition Scores and Total Vanadium Indices for Each Subject Ranked by MBMAX Values.	70
9. Seven-Day Energy and Nutrient Intake Means for Individual Subjects	72
10. Mean Energy and Nutrient Intakes as Percentages of 1980 Recommended Dietary Allowances	73
11. Percentage of Mean Energy Intakes from Protein, Fat, and Carbohydrate	75
12. Analysis of Blood Pressure (MBPIS) with Various Models . . .	82

CHAPTER I

INTRODUCTION

Essential hypertension has become a predominant health problem in the United States, with an estimated 60 million Americans suffering from the disease. Individuals classified as hypertensive have blood pressures exceeding 140/90 mm Hg (Garay and Meyer, 1979; Meyer, 1980). By definition, the cause of elevated arterial blood pressure associated with essential hypertension is unknown, not attributable to any disease state (Garay and Meyer, 1979). Frohlich (1982) stated that "this disease [essential hypertension] of multifactorial origin must reflect an imbalance of a variety of mechanisms that normally serve to control arterial pressure." The factors that affect arterial pressure are both genetic and environmental in nature (De Mendonca et al., 1981; Frohlich, 1982; Hunt, 1983).

Abnormalities have been found in Na^+ and K^+ transport systems of erythrocytes and leucocytes of essential hypertensive subjects (Fregly and Kare, 1982; Garay et al., 1980a,b; Garay and Meyer, 1979; Poston et al., 1981; Postnov et al., 1977). Research indicates an association between Na^+, K^+ -ATPase, a system that transports Na^+ and K^+ ions across cell membranes, and essential hypertension (Fregly and Kare, 1982; Hamlyn et al., 1982; Morgan et al., 1981; Poston et al., 1981; Stokes et al., 1983; Woods et al., 1981). Na^+, K^+ -ATPase inhibitors in the diet may be responsible for the irregular Na^+ and

K^+ transport activity, which has been observed (Poston et al., 1981). In vitro Na^+, K^+ -ATPase inhibitors have been found in foods (Harlan and Mann, 1982; Sagnella and MacGregor, 1984). Also the consumption of foods that contain vanadium (Myron et al., 1977; Soremark, 1967), an inhibitor of Na^+, K^+ -ATPase activity (Cantley and Aisen, 1979), may have a role in the etiology of hypertension (Hom et al., 1982; Steffen et al., 1981). Other dietary factors that have been recognized as possible hypertensive or hypotensive agents include sodium (Dahl, 1977), potassium (Morgan and Myers, 1982), calcium (McCarron et al., 1984), and magnesium (Altura, 1983).

No studies relating diet and the Na^+, K^+ -ATPase activity of essential hypertensive subjects have been found in the literature. The objectives of this pilot study with normotensive and hypertensive female subjects were:

1. To measure blood pressure and erythrocyte ouabain binding, a measure of Na^+, K^+ -ATPase activity.
2. To assess nutrient intakes and the consumption of vanadium and/or Na^+, K^+ -ATPase inhibitors in foods.
3. To determine if there is a relationship between erythrocyte Na^+, K^+ -ATPase (ouabain binding) activity and blood pressure.
4. To determine if associations exist among erythrocyte Na^+, K^+ -ATPase activity and/or blood pressure and the dietary intake of calories and nutrients, (e.g., protein, fat, carbohydrate, Na^+, K^+ , Ca^{2+} , and vanadium).

5. To determine if there are relationships between the consumption of foods containing in vitro Na^+, K^+ -ATPase inhibitors (Harlan and Mann, 1982), erythrocyte Na^+, K^+ -ATPase activity (ouabain binding) and/or blood pressure.

It was hypothesized that the women with essential hypertension would have increased Na^+, K^+ -ATPase activities (Stokes et al., 1983; Woods et al., 1981) when compared to normotensive controls. It was predicted that the diets of the essential hypertensive women would be different from those of the normotensive control group.

CHAPTER II

REVIEW OF LITERATURE

Na⁺ and K⁺ Transport in Essential Hypertension

It has been postulated that essential hypertension is related to abnormalities in Na⁺ and K⁺ transport systems that regulate physiological Na⁺ and K⁺ concentrations (De Mendonca et al., 1981; Fregly and Kare, 1982; Garay et al., 1980a,b; Garay and Meyer, 1979; Hamlyn et al., 1982; Morgan et al., 1981; Poston et al., 1981; Stokes et al., 1983; Woods et al., 1981). Potassium is more concentrated than Na⁺ inside cells whereas the opposite is found in extracellular fluids. These concentration differences create an electrochemical gradient (DeLuise et al., 1980; Sweadner and Goldin, 1980). Many systems are responsible for maintaining proper physiological Na⁺ and K⁺ concentrations: Na⁺,K⁺ countertransport (Na⁺,K⁺-adenosine triphosphatase, Na⁺,K⁺-ATPase, or the Na⁺,K⁺-"pump") (Skou, 1965; Sweadner and Goldin, 1980), Na⁺,K⁺ cotransport (Fregly and Kare, 1982; Garay et al., 1980a), Na⁺,Li⁺ countertransport (Woods et al., 1982), and Na⁺,Ca²⁺ transport (McCarron, 1983a). Of these transport systems, only Na⁺,K⁺-ATPase was measured in this study.

Ion Transport in Human Erythrocytes and Leucocytes

Differences in Na⁺ and K⁺ transport in human erythrocytes and leucocytes have been found between hypertensive and normotensive

subjects. These differences in ion transport between hypertensive and normotensive subjects have been detected by studying the influx (inward movement of ions) and efflux (outward movement of ions) of Na^+ and K^+ in red and white blood cells. Poston et al. (1981) took erythrocytes and leucocytes from normotensive subjects and incubated them in the blood and serum of hypertensive patients to determine if sodium transport would be altered. Sodium efflux from the white blood cells was decreased. Edmondson and co-workers (1975) studied sodium efflux from leucocytes of essential hypertensive and normotensive patients. The sodium efflux was decreased for the hypertensive subjects. Edmondson et al. (1975) concluded that irregularities in cellular sodium transport were "directly related to hypertension." In addition, Na^+ and water contents of the leucocytes from the hypertensive subjects were higher than those of the normotensive controls (Edmondson et al., 1975).

Garay and Meyer (1979) studied the Na^+ and K^+ fluxes in the erythrocytes of essential hypertensive subjects. In the experiment, the erythrocytes were manipulated in order to induce higher intracellular sodium concentrations and low potassium concentrations. When the net Na^+/K^+ flux ratio was calculated it was found to be "consistently low" relative to the net flux ratio of the normotensive control group studied. The net Na^+/K^+ flux ratio is defined as the net Na^+ efflux/net K^+ influx (De Mendonca et al., 1981).

Garay et al. (1980b) used the same technique of determining net Na^+/K^+ flux ratios as Garay and Meyer (1979) in the development

of a procedure for identifying essential hypertension in a clinical setting. The essential hypertensive subjects had reduced Na^+/K^+ fluxes. The subjects in the study with secondary hypertension had normal net flux ratios. Thus with this technique, it may be possible to differentiate between essential and secondary hypertension (Garay et al., 1980b).

De Mendonca et al. (1981) placed the erythrocytes of normotensive and hypertensive subjects in a medium high in Na^+ and low in K^+ to study net Na^+/K^+ fluxes. The relatives of hypertensives were studied to determine the possible genetic transmission of hypertension (De Mendonca et al., 1981). Net Na^+/K^+ fluxes were reduced in the hypertensive subjects. Net Na^+/K^+ fluxes also were lower for children with two hypertensive parents, compared to those of children with only one or no hypertensive parents. Decreased net Na^+/K^+ fluxes also were noted in hypertensive parents of hypertensive subjects in the study. Net Na^+/K^+ flux ratios may serve as an indicator of someone's predisposition for developing hypertension, since consecutive generations with hypertension were found to have reduced net Na^+/K^+ fluxes (De Mendonca et al., 1981).

The work of Woods et al. (1981) and Stokes et al. (1983) also indicated abnormal ion transport in hypertensive subjects. Woods et al. (1981) and Stokes et al. (1983) used ^{86}Rb as an indicator of potassium uptake in the erythrocytes of normotensive and hypertensive subjects. In both studies Woods et al. (1981) and Stokes et al. (1983) found ^{86}Rb uptake to be greater in hypertensives than in the

normotensive controls. Morgan et al. (1981) suggested that Na^+ intake may have an effect on Na^+ efflux in persons with high blood pressure. Morgan et al. (1981) used ^{22}Na to monitor Na^+ efflux and found that in hypertensive men, blood pressure increased and Na^{22} efflux decreased as Na^+ intake increased. These studies confirmed other findings of abnormal sodium and potassium fluxes in hypertensive individuals.

Na^+, K^+ -ATPase and Essential Hypertension

Because Na^+, K^+ -ATPase is one of the transport systems that regulates Na^+ and K^+ concentrations in cells, it is reasonable to speculate that Na^+, K^+ -ATPase may have an involvement in the altered Na^+ and K^+ fluxes, which have been found in hypertensive subjects. Na^+, K^+ -ATPase functions by hydrolyzing ATP, which provides the energy necessary to transport Na^+ and K^+ ions across cell membranes (Dahl and Hokin, 1974; Glynn, 1968; Skou, 1965; Sweadner and Goldin, 1980). ATP will serve as the substrate for the reaction only when it is located inside the cell, and only if Na^+ , K^+ , and Mg^{2+} are present (Dahl and Hokin, 1974; Glynn, 1968; Sweadner and Goldin, 1980). For each ATP molecule hydrolyzed in the cell, three Na^+ ions are exchanged for two K^+ ions (Glynn, 1968; Sweadner and Goldin, 1980). Cardiac glycosides such as ouabain, when located outside the cell, will inactivate Na^+, K^+ -ATPase (Dahl and Hokin, 1974; Glynn, 1968; Sweadner and Goldin, 1980), resulting in an increased extracellular sodium concentration (Blaustein, 1977).

Woods et al. (1981) and Stokes et al. (1983) concluded from their work with ^{86}Rb influxes in the erythrocytes of hypertensive

subjects that Na^+, K^+ -ATPase activity was elevated in the hypertensive subjects. The researchers based their conclusions on the results obtained when the ^{86}Rb assay was conducted with and without the addition of ouabain. The ouabain-sensitive ^{86}Rb uptake was greater in the hypertensive subjects, indicating increased K^+ influx, and consequently increased Na^+, K^+ -ATPase activity. Garay et al. (1980a) measured Na^+/K^+ fluxes of Na^+ -loaded/ K^+ -depleted erythrocytes with the addition of ouabain. Because K^+ influx was increased in hypertensive subjects, the researchers inferred that Na^+, K^+ -ATPase also was increased in hypertensive subjects. Garay et al. (1980a) attributed the elevated activity to a defect in the Na^+, K^+ co-transport system. It was postulated that the Na^+, K^+ -pump is attempting to compensate for the Na^+, K^+ co-transport defect (Garay et al., 1980a).

Na^+, K^+ -ATPase Inhibitors and Essential Hypertension

After incubating leucocytes of normotensive subjects in the blood and serum of hypertensive subjects, and finding a decrease in sodium efflux, Poston et al. (1981) postulated that abnormalities in sodium efflux may be attributed to an increase in a Na^+, K^+ -ATPase inhibitor. Other researchers also have indicated the possible involvement of a Na^+, K^+ -ATPase inhibitor in the etiology of hypertension (Hamlyn et al., 1982; MacGregor and de Wardener, 1981; MacGregor et al., 1981; Morgan et al., 1981). Hamlyn et al. (1982) investigated the presence of a Na^+, K^+ -ATPase inhibitor in the plasma of hypertensive subjects. The assay used by Hamlyn et al. (1982) involved

monitoring the effects of deproteinized plasma of hypertensive and normotensive subjects, on Na^+, K^+ -ATPase derived from canine kidney. Inhibition of Na^+, K^+ -ATPase was measured by "coupling" NADH absorption with the hydrolysis of ATP. If ATP hydrolysis decreased, inhibition of the enzyme (Na^+, K^+ -ATPase) was assumed to increase. In the experiment there was no significant difference in ATPase activity between the plasma of normotensive and the saline control. However, 14% inhibition of Na^+, K^+ -ATPase was noted in tests on the plasma of the hypertensive subjects. Comparisons of inhibition were made for ouabain, vanadate ions, and the plasma of the hypertensives. The "onset" of inhibitory activity exhibited from the plasma of hypertensives was faster than both ouabain and vanadate (Hamlyn et al., 1982).

MacGregor and de Wardener (1981) and MacGregor et al. (1981) postulated that a defect in the kidneys of hypertensive subjects may be responsible for an increase in a circulating Na^+, K^+ -ATPase inhibitor. The inherited defect in the kidney results in the kidney's inability to excrete sodium as it should, causing extracellular volume to increase. Increased extracellular volume stimulates the excretion of a Na^+, K^+ -ATPase inhibitor. The Na^+, K^+ -ATPase inhibitor functions by increasing sodium excretion, but at the same time inhibits sodium transport within cells other than the kidney, resulting in intracellular volume increases. Based on the work of Blaustein (1977), MacGregor and de Wardener (1981) concluded that the increased intracellular volume is due to increased intracellular calcium concentration, which ultimately increases smooth muscle tone and causes increased peripheral resistance and high blood pressure.

Dietary Intake of Essential Nutrients and Essential Hypertension

Dietary intake is one of the environmental factors that has been studied as a cause of essential hypertension. The relationship between diet and hypertension remains a controversial issue. In evaluating the Coronary Prevention Evaluation Program (CPEP), Stamler et al. (1980) concluded that alterations in the diet (without medication) were effective in reducing and controlling the hypertension of "middle-aged coronary-prone obese men." Dietary components thought to influence blood pressure include the essential nutrients Na^+ , K^+ , Ca^{2+} , and Mg^{2+} .

Sodium and Potassium

Allen (1920) was one of the first investigators to suggest that a positive relationship existed between salt intake and hypertension. A positive relationship between sodium intake and the prevalence of hypertension has been found in numerous epidemiological studies. In cultures where sodium consumption is low, hypertension is at a minimum (Dahl, 1977; Hunt, 1983). Freis (1976) advocated the necessity for reducing dietary sodium. It was his opinion that excessive salt intake is the cause of hypertension because increased salt intake results in increased extracellular fluid volume, accompanied by an increase in hypertension. An increase in potassium has been advocated for treating hypertension (Dustan, 1983; Morgan and Myers, 1982; Skrabal et al., 1981). This theory deserves

consideration because epidemiological studies indicate that in populations with a low sodium intake and where the incidence of hypertension is rare, the consumption of dietary potassium is high (Hunt, 1983).

In an early study by Dahl and Love (1957), a positive correlation was found between salt usage and hypertension. It was concluded that high salt users were at a greater risk for developing hypertension, and that overweight subjects were more likely to have hypertension than were non-overweight subjects. It must be noted however, that the method used by Dahl and Love (1957) to estimate salt usage was crude at best. Evaluation of salt usage was based on whether the subject salted food before or after tasting (Dahl and Love, 1957). Contrary to these findings, McCarron et al. (1984) found when examining a subgroup from the Health and Nutrition Examination survey I (HANES I), that a negative correlation existed between sodium intake and hypertension.

Morgan et al. (1978) also found a positive relationship between salt intake and hypertension. The researchers compared 31 patients who had diastolic blood pressures between 95 and 100 mm Hg, and who were receiving various treatments for their hypertensive conditions. The subjects were either under no treatment, following a moderately salt restricted diet, or taking antihypertensive drugs. The patients on the salt-restricted diet (for a two-year period) showed the greatest decrease in diastolic blood pressure. Patients on the antihypertensive drug regime had blood pressure reductions

similar to those of the salt-restricted group, whereas the untreated patients experienced an increase in hypertension. Morgan et al. (1978) thought that an even greater reduction in blood pressure would have been produced if the salt-restricted diet had been followed with greater precision. They recommended that individuals with diastolic blood pressures between 90 and 105 mm Hg attempt a salt-restricted diet before resorting to drug therapy. Morgan and Myers (1982) suggested that subjects with diastolic blood pressure between 96 and 105 mm Hg try to regulate blood pressure by reducing salt intake, prior to the administration of blood pressure medications. The researchers also reported that diets low in sodium and high in potassium were successful treatments for reducing high blood pressure (Morgan and Myers, 1982).

In opposition to Morgan et al. (1978) and Morgan and Myers (1982), Laragh and Pecker (1983) suggested that sodium restriction be administered as therapy only to patients "in whom its effectiveness has been established," since salt restriction is not effective in all subjects. Only 30%-50% of essential hypertensive subjects experience a response to sodium reduction (Laragh and Pecker, 1983). Dahl (1977) and co-workers were among the first researchers to postulate that genetic factors must exist relative to hypertension and salt ingestion, since not all hypertensive rats (or humans) are affected by salt intake. Kawasaki et al. (1978) illustrated the presence of genetic factors by classifying 19 essential hypertensives as salt-sensitive or non-salt sensitive according to their blood

pressure responses to diet regimes ranging from 9 to 240 meq of sodium per day. Differences in responses were noted between the subjects, and thus they were classified accordingly as salt-sensitive or non-salt sensitive. Sodium loading increased the blood pressures of the salt-sensitive subjects studied, but not the blood pressures of the non-salt sensitive subjects studied. In addition, the salt-sensitive patients excreted less sodium than the non-salt sensitive subjects, during periods of sodium loading and sodium deprivation.

Parfrey et al. (1981) studied the effects of different sodium and potassium intakes on the blood pressure of young men with and without hypertensive parents. The subjects were classified according to whether one or both of their parents had hypertension (PHT group), or neither of their parents had hypertension (PNT group). Increased Na^+ intake caused the blood pressure in both groups (PHT and PNT) to increase. Lowering Na^+ intake and supplementing the subjects' diets with K^+ caused the blood pressure of the PHT group to fall, whereas a decrease in the blood pressure of the PNT group was not noted. The researchers concluded that sodium caused an increase in blood pressure for both subjects predisposed and not predisposed to hypertension. It was hypothesized that genetic factors may influence one's response to change in intake because the subjects predisposed to hypertension were the only subjects who responded to increased K^+ intake. Parfrey et al. (1981)

also concluded that persons predisposed to high blood pressure may be more inclined to develop hypertension if their intake of K^+ is not sufficient.

Myers et al. (1983) studied the effects of increased Na^+ intake on erythrocyte Na^+ concentration and blood pressure of normotensive and hypertensive subjects. "The subjects were given reduced or high sodium intakes for 2 week periods using a randomized crossover design." The amounts of the sodium intakes were not indicated in the study. Normotensive subjects had no change in systolic or diastolic blood pressure with increased sodium intake, but the sodium concentration in the red blood cells did increase significantly. For hypertensives, systolic blood pressure rose as sodium intake was increased, and intracellular sodium concentration increased with increasing sodium intake. These results support the theory that increased sodium intake affects ion transport systems in the body (Myers et al., 1983).

Today, disagreement exists as to what advice should be given to the general public concerning sodium consumption. Kolata (1982) quoted Graham Ward, coordinator for the National Blood Pressure Evaluation Program of the National Institute of Health, as stating that not enough scientific evidence exists to warrant the establishment of a maximum level of sodium intake for the general public. On the other hand, it was the opinion of Arthur Hull Hayes, Commissioner of the FDA, that there should be a national campaign for sodium reduction (Kolata, 1982). McCarron et al. (1984) found no relationship

between the sodium intake of hypertensive and normotensives, but when the entire population was studied (10,372 individuals), a negative relationship existed between Na^+ intake and blood pressure. It is evident that research needs to continue until the role of sodium in hypertension is clarified.

Ophir et al. (1983) studied the prevalence of high blood pressure among vegetarians and non-vegetarians living in the Tel Aviv area. Persons were considered hypertensive if their blood pressure exceeded 160/95 mm Hg. For a significant time period, the vegetarians had been following an established vegetarian diet, which included no meats and an abundance of fruits, vegetables, and nuts. Urinary sodium and potassium excretions were the bases for estimating sodium and potassium intakes. Sodium excretion was similar for all subjects, but the vegetarian group excreted 26% more potassium than the non-vegetarian group. An inverse correlation was found for the vegetarian group between K^+ excretion and blood pressure. Two percent of the vegetarians were hypertensive, whereas 26% of the non-vegetarian group were hypertensive. Ophir et al. (1983) concluded that the non-vegetarians and the vegetarians were at the same risk for developing hypertension, but that the vegetarians' high dietary intake of potassium produced a "protective" effect against developing high blood pressure.

On the other hand, Gros et al. (1971) indicated that potassium did not affect hypertension. The diets of seven untreated essential hypertensives were supplemented with 6 g of sodium per day for two

to nine weeks. In a subsequent experiment the subjects were given a mixture of 6 g sodium and 6 g potassium to consume for four weeks. In both cases the dietary supplements did not affect body weights or blood pressures of the subjects studied. Also the supplements had no effect on blood pressure even when the patients were taking anti-hypertensive drugs (Gros et al., 1971). It was noted in the study that the levels of sodium and potassium administered were almost unpalatable. Possible explanations of the results include that:

(1) the dosages of sodium and potassium used may have reached a saturation level at which point the effects of sodium and potassium on high blood pressure are no longer noticeable; (2) the sample size used was small; (3) the subjects may not have been salt-sensitive; and (4) the experimental design did not include the administration of potassium without sodium.

In reviewing the role of potassium in hypertension, Treasure and Ploth (1983) attempted to explain the hypotensive activity of potassium. Potassium may function as a diuretic agent which causes blood pressure to decline, especially in the "salt-dependent" forms of hypertension (Treasure and Ploth, 1983). Treasure and Ploth (1983) suggested that potassium may act as a vasodilator in conjunction with Na^+, K^+ -ATPase activity. Their hypothesis is based on the fact that ouabain inhibits both "potassium-induced vasodilation" and Na^+, K^+ -ATPase activity (Chen et al., 1972). Based on the results of Blaustein (1977), Treasure and Ploth (1983) speculated that increasing potassium intake may initiate Na^+, K^+ -ATPase activity, which

results in Na^+ extrusion from cells with high intracellular Na^+ concentrations. As a result, Na^+ is able to passively enter cells and Ca^{2+} will simultaneously leave cells, causing a decrease in peripheral resistance (because of decreased intracellular calcium) and a reduction in blood pressure (Treasure and Ploth, 1983).

Calcium

Recent evidence indicates a possible association between calcium and hypertension (McCarron, 1983a; McCarron et al., 1984). It is reasonable to speculate that calcium has an influence on blood pressure, since it can affect peripheral resistance and cardiac output (McCarron, 1983b). McCarron (1982) noted that, in untreated essential hypertensive subjects, serum calcium levels were normal, but levels of serum ionized calcium were low. The low serum ionized calcium levels were attributed to increased protein binding of the extracellular calcium. In a letter to the New England Journal of Medicine, Schussler (1982) disagreed with the conclusions of McCarron (1982). Schussler (1982) stated that a low concentration of serum ionized calcium and increased protein binding were not the causes of hypertension, since it has not been proven that hypertension may be caused by "hypocalcemia due to malabsorption, renal tubular acidosis, or hypothyroidism." In an epidemiological study evaluating dietary intake, McCarron et al. (1982) reported that hypertensive subjects consumed less calcium in the form of non-fluid dairy products than did normotensive controls. "The data suggest that inadequate calcium

intake may be a previously unrecognized factor in the development of hypertension" (McCarron et al., 1982). McCarron et al. (1984) used data from the Health and Nutrition Examination Survey I (HANES I) to examine the relationship among nutrient intakes, blood pressure, and body mass index (BMI). When age, sex, and race were controlled for in the study population (10,372 subjects), the hypertensive subjects had lower nutrient intakes of Ca^{2+} as well as vitamin A and vitamin C, than did the normotensives. Before age, sex, and race were controlled for in the analysis, and with hypertension being defined as a systolic blood pressure greater than or equal to 160 mm Hg, the intakes of linoleic acid and Ca^{2+} were lower for hypertensive subjects than for normotensive subjects. McCarron et al. (1984) also noted that as body mass index (BMI) increased, calcium consumption decreased. When the study-population data were divided into groups based on the subjects' daily intakes of Ca^{2+} , K^+ , and Na^+ , the intake of each nutrient was negatively correlated with systolic blood pressure. McCarron et al. (1984) concluded that "the risk of being hypertensive increased" as the consumption of Ca^{2+} , K^+ , and Na^+ decreased.

Kesteloot and Geboers (1982) investigated the relationship between serum calcium and blood pressure among men (9321) and women (421) in the Belgian army. Blood samples were taken for the determination of serum calcium. Urinary calcium excretion was estimated from 24-hour urine collections for a sub-group of the original population. A positive correlation was found between serum calcium and systolic and diastolic blood pressure only for the men in the study.

Urinary calcium excretion correlated positively with diastolic blood pressure. These results of positive relationships between calcium and blood pressure are in opposition to those of McCarron et al. (1984). Serum ionized calcium was not measured (Kesteloot and Geboers, 1982).

The work of Belizan et al. (1983) is an example of a controlled double-blind study that illustrates the effects of calcium supplementation on blood pressure. A study population was made up of men and women who were given either calcium tablets (equivalent to 1 g of elemental calcium) or a placebo to take daily for a 22-week period. Total calcium intake was assessed using a 1-day dietary record, and through interviews in which the examiner determined the consistency with which the subjects followed the prescribed treatment. Belizan et al. (1983) found a decrease in diastolic blood pressure of the calcium-supplemented men and women studied. However, it would be inappropriate to extrapolate these results to hypertensive subjects, because the study population included only normotensive individuals.

An inverse relationship between calcium consumption and blood pressure had been observed in a Lipid Research Clinic prevalence study conducted between 1972-1974. The study was originally designed to assess risk factors in heart disease. Ackley et al. (1983) re-examined the data collected in 1972-1974 to determine if the results were independent of age, obesity, and alcohol consumption. The data collected for the study were divided into two parts. First the

entire population of 5050 adults was examined, referred to as Visit 1. Secondly, a 15% randomized sample was chosen from the original population to participate in a more involved evaluation, designated as Visit 2. Ackley et al. (1983) used data collected from Visit 1 on milk consumption and data from a 24-hour dietary recall taken in Visit 2 to assess calcium intake from dairy products.

Following adjustments for age, obesity, and alcohol consumption, a significant decrease in the diastolic blood pressure was noted for the men as their whole milk consumption increased. No significant relationships were observed between the blood pressures of the women and their milk consumption. When additional analyses were conducted, a significant negative correlation existed between the men's calcium intake from all dairy sources and diastolic blood pressure (Ackley et al., 1983). It was noted that the inclusion of non-dairy products would have affected the calcium intake, possibly producing different results. Also other components in the diet that may have affected blood pressure levels were not examined.

Magnesium

Altura and Altura (1981) have identified several modes of action by which a deficiency in magnesium may result in the elevation of blood pressure. A decreased level of serum Mg^{2+} may stimulate the activity of smooth muscle constrictors, such as angiotension and epinephrine. Since vascular resistance would be increased, an elevation in blood pressure could result. Decreased concentrations of

serum and tissue magnesium could affect blood pressure by acting on neuro-humoral substances which normally function by initiating relaxation of peripheral blood vessels. Prostaglandins are examples of neuro-humoral substances. Another mode by which Mg^{2+} may function in preventing hypertension is through its competition with Ca^{2+} at smooth muscle cellular membrane binding sites. By competing with Ca^{2+} for the binding sites, Mg^{2+} would decrease the concentration of Ca^{2+} in the cell membrane. The substitution of Mg^{2+} for Ca^{2+} would be effective because increases in Ca^{2+} instigate the contraction of vascular smooth muscle (Altura, 1983; Altura and Altura, 1981; Altura et al., 1981).

Other Environmental Factors and Essential Hypertension

Other factors that may influence hypertension are caloric intake (Young et al., 1978), sugar intake (Buñag et al., 1983; Rebello et al., 1983), fat intake (Iacono et al., 1983; Smith-Barbaro and Pucak, 1983), body weight (Reisin et al., 1978; Stamler et al., 1978), and alcohol consumption (Arkwright et al., 1982). In vitro Na^+, K^+ -ATPase inhibitors and enhancers found in food (Harlan and Mann, 1982) and dietary vanadium consumption also may affect essential hypertension (Steffen et al., 1981).

Caloric Intake, Sucrose Intake, Fat Intake, Body Weight, Alcohol Consumption, and Essential Hypertension

Caloric and sugar intakes. Young et al. (1978) illustrated using spontaneously hypertensive rats that a decrease in caloric

intake (with no sodium restriction) resulted in a reduction in blood pressure. Buñag et al. (1983) found that administering an 8% sucrose solution for 5 weeks to male weanling rats caused systolic pressure to become elevated. Rebello et al. (1983) demonstrated using 20 normotensive males that the consumption of various sugar solutions caused elevation of systolic blood pressure. Sugar solutions (20% w/v) were prepared based on the subject's body weight (1 g sugar/kg body weight). The experiment lasted for 6 hours, beginning after a 12-hour fast. The 6-hour period was divided into three 2-hour intervals: the presugar period, the sugar period, and the postsugar period. Thus, the sugar solutions were administered 2 hours after the experiment began. Consumption of sucrose solutions and glucose solutions caused an elevation in blood pressure, but fructose, galactose, and lactose solutions had no significant effect on blood pressure (Rebello et al., 1983).

Fat intake. In a review article by Smith-Barbaro and Pucak (1983), it was suggested that the type of fat consumed influences blood pressure. The consumption of polyunsaturated fats, especially linoleic acid may aid in the reduction in blood pressure (Smith-Barbaro and Pucak, 1983). Iacono et al. (1983) also studied fat intake in relation to blood pressure. Thirty normotensive Finnish couples in a dietary intervention study were instructed to decrease their fat consumption and increase their polyunsaturated/saturated fat ratio. The subjects' dietary fat modifications were accomplished by substituting foods similar to those found in their normal diet, but

with lower fat contents. For example, during the intervention period, whole milk was replaced by skim milk, and low fat cheeses, leaner meats, and margarine rather than butter were consumed. The study lasted a total of 13 weeks, which included one week of baseline evaluation, a 6-week intervention period, and a 6-week follow-up period in which the subjects resumed eating normally. Food consumption records were kept, and proximate analyses of food composites were determined to assess dietary intakes. Both systolic and diastolic blood pressure decreased significantly during the low fat consumption period. Once the subjects began consuming their normal diet with increased fat intake and a decreased polyunsaturated/saturated fat ratio, blood pressure was increased compared to the blood pressure measurements at the end of the intervention period. From these results it is possible to conclude that fat intake may alter blood pressure. However, other components of the diet were altered simultaneously with fat. Notably, as fat intake decreased, protein and carbohydrate intakes increased.

Body weight. Stamler et al. (1978) noted an association between hypertension and body weight. The results of the screening of one million Americans by the Community Hypertension Evaluation Clinic showed that the incidence of high blood pressure was 50%-300% greater in overweight subjects compared to normal and underweight subjects (Stamler et al., 1978). Reisin et al. (1978) studied overweight hypertensive subjects and changes in blood pressure

following weight loss. The subjects were categorized according to whether they were taking antihypertension drugs and/or whether they were participating in the weight reduction program. All subjects (medicated and unmedicated) in the weight reduction group lost at least 3 kg, and 75% of the non-medicated group and 61% of the medicated group reduced their blood pressure to less than 140/90 mm Hg. An increase in blood pressure was noted for 27% of the group not participating in weight reduction. Thus, Reisin et al. (1978) concluded that a positive relationship existed between weight loss and blood pressure. The results of Reisin et al. (1978) occurred without any specified alterations in salt intake.

Alcohol consumption. The prevalence of hypertension is thought to increase in conjunction with alcohol consumption (Arkwright et al., 1982). Arkwright et al. (1982), when studying a population of working men in Australia, found that the prevalence of elevated systolic blood pressure in heavy and moderate drinkers was four times that of non-drinkers. However, the investigators concluded that the effect of alcohol on blood pressure was reversible, since individuals who were once heavy drinkers had blood pressures comparable to the non-drinkers (Arkwright et al., 1982).

Diet, Na^+ , K^+ -ATPase Inhibitors/Enhancers and Essential Hypertension.

Another means by which the diet may influence hypertension is through the inhibition of Na^+ , K^+ -ATPase because abnormalities in cation transport (Garay et al., 1980a; Stokes et al., 1983; Woods

et al., 1981) or ouabain binding (Erdmann et al., 1979) have been associated with essential hypertension. Harlan and Mann (1982) identified the presence of in vitro Na^+, K^+ -ATPase inhibitors and enhancers in foods. Samples of foods were dried and extracted in acetone, methanol, and water. Different levels of inhibitors were detected in the food extracts, depending on the solvents used, indicating the presence of "multiple inhibitory compounds" (Harlan and Mann, 1982). Extracts that contained potent inhibitors were obtained from tea, cocoa, Sanka, yellow squash, pineapple, and lettuce. Pepsi Cola and Coca Cola extracts were found to stimulate Na^+, K^+ -ATPase activity. Harlan and Mann (1982) suggested that the inhibitors/enhancers in these foods were polyphenol-type compounds.

Sagnella and MacGregor (1984) studied the effects of tea extracts on: (1) human erythrocytes and Na^+, K^+ -ATPase isolated from hog brain, (2) the ability of tritiated ouabain ($^3\text{H}\text{O}$) to bind at red blood cell membrane Na^+, K^+ -ATPase sites, and (3) the entry of radioactive rubidium (^{86}Rb) into red blood cells. The tea extracts had an inhibitory effect (up to 100% inhibition) on the Na^+, K^+ -ATPase derived from hog brain. Erythrocyte ATPase activity also was inhibited by the tea extracts, but the researchers concluded that the extracts must inhibit other ATPases, perhaps in addition to Na^+, K^+ -ATPase, since the percentage of inhibition was greater than that exhibited by ouabain, which is specific for Na^+, K^+ -ATPase. The tea extracts did not affect the binding of tritiated ouabain to red blood cell membranes, indicating that the Na^+, K^+ -ATPase inhibitors

in the tea extracts were chemically different from ouabain. ^{86}Rb influx decreased 25% in the presence of the tea extracts, which led Sagnella and MacGregor (1984) to conclude that the Na^+, K^+ -ATPase inhibitors in tea may interfere with Na^+, K^+ -ATPase at internal cellular sites, rather than external cellular sites as is the case with ouabain.

Vanadate, the +5 oxidation state of vanadium (Cantley and Aisen, 1979) inhibits Na^+, K^+ -ATPase activity (Cantley and Aisen, 1979; Cantley et al., 1978; Cantley et al., 1977; Quist and Hokin, 1978). It has been postulated, based on the results of rat experiments, that an association exists between vanadium and the etiology of hypertension (Hom et al., 1982; Steffen et al., 1981). Steffen et al. (1981) conducted a study with a group of 27 rats in which one kidney had been removed. The rats were divided into three groups, based on the amount of vanadium supplemented to the normal diet. One group ate regular rat chow, another group ate rat chow containing 100 ppm of vanadate, and the third group consumed rat chow supplemented with 200 ppm of vanadate.

Systolic blood pressure of the rats in the high (200 ppm vanadate) and the low (100 ppm vanadate) diet groups were higher than those of the control group (Steffen et al., 1981). Before the termination of the study, the systolic blood pressure of the high vanadium rat group was higher than that of the low vanadium diet group, indicating a dose-dependent effect of vanadium on blood pressure. In addition Steffen et al. (1981) noted that plasma

vanadium levels were higher in the two vanadium supplemented groups, than in the control group.

In another rat study (Bogden et al., 1982), various levels of vanadium in the form of sodium metavanadate (0 ppm, 5.0 ppm, and 25.0 ppm) were added to the diets of rats for a 2-week period. After the rats consumed the diet for 2 weeks, blood, plasma, kidney, brain, and liver samples were analyzed for vanadium content. Increased vanadium intake resulted in greater retention of vanadium in the blood and tissues of the rats. Based on the results of Cantley et al. (1977), Bogden et al. (1982) concluded that the tissue vanadium concentrations were high enough to inhibit more than 50% of Na^+, K^+ -ATPase activity in vitro. However, as pointed out by Bogden et al. (1982), it has been shown (Higashino et al., 1982) that vanadium tissue concentrations, comparable to those found by Bogden et al. (1982) were not effective in inhibiting Na^+, K^+ -ATPase activity. It is possible that the vanadium in the tissues is not in the proper oxidation state to inhibit Na^+, K^+ -ATPase (Johnson et al., 1974).

Vanadium is an essential micronutrient in the growth and development of chicks and rats (Hopkins and Mohr, 1974). Dietary sources high in vanadium content include dill, lettuce, parsley, radishes, and lobster (Myron et al., 1977; Soremark, 1967). Based on vanadium's ability to inhibit Na^+, K^+ -ATPase activity and the results of Steffen et al. (1981), it is reasonable to speculate that dietary sources of vanadium may affect hypertension.

Dietary Survey Techniques

Nutritional assessment is a means for investigating possible relationships between diet and disease. Examples of nutritional assessment methods include dietary survey techniques, clinical evaluations, and biochemical assays (Krehl and Hodges, 1965). Dietary survey techniques or methods are "tools" used in the assessment of dietary intake data collected from individuals or populations (Burke, 1947) and aid in the identification of nutrient deficiencies in the diet (Burke, 1947; McHenry et al., 1945; Pekkarinen, 1970).

Important in the selection of a dietary survey technique are the validity and the reliability of the method. Young (1981) defined validity as "the degree to which the method is a true measure of what the investigator wishes to describe"; reliability is defined as "the repeatability or true reproducibility or the error variation in collecting and processing dietary data." Other factors that influence the choice of a dietary survey method include the objectives of the study (Linusson et al., 1974; Pekkarinen, 1970), e.g., whether qualitative or quantitative data are desired; the level of accuracy that is needed; available funding; personnel requirements; the sample size needed; subject characteristics such as literacy, memory and variability of the diet (Pekkarinen, 1970); and the enthusiasm of the participants (Cellier and Hankin, 1963). Examples of dietary survey techniques are (1) the diet or dietary history, sometimes referred to as a dietary interview, (2) the dietary questionnaire, (3) diet, dietary, or food records, (4) 24-hour or 1-day dietary recalls, and lastly, (5) food frequencies.

Diet History/Dietary Questionnaire

The diet history is an interviewing technique in which a trained interviewer questions the subject about his/her present and past food habits. Typical questions that may be included in a diet history are (1) number and time of daily meals, (2) use of salt, (3) use of diet products, (4) use of convenience foods, and (5) consumption of alcoholic beverages (Sanjur, 1982). The diet history is often used in epidemiological studies (Pekkarinen, 1970) and often includes a 24-hour recall (Young, 1981). It is not effective when quantitative data from individuals are needed (Pekkarinen, 1970). The dietary questionnaire is similar to the dietary history, but no interviewer is required. The subject completes and returns a dietary questionnaire form without any assistance (Pekkarinen, 1970).

Huenemann and Turner (1942) did not support the use of diet histories when determining the nutrient intakes of a small number of subjects. Using diet histories and dietary records Huenemann and Turner (1942) studied the dietary intakes of 25 6- to 12-year-old children. Diet records were used to test the reliability of the diet histories. "Average daily intakes" of calories and nutrients (calcium, phosphorus, iron, vitamin A, vitamin D, thiamin, ascorbic acid, riboflavin, carbohydrates, protein, and fat) were the bases for comparing the two dietary survey techniques (Huenemann and Turner, 1942). The diet histories included a 24-hour recall, usually conducted in the presence of the child's mother. In addition, the subjects were questioned about the frequencies and quantities of food

consumption, in order that "average daily food intakes" could be computed (Huenemann and Turner, 1942). Diet records were kept for 10 to 14 days, three or four times throughout the study. During record-keeping, foods were weighed using gram scales; preparation methods and recipes were reported also. Comparisons of each child's "average daily" intake calculated from diet history and diet record data, indicated lack of agreement between the two dietary survey methods. The lack of agreement was attributed to the subjects' not being able to identify their usual food intakes (Huenemann and Turner, 1942).

In agreement with Huenemann and Turner (1942), Burke (1947) stated that diet histories are not beneficial to the researcher if the subject cannot identify his normal food consumption patterns. Huenemann and Turner (1942) concluded that diet histories are of little quantitative value; and that when small sample sizes are involved in nutritional assessment studies, quantities of foods need to be reported in specific amounts. Blecha (1951), like Huenemann and Turner (1942), stated that dietary histories are not an appropriate dietary method to use when quantitative data are desired. Blecha's (1951) recommendation is based on the problems associated with remembering food intakes, seasonal variations in the diet, and the disruption of routine patterns due to snacking and/or eating meals away from home. However, diet history data may be beneficial in the clinical setting if the diet history is conducted prior to issuing dietary instructions (Blecha, 1951).

Trulson (1954) suggested that diet histories be used in the clinical setting. Trulson (1954) used dietary interviews, 7-day records, and 3 or more 24-hour recalls to determine the variability of the eating patterns of 10 to 12-year-old children. Even though differences in the mean consumption of nutrients and the food commodities studied (milk, eggs, and protein) were found when data from the three survey techniques were compared, the dietary interview and the 7-day record did produce similar results. Trulson (1954) concluded that it is difficult to determine which dietary method is a correct estimation of food intake, especially if the subject does not have any recognizable food consumption patterns. It was the opinion of the researcher that the dietary interview (history) is the most desirable method for use in the clinical setting because present and past food habits are revealed. However, if an interview is not feasible, Trulson (1954) recommended the substitution of a 7-day record.

Dietary Record

The diet (dietary) record or food record involves the recording of foods consumed for a specific time period. The most commonly used dietary records are 1-day, 3-day, and 7-day records. Food consumption can either be recorded in portions, slices, glasses, recorded in household measures (i.e., measuring cups and spoons), or it can be weighed for more precise results. Longer recording periods are necessary when determining the intake of individuals as opposed to group surveys (Pekkarinen, 1970).

Results of dietary survey techniques have revealed that the number of days included in a dietary record is of extreme importance. Enough days must be included to provide adequate data on a subject's nutrient intakes, but the number of days must be limited in order to maintain a subject's interest (Cellier and Hankin, 1963; Chalmers et al., 1952). According to Marr (1971), longer recording periods produce greater validity. However, Stuff et al. (1983) pointed out that there needs to be a balance between accuracy and burdens placed on subjects. Too much "interference" in a subject's lifestyle such as a lengthy recording period, produces results that do not reflect usual dietary intakes (Stuff et al., 1983). A longer recording period is necessary when determining the dietary intakes of individuals than when determining the dietary intakes of a group (Shapiro, 1979). Cellier and Hankin (1963) advocated the use of 4-day records (3 weekdays and 1 weekend day) when groups of 100 or more are being studied. The use of 1-day records is not recommended for individuals or small groups (Garn et al., 1978; Garn et al., 1976; Todd et al., 1983).

Todd et al. (1983) stated that 1-day records are not accurate when evaluating food intakes. It would be erroneous to assess whether an individual is at a nutritional risk based on a 1-day record alone (Garn et al., 1978). One-day records may be beneficial when conducting field studies, when the subject's have a short memory span, or if the group under study is of substantial size (Garn et al., 1976).

To obtain representative dietary intakes of individuals the use of 7-day records has been proposed (Acheson et al., 1980; Chalmers et al., 1952; Krantzler et al., 1982; Stuff et al., 1983; Todd et al., 1983; Young et al., 1952a). Krantzler et al. (1982) in a study involving college students, concluded that of the methods studied, 7-day diet records were most accurate when determining the dietary intake of individuals. The next most accurate methods (in descending order of accuracy) were the 3-day record, 6-hour recall, and the 24-hour recall. The criteria used to assess the validity of the dietary recall and records were based on the observation of student intakes of cafeteria food. At each meal, the students were provided with a form on which they indicated which foods were chosen and the number of servings of each. Once the students' trays with the attached forms were returned to the kitchen, the investigators visually examined the forms to see if discrepancies existed between what was recorded and actual consumption. The validity of the dietary recalls and records were evaluated by determining "a food item agreement score, expressed as the number of foods correctly identified/number of foods reported x 100" (Krantzler et al., 1982). In this manner the validity of the observational and methods data were compared by meal, by food groups, and by individual. The results of Krantzler et al. (1982) indicated that if feasible, 7-day dietary records should be used when collecting dietary intake data from individuals, since it was found to be the most accurate method tested.

In a study by Stuff et al. (1983) comparisons were made among food frequency forms, 1-day records, 3-day records, and 7-day records. The study involved 40 lactating women, 3-6 weeks post-partum. All participants were interviewed at home by a trained nutritionist, at which time a medical history questionnaire was completed, and data were collected for the food frequency form. The subjects were asked to keep a 7-day dietary record, recording in household measures all foods consumed. From the 7-day record data, days were randomly chosen to represent a 1-day record and a 3-day record. The 1-day record was randomly selected from 1 of 5 weekdays, whereas the 3-day record consisted of 2 weekdays and 1 weekend day randomly chosen. The validity of the 1-day records, 3-day records, and the food frequency forms were compared to the results of the 7-day record. The 7-day record was used as a standard because it was thought to be "the best compromise between obtaining accurate information with 'minimum' imposition on the subject's lifestyle" (Stuff et al., 1983).

No differences in means for calories, protein, fat, carbohydrate, calcium, phosphorus, and iron were found when results from the four dietary methods were compared (Stuff et al., 1983). Significant differences were noted among the dietary techniques used to collect the dietary intakes of the lactating women, when "the degree of agreement between the methods" was assessed using intra-class correlation coefficients. All the methods differed from the 7-day records, but the best agreement was between the 3-day records and the 7-day records. The most disagreement resulted

when the 7-day record and the food frequency data were compared. Stuff et al. (1983) concluded that the 3-day records can be used for the determination of population intakes; but 3-day records are only of qualitative value when used to assess the nutrient intake of individuals. However, the researchers stated that 3-day records could be used to assess the quality of the diet (Stuff et al., 1983). Since none of the dietary techniques studied (1-day records, 3-day records, or food frequency forms) produced results comparable to those of the 7-day record, Stuff et al. (1983) recommended that the 7-day record be used to estimate an individual's nutrient intakes.

Todd et al. (1983) altered the conventional methods of dietary intake record-keeping by asking subjects to tape-record weighed food intakes that were then compared to estimated food intakes (in household measures) recorded in a notebook (diary method), and 24-hour recalls. The study included 30 days (six 5-consecutive day periods) and an average of two 24-hour recalls per subject. The diary record method, and the tape-recording and weighing method were both determined to be accurate means for recording energy and protein intakes, since no significant differences in means were found using Analysis of Variance. As determined from the analysis, the weighing of food intakes and measuring food intakes by volume produce similar results (Todd et al., 1983). These results support the use of volume measurements in the determination of food intakes (Todd et al., 1983). From the data collected for the group, the intakes of individuals

were estimated using one day of recorded intake on tape. One-day records were found to be incorrect measures of individuals' intakes, based on "percentage deviations of sample means from usual (30 day) mean intakes," using 95% confidence intervals (Todd et al., 1983).

24-Hour Dietary Recalls

The 24-hour dietary recall or 1-day recall, like the diet history, is an interview technique, but specific time periods are involved. An interviewer asks the subject to recall foods consumed during the previous day, sometimes beginning with the meal he/she remembers best (Pekkarinen, 1970). The dietary recall can represent longer periods of time than one day, but longer periods of time tend to produce erroneous results because it is more difficult for the subject to remember what he/she has eaten (Pekkarinen, 1970). The 24-hour recall is a popular method because it is not as time-demanding as some of the other methods, and it can be used when subject cooperation is minimal (Young et al., 1952b).

Beaton et al. (1983) used data that had been collected from a previous experiment (Beaton et al., 1979) to determine the sources of variance in the 24-hour dietary recall. The sources of variance included subject, sequence of interviews, interviewer, and day of the week. The study involved 30 males and 30 females from 25 to 44 years old. Six 24-hour recalls were taken per subject within a period of 18 to 99 days.

No day-of-the-week variations were found when the males' dietary intakes were analyzed. Day-of-the-week variations were evident for females when energy, total carbohydrates, starch, other carbohydrates, thiamin, niacin, iron, and caffeine intakes were evaluated. When the nutrients were expressed as a percentage of total energy, the day-of-the-week variation was minimized. The intakes of the females increased on the weekend days compared to weekdays. Exceptions to this observation were for calcium intake, reflected by a decreased calcium/energy ratio, and caffeine. Intraindividual and interindividual variations in intake also occurred. Beaton et al. (1983) concluded, because of the intraindividual variations which exist, that "one day data provide a very inadequate estimate of usual intake of individuals."

Young et al. (1952b) compared results from dietary histories, 7-day records, and 24-hour recalls. The investigators were interested in the mean intakes of college students, grade-school students, and pregnant women. On an individual basis, the 24-hour recall did not produce the same results as the dietary history or the 7-day diet record; indicating that the 24-hour recall could not be substituted for the 7-day record (Young et al., 1952b). In the study by Todd et al. (1983) 24-hour recalls also were found to be poor means for estimating an individual's protein and energy intakes.

Food Frequency

Food frequencies or food frequency questionnaires are another means for collecting dietary intake information. They are inexpensive

as well as easy to process and distribute (Axelson and Csernus, 1983). A food frequency is a form that includes a group of selected foods or food categories (Campbell et al., 1982) and corresponding frequency categories. Subjects are asked to indicate the frequency in which the food item is consumed, for example: never, rarely, once a year, once a month, once a week (Sanjur, 1982). Food frequencies provide qualitative information that reveals intake patterns and the degree of diet diversity (Campbell et al., 1982). When food frequencies are used, the investigator is assuming that serving sizes for individuals are similar (Marr, 1971), unless of course questions about the quantities of the foods consumed are asked (Campbell et al., 1982). Mullen et al. (1984) found that many individuals "could accurately estimate their intake" using a food frequency questionnaire. Assessment of accuracy was based on comparisons with actual intakes. Mullen et al. (1984) noted that it was easier for subjects to predict their consumption of food items that are main dishes at meals, such as entrees and meats, rather than foods that are considered to be accompaniments.

CHAPTER III

METHODS

General Plan

Seventeen female subjects ages 23-58 were recruited through personal contacts, advertisements in The University of Tennessee newspaper, and flyers distributed throughout campus, to participate in blood pressure measurements, blood sampling (2 days), and dietary assessment using 7-day dietary records and/or food frequency forms. Procedures and experimental design were approved by The University of Tennessee Committee on Research Participation. The University of Tennessee, Knoxville, faculty, staff, and graduate students were included in the study population. Subjects were asked to refrain from eating or drinking (except water) for 10 hours prior to blood sampling. Testing was conducted in the Human Studies Facility of the Department of Nutrition and Food Sciences between 7 and 9 a.m. Subjects were paid \$10 for each blood sample (\$20 per sampling day). An outline of the project's sequence of activities is given in Appendix A. The memorandum describing the project to prospective subjects is shown in Appendix B.

Screening Process and Description of Subjects

The candidates were screened prior to acceptance. The screening process involved the completion of a Subject

Questionnaire (Appendix C) that included various medical history questions. Persons not familiar with their normal blood pressure measurements, and/or who had diabetes, heart disease, kidney disease and/or thyroid disorders were automatically eliminated as prospective subjects. Secondary hypertensive subjects were not included in the study population. Consent forms (Appendix D) were obtained from the subjects before the beginning of the experiment, after the subjects had been informed of the project's purposes and procedures.

Six medicated hypertensive females and seven normotensive females with no history of hypertension were included in the study. The remaining subjects (4) were classified as normotensive, but one had previously been hypertensive and medicated (784). Three (633, 869 and 921) had had elevated blood pressure on several occasions, but blood pressure reduction was accomplished through dietary modifications (633 and 921), or the discontinued use of oral contraceptives (869). Fifteen subjects were within the normal weight/height ranges; the other two subjects (839 and 414) were 17% and 35% above upper weight/height limits, respectively (NAS/NRC, 1980) (Appendix E). Subject characteristics including height, weight, age, body mass index (BMI), and family history are given in Appendix E.

Blood Pressure Measurement

Blood pressure was measured using a standard mercury sphygmomanometer with the identification and disappearance of Korotkoff sounds indicating systolic and diastolic blood pressure

levels, respectively. Upon arrival on the day of testing, the subjects were weighed without shoes, and then were asked to rest in a semi-reclining position for 15 minutes before blood pressure measurements were taken. Blood pressure was measured a second time in a semi-reclining position, 15 minutes following blood sampling. The phlebotomist who was responsible for the blood sampling also was responsible for making the blood pressure measurements.

Blood Sampling and Red Blood Cell Counting

An experienced phlebotomist was employed to take the blood samples. Two 10-ml fasting blood samples were obtained from the subjects on two separate occasions. The two sampling days were approximately one month apart, both within the first 8 to 10 days of the follicular phase of the menstrual cycle for the premenopausal women, in order to minimize variations in hormonal levels (Khaw and Barrett-Connor, 1984; Morgan, 1973). For the post-menopausal subjects, and for the women who had had hysterectomies (155, 588, 727 and 839) and ovariectomies (839), the two sampling days were scheduled approximately one month apart at the subject's convenience. Several of the subjects whose erythrocyte Na^+, K^+ -ATPase activity data (maximum ouabain binding values) were not usable due to unavoidable problems with the assay were asked to return for blood sampling a third time. Ten-ml heparinized Vacutainers (Becton-Dickinson and Co., Rutherford, NJ) with 21-gauge needles were used to collect the two 10-ml blood samples. The second 10-ml blood sample was used as

a back-up in the event that the Na^+, K^+ -ATPase assay needed to be repeated, due to technical problems. The concentration of red blood cells was determined by diluting the red blood cell suspension (0.5 ml) to a volume of 50 ml. Cells were counted using a hemacytometer with two Neubauer counting chambers (Bauer, 1982). A total of four counts were made for each subject (2 dilutions x 2 chambers). The values obtained were equivalent to half the "normal" counts, due to the dilution factor.

Determination of Maximum Ouabain Binding: An Indicator
of Erythrocyte Na^+, K^+ -ATPase Activity

A measure of the Na^+, K^+ -ATPase activity for intact red blood cells was determined using a tritiated ouabain ($^3\text{H}\text{O}$) binding technique (Akeru and Cheng, 1977; DeLuise et al., 1980), as modified by Kennedy et al. (1982). The active number of enzyme units per cell and enzyme activity are proportional to the number of ouabain binding sites per cell (Gelbart and Goldman, 1977; Schmalzing et al., 1981). Laboratory technicians familiar with the Na^+, K^+ -ATPase procedure were hired to perform the assays.

Erythrocytes were separated from whole blood by centrifugation (2000 g), resuspended in isotonic saline, washed three times with choline chloride (140 mM), and finally suspended in red blood cell buffer (150 mM NaCl, 30 mM Hepes, 10 mM glucose, pH 7.4). Aliquots (500 μl) of red blood cell suspension were incubated for 3 hours (37°C) with one of six concentrations of $^3\text{H}\text{O}$ (1 to 100 nM), and 50 μl

of either unlabeled ouabain (10 mM) or red blood cell buffer. The reaction was quenched and the cells were washed with aliquots of cold choline chloride (3 x 1 ml). Bound ^3H O was resolubilized with 500 μl trichloroacetic acid (5%). Ten ml of Biofluor (New England Nuclear, Boston, MA) were added and radioactivity was counted in a Beckman LS100C Scintillation counter. Specific ouabain binding was calculated by taking the difference between binding measured in the presence (non-specific) and absence (total binding) of 10 mM ouabain. Maximum binding capacity (BMAX) was calculated from the double reciprocal plot of concentration versus specific binding (Akeru and Cheng, 1977).

Dietary Analysis

The dietary survey techniques used in the study were a food frequency form and a 7-day dietary record. During the initial screening process, subjects were asked to complete a food frequency form (Appendix F). The dietary records (Appendix G) were kept by 15 of the 17 subjects for a period of 7 consecutive days, beginning with the day of the first or second (subject 342) blood sampling.

Food Frequency Form

A variety of foods were listed on the food frequency form (Appendix F), including foods that have been found to contain in vitro Na^+, K^+ -ATPase inhibitors (Harlan and Mann, 1982; Sagnella and MacGregor, 1984) and foods that contain various levels of

vanadium because vanadium also is an inhibitor of Na^+, K^+ -ATPase activity (Cantley and Aisen, 1979; Cantley et al., 1978; Cantley et al., 1977; Quist and Hokin, 1978). A Total Na^+, K^+ -ATPase Inhibition Score (Appendix H, I) and a Total Vanadium Index (Appendix H, II) were determined for each subject based on the frequency of foods consumed. Consumption was calculated by assigning numerical values (Frequency Factors) (Table 1) to categories representing frequency of consumption (Table 1 and Appendix F). Frequency Factors (Table 1) were adjusted to reflect the consumption of seasonal foods (as indicated by the subject; see Appendix F), e.g., cantaloupe consumed only in summer months. Also the consumption of raw and cooked/processed foods was combined to yield one consumption figure (e.g., raw and canned tomatoes). This was necessary because Harlan and Mann (1982) did not specify the form in which foods containing in vitro Na^+, K^+ -ATPase inhibitors were analyzed. The form in which some of the foods containing vanadium were analyzed is also unknown.

Calculation of Total Na^+, K^+ -ATPase Inhibition Score. The units of inhibition for each of the 26 foods showing in vitro inhibitory activity (Harlan and Mann, 1982) were calculated by first converting units of the ATPase inhibitor(s) of a food from units of inhibitor/g dry weight to units of inhibitor/g wet weight (Appendix H, I, step A) and Table 2. The quantity of units/g wet weight multiplied by a food's serving size (Appendix I) represents the units of ATPase inhibitor found in the food consumed (Food Item Inhibition

TABLE 1
SCALING FACTORS REPRESENTING THE FREQUENCY OF FOOD CONSUMPTION^{a,b}

Frequency of Consumption	Factor
Never	0
Rarely or occasionally	1
Once a month	5
Two or three times a month	10
Once a week	20
Several times a week	60
Once a day	150
Several times a day	450

^aFactors calculated relative to an arbitrary base of "once a month" = 5; e.g., "once a day" = 5 x 30.

^bFactors used in the calculations of Na⁺,K⁺-ATPase Inhibition Scores and Vanadium Indices. See Appendix H.

TABLE 2
IN VITRO Na^+, K^+ -ATPase INHIBITOR LEVELS IN FOODS

Food Item	Units of Inhibitor ^a g wet weight	Units of Inhibitor ^b g dry weight
Cucumber	3.9	60
Turnip Greens ^C	3.9	60
Pear	3.5	21
Banana	3.2	13
Beef Liver ^C	3.1	7
Pork Liver ^C	2.6	6
Carrot	2.4	20
Cabbage ^C	2.0	36
Onion	2.0	18
Peach	1.3	12
Orange	1.1	8
Apple	0.8	5
Tomato	0.8	12
Radish	0.7	13
Cantaloupe	0.6	7
Corn ^C	0.5	2
Potato ^C	0.5	2
Garlic	0.4	1
Pineapple	0.4	3
Lettuce	0.3	7
Sanka	0.2	16
Eggplant ^C	0.2	4
Yellow Squash ^C	0.2	4
Watermelon	0.2	3
Coffee	0.1	10
Sweet Potato ^C	0.1	0.4

^aConversion of dry to wet weights calculated by Gaskin (see Appendix H, I, Step A), using USDA Handbook No. 456 (Adams 1975); unless indicated otherwise, the conversion was based on the water contents of raw foods.

^bHarlan and Mann, 1982. One inhibitor unit = activity to inhibit one unit of ATPase by 50%.

^cWater content of cooked food used in the conversion of dry to wet weights.

Rating) multiplied by the frequency of consumption (Frequency Factor, Table 1) equals the Inhibition Score for that particular food (Appendix H, I, step C). The Total ATPase Inhibition Score for each subject was determined by summing the Inhibition Scores obtained for each of the foods consumed by the subject. The calculation of an Inhibition Score for an apple consumed once a week is shown in Appendix H. Units of Na^+, K^+ -ATPase in vitro inhibitors (per ml) in selected beverages also used in the calculation of Total Na^+, K^+ -ATPase Inhibition Scores are shown in Table 3.

Calculation of Total Vanadium Index. Foods that contain vanadium were classified into six categories (very low, low, medium, medium-high, high, and very high) based on published values for vanadium levels in foods (Myron et al., 1977; Soremark, 1967). A numerical value (Vanadium Content Score) (Table 4) was assigned to each category, relative to the vanadium range of 1-20 ppb = 3 (e.g., the vanadium range of 20-100 ppb = 15); see Table 4. Vanadium Content Scores were adjusted for foods with reported concentrations in more than one category (e.g., radish = 9, see Table 4). Categories and Vanadium Content Scores were used instead of the reported vanadium concentrations because of the uncertainty in the degree of accuracy for quantitative data obtained from food frequency forms, and because literature values for vanadium are not in agreement (Myron et al., 1977; Soremark, 1967).

TABLE 3
IN VITRO Na^+ , K^+ -ATPase INHIBITOR LEVELS (UNITS/ML)^a
 IN SELECTED BEVERAGES^b

Beverage	u/ml
Tea (Lipton)	13.6
Cocoa (Hershey)	2.8
White wine (Italian)	0.0
Rose wine (Italian)	Trace
Red wine (American)	4.1
Grape juice	8.0

^aOne inhibitor unit = activity to inhibit one unit of ATPase by 50%.

^bHarlan and Mann (1982).

TABLE 4
VANADIUM CONCENTRATIONS AND VANADIUM CONTENT SCORES OF SELECTED FOODS

Food Category	Vanadium Concentration (ppb, wet wt.) ^a	Category ^b	Vanadium Range, ppb ^b	Vanadium Content Score ^b
<u>Beverages</u>				
Beer, Canned	11	low	1-20	3
Coffee	1.6	low	1-20	3
Tea	1.3	low	1-20	3
Cola, Canned	0.7	very low	<1	0.5
<u>Cereals, Grains and Seeds</u>				
Black Pepper	987	very high	>600	150
Dill Seed	431	high	300-600	90
Dill	140	medium-high	100-300	45
White Rice	21	medium	20-100	15
Navy Beans	14	low	1-20	3
Field Peas	7	low	1-20	3
Oatmeal	6	low	1-20	3
Corn Meal	2	low	1-20	3
Brown Rice	<1	very low	<1	0.5
<u>Fresh Fruits and Vegetables</u>				
Parsley	790 ^C	very high	>600	150
Radish	5, 52.1 ^C	low/medium	1-20 & 20-100	9
Lettuce	4, 21 ^C	low/medium	1-20 & 20-100	9
Squash	4	low	1-20	3

TABLE 4 (Continued)

Food Category	Vanadium Concentration (ppb, wet wt.) ^a	Category ^b	Vanadium Range, ppb ^b	Vanadium Content Score ^b
<u>Fresh Fruits and Vegetables</u>				
Apple	1.1, ^c 4	low	1-20	3
Banana	3 ^c	low	1-20	3
Cucumber		low	1-20	3
Cabbage	2	low	1-20	3
Carrot	1	low	1-20	3
Cauliflower	<1, ^a 0.08 ^c	very low	<1	0.5
<u>Meat and Dairy Products</u>				
Chicken (Light)	22	medium	20-100	15
Egg Yolk	21	medium	20-100	15
Chicken (Dark)	12	low	1-20	3
Bologna	8	low	1-20	3
Beef Liver	6	low	1-20	3
Bacon	5	low	1-20	3
Calf Liver	2.4 ^c	low	1-20	3
Ground Beef	1	low	1-20	3
Pork Chop	1	low	1-20	3
Skim Milk, Dried	0.21, ^{ce} 25	very low/medium	<1 & 20-100	3
Whole Milk	0.08, ^{ce} 3	very low/low	<1 & 1-20	1.5
<u>Seafood</u>				
Codfish	28	medium	20-100	15
Scallops	22	medium	20-100	15

TABLE 4 (Continued)

Food Category	Vanadium Concentration (ppb, wet wt.) ^a	Category ^b	Vanadium Range, ppbb	Vanadium Content Score ^b
<u>Seafood</u>				
Tuna, Canned	11	low	1-20	3
Lobster	5, 43 ^c	low/medium	1-20 & 20-100	9
Sardines	9.5 ^{cd}	low	1-20	3
Mackerel, Fresh	2.6	low	1-20	3
Trout, Fresh	0.4 ^c	low	<1	0.5
<u>Fats and Oils</u>				
Margarine	4	low	1-20	3
Lard (Commercial)	2	low	1-20	3
Butter	1	low	1-20	3
Corn Oil (Supermarket)	1	low	1-20	3
Soybean Oil	1	low	1-20	3
<u>Prepared Foods</u>				
Breakfast Cereal, Natural	93	medium	20-100	15
Gelatin (Sweden)	44 ^c	medium	20-100	15
Peanut Butter	44	medium	20-100	15
Milkshake, Chocolate	21	medium	20-100	15
White Bread	20	medium	20-100	15
Dill Pickle	13	low	1-20	3
Whole Wheat Bread	11	low	1-20	3

TABLE 4 (Continued)

^aParts per billion (ppb); unless indicated otherwise, vanadium concentrations are from Myron et al. (1977); for skim milk ppb, dry weight.

^bAssigned by Gaskin, based on vanadium concentration (ppb, wet wt.); used in the calculation of vanadium indices (see Appendix H).

^cSoremark (1967).

^dMean for varieties given (Soremark, 1967).

^eMean for USA varieties given (Soremark, 1967).

A Vanadium Index (Appendix H, II) was determined for each food consumed by the individual subjects. The Vanadium Index (Appendix H, II) was computed by multiplying the Vanadium Content Score of a food (Table 4) by the Frequency Factor (Table 1, page 45) and the serving size (g) (Appendix I). Subsequently, a Total Vanadium Index for each subject was calculated by summing the Vanadium Indices for all foods consumed. An example of the calculation for a dill pickle consumed once a week is shown in Appendix H, II.

7-Day Dietary Record

For the purposes of this study, the 7-day dietary record was chosen as the dietary survey technique because the dietary intake of individuals was being assessed, the sample included a small number of subjects, and quantitative rather than qualitative data were necessary. The use of 7-day records has been recommended when determining the nutrient intakes of individuals (Acheson, 1980; Chalmers et al., 1952; Krantzler et al., 1982; Stuff et al., 1983; Todd et al., 1983; Young et al., 1952a). In addition the 7-day record provides accurate data without placing too many demands on the subjects (Stuff et al., 1983), and is representative of 28 days of dietary intake (Jeor et al., 1983).

Subjects were asked to keep a 7-day dietary record beginning the day of the first or second blood sampling. Subjects received explicit instructions on the recording of their dietary intake. Brand-names of items and food preparation methods were to be indicated

on the dietary record forms. Participants were asked to measure their dietary intakes in household measures rather than weighing their intakes, since no differences were found by Todd et al. (1983) between intakes measured by volume and weighed intakes. Secondly, the weighing of food intakes is thought to alter normal consumption patterns (Pekkarinen, 1970). To aid in the recording of portion sizes, the participants were given measuring spoons, measuring cups, and a ruler. The subjects were instructed not to change their eating habits. A list of responsibilities and reminders were given to subjects to aid them in the recording of intakes (Appendix J). A reduced version of the dietary intake record form is shown in Appendix G.

After the week of record-keeping was completed, each subject met with the investigator to discuss the dietary records. The investigator questioned the subject about information that was omitted from the records. Subjects were contacted during the coding process if more information was needed in order for the foods to be appropriately coded and converted into gram amounts.

Dietary records were processed as described by Marshall and Judd (1982). Items in the records were assigned codes from listings in the tape version of the revised sections of the USDA Handbook No. 8 on dairy and egg products (Posati and Orr, 1979), spices and herbs (Marsh et al., 1977), fats and oils (Reeves and Weihrauch, 1979), poultry products (Posati, 1979), soups, sauces, and gravies

(Marsh, 1980), sausages and luncheon meats (Richardson et al., 1980), supplemented with USDA Handbook No. 456 (Consumer and Food Economics Institute, 1977), data compiled by USDA's Lipid Nutrition Laboratory, and data obtained from manufacturers of specific products. Nutrient information received from manufacturers in the form of U. S. RDA percentages was converted to the amount of the nutrient per 100 g. Nutrient contents of the diets were calculated by computer using the Statistical Analysis System (SAS) (SAS Institute, Cary, NC). Nutrient intakes were summed for each subject by day; and nutrient means for the week also were computed.

Statistical Analysis

In choosing the statistical analysis to be used, the investigator recognized that the number of candidate variables hypothesized to be related to blood pressure exceeded the number of observations. In an attempt to explore possible relationships among blood pressure (the dependent variable) and the candidate variables in this pilot study, stepwise regression procedures in the Statistical Analysis System (SAS Institute, 1982b) were used. PROC STEPWISE (SAS Institute, 1982b) involves the selection of variables, through a series of sequential steps, which best account for variations in blood pressure (i.e., cause the greatest increase in R^2 , the squared multiple correlation coefficient).

Candidate variables hypothesized to be related to blood pressure were mean maximum ouabain binding (MBMAX), dietary intake

variables (protein, fat, carbohydrate, sodium, potassium, calcium, iron, vitamin A, vitamin C, thiamin, riboflavin, niacin, cholesterol, and crude fiber), body mass index (BMI), and age. As an option in PROC STEPWISE (SAS Institute, 1982b) MBMAX was included as the first variable in each step of the procedure, in order that the effects of the other candidate variables on the relationship between blood pressure and MBMAX could be examined. Also the maximum number of candidate variables to be introduced into the stepwise model was arbitrarily set at 5, in order that the number of variables used would not exceed more than 50% of the number of observations. The level of significance was defined as $p < 0.05$. From this preliminary work and in conjunction with factor analysis (PROC FACTOR) (SAS Institute, 1982a), a regression model was chosen that included MBMAX and dietary intake variables found to be related to blood pressure.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, subjects are described based on blood pressure measurements, maximum erythrocyte ouabain binding (BMAX), one-week nutrient and energy intake means (15 subjects), body mass index (BMI), age, Total Inhibition Scores, and Total Vanadium Indices. The relationships among these variables are discussed. The consumption of foods containing vanadium and/or in vitro Na^+, K^+ -ATPase inhibitors also is considered. To preserve confidentiality, subjects are referred to by 3-digit random numbers.

Mean maximum ouabain binding (MBMAX) and blood pressure measurements were calculated for each subject, and mean 7-day dietary intakes were calculated for 15 of the 17 subjects. Original data from which the MBMAX and blood pressure means were derived, and other subject characteristics (height, weight, age, body mass index (BMI), and family history) are included in Appendix E.

Blood Pressure Measurements

As determined from factor analysis (PROC FACTOR) (SAS Institute, 1982a), mean systolic and diastolic blood pressures taken before and after blood sampling on the two sampling days were highly correlated. For this reason mean systolic blood pressure taken prior to sampling (MBP1S) was chosen for use in statistical analyses, it being the most complete set of systolic values. In addition, it has been suggested

that systolic blood pressure is a better indicator than diastolic blood pressure of the predisposition to ischemic heart disease (Rabkin et al., 1978).

MBPIS ranged from 98 to 150 mm Hg for the study population (Table 5). Based on blood pressure measurements, subjects were categorized as normotensive (11 subjects), medicated and controlled hypertensives (5 subjects), or medicated and moderately uncontrolled hypertensives (1 subject). All but one of the six medicated hypertensive subjects had mean systolic blood pressures below the "normal" limit of 140 mm Hg (Garay and Meyer, 1979; Meyer, 1980). Despite medication, subject 155 had a MBPIS of 150 mm Hg. This may be due to a self-reported inconsistency in taking the diuretic agent as prescribed for treatment (Table 5) or to the effect of arthritis medication (see section following).

Maximum Ouabain Binding and Blood Pressure

Mean maximum ouabain binding (MBMAX), an indicator of Na^+, K^+ -ATPase activity (Gelbart and Goldman, 1977; Schmalzing et al., 1981), is presented by blood pressure grouping in Table 5. MBMAX ranged from 0.295 (subject 414) to 0.820 pmoles/ 10^9 cells (subject 869) (Table 6). The number of ouabain binding sites per cell was calculated from MBMAX for each subject, assuming one binding site per bound molecule of ouabain. The number of ouabain binding sites per cell for the group studied ranged from 178 to 494 ($\bar{X} = 313$). Similar results were calculated by Erdmann et al. (1979) for

TABLE 5
 MEAN SYSTOLIC BLOOD PRESSURE (MBP1S),^a MEAN MAXIMUM OUABAIN BINDING (MBMAX),^b AND
 MEDICATION DESCRIPTIONS^c FOR NORMOTENSIVE CONTROL, MEDICATED AND CONTROLLED
 HYPERTENSIVE, AND MEDICATED MODERATELY UNCONTROLLED HYPERTENSIVE SUBJECTS

Blood Pressure Category and Subject Number	MBP1S ^a	MBMAX ^b	Medication	Comments
Normotensive				
339	102	0.300		
342	100	0.370		
495	115	0.400		
633	135	0.465		Elevated blood pressure during physical examination in the past; advised to modify diet.
643	114	0.510		
672	98	0.610		
727	106	0.540		
784	115	0.450		Previously hypertensive and medi- cated.
869	133	0.820		Experienced elevated blood pressure when using oral contraceptives; with discontinued use of oral con- traceptives blood pressure has not returned to levels prior to usage.

TABLE 5 (Continued)

Blood Pressure Category and Subject Number	MBP1S ^a	MBMAX ^b	Medication	Comments
921	109	0.530		Elevated blood pressure during physical examination in the past; advised to modify diet.
991	110	0.620		
Medicated/Controlled Hypertensive				
276	133	0.670	Corgard (20 mg/day)	Nonselective, β -adrenergic blocking agent.
414	127	0.295	Corgard (40 mg/day)	Nonselective, β -adrenergic blocking agent.
			Dyazide	Triamterine + thiazide; a diuretic to achieve potassium balance. ^d
			Feldene	Rheumatoid arthritis medication.
588	134	0.720	HCTZ (50 mg/day)	Hydrochlorothiazide; a potassium-wasting diuretic. ^d
			Premarin (0.625 mg)	Estrogen medication; taken in three-week cycles per month.

TABLE 5 (Continued)

Blood Pressure Category and Subject Number	MBP1S ^a	MBMAX ^b	Medication	Comments	
Medicated/ Uncontrolled Hypertensive	813	126	0.590	Tenormin 50	β -selective adrenergic blocking agent
				Hygroton	Chlorthalidone; a potassium-wasting diuretic. ^d
	839	120	0.585	Esidrix (25 mg, 2X daily)	A thiazide potassium-wasting diuretic. ^d
				Klorvess (20 mg, 2X daily)	A potassium supplement.
	155	150	0.365	Esidrix (25 mg/day)	A thiazide potassium-wasting diuretic. ^d
				Naprosyn	Arthritis medication.

^aMean systolic blood pressure (mm Hg) taken prior to blood sampling. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

TABLE 5 (Continued)

^bMean maximum ouabain binding (pmoles/10⁹ cells). N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^cUnless indicated otherwise, medication descriptions are from Physicians' Desk Reference (Angel, 1984).

^dWilber (1982).

TABLE 6
SUBJECTS RANKED ACCORDING TO MBMAX^a WITH CORRESPONDING MBP1S^b

Subject Number	MBMAX	MBP1S
414	0.295	127
339	0.300	102
155	0.365	150
342	0.370	100
495	0.400	115
784	0.450	115
633	0.465	135
643	0.510	114
921	0.530	109
727	0.540	106
839	0.585	120
813	0.590	126
672	0.610	98
991	0.620	110
276	0.670	133
588	0.720	134
869	0.820	133

^aMean maximum ouabain binding (pmoles/10⁹ cells). N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^bMean systolic blood pressure (mm Hg) taken prior to blood sampling. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

normotensive subjects (259 ± 60) and hypertensive subjects (452 ± 84).

Subjects are ranked according to MBMAX (low to high MBMAX) with corresponding MBPIS's in Table 6. Generally, as MBPIS increased, MBMAX increased. A plot of MBPIS versus MBMAX is presented in Figure 1. These results are consistent with those of Stokes et al. (1983) and Woods et al. (1981) who concluded when measuring ^{86}Rb influxes in the presence of ouabain, that Na^+, K^+ -ATPase activity was higher in hypertensive subjects than in normotensives. In subjects who had relatively high MBMAX and elevated MBPIS, the increased activity of Na^+, K^+ -ATPase may be a reflection of a defect in another transport system, such as Na^+, K^+ -cotransport. It has been suggested that Na^+, K^+ -ATPase could be compensating for the defect by increased activity (Garay et al., 1980a; Haddy and Pamnani, 1983). Increased Na^+, K^+ -ATPase activity also may occur if the subject has a Na^+ leak (increased permeability) in the red blood cell membrane (Haddy and Pamnani, 1983).

Four subjects (414, 155, 633, and 672) had MBPIS-MBMAX measurements that deviated substantially from the general pattern. Subjects 414 and 155 had unusually low MBMAX's of 0.295 and 0.365, relative to their MBPIS's of 127 mm Hg and 150 mm Hg, respectively (Table 6 and Figure 1). Both of these subjects (414 and 155) in addition to taking blood pressure medications, were taking medications for arthritis (Table 5) that inhibit prostaglandin synthesis (Angel, 1984). Inhibited prostaglandin synthesis may account for the elevated blood pressures in the two subjects, since

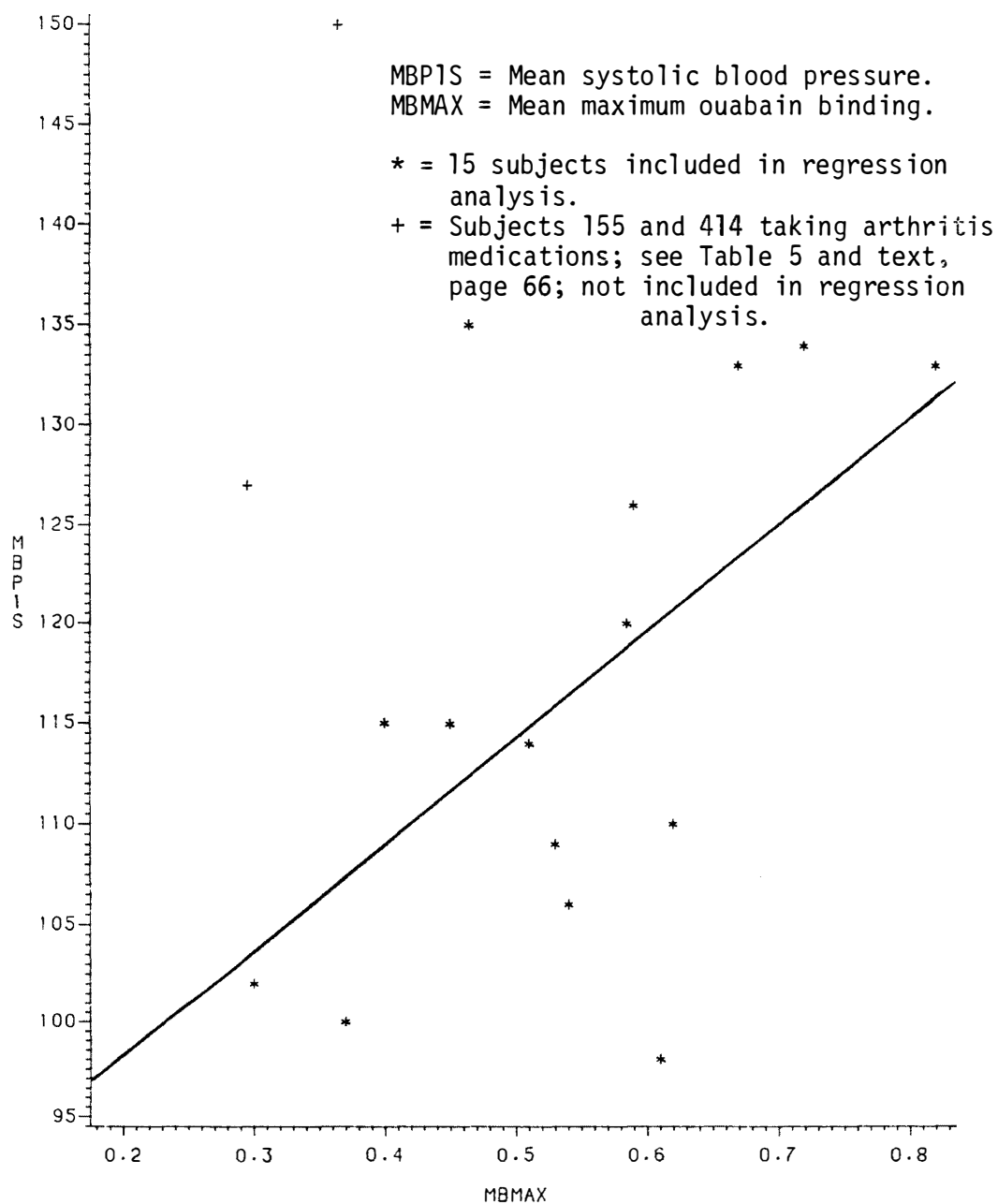


Figure 1. Blood pressure as a function of maximum ouabain binding; regression line $MBP1S = 53.47 (MBMAX) + 87.51$ for all * values ($R^2 = 0.32$, $p < 0.03$; adjusted $R^2 = 0.27$).

prostaglandins (e.g., prostacyclin) act by relaxing coronary arteries (Dusting et al., 1977; Weber et al., 1981). Subject 633 had high MBP1S (135 mm Hg), relative to MBMAX (0.495), and subject 672 had low MBP1S (98 mm Hg) relative to MBMAX (0.610) (Table 6 and Figure 1). There is no apparent reason for the results of subject 633. The results for subject 672 (relatively low MBP1S with relatively high MBMAX) may be related to the subject's family history of hypertension (Appendix E). Increases and decreases in sodium transport have been found in normotensive relatives of hypertensive subjects, compared to normotensive subjects without a family history of hypertension (De Mendonca et al., 1981; Garay et al., 1980a; Garay and Meyer, 1979; Woods et al., 1981). Garay et al. (1980a), by studying Na^+ and K^+ fluxes in Na^+ -loaded erythrocytes (with and without ouabain), concluded that Na^+, K^+ -ATPase activity was increased in the normotensive relatives of hypertensives.

In a regression procedure (PROC REG) (SAS Institute, 1982b), the relationship between the dependent variable MBP1S and MBMAX was identified. There was no correlation between MBP1S and MBMAX when all 17 subjects were included in the analysis. However, when data from the two subjects taking arthritis medications (subjects 414 and 155) were omitted from the analysis, a significant ($p < 0.03$) positive correlation was observed between MBP1S and MBMAX. The mean square, the significance of the F ratio, and partial regression coefficients for the regression with 15 subjects are given in Table 7. Because of the possible effect of the arthritis medications,

TABLE 7
MEAN SQUARE, SIGNIFICANCE OF F RATIO, AND PARTIAL
REGRESSION COEFFICIENTS FOR MBP1S^a VERSUS
MBMAX^b WITH 15 SUBJECTS^{c,d}

	df	Mean Square	P	Partial Regression Coefficients
Intercept	1			87.51
MBMAX	1	754.53	0.027	53.47
Error	13			
Total	14			

^aMean systolic blood pressure (mm Hg) taken prior to blood sampling. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^bMean maximum ouabain binding (pmoles/10⁹ cells). N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^cData from subjects (155 and 414) taking arthritis medications omitted from analyses; see text, page 66 and Table 5, page 59. Subjects 155 and 414 not included in subsequent statistical analyses.

^dRegression: $R^2 = 0.32$; adjusted $R^2 = 0.27$.

subsequent statistical analyses were limited to 13 subjects (15 subjects with dietary intake data excluding subjects 414 and 155). It must be recognized, however, that the effect of the arthritis medication on the relationship between MBPIS and MBMAX may not occur with a larger number of subjects.

Body Mass Index, Age, Blood Pressure, and Maximum
Ouabain Binding

Body mass index (weight/height^2) as calculated for the study population ranged from 18.6 to 31.9 kg/M^2 (Appendix E). PROC STEPWISE (SAS Institute, 1982b) was used to determine relationships among mean systolic blood pressure (MBPIS), mean maximum ouabain binding (MBMAX), body mass index (BMI), and age. Body mass index has been found to be inversely related to blood pressure (Havlik et al., 1983). In the present study no association was apparent between MBPIS and BMI. Age was not related to either MBPIS or MBMAX.

With MBMAX as the dependent variable in PROC STEPWISE (SAS Institute, 1982b), no relationship was observed between MBMAX and MBI for 13 or 15 subjects (all subjects except 155 and 414). These results are not consistent with those of DeLuise et al. (1980) and Narayanareddy and Kaplay (1983). DeLuise et al. (1980) noted a reduction in the number of ouabin binding sites in the erythrocytes of obese subjects, compared to normal weight subjects. Narayanareddy and Kaplay (1983) demonstrated that the number of ouabain binding sites on red blood cells were indirectly related to BMI in a group of healthy male subjects.

Total Inhibition Scores, Total Vanadium Indices,
Blood Pressure, and Maximum Ouabain Binding

Inhibition Scores and Vanadium Indices were calculated for each subject as illustrated in Appendix H. Total Inhibition Scores and Total Vanadium Indices were determined by summing the Inhibition Scores and Vanadium Indices of the foods consumed by each subject. Total Inhibition Scores and Total Vanadium Indices are shown in Table 8 with corresponding MBMAX values. Factors which influenced the magnitude of the Total Inhibition Scores and Total Vanadium Indices included the frequency of consumption, serving size, the in vitro inhibitory activity of the food consumed (Table 2, page 46 and Table 3, page 48) or the Vanadium Content Scores of the foods consumed (Table 4, page 49), and the diversity of the diet. Based on these factors, foods that contributed to relatively high Total Inhibition Scores were coffee, tea, lettuce, bananas, apples, and potatoes. The consumption of coffee, tea, white and whole wheat breads, breakfast cereals, lettuce, white rice, and chicken (white meat) by subjects contributed to increased Total Vanadium Indices.

As determined from regression analysis (PROC REG) (SAS Institute, 1982b), there is no apparent trend between either Total Inhibition Scores or Total Vanadium Indices and MBPIS. Also no trends were apparent between Total Inhibition Scores or Total Vanadium Indices and MBMAX, according to PROC REG analysis (SAS Institute, 1982b). The fact that no associations were found may be related to

TABLE 8
TOTAL INHIBITION SCORES^a AND TOTAL VANADIUM INDICES^a
FOR EACH SUBJECT RANKED BY MBMAX^b VALUES

Subject Number	MBMAX	Total Inhibition Score x 10 ⁻³	Total Vanadium Index x 10 ⁻³
414	0.295	228	693
339	0.300	114	664
155	0.365	533	750
342	0.370	290	784
495	0.400	128	595
784	0.450	358	394
633	0.465	130	360
643	0.510	1627	645
921	0.530	538	623
727	0.540	83	376
839	0.585	635	570
813	0.590	68	462
672	0.610	244	404
991	0.620	210	307
276	0.670	103	450
588	0.720	1551	776
869	0.820	1076	349

^aSee Appendices F and H.

^bMean maximum ouabain binding (pmoles/10⁹ cells). N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

the scarcity of published data on in vitro Na^+, K^+ -ATPase inhibitors or vanadium contents in foods, the questionable accuracy of quantitative data obtained from food frequencies, or the possible inability of subjects to determine the quantities and frequencies of consumption. However, even if an association had been found, it would be subject to further investigation, since the in vivo effects of foods containing in vitro Na^+, K^+ -ATPase inhibitors and /or vanadium have not been studied. It has been suggested that vanadium is reduced from the +5 to the +4 oxidative state in vivo (Johnson et al., 1974). The bioavailability of vanadium in foods also is unknown (Myron et al., 1977). In addition, cooking and processing of foods containing Na^+, K^+ -ATPase inhibitors and/or vanadium may have a role in the effectiveness of Na^+, K^+ -ATPase inhibitors and/or vanadium in vivo, possibly enhancing or diminishing in vitro effects.

Evaluation of Dietary Intakes

Energy, Protein, Fat, and Carbohydrate Intakes

Mean energy intakes are shown in Table 9. Mean intakes expressed as percentages of the recommended energy intakes are included in Table 10 (NAS/NRC, 1980). The midpoints of the suggested recommended energy intakes for the appropriate age group (23-50 years = 2000 kcal; > 50 years old = 1800 kcal) were the bases for the percentage intake evaluations. The percentage of calories derived from protein, fat, and carbohydrate also were calculated for each subject (Table 11).

TABLE 9
SEVEN-DAY ENERGY AND NUTRIENT INTAKE MEANS FOR INDIVIDUAL SUBJECTS

Blood Pressure Category and Subject Number	MBPIS ^a	Energy (Kcal)	Protein (g)	Fat (g)	Carbo-hydrate (g)	Calcium (mg)	Phosphorus (mg)	Iron (mg)	Potassium (mg)	Sodium (mg)	Vitamin A (IU)	Crude Fiber (g)	Thiamin (mg)	Ribo-flavin (mg)	Niacin (mg)	Ascorbic Acid (mg)	Cholesterol (mg)
Normotensive^a																	
672	98	1813	56	63	264	742	1069	15	2377	1984	6253	7.2	1.5	1.6	19	134	256
342	100	2045	83	94	191	1062	1399	15	3594	2340	6512	4.4	1.5	5.6	24	194	324
339	102	1527	62	59	191	789	959	11	2114	2264	5962	3.3	1.5	1.5	15	102	251
727 ^e	106	2036	86	70	252	958	1268	16	3109	2772	12031	3.7	1.9	2.6	28	137	312
921	109	1342	66	48	166	828	1144	12	2680	1787	4535	4.3	1.0	1.4	19	124	209
991	110	1720	72	69	212	1221	1406	11	3062	2463	5639	5.7	1.2	1.9	14	163	216
643 ^b	114	1854	54	67	251	968	979	11	2410	1970	13257	4.1	2.6	3.2	30	305	203
495 ^b	115	1052	51	30	148	1019	950	32	2113	2161	11594	3.2	16	11	119	632	113
784	115	1002	39	35	129	305	382	7	991	1669	1560	1.9	0.75	0.86	12	81	148
869 ^c	133	2262	74	100	293	875	1035	18	2342	2588	8623	3.6	2.2	2.2	21	174	300
Medicated/ Controlled Hypertensive^a																	
839 ^d	120	1329	57	60	149	441	676	9	3505	1559	8192	4.2	0.96	0.95	16	197	184
813	126	1955	78	65	200	640	1022	16	2813	3097	5687	4.5	1.2	1.5	24	156	240
414	127	1334	58	57	146	556	765	10	2614	3777	5775	4.3	0.87	1.2	14	152	156
568 ^b	134	1603	52	63	204	633	849	28	2061	2227	11073	3.7	2.7	3.4	33	236	91
Medicated/ Uncontrolled Hypertensive^a																	
155	150	1862	53	84	227	419	735	11	1735	2334	4560	2.9	0.85	1.0	15	94	156

^aMean systolic blood pressure (mm Hg) taken prior to blood-sampling. Subjects ranked by MBPIS within blood pressure categories. N=2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n=1; see Appendix E.

^bTook multiple vitamin supplement daily; see Appendix K.

^cTook multiple vitamin supplement 2 of 7 days; see Appendix K.

^dTook 500 mg of ascorbic acid 1 of 7 days. Took Klorvess, a potassium supplement daily; see Table 5, page 59, and Appendix K.

^eTook Metamucil daily; dosage accounted for 112 kcal per day.

TABLE 10

MEAN ENERGY AND NUTRIENT INTAKES AS PERCENTAGES OF 1980 RECOMMENDED DIETARY ALLOWANCES^{a,b}

Blood Pressure Category and Subject Number	MBPIS ^c	Energy	Calcium	Phos- phorus	Iron	Vitamin A	Thiamin	Ribo- flavin	Niacin	Ascorbic Acid
Normotensive ^c										
672 _d	98	91	93	134	83	156	150	133	146	223
342 _d	100	114	133	167	150	163	150	467	185	323
339	102	76	99	120	61	149	150	125	115	170
727 _g	106	102	120	159	89	301	190	217	215	228
921 _d	109	75	104	143	120	115	100	117	146	207
991	110	86	153	176	61	141	120	158	108	272
643 _e	114	93	121	122	61	331	260	267	231	508
495 _e	115	53	127	119	178	290	1600	917	915	1153
784 _d	115	56	38	48	70	39	75	72	92	135
869 _f	133	113	109	129	100	216	220	183	162	290
Medicated/ Controlled Hypertensive ^c										
839 _{dh}	120	74	55	85	90	205	96	79	123	328
813	126	98	80	128	89	142	120	125	185	260
414 _d	127	74	70	96	100	144	87	100	108	253
588 _{de}	134	89	79	106	280	277	270	283	254	393

TABLE 10 (Continued)

Blood Pressure Category and Subject Number	MBPIS ^C	Energy	Calcium	Phos- phorus	Iron	Vitamin A	Thiamin	Ribo- flavin	Niacin	Ascorbic Acid
Medicated/ Uncontrolled Hypertensive ^C										
155 ^d	150	103	52	92	110	114	85	83	115	163

^aNAS/NRC "Recommended Dietary Allowances," 1980.

^bUnless indicated otherwise subjects are age 23-50; Recommended Energy Allowance midpoint = 2000 kcal; Iron RDA = 18 mg.

^cMean systolic blood pressure (mm Hg) taken prior to blood sampling. Subjects ranked by MBPIS within blood pressure categories. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^dOver age 50; Recommended Energy Allowance midpoint = 1800 kcal, Iron RDA = 10 mg.

^eTook multiple vitamin supplement daily; see Appendix K.

^fTook multiple vitamin supplement 2 of 7 days; see Appendix K.

^gTook Metamucil daily; dosage accounted for 112 kcal per day.

^hTook 500 mg of ascorbic acid 1 of 7 days. Took Klorvess, a potassium supplement daily; see Table 5, page 59, and Appendix K.

TABLE 11
PERCENTAGE OF MEAN ENERGY INTAKES FROM PROTEIN, FAT, AND CARBOHYDRATE^a

Blood Pressure Category and Subject Number	MBP1S ^b (mm Hg)	Energy (kcal)	Protein (%)	Fat (%)	Carbohydrate (%)
Normotensive ^b					
672	98	1813	12	30	58
342 ^{cd}	100	2045	16	41	37
339	102	1527	16	34	50
727 ^e	106	2036	17	31	50
921 ^c	109	1342	19	32	49
991	110	1720	16	36	48
643 ^d	114	1854	12	32	54
495	115	1052	19	25	56
784 ^c	115	1002	16	31	51
869	133	2262	12	38	50
Medicated/ Controlled Hypertensive ^b					
839 ^c	120	1329	16	40	44
813 ^d	126	1955	16	30	41
414 ^{cd}	127	1334	17	38	44
588 ^c	134	1603	13	36	51

TABLE 11 (Continued)

Blood Pressure Category and Subject Number	MBP1S ^b (mm Hg)	Energy (kcal)	Protein (%)	Fat (%)	Carbohydrate (%)
Medicated/ Uncontrolled Hypertensive ^b 155 ^c	150	1862	11	41	48

^aUnless indicated otherwise subjects are age 23-50; Recommended Energy Allowance midpoint = 2000 kcal (NAS/NRC, 1980).

^bMean systolic blood pressure (mm Hg) taken prior to blood sampling. Subjects ranked by MBP1S within blood pressure categories. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^cOver age 50; Recommended Energy Allowance midpoint = 1800 kcal (NAS/NRC, 1980).

^dAlcohol consumption accounted for a portion of mean energy intake.

^eTook Metamucil daily; dosage accounted for 112 kcal per day.

Mean protein intakes are listed for each subject in Table 9. Protein calories accounted for 11% to 19% of mean energy intakes (Table 11). The Recommended Dietary Allowance for protein for a 120 lb. woman age 19 or older is 44 grams (NAS/NRC, 1980). All subjects consumed more than 44 g of protein, except for subject 784 who consumed a mean of 39 g, which as reported by Pao and Mickle (1981) is probably related to a low energy intake (Tables 9 and 10).

Mean fat intakes are shown in Table 9. Twenty-five percent to 41% of mean energy intakes were derived from fat. Fat was the primary energy source for subjects 342 and 155 (Table 11). Increased fat intake in these subjects is related to increased meat consumption and/or snack foods with high fat contents. The highest percentage (41%) is similar to the percent energy derived from fat as reported for adult female subjects in the Nationwide Food Consumption Survey (NFCS) (Pao and Mickle, 1981). Several subjects (991, 839, 414, and 155) exceeded the suggested 35% maximum (for individuals with energy intakes under 2000 kcal) of calories derived from fat (NAS/NRC, 1980) (Table 11).

Mean carbohydrate intakes are given in Table 9. Percentages of mean energy intakes from carbohydrate ranged from 37% to 58% (Table 11). The Senate Select Committee on Nutrition and Human Needs (1977) suggested that carbohydrate represent 55-60% of energy intake. Subjects 672 and 495 were within the 55-60% range; six subjects (339, 727, 643, 784, 869, and 588) were between 50% and 55%. Carbohydrate accounted for less than 50% of the energy intakes of the

remaining subjects (342, 921, 991, 839, 813, 414, and 155) (Table 11). Based on the dietary guidelines developed by the Senate Select Committee on Nutrition and Human Needs (1977), the majority of the subjects probably should increase the proportion of carbohydrates in their diets. In evaluating mean energy, protein, fat, and carbohydrate intakes, there is no apparent relationship between mean intakes and blood pressure among the subjects studied, as determined from PROC STEPWISE (SAS Institute, 1982b).

Calcium and Iron Intakes

Four subjects (784, 839, 414, and 155) had mean dietary intakes of calcium $\leq$ 70% of the suggested 800 mg RDA (NAS/NRC, 1980) (Table 10). All of the medicated (controlled and uncontrolled) hypertensive subjects' diets were low in calcium ($\leq$ 80% of the RDA), possibly due to a lack of dairy products in the diet as observed by McCarron et al. (1984). This finding of low calcium intake in hypertensive subjects is consistent with the results of McCarron et al. (1984). An inverse relationship was noted between calcium intake and high blood pressure when McCarron et al. (1984) evaluated HANES I (Health and Nutrition Examination Survey I) data. Dietary intakes of iron also were $\leq$ 70% of the RDA (18 mg, women < 50 years old; 10 mg, women > 50 years old) (NAS/NRC, 1980) for several subjects (339, 991, 784, and 643). Women's diets are often lacking in calcium and/or iron (Pao and Mickle, 1981). No

correlations were found between mean calcium or iron intakes and MBPIS, as observed from PROC STEPWISE (SAS Institute, 1982b); however, different results may have occurred if some of the women had not been taking multiple vitamin/mineral supplements (Appendix K).

Sodium Intake

According to the NAS/NRC (1980), "estimated safe and adequate" daily sodium intake is between 1100 and 3300 mg (2.8-8.4 g sodium chloride). On the basis of sodium intakes calculated from dietary records, only one subject (414) exceeded this amount by consuming a mean daily sodium intake of 3777 mg (Table 9, page 72). This mean sodium intake may not accurately reflect the subject's normal intake. The subject reported that the week of record-keeping was atypical, because she was ill during the period. The subject consumed high-sodium bouillon on several occasions (Marsh, 1980); thus an over-estimation of her usual mean sodium intake may have occurred.

The Select Committee on GRAS Substances (1979) reported that the average intake of sodium per day by American adults is between 3900 and 4700 mg (10 and 12 g sodium chloride). All subjects had mean sodium intakes below this range. The fact that the medicated hypertensive subjects may have been advised to modify their sodium intake, may account for their reduced sodium intakes. However, it must be recognized that sodium intake is difficult to assess and is easily underestimated (McCarron et al., 1984). It is possible that the mean sodium intakes of women in the study were underestimated, since salt

added at the table (discretionary sodium) (McCarron et al., 1984) is difficult to quantify, the sodium content of foods purchased away from home is often unknown, and the sodium content of manufactured products frequently is not included as nutritional data on the product. Since all of these factors affect the calculation of sodium intake, actual mean sodium intake may be higher or lower than that reported (Table 9, page 72). As determined by stepwise regression (PROC STEPWISE) (SAS Institute, 1982b), in the present study, no correlation was found between sodium intake and MBP1S.

Potassium Intake

The "estimated safe and adequate" intake of potassium is between 1875 and 5625 mg per day (NAS/NRC, 1980). Two subjects (784, and 155) were below this range and no subject exceeded the 5625 mg per day upper-limit (Table 9). Increased potassium intake may be related to decreases in blood pressure (Dustan, 1983; Khaw and Barrett-Connor, 1984; Ophir et al., 1983; Treasure and Ploth, 1983). However, in the present study, no association was apparent between MBP1S and mean potassium intake, as determined using PROC STEPWISE (SAS Institute, 1982b).

Vitamin A, Vitamin C, Thiamin, Riboflavin, and Niacin Intakes

The mean daily intakes of vitamin A, vitamin C, thiamin, riboflavin, and niacin are included in Table 9, and mean intakes as percentages of the Recommended Dietary Allowances (NAS/NRC, 1980) are given in Table 10, page 73. Intakes for all subjects exceeded the RDA

of 60 mg of vitamin C. None of the subjects consumed $\leq 70\%$ of the RDA for thiamin, riboflavin, or niacin (Table 9, page 72 and Table 10, page 73). The mean dietary intakes of subject 784 were below the RDA for most of these nutrients (vitamin A, thiamin, riboflavin, and niacin), which is probably attributable to her low mean energy intake (1002 kcal/day) (Table 9). It is difficult to achieve adequate nutrient intakes when energy intake is minimal (Pao and Mickle, 1981). Other subjects might have been below the RDA for these nutrients if they had not taken multiple vitamin/mineral supplements (Appendix K). None of these nutrients were found to be related to MBP1S when included as candidate variables in stepwise regression (PROC STEPWISE) (SAS Institute, 1982b).

Fiber and Cholesterol Intakes

Mean intakes of crude fiber, as shown in Table 9, ranged from 1.9 g to 7.2 g for the subjects studied. Crude fiber, as analyzed, is a component of dietary fiber (Southgate et al., 1976) and is an "imprecise index of carbohydrates in plant foods which are not digested by man" (Robertson, 1972). Dietary fiber intake cannot be estimated from crude fiber values (Schaller, 1978). Thus, the dietary fiber intakes of the subjects studied is unknown. As determined by stepwise regression (PROC STEPWISE) (SAS Institute, 1982b), crude fiber was found to have a significant inverse relationship with MBP1S (model 2A, Table 12) (see next section).

The mean daily intake of cholesterol for the group of women studied was between 91 and 324 mg (Table 9). Eggs and meats

TABLE 12
ANALYSIS OF BLOOD PRESSURE (MBPIS)^a WITH VARIOUS MODELS^{b,c}

	Model 1A ^{b,d}			Model 2A ^{b,e}			Model 3A ^b			Model 1B ^c		
	df	Mean Squares	Partial Regression Coefficients	df	Mean Squares	Partial Regression Coefficients	df	Mean Squares	Partial Regression Coefficients	df	Mean Squares	Partial Regression Coefficients
Intercept	1		83.90	1		99.09	1		105.88	1		94.61
MBMAX ^f	1	746.00*	55.55	1	1076.82**	69.64	1	993.48**	67.33	1	977.95**	75.64
Fiber				1	540.60**	-5.52	1	358.00**	-4.74			
Cholesterol							1	90.04	-0.04			
Carbohydrate										1	235.50	-0.11
Residual	11	81.64		10	35.74		9	29.71		10	66.25	

* $p < 0.02$.

** $p < 0.01$.

^aMean systolic blood pressure (mm Hg) taken prior to blood sampling. N = 2 except for subjects 339, 643, 672, 727, 813 and 991 for whom n = 1; see Appendix E.

^bCandidate independent variables included in PROC STEPWISE (SAS Institute, 1982b) for models 1A, 2A and 3A were: MBMAX, cholesterol (mg), sodium (mg), vitamin C (mg), and crude fiber (g).

^cCandidate independent variables included in PROC STEPWISE (SAS Institute, 1982b) for model 1B were: MBMAX, carbohydrate (g), energy (kcal), calcium (mg), sodium (mg), riboflavin (mg), vitamin A (IU), and fat (g).

^dModel 1A: $R^2 = 0.45$, $p = 0.0116$.

^eModel 2A: $R^2 = 0.78$, $p = 0.0005$.

^fMaximum ouabain binding (pmoles/ 10^9 cells). N = 2 except for subjects 339, 643, 672, 727, 813, and 991 for whom n = 1; see Appendix E.

contributed to increased cholesterol consumption. Subjects 342 and 727 were the only subjects who exceeded the 300 mg per day cholesterol level that has been recommended as a dietary guideline (Select Committee on Nutrition and Human Needs, 1977). It must be noted however that the dietary guidelines presented in 1977 are a controversial issue (American Dietetic Association, 1977). According to stepwise regression (PROC STEPWISE) (SAS Institute, 1982b), MBP1S and mean cholesterol intake were not related (model 3A, Table 12).

Effect of Maximum Ouabain Binding and Dietary Intake
on Mean Systolic Blood Pressure

As previously discussed, stepwise regression (PROC STEPWISE) (SAS Institute, 1982b) was used to identify the statistical model that best describes the relationships among MBP1S, MBMAX, and dietary intake. As an option in PROC STEPWISE (SAS Institute, 1982b), MBMAX was forced as the first variable in each step of the procedure, in order that changes in the relationship between MBP1S and MBMAX could be examined as dietary variables were introduced into the procedure. The inclusion of variables in the final model was dependent upon the significance of F ratios ($p < 0.05$) as observed from preliminary stepwise regressions using candidate variables. An example of stepwise regression (PROC STEPWISE) (SAS Institute, 1982b) in which an independent variable (carbohydrate) was the first dietary variable to enter the model, but which did not reach significance ($p > 0.05$), and thus was not included in the final model (model 2A, Table 12) is shown in Table 12 (model 1B).

The final model included MBPIS as the dependent variable; MBMAX and crude fiber were independent variables. MBPIS and MBMAX were significantly related in all stepwise procedures. Crude fiber was the only dietary variable that entered with significance in a 2-variable model (model 2A, Table 12). No other dietary variables were significant in a 3-variable model, as illustrated using cholesterol (model 3A, Table 12). Mean squares, significance of F ratios, and partial regression coefficients are shown for the final model in Table 12 (model 2A). The regression equation for the final model (model 2A, Table 12) with MBMAX and crude fiber as independent variables is as follows:

$$\text{MBPIS} = 69.64 (\text{MBMAX}) - 5.52 (\text{Fiber}) + 99.09 \quad .$$

This equation predicts that as MBMAX increases by one pmoles/ 10^9 cells, MBPIS will increase approximately 69 mm Hg, and as crude fiber intake increases by one gram, MBPIS will decrease about 5 mm Hg. The mean squares or type II sum of squares for MBMAX (i.e., partial relationship of MBPIS with MBMAX and crude fiber) increased when crude fiber was introduced into the model, indicating that the two variables (MBMAX and crude fiber) are inversely correlated, and that dietary intake is related to MBPIS via MBMAX. In other words, the inverse relationship found between MBMAX and crude fiber suggests that dietary intake may influence MBMAX (Na^+, K^+ -ATPase activity), which in turn may alter blood pressure (MBPIS). If no relationship existed between MBMAX and crude fiber, the type II sum of squares (mean squares) would not change, which would indicate that the variables are independently associated with blood pressure.

Because only crude fiber was calculated in the present study, it is impossible to compare these results with the dietary fiber studies. It is difficult to understand why crude fiber should be related to blood pressure, since crude fiber is not considered to be of physiological significance (Schaller, 1978). Conflicting results have been found relative to the influence of dietary fiber intake on blood pressure (Brussaard et al., 1981; Wright et al., 1979). Brussaard et al. (1981) found blood pressure and fiber intake to be unrelated in a group of normotensive subjects, whereas Wright et al. (1979) observed decreases (insignificant) in blood pressure as fiber intake increased. It is possible that crude fiber appears as a proxy for a combination of variables that do not significantly contribute to the model for these few subjects (see below). Such variables that possibly should be given important consideration in subsequent research studies, in addition to crude fiber, are cholesterol, niacin, thiamin, and carbohydrate. The relationship between MBP1S and cholesterol, in the preliminary stepwise regressions, did not reach significance, but when cholesterol was included as a candidate variable in the stepwise procedure, it was the second dietary variable to enter the model (model 3A, Table 12). Carbohydrate was often the third dietary variable to enter the model. Niacin and thiamin should be considered as factors in future studies because of a trend toward a positive relationship with fiber. As each of these variables were introduced into the

regression procedure, the type II sum of squares for fiber decreased.

It must be noted that potential relationships between MBPIS and some of the dietary variables may have been obscured, due to the increased vitamin and mineral intakes of subjects taking multiple vitamin/mineral supplements (Appendix K). The response, if any, of MBPIS to vitamin or other nutrient intakes must plateau at some level. Under these circumstances nonlinear regression techniques would be appropriate to investigate the relationships among MBPIS, MBMAX, and dietary variables. When a larger study population is involved, it would be feasible to consider such an evaluation.

CHAPTER V

SUMMARY

The relationships among blood pressure, erythrocyte Na^+, K^+ -ATPase activity (ouabain binding) and dietary intake were investigated in a pilot study involving normotensive (11) and medicated-hypertensive (6) female subjects. The relationships among blood pressure, maximum ouabain binding, and the consumption of foods containing in vitro Na^+, K^+ -ATPase inhibitors and/or vanadium were examined. Subjects (15) participated in dietary assessment (7-day dietary records) and blood sampling (17 subjects) for the determination of ouabain binding to erythrocytes, an indirect measure of Na^+, K^+ -ATPase activity.

Mean systolic blood pressure taken prior to blood sampling (MBP1S) and mean maximum ouabain binding (MBMAX) were directly related. The MBMAX-MBP1S values for several subjects (414, 155, 633, and 672) deviated substantially from the general pattern (Table 6, page 63). Subjects 414 and 155 had unusually low MBMAX's relative to elevated blood pressure. This may be related to their use of arthritis medications (Table 5, page 59) that inhibit prostaglandin synthesis. Inhibited prostaglandin synthesis may account for the subjects' elevated blood pressures because prostaglandins have demonstrated involvement in vasodilation. MBP1S and MBMAX were not related when data from these two subjects were included in linear

regression analysis. Because of the effects of the arthritis medications on blood pressure, data for subjects 414 and 155 were not included in subsequent statistical analysis.

In stepwise regression procedures (PROC STEPWISE) the relationships among age, body mass index (BMI), MBMAX, and MBP1S were evaluated. Age was not associated with either MBP1S or MBMAX. BMI was not related to MBMAX or MBP1S.

Food frequency data collected from subjects (17) were used to evaluate possible associations among MBP1S, MBMAX, and the consumption of foods containing in vitro Na^+, K^+ -ATPase inhibitors and/or foods containing vanadium (an inhibitor of Na^+, K^+ -ATPase activity). Total Inhibition Scores and Total Vanadium Indices, indicators of the consumption of foods containing Na^+, K^+ -ATPase inhibitors and/or vanadium, were calculated per subject. As determined from regression analysis no apparent trends were detected between MBP1S and either Total Inhibition Scores or Total Vanadium Indices. No associations were found between MBMAX and either Total Inhibition Scores or Total Vanadium Indices, as analyzed using linear regressions.

Energy and nutrient means were calculated for the 15 subjects who kept 7-day dietary records. Mean energy intakes ranged from 1002 to 2262 kcal. The majority of the mean energy intakes were derived from carbohydrate intakes. Calcium and iron were problem nutrients since several subjects consumed $\leq 70\%$ of the Recommended Dietary Allowance (RDA) for one or both of these nutrients. All of the medicated-hypertensive subjects studied had low calcium intakes

($\leq 80\%$) relative to the RDA of 800 mg. All subjects exceeded the RDA of 60 mg for vitamin C, and none of the subjects consumed $\leq 70\%$ of the RDA for thiamin, niacin, and riboflavin. One subject's mean intakes were below the RDA for vitamin A, thiamin, riboflavin, and niacin, which may have resulted from the subject's low mean energy intake (1002 kcal).

Analysis of dietary intake data by PROC STEPWISE (SAS Institute, 1982b) indicated that the intake of crude fiber is negatively associated with MBPIS, but only when MBMAX is included in the regression equation. Crude fiber and MBMAX were also found to be inversely related. Of the other dietary variables analyzed (energy, protein, fat, carbohydrate, calcium, iron, vitamin A, vitamin C, thiamin, riboflavin, niacin, and cholesterol), no significant relationships with blood pressure were apparent. It is possible that crude fiber is serving as a proxy for a combination of variables that independently do not alter blood pressure. Variables that may be included in this category and should be considered in future research because of their frequent appearance in stepwise regressions, in addition to crude fiber, are cholesterol, thiamin, niacin, and carbohydrate. It must be noted that increased vitamin and mineral intakes by subjects taking multiple vitamin supplements may have masked the true relationship of some of the dietary variables and mean systolic blood pressure. This also should be a consideration in future studies conducted with larger sample sizes.

REFERENCES

.

REFERENCES

- Acheson, K. J., Campbell, I. T., Edholm, O. G., Miller, D. S. and Stock, M. J. 1980. The measurement of food and energy intake in man—an evaluation of some techniques. *Amer. J. Clin. Nutr.* 33: 1147.
- Ackley, S., Barrett-Connor, E. and Suarez, L. 1983. Dairy products, calcium, and blood pressure. *Amer. J. Clin. Nutr.* 38: 457.
- Adams, C. F. 1975. Nutritive value of American foods in common units. USDA Agric. Handbook No. 456, Agric. Res. Service, Washington, DC.
- Akera, T. and Cheng, V-J. K. 1977. A simple method for the determination of affinity and binding site concentration in receptor binding studies. *Biochim. Biophys. Acta.* 470: 412.
- Allen, F. M. 1920. Arterial hypertension. *JAMA* 74: 652. Quoted in Dahl, L. K. 1977. Salt intake and hypertension. In "Hypertension Physiopathology and Treatment," p. 550. Genest. J., Koiv, E. and Kuchel, O. (Eds.). McGraw Hill, NY.
- Altura, B. M. 1983. Role of magnesium in hypertension and ischemic vascular disorders. *Proc. Stokely-Van Camp Annual Symposium*, p. 87. Dept. of Nutr. and Food Sci., The Univ. of Tennessee, Knoxville.
- Altura, B. M. and Altura, B. J. 1981. Magnesium ions and contraction vascular smooth muscles: relationship to some vascular diseases. *Fed. Proc.* 40: 2672.
- Altura, B. M., Altura, B. J., Carella, A. and Turlapaty, P. 1981. Hypomagnesemia and vasoconstriction: Possible relationship to etiology of sudden death ischemic heart disease and hypertensive vascular diseases. *Artery* 9(3): 212.
- American Dietetic Association. 1977. Dietary goals. A statement by the American Dietetic Association. *J. Amer. Diet. Assoc.* 71: 227.
- Angel, J. E. 1984. "Physicians' Desk Reference," 38th ed. Medical Economics Co., Inc., Oradell, NJ.
- Arkwright, P. D., Beilin, L. J., Rouse, I., Armstrong, B. K., Phil, D. and Vandongen, R. 1982. Effects of alcohol use and other aspects of lifestyle on blood pressure levels and prevalence of hypertension in a working population. *Circulation* 66(1): 60.

- Axelsson, J. M. and Csernus, M. M. 1983. Reliability and validity of a food frequency checklist. *J. Amer. Diet. Assoc.* 83: 152.
- Bauer, J. D. 1982. "Clinical Laboratory Methods," C. V. Mosby Co., St. Louis.
- Beaton, G. H., Milner, J., McGuire, V., Feather, T. E. and Little, J. A. 1983. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. *Amer. J. Clin. Nutr.* 37: 986.
- Beaton, G. H., Milner, J., Corey, P., McGuire, V., Cousins, M., Stewart, E., de Ramos, M., Hewitt, D., Grambsch, P. V., Kassim, N. and Little, J. A. 1979. Sources of variance in 24-hour recall data: implications and interpretations. *Amer. J. Clin. Nutr.* 32: 2546.
- Belizan, J. M., Villar, J., Pineda, O., Gonzalez, A. E., Sainz, E., Garrera, G. and Sibrian, R. 1983. Reduction in blood pressure with calcium supplementation in young adults. *JAMA* 249: 1161.
- Blaustein, M. P. 1977. Sodium ions, calcium ions, blood pressure regulation, and hypertension: a reassessment and a hypothesis. *Amer. J. Physiol.* 232(3): C165.
- Blecha, E. E. 1951. Dietary study methods III. The dietary history for use in diet therapy. *J. Amer. Diet. Assoc.* 27: 968.
- Bogden, J. D., Higashino, H., Lavenhar, M. A., Bauman, J. W., Kemp, F. W. and Aviv, A. 1982. Balance and tissue distribution of vanadium after short-term ingestion of vanadate. *J. Nutr.* 112: 2279.
- Brussaard, J. H., van Raaij, J. M. A., Stasse-Walthuis, M., Katan, M. B. and Hautvast, J. G. A. J. 1981. Blood pressure and diet in normotensive volunteers: absence of an effect of dietary fiber, protein, or fat. *Amer. J. Clin. Nutr.* 34: 2023.
- Buñag, R. D., Tomita, T. and Sasaki, S. 1983. Chronic sucrose ingestion induces mild hypertension and tachycardia. *Hypertension* 5(2): 218.
- Burke, B. S. 1947. The dietary history as a tool in research. *J. Amer. Diet. Assoc.* 23: 1041.
- Campbell, C., Roe, D. A. and Eickwort, K. 1982. Qualitative diet indexes: A descriptive or an assessment tool? *J. Amer. Diet. Assoc.* 81: 687.

- Cantley, L. C. and Aisen, P. 1979. The fate of cytoplasmic vanadium. Implication on (Na,K)-ATPase inhibition. *J. Biol. Chem.* 254: 1781.
- Cantley, L. C., Cantley, L. B. and Josephson, L. 1978. A characterization of vanadate interactions with (Na,K)-ATPase. Mechanistic and regulatory implications. *J. Biol. Chem.* 253: 7361.
- Cantley, L. C., Josephson, L., Warner, R., Yanagisawa, M., Lechene, C. and Guidotti, G. 1977. Vanadate is a potent (Na,K)-ATPase inhibitor found in ATP derived from muscle. *J. Biol. Chem.* 252: 7421.
- Cellier, K. M. and Hankin, M. E. 1963. Studies of nutrition in pregnancy. I. Some considerations in collecting dietary information. *Amer. J. Clin. Nutr.* 13: 55.
- Chalmers, F. W., Clayton, M. M., Gates, L. O., Tucker, R. E., Wertz, A. W., Young, C. M. and Foster, W. D. 1952. The dietary record—how many and which days? *J. Amer. Diet. Assoc.* 28: 711.
- Chen, W. T., Brace, R. A., Scott, J. B., Anderson, D. K. and Haddy, F. J. 1972. The mechanism of the vasodilator action of potassium. *Proc. Soc. Exp. Biol. Med.* 140: 820.
- Consumer and Food Economics Institute. 1977. Expansion of data published in nutritive value of American foods in common units. USDA Data Set No. 456-3, Washington, DC.
- Dahl, J. L. and Hokin, L. E. 1974. The Sodium Potassium Adenosinetriphosphatase. In *Annual Review of Biochemistry*. 43: 327. Annual Reviews, Inc., Palo Alto, CA.
- Dahl, L. K. 1977. Salt intake and hypertension. In "Hypertension Physiopathology and Treatment," Genest, J., Koiw, E. and Kuchel, O. (Eds.), McGraw Hill, NY.
- Dahl, L. K. and Love, R. A. 1957. Etiological role of sodium chloride intake in essential hypertension in humans. *JAMA* 164: 397.
- DeLuise, M., Blackburn, G. R. and Flier, J. S. 1980. Reduced activity of the red-cell sodium pump in human obesity. *N. Engl. J. Med.* 303: 1017.
- De Mendonca, M., Garay, R. P., Ben-Ishay, D. and Meyer, P. 1981. Abnormal erythrocyte cation transport in primary hypertension. Clinical and experimental studies. *Hypertension* 3 (suppl I): I-179.

- Dustan, H. P. 1983. Role of nutrition in hypertension. Its control—experimental aspects. *Prog. Biochem. Pharmacol.* 19: 177.
- Dusting, G. L., Moncada, S. and Vane, J. R. 1977. Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachidonic acid. *Prostaglandins* 13(1): 3.
- Edmondson, R. P. S., Hilton, P. J., Thomas, R. D., Patrick, J. and Jones, N. F. 1975. Abnormal leucocyte composition and sodium transport in essential hypertension. *Lancet* 1: 1003.
- Erdmann, E., Kraweitz, W. and Koch, M. 1979. Cardiac glycoside receptors in disease: the number of ouabain binding sites in human erythrocytes is subject to regulation. In " Na^+ , K^+ -ATPase Structure and Kinetics," Skou, J. C. (Ed.). Academic Press, NY.
- Fregly, M. J. and Kare, M. R. 1982. "The Role of Salt in Cardiovascular Hypertension," Academic Press, NY.
- Freis, E. D. 1976. Salt, volume and prevention of hypertension. *Circulation* 53: 589.
- Frohlich, E. D. 1982. Physiological observations in essential hypertension. *Amer. J. Clin. Nutr.* 80: 18.
- Garay, R. P., Dagher, G. and Meyer, P. 1980a. An inherited sodium-ion potassium-ion cotransport defect in essential hypertension. *Clin. Sci.* 59: 191s.
- Garay, R. P., Elghozi, J-L., Dagher, G. and Meyer, P. 1980b. Laboratory distinction between essential and secondary hypertension by measurement of erythrocyte cation fluxes. *N. Engl. J. Med.* 302: 769.
- Garay, R. P. and Meyer, P. 1979. A new test showing abnormal Na^+ K^+ fluxes in erythrocytes of essential hypertensive patients. *Lancet* 1: 349.
- Garn, S. M., Larkin, F. A. and Cole, P. E. 1978. The real problem with 1-day diet records. *Amer. J. Clin. Nutr.* 31: 1114.
- Garn, S. M., Larkin, F. A. and Cole, P. E. 1976. The problem with one-day dietary intakes. *Ecol. Food Nutr.* 5: 245.
- Gelbart, A. and Goldman, R. H. 1977. Correlation between microsomal (Na^+ + K^+)-ATPase activity and (^3H) ouabain binding of heart tissue homogenates. *Biochim. Biophys. Acta.* 481: 689.

- Glynn, J. M. 1968. Membrane adenosine triphosphatase and cation transport. *Br. Med. Bull.* 24: 165.
- Gros, G., Weller, J. M. and Hoobler, S. W. 1971. Relationship of sodium and potassium intake to blood pressure. *Amer. J. Clin. Nutr.* 24: 605.
- Haddy, F. J. and Pamnani, M. B. 1983. The role of a humoral sodium-potassium inhibitor in low-renin hypertension. *Fed. Proc.* 42: 2673.
- Havlik, R. J., Hubert, H. B., Fabsitz, R. R. and Feinleib, M. 1983. Weight and hypertension. *Ann. Intern. Med.* 98(5, Part 2): 855.
- Hamlyn, J. M., Ringel, R., Schaeffer, J., Levinson, P. D., Hamilton, B. P., Kowarski, A. A. and Blaustein, M. P. 1982. A circulating inhibitor of $(\text{Na}^+ + \text{K}^+)$ -ATPase associated with essential hypertension. *Nature* 30: 650.
- Harlan, D. M. and Mann, G. V. 1982. A factor in food which impairs Na^+ , K^+ -ATPase in vitro. *Amer. J. Clin. Nutr.* 36: 250.
- Higashino, H., Bogden, J. D., Lavenhar, T. and Aviv, A. 1982. Vanadium, Na^+ , K^+ -ATPase, and potassium adaptation in the rat. *Amer. J. Physiol.* 244 (Renal, Fluid and Electrolyte Physiol. 13): F105.
- Hom, G. J., Chelly, J. and Jandhyala, B. S. 1982. Evidence for centrally mediated effects of vanadate on the blood pressure and heart rate of anesthetized dogs. *Proc. Soc. Exp. Biol. Med.* 169: 401.
- Hopkins, L. L. and Mohr, H. E. 1974. Vanadium as an essential nutrient. *Fed. Proc.* 33: 1773.
- Huenemann, R. L. and Turner, D. 1942. Methods of dietary investigation. *J. Amer. Diet. Assoc.* 18: 562.
- Hunt, J. C. 1983. Sodium intake and hypertension: A cause for concern. *Ann. Intern. Med.* 98(5, Part 2): 724.
- Iacono, J. M., Puska, P., Dougherty, B. A., Pietinen, P., Vartiainen, E., Leino, U., Mutanen, M. and Moisio, S. 1983. Effect of dietary fat on blood pressure in a rural Finnish population. 1983. *Amer. J. Clin. Nutr.* 38: 860.
- Jeor, S. T., Guthrie, H. A. and Jones, M. B. 1983. Variability in nutrient intake in a 28-day period. *J. Amer. Diet. Assoc.* 83: 155.

- Johnson, J. L., Cohen, H. J. and Rajagopalan, K. V. 1974. Studies of vanadium toxicity in the rat—lack of correlation with molybdenum utilization. *Biochem. Biophys. Res. Commun.* 56: 940.
- Kawasaki, T., Delea, C. S., Bartter, F. C. and Smith, H. 1978. The effect of high-sodium and low-sodium intakes on blood pressure and other related variables in human subjects with idiopathic hypertension. *Amer. J. Med.* 64: 193.
- Kennedy, B. W., Conway, J. M., Lipton, S. H., Lieberman, W. P., Marcus, W. M. and Marable, N. L. 1982. Na^+ , K^+ -ATPase activities in dog erythrocytes with and without saline infusions of unanesthetized beagles: a preliminary study. *Fed. Proc.* 41: 401.
- Kesteloot, H. and Geboers, J. 1982. Calcium and blood pressure. *Lancet* 1: 813.
- Khaw, K. T. and Barrett-Connor, E. 1984. Dietary potassium and blood pressure in a population. *Amer. J. Clin. Nutr.* 39: 963.
- Kolata, G. 1982. Value of low sodium diets questioned. *Science* 216: 38.
- Krantzler, N. J., Mullen, B. J., Schutz, H. G., Grivetti, L. E., Holden, C. A. and Meiselman, H. L. 1982. Validity of telephoned diet recalls and records for assessment of individual food intakes. *Amer. J. Clin. Nutr.* 36: 1234.
- Krehl, W. A. and Hodges, R. E. 1965. The interpretation of nutritional survey data. *Amer. J. Clin. Nutr.* 17: 191.
- Laragh, J. H. and Pecker, M. S. 1983. Dietary sodium and essential hypertension: some myths, hopes, and truths. *Ann. Intern. Med.* 98(5, Part 2): 735.
- Linusson, E., Sanjur, D. and Erickson, E. 1974. Validating the 24-hour recall. *Archivos Latinoamericanos de Nutricion.* 24(2) (June, Caracas, Venezuela). Quoted in Sanjur, D. 1982. "Social and Cultural Perspectives in Nutrition," p. 172. Prentice-Hall, Englewood Cliffs, NJ.
- MacGregor, G. A. and de Wardener, H. E. 1981. Is a circulating sodium transport inhibitor involved in the pathogenesis of essential hypertension? *Clin. Exp. Hypertens.* 3(4): 815.
- MacGregor, G. A., Fenton, S., Alaghband-Zadeh, J., Markandu, N. D., Roulston, J. E. and de Wardener, H. E. 1981. An increase in a circulating inhibitor of Na^+ , K^+ -dependent ATPase: a possible link between salt intake and the development of essential hypertension. *Clin. Sci.* 61: 17s.

- Marr, J. W. 1971. Individual dietary surveys purposes and methods. *World Rev. Nutr. Diet.* 13: 105.
- Marsh, A. C. 1980. Composition of foods: Soups, sauces and gravies. Raw, processed, prepared, USDA Agric. Handbook No. 8-6, Consumer and Food Economics Institute, Washington, DC.
- Marsh, A. C., Moss, M. K. and Murphy, E. W. 1977. Composition of Foods: spices and herbs. Raw, processed, prepared, USDA Agric. Handbook No. 8-2, Consumer and Food Economics Institute, Washington, DC.
- Marshall, M. W. and Judd, J. T. 1982. Calculated vs. analyzed composition of four modified diets. *J. Amer. Diet. Assoc.* 80: 537.
- McCarron, D. A., Morris, C. D., Henry, H. J. and Stanton, J. L. 1984. Blood pressure and nutrient intake in the United States. *Science* 224: 1392.
- McCarron, D. A. 1983a. Calcium and magnesium nutrition in human hypertension. *Ann. Intern. Med.* 98(5, Part 2): 800.
- McCarron, D. A. 1983b. Nutritional and physiological aspects of dietary calcium in human hypertension. *Proc. Stokely-Van Camp Annual Symposium*, p. 41. Dept. of Nutr. and Food Sci., The Univ. of Tennessee, Knoxville.
- McCarron, D. A. 1982. Low serum concentrations of ionized calcium in patients with hypertension. *N. Engl. J. Med.* 307: 226.
- McCarron, D. A., Morris, C. D. and Cole, C. 1982. Dietary calcium in human hypertension. *Science* 217: 267.
- McHenry, E. W., Fergunson, H. P. and Gurland, J. 1945. Sources of error in dietary surveys. *Can. J. Public Health* 36: 355.
- Meyer, P. 1980. "Hypertension, Mechanisms and Clinical and Therapeutic Aspects," Oxford University Press, New York.
- Morgan, H. E. 1973. Endocrine control systems. In "Best and Taylors Physiological Basis of Medical Practice," J. R. Brobeck (Ed.). Williams and Wilkins Co., Baltimore, MD.
- Morgan, T. and Myers, J. 1982. Dietary salt and hypertension. *Aust. Fam. Physician* 11: 264.
- Morgan, T., Myers, J. and Fitzgibbon, W. 1981. Sodium intake, blood pressure and red cell sodium efflux. *Clin. Exp. Hyperten.* 3(4): 641.

- Morgan, T., Gillies, A., Morgan, G., Adam, W., Wilson, M., Carney, S. 1978. Hypertension treated by salt restriction. *Lancet* 1: 227.
- Mullen, B. J., Krantzler, N. J., Grivetti, L. E., Schutz, H. G. and Meiselman, H. L. 1984. Validity of a food frequency questionnaire for the determination of individual food intake. *Amer. J. Clin. Nutr.* 39: 136.
- Myers, J. B., Morgan, T. O. and Edwards, K. 1983. Effect of sodium intake on intraerythrocyte sodium concentration in normotensive subjects and patients with untreated mild hypertension. *Clin. Exper. Pharmacol. Physiol.* 10: 361.
- Myron, D. R., Givand, S. H. and Nielsin, F. H. 1977. Vanadium content of selected foods as determined by flameless atomic absorption spectrometry. *J. Agric. Food Chem.* 25: 297.
- Narayanareddy, K. and Kaplay, S. S. 1983. Inverse relationship between ouabain sites on human erythrocytes and body mass index in normal healthy subjects. *Metabolism* 32: 722.
- NAS/NRC. 1980. "Recommended Dietary Allowances," 19th ed. National Academy of Sciences, Washington, DC.
- Ophir, O., Peer (Peresecenschi) G., Gilad, J., Blum, M. and Aviram, A. 1983. Low blood pressure in vegetarians: the possible role of potassium. *Amer. J. Clin. Nutr.* 37: 755.
- Pao, E. M. and Mickle, S. J. 1981. Problem nutrients in the United States. *Food Technol.* 35: 58.
- Parfrey, P. S., Wright, P. Holly, J. M. P., Evans, S. J. W., Condon, K., Vandenburg, M. J., Goodwin, F. J., and Ledingham, J. M. 1981. Blood pressure and hormonal changes following alteration in dietary sodium and potassium in young men with and without a familial predisposition to hypertension. *Lancet* 1: 113.
- Pekkarinen, M. 1970. Methodology in the collection of food consumption data. *World Rev. Nutr. Diet.* 12: 145.
- Posati, L. P. 1979. Composition of foods: poultry products. Raw, processed, prepared, USDA Agric. Handbook No. 8-5, Consumer and Food Economics Institute, Washington, DC.
- Posati, L. P. and Orr, M. L. 1979. Composition of foods: dairy and egg products. Raw, processed, prepared, USDA Agric. Handbook No. 8-1, Consumer and Food Economics Institute, Washington, DC.

- Poston, L., Sewell, R. B., Wilkinson, S. P., Richardson, P. J., Williams, R., Clarkson, E. M., MacGregor, G. A. and de Wardener, H. E. 1981. Evidence for circulating sodium transport inhibitor in essential hypertension. *Br. Med. J.* 282: 847.
- Postnov, Y. V., Orlov, S. N., Shevchenko, A. and Adler, A. M. 1977. Altered sodium permeability, calcium binding and Na-K-ATPase activity in the red blood cell membrane in essential hypertension. *Pflugers. Arch.* 371: 263.
- Quist, E. E. and Hokin, L. E. 1978. The presence of two (Na^+, K^+)-ATPase inhibitors in equine muscle ATP: Vanadate and a dithioerythritol-dependent inhibitor. *Biochim. Biophys. Acta.* 511: 202.
- Rabkin, S. W., Mathewson, F. A. L., Tate, R. B. 1978. Predicting risk of ischemic heart disease from systolic and diastolic blood pressures. *Ann. Intern. Med.* 88: 342.
- Rebello, T., Hodges, R. E. and Smith, J. L. 1983. Short-term effects of various sugars on antinatriuresis and blood pressure changes in normotensive young men. *Amer. J. Clin. Nutr.* 38: 84.
- Reeves, J. B. and Weihrauch, J. L. 1979. Composition of foods: fats and oils. Raw, processed, prepared, USDA Agric. Handbook No. 8-4, Consumer and Food Economics Institute, Washington, DC.
- Reisin, E., Abel, R., Modan, M., Silverberg, D. S., Eliahou, H. E. and Modan, B. 1978. Effect of weight loss without salt reduction of blood pressure in overweight hypertensive patients. *N. Engl. J. Med.* 298: 1.
- Richardson, M., Posati, L. P. and Anderson, B. A. 1980. Composition of foods: sausages and luncheon meats. Raw, processed, prepared, USDA Agric. Handbook No. 8-7, Consumer and Food Economics Institute, Washington, DC.
- Robertson, J. 1972. Changes in fibre content of the British diet. *Nature* 238: 290.
- Sagnella, G. A. and MacGregor, G. A. 1984. Characteristics of a ($\text{Na}^+ - \text{K}^+$)-ATPase inhibitor in extracts of tea. *Amer. J. Clin. Nutr.* 40: 36.
- Sanjur, D. 1982. "Social and Cultural Perspectives in Nutrition," Prentice-Hall, Englewood, NJ.
- SAS Institute. 1982a. "SAS User's Guide: Basic," SAS Institute, Inc., Cary, NC.

- SAS Institute. 1982b. "SAS User's Guide: Statistics," SAS Institute, Inc., Cary, NC.
- Schaller, D. 1978. Fiber content and structure in foods. *Amer. J. Clin. Nutr.* 31: S99.
- Schmalzing, G., Pfaff, E. and Breyer-Pfaff, U. 1981. Red cell ouabain binding sites, Na^+ , K^+ -ATPase, and intracellular Na^+ as individual characteristics. *Life Sciences* 29: 371.
- Schussler, G. C. 1982. A letter to the editor. *N. Engl. J. Med.* 304: 1525.
- Select Committee on GRAS Substances. 1979. Evaluation of the health aspects of sodium chloride and potassium chloride as food ingredients, SCOGS 102 Federation of American Societies for Experimental Biology, Life Sciences Research Office, Bethesda, MD.
- Select Committee on Nutrition and Human Needs, United States Senate. 1977. "Dietary Goals for the United States," US Printing Office Washington, DC. [In *J. Amer. Diet. Assoc.* 1977. 70: 360.]
- Shapiro, L. R. 1979. Streamlining and implementing nutritional assessments. *J. Amer. Diet. Assoc.* 75: 230.
- Skou, J. C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiological Reviews* 45: 596.
- Skrabal, F., Aubock, J. and Hortnagl, H. 1981. Low sodium/high potassium diet for prevention of hypertension: Probable mechanisms of action. *Lancet* 2: 895.
- Smith-Barbaro, P. A. and Pucak, G. J. 1983. Dietary fat and blood pressure. *Ann. Intern. Med.* 98(5, Part 2): 828.
- Soremark, R. 1967. Vanadium in some biological specimens. *J. Nutr.* 92: 183.
- Southgate, D. A. T., Baily, B., Collinson, E. and Walker, A. F. 1976. A guide to calculating intakes of dietary fibre. *J. Human Nutr.* 30: 303.
- Stamler, J., Farinaro, E., Mojonier, L. M., Hall, Y., Moss, D. and Stamler, R. 1980. Prevention and control of hypertension by nutritional-hygienic means. *JAMA* 243: 1819.

- Stamler, R., Stamler, J., Riedlinger, W. F., Algera, G., Roberts, R. H. 1978. Weight and blood pressure findings in hypertension screening of 1 million Americans. *JAMA* 240: 1607.
- Steffen, R. P., Pamnani, M. B., Clough, D. L., Huot, S. J., Muldoon, S. M. and Haddy, F. J. 1981. Effect of prolonged dietary administration of vanadate on blood pressure. *Hypertension* 3(Suppl 1): I-173.
- Stokes, G. S., Monaghan, J. C. and Middleton, A. T. 1983. Erythrocyte cation fluxes in normal and hypertensive human subjects. *Clin. Exp. Pharmacol. and Physiol.* 10: 279.
- Stuff, J. E., Garza, C., Smith, E. O., Buford, L. N., Nichols, B. L. and Montandon, C. M. 1983. A comparison of dietary methods in nutritional studies. *Amer. J. Clin. Nutr.* 37: 300.
- Sweadner, K. J. and Goldin, S. M. 1980. Active transport of sodium and potassium and ions. Mechanism, function and regulation. *N. Engl. J. Med.* 302: 777.
- Todd, K. S., Hudes, M. and Calloway, D. H. 1983. Food intake measurement: problems and approaches. *Amer. J. Clin. Nutr.* 37: 139.
- Treasure, J. and Ploth, D. 1983. Role of dietary potassium in the treatment of hypertension. *Hypertension* 5: 864.
- Trulson, M. F. 1954. Assessment of dietary study methods. I. Comparisons of methods for obtaining data for clinical work. *J. Amer. Diet. Assoc.* 30: 991.
- Weber, P. C., Siess, W., Lorenz, R. and Scherer, B. 1981. The role of prostaglandins in essential hypertension. *Int. J. Obesity* 5(suppl 1): 125.
- Wilber, J. A. 1982. The role of diet in the treatment of high blood pressure. *J. Amer. Diet. Assoc.* 80: 25.
- Woods, J. W., Falk, R. J., Pittman, A. W., Klemmer, P. J., Watson, B. S. and Namboodiri, K. 1982. Increased red cell sodium-lithium countertransport in normotensive sons of hypertensive parents. *N. Engl. J. Med.* 306: 593.
- Woods, K. L., Beevers, D. G. and West, M. 1981. Familial abnormality of erythrocyte cation transport in essential hypertension. *Br. Med. J.* 282: 1181.
- Wright, A., Burstyn, P. G. and Gibney, M. J. 1979. Dietary fibre and blood pressure. *Brit. Med. J.* 2: 1541.

- Young, C. 1981. Dietary methodology. In "Assessing Changing Food Consumption Patterns," National Academy Press. Washington, DC.
- Young, C. M., Chalmers, F. W., Church, H. N., Clayton, M. M., Tucker, R. E., Werts, A. W., and Foster, W. D. 1952a. A comparison of dietary study methods. I. Dietary history vs. seven-day record. J. Amer. Diet. Assoc. 28: 124.
- Young, C. M., Hagan, G. C., Tucker, R. C. and Foster, W. D. 1952b. A Comparison of dietary study methods. II. Dietary history vs. seven-day record vs. 24-hour recall. J. Amer. Diet. Assoc. 28: 218.
- Young, J. B., Mullen, D. and Landsberg, V. 1978. Calorie restriction lowers blood pressure in the spontaneously hypertensive rat. Metabolism 27: 1711.

APPENDICES

"

APPENDIX A

ACTIVITY TIMETABLE: HYPERTENSION, ERYTHROCYTE
 Na^+, K^+ -ATPase, AND DIET STUDY

ACTIVITY TIMETABLE: HYPERTENSION, ERYTHROCYTE
 Na^+, K^+ -ATPase AND DIET STUDY

1. Developed recruiting materials for recruiting prospective subjects
2. Prospective subjects completed Medical History Questionnaires and Food Frequency Forms.
3. Selected normotensive and hypertensive subjects.
4. Subjects submitted Consent Forms.
5. Subjects donated two 10-ml blood samples.
6. Subjects recorded 7-day dietary intakes.
7. Subjects returned 7-day dietary intake records.
8. Analyzed subjects' erythrocyte Na^+, K^+ -ATPase activities.
9. Updated data base with nutrient information provided by manufacturers.
10. Coded 7-day dietary records.
11. Entered subjects' dietary intake data on the computer.
12. Analyzed nutrient and energy intakes of subjects, using SAS^a programs.
13. Made conclusions, summarized and presented results.

^aStatistical Analysis System (SAS Institute, Cary, NC).

APPENDIX B

MEMORANDUM TO PROSPECTIVE SUBJECTS

MEMORANDUM

TO: Prospective Subjects

FROM: Dr. Nina Marable
Gloria Gaskin

RE: Human Erythrocyte Na^+, K^+ -ATPase, Hypertension, and Diet Study

The purpose of this project is to study the relationship in adult humans between blood pressure, an enzyme in red blood cells (Na^+ , K^+ -ATPase), and diet. Persons with and without high blood pressure are needed as participants in the study.

Persons interested in participating in the study will be required to have blood pressure measurements taken, complete a medical history questionnaire, and complete a diet frequency form. Collecting this information will enable us to select the most suitable subjects to participate in the study.

Once chosen to participate in the project, subjects will be required to give two 10-ml blood samples on each of two occasions. Blood sampling will be conducted by a registered medical technologist or qualified hospital staff. Subjects will also be required to keep dietary records for seven consecutive days.

The blood sampling days will be approximately one month apart, according to the beginning of the subject's menstrual period when appropriate. For example, if the first sampling is on or about the 28th of the month, the second sampling day will be on or about the 28th of the following month, depending on the length of the subject's menstrual period.

It will be necessary for subjects not to eat or drink anything (except water) for 10 hours prior to the donation of the blood samples. Sampling will take place between 6 and 9 a.m. in the Human Studies Facility of the Nutrition and Food Sciences Department. Subjects will be weighed, without shoes upon arrival. Blood pressure measurements will also be taken several times during the blood sampling sessions.

Prospective Subjects
Page 2

Subjects will be required to keep explicit dietary records, recording all food and drinks consumed for a period of seven days. Instructions will be given explaining dietary record keeping procedures, after the first blood sampling session.

This study has been approved by the University Committee on Research Participation at The University of Tennessee. Subject identity will remain confidential at all times. If you have any questions regarding the procedures of the project or would like more information, CONTACT:

Gloria Gaskin
318 Harris Home Economics Building
974-6252

Dr. Nina L. Marable
301 C Harris Home Economics Building
974-6256

APPENDIX C

SUBJECT QUESTIONNAIRE

CODE NUMBER _____

SUBJECT QUESTIONNAIRE

Name: _____ Date: _____

Address: _____

Telephone (Business): _____ (Home): _____

Best time to call _____

Birthdate: _____

Height: _____

Weight: _____

Name of Physician: _____

Address: _____

1. When was the last time you had your blood pressure checked: _____
Date

2. Do you have high blood pressure: _____
Yes or No

If yes, what medications have been used to control your blood pressure:

NAME OF MEDICATION

DATES OF USE

If yes, what was your blood pressure before medication? _____

What is your usual blood pressure with medication? _____

3. Do you have:

Diabetes?

Heart Disease?

Kidney problems?

Thyroid problems? _____

Have you been checked for these problems by your physician?

Yes or No

4. Are you presently taking any prescription or nonprescription medications, including oral contraceptives?

Yes or No

Prescription or Nonprescription

NAME OF MEDICATION

REASON FOR USE

5. Are you presently under the care of a physician for any medical problem other than high blood pressure? Yes or No If yes, what is the problem?

Yes or No

6. Are you pregnant? Yes or No

Yes or No

7. Has your weight remained stable for the past two years? Yes or No

Yes or No

If not, please explain.

8. Do you menstruate? Yes or No

Yes or No

If yes, are your periods regular? _____
Yes or No

Yes or No

When did your last period begin? _____
Date

Date _____

When do you expect your next period to begin? _____
Date

If no, have you had a hysterectomy? _____; ovariectomy?
Yes or No

Yes or No

9. Do you salt foods during food preparation? _____
Yes or No

Do you salt foods at the table? _____
Yes or No

10. Is there a history of high blood pressure in your family
(immediate family i.e. parents, sisters, brothers, grandparents)?
_____ (specify)
Yes or No

APPENDIX D

CONSENT FORM

CONSENT FORM

I, _____, freely and voluntarily and without undue inducement, consent to participate in a nutrition research project entitled "Human Erythrocyte Na, K-ATPase and Hypertension," to be conducted in the Department of Nutrition and Food Sciences, The University of Tennessee, between October 1, 1983 and March 31, 1984. The purpose of the study is to investigate the relationship between blood pressure, an enzyme which occurs in red blood cells, and diet. For some people, there appears to be a relationship between high blood pressure and the way the red blood cell enzyme works; the enzyme in turn may be affected by diet. An understanding of how diet may affect the enzyme could contribute to the improvement of treatment and control of high blood pressure.

RESPONSIBILITIES: Subjects will be asked to do the following things:

1. have their blood pressure measured several times.
2. fill out a medical history form and a general dietary questionnaire.
3. give two 10-ml blood samples between 6 and 9 AM on each of two occasions after fasting (except for water) for 10 hours. Scheduling of the blood-sampling sessions will be determined, for menstruating women, by the timing of the subject's menstrual cycle.
4. keep complete dietary intake records for seven specified consecutive days after the first blood sampling session. Some instruction on the keeping of records will be provided.

CONFIDENTIALITY: The identity of the subjects will remain confidential at all times, and will not be revealed in oral or written reports of results. Medical history information will be available only to the Principal Investigator or to other project personnel on a need-to-know basis; it will be held in strict confidence by all personnel. Records which include the names of subjects, including consent forms, will be kept in a locked file in the Principal Investigator's office.

WITHDRAWAL OF CONSENT: Subjects may withdraw consent and drop out of the study at any time without penalty. Subjects also may be dropped from the study by the investigators without penalty.

APPROVAL AND INQUIRIES: The study has been reviewed by the Committee on Research Participation at The University of Tennessee. Inquiries regarding the study and the stated procedures should be directed to the Principal Investigator:

Dr. Nina Marable
301C Harris Hall
Department of Nutrition and Food Sciences
The University of Tennessee
Knoxville, TN 37996-1900
Telephone: 974-3491

RISK: No significant risks to subjects are associated with the study except those associated with blood sampling such as would be performed during a routine physical examination. These risks are considered minimal.

COMPENSATION FOR INJURY: If a subject suffers injury as a result of participation in the study, he/she can expect no compensation.

BENEFITS: Subjects will be given a complete nutrient intake profile calculated from their seven days of dietary intake records. Subjects will also be given their blood pressure results; the results from the enzyme measurements will be discussed with them at the conclusion of the study. Subjects will be paid \$10 per blood sample. The benefits to knowledge will include new information about the relationship of diet to hypertension and about the role of the enzyme Na, K-ATPase in the etiology of hypertension.

I understand the above explanations. I have been shown the diet and medical history questionnaires and the record form for complete intake records. Details of the blood sampling procedures have been explained to me, and I have had the opportunity to ask questions. I agree to participate in the study to be conducted at The University of Tennessee between October 1, 1983 and March 31, 1984.

Date

Signature of Subject

Date

Signature of Witness

APPENDIX E

SUBJECT CHARACTERISTICS

SUBJECT CHARACTERISTICS FOR WOMEN PARTICIPATING IN HYPERTENSION, ERYTHROCYTE Na⁺,K⁺-ATPase AND DIET STUDY

Subject	Age (yrs.)	Height (cm)	Weight ^a (kg)	BMI ^b	Day ^c	BP1S ^d	BP1D ^e	BP2S ^f	BP2D ^g	BMAX ^h	Family History ⁱ
155 ⁺	52	165.1	70.0	25.7	1 2	150 150	90 88	142 144	84 84	0.380 0.350	Yes
276 [†]	41	169.0	63.4	22.2	1 2	138 128	* 82	* *	* *	0.650 0.690	Yes
339	41	153.7	60.3	25.5	1	102	74	100	78	0.300	Yes
342	54	167.6	66.4	23.6	1 2	102 98	76 68	102 95	68 65	0.370 0.370	No
414 [†]	58	167.6	89.5	31.9	1 2	118 136	88 94	116 139	84 94	0.330 0.260	Yes
495	41	164.5	60.3	22.3	1 2	118 112	78 74	110 106	70 72	0.480 0.320	Yes
588 [†]	55	156.2	57.4	23.5	1 2	134 134	84 90	* 134	* 90	0.620 0.820	No
633	51	160.1	62.5	24.4	1 2	132 138	90 94	134 136	90 94	0.500 0.430	Yes
643	23	180.3	70.7	21.7	1	114	60	116	66	0.510	Yes
672	26	174.0	59.5	19.6	1	98	58	*	*	0.610	Yes
727	41	172.7	62.3	20.9	1	106	72	106	68	0.540	No
784	58	157.5	60.3	24.3	1 2	112 118	80 76	112 *	80 *	0.450 0.450	Yes
813 [†]	48	174.0	65.5	21.6	1	126	88	128	88	0.590	No
839 [†]	51	157.5	69.4	28.0	1 2	120 120	82 86	120 120	86 84	0.650 0.520	Yes
869	23	174.0	56.3	18.6	1 2	134 132	86 94	130 138	84 96	0.880 0.760	Yes
921	55	161.3	63.8	24.5	1 2	116 102	78 72	110 100	92 68	0.630 0.430	Yes
991	43	172.7	56.8	19.0	1	110	76	112	78	0.620	No

^aWeight taken without shoes, prior to blood-sampling. N = 2 except for subjects 276, 643 and 672 for whom n = 1; and subjects 839 and 869 for whom n = 3.

$$^b\text{Body Mass Index} = \frac{\text{Weight (kg)}}{(\text{Height (M)})^2}.$$

^cBlood-sampling day #1 or day #2.

⁺Medicated moderately uncontrolled hypertensive.

^dSystolic blood pressure (mm Hg) before blood sampling.

[†]Medicated controlled hypertensive.

^eDiastolic blood pressure (mm Hg) before blood sampling.

*Missing data.

^fSystolic blood pressure (mm Hg) after blood sampling.

^gDiastolic blood pressure (mm Hg) after blood sampling.

^hMaximum ouabain binding (pmoles/10⁹ cells).

ⁱFamily history of hypertension.

APPENDIX F

FOOD FREQUENCY FORM

NAME _____

Code _____

This is a Food Frequency form. Please indicate by placing a check in the column which best represents your "average" consumption during the year of the foods and food items listed, except for those you consider to be "seasonal." Please note which foods you consider to be seasonal, indicate the periods during the year the foods are eaten, and check the column describing your "average" consumption during that period. See examples 1 and 2 below. If fruits and vegetables are not prepared from the "fresh" state, indicate the processed form used (i.e. - canned, frozen, dried, etc.) in the comments section. See Example 3 below. If a larger quantity of food (than what is given) is consumed once a day - i.e. at one sitting, check the "several times a day" column. See example 4 below. If a smaller quantity of food (than what is given) is consumed once a day, then check the "once a day" column, cross out the amount given, and indicate in the comments section the amount actually consumed. See example 5 below.

EXAMPLES

		Several times a week	Comments
1. Cocoa, hot	1 C	X	Nov. to Feb.
2. Strawberries	1 C	X	June to Aug.
3. Broccoli	1 C	X	Fresh - 1/2 time Frozen - 1/2 time

		Several times a day	Comments
4. Eggs	1	X	2 at Breakfast

		Once a day	Comments
5. Orange Juice	~~1~~ -c-	X	1/2 c at Breakfast

FOOD FREQUENCY

	Quantity	Never	Rarely or Occasionally	Once a Month	Two or Three Times a Month	Once a Week	Several Times a Week	Once a Day	Several Times a Day	Comments
Beverages										
1. Tea										
Hot	1 c									
Iced	1 c									
2. Cocoa, Hot	1 c									
3. Decaffeinated Coffee	1 c									
4. Coffee	1 c									
5. Milk										
Whole	1 c									
Lowfat (2%)	1 c									
Skim (1%)	1 c									
Dried										
Whole	1 c									
Non-Fat	1 c									
Buttermilk	1 c									
Chocolate										
Lowfat (2%)	1 c									
Skim (1%)	1 c									
6. Pepsi Cola, 1 can	12 oz.									
7. Coca Cola, 1 can	12 oz.									
8. Other Soft Drinks (Specify)										
9. White Wine	1/2 c									
10. Rose Wine	1/2 c									
11. Red Wine	1/2 c									
12. Beer, 1 can	12 oz.									
13. Other Alcoholic beverages (Specify)										
14. Grape Juice	1 c									
15. Orange Juice	1 c									
16. Grapefruit Juice	1 c									
17. Milkshake										
Chocolate	1									
Vanilla	1									

	Quantity	Never	Rarely or Occasionally	Once a Month	Two or Three Times a Month	Once a Week	Several Times a Week	Once a Day	Several Times a Day	Comments
<u>Fruits</u>										
18. Apples	1									
19. Bananas	1									
20. Cantaloupe	1/2									
21. Grapes										
Red	10									
White	10									
22. Honeydew Melon	1/2									
23. Peaches, Fresh	1									
canned	1/2 c									
24. Pears, Fresh	1									
canned	1/2 c									
25. Pineapple, Fresh	1/2 c									
canned	1/2 c									
26. Grapefruit	1/2									
27. Oranges	1									
28. Strawberries	1/2 c									
29. Tangerines	1									
30. Watermelon	1 c									
31. Other Fruits										
- (Specify)										
<u>Vegetables</u>										
32. Broccoli	1 c									
33. Cabbage	1/4 head									
34. Carrots	1									
35. Cauliflower	1/2 c									
36. Corn	1/2 c									
37. Cucumbers, Slices	6									
38. Celery, Stalk	1									
39. Eggplant	1/2 c									
40. Green Pepper	1									
41. Lettuce	1/4 head									
42. Lima Beans	1/2 c									
43. Mushrooms	1/4 c									
44. Onions, Chopped	1 t									
45. Potatoes	1									
46. Sweet Potatoes	1									
47. Radishes	5									
48. Tomatoes, Fresh	1									
canned	1/2 c									
49. Turnip Greens	1/2 c									
50. Spinach	1/2 c									
51. Yellow Summer Squash	1/2 c									
52. Zucchini Squash	1/2 c									
53. Green Beans, Fresh	1/2 c									
canned	1/2 c									

	Quantity	Never	Rarely or Occasionally	Once a Month	Two or Three Times a Month	Once a Week	Several Times a Week	Once a Day	Several Times a Day	Comments
54. Green Peas, Fresh	1/2 c									
canned	1/2 c									
55. Blackeyed Peas	1/2 c									
56. Dried Beans and Peas										
Field Peas	1/2 c									
Navy Beans	1/2 c									
57. Other Vegetables (Specify)										
<u>Eggs and Dairy Products</u>										
58. Eggs	1									
59. Cheese	1 c									
60. Cottage Cheese	1 c									
61. Ice Cream										
Chocolate	1 c									
Other (Specify)	1 c									
<u>Grains and Cereals</u>										
62. Oatmeal	1 c									
63. Cream of Wheat	1 c									
64. Natural (Whole Grain, Ready to Eat)	1 c									
65. Macaroni, Cooked	1 c									
66. Spaghetti, Cooked	1 c									
67. Rice, White	1 c									
Brown	1 c									
68. Other Grains and Cereals (Specify)										
<u>Meats</u>										
69. Bacon, Slices	2									
70. Sausage, Patties	2									
71. Ham, Slices	2									
72. Other Cuts of Pork (Specify)	3 oz.									
73. Chicken										
White	3 oz.									
Dark	3 oz.									
74. Turkey										
White	3 oz.									
Dark	3 oz.									
75. Beef										
Ground	3 oz.									
Cuts of Beef (Specify)	3 oz.									

	Quantity	Never	Rarely or Occasionally	Once a Month	Two or Three Times a Month	Once a Week	Several Times a Week	Once a Day	Several Times a Day	Comments
76. Liver										
Beef, Slice	1									
Pork, Slice	1									
Calf, Slice	1									
Chicken	6									
77. Bologna, Slice	1									
78. Other Processed Meats (Specify)										
79. Codfish	1									
80. Frozen Breaded Fish Products (Specify)										
81. Lobster	1 c									
82. Sardines	4									
83. Scallops	6									
84. Shrimp	6									
85. Oysters	7									
86. Tuna	1/2 c									
87. Flounder, Fillet	1									
88. Mackerel, Fillet (Fresh)	1									
89. Trout, Fillet (Fresh)	1									
90. Salmon	1 c									
Baked Products										
91. Bread, White, Slices	2									
Whole Wheat, Slices	2									
92. Cakes, Yellow, Slice	1									
Chocolate, Slice	1									
93. Pancakes	2									
94. Waffles	1									
95. Cookies, Chocolate Chip	1									
Oatmeal	1									
Other (Specify)	1									
96. Muffins, Blueberry	1									
Corn (Cornbread)	1									
Bran	1									
97. Doughnuts	1									
98. Brownies	1									
99. Fudge, Square	1									
100. Other Baked Products (Specify)										

		Quantity	Never	Rarely or Occasionally	Once a Month	Two or Three Times a Month	Once a Week	Several Times a Week	Once a Day	Several Times a Day	Comments
<u>Snack Foods</u>											
101.	Popcorn, Salted	1 c									
	Unsalted	1 c									
102.	Pretzels	10									
103.	Potato Chips	10									
104.	Saltines	4									
105.	Peanuts	1/2 c									
106.	Other Snack Foods (Specify)										
<u>Fats and Oils</u>											
107.	Margarine	1 T									
108.	Butter	1 T									
109.	Corn Oil	1 T									
110.	Lard	1 T									
111.	Vegetable Shortening	1 T									
112.	Soybean Oil	1 T									
113.	Other Fats and Oils (Specify)										
<u>Miscellaneous</u>											
114.	Pudding, Chocolate	1 c									
	Other (Specify)										
115.	Gelatin	1 c									
116.	Chocolate Bar	1-6"									
117.	Carob Candy Bar (Cocoa Substitute)	1-6"									
118.	Tootsie Roll	1 med.									
119.	Peanut Butter Cups	2									
120.	Other Candy (Specify)										
121.	Chocolate Syrup	2 T									
122.	Peanut Butter	2 T									
123.	Black Pepper	Dash									
124.	Dill Weed	Dash									
125.	Dill Seed	Dash									
126.	Dill Pickle	1									
127.	Garlic, Clove	1/4									
128.	Horseradish	1/4 t									
129.	Jelly/Jam	1 T									
130.	Parsley	Dash									
131.	Canned Soups (Specify)	1 c									

APPENDIX G

DIETARY INTAKE RECORD FORM

APPENDIX H

Na^+, K^+ -ATPase INHIBITION SCORE AND VANADIUM INDEX CALCULATIONS

I. Calculation of Na⁺,K⁺-ATPase Inhibition Score.

A. Convert units of inhibitor/g dry weight to units of inhibitor/g wet weight:

$$\frac{\text{units}^a}{\text{g dry wt.}} \times \frac{\text{g dry wt.}^b}{\text{g wet wt.}} = \frac{\text{units}}{\text{g wet wt.}}$$

B. Calculate Inhibition Rating (units of inhibitor) for the food item:

$$\text{Serving Size}^c \times \frac{\text{units}}{\text{g wet wt.}} = \frac{\text{units of inhibitor}}{\text{food item}}$$

C. Calculate Inhibition Score for the food item based on the frequency of consumption by the subject:

$$\text{Inhibition Rating} \times \text{Frequency Factor}^d = \text{Inhibition Score}$$

Example of Na⁺,K⁺-ATPase Inhibition Score calculation for a subject who consumed an apple once a week:

1. Dry matter/apple = 15.6%
Water content/apple = 84.4%

$$\frac{5 \text{ units}^a}{\text{g dry wt.}} \times \frac{0.156 \text{ g dry wt.}^b}{\text{g wet wt.}} = \frac{0.8 \text{ units}}{\text{g wet wt.}}$$

2. Serving Size^c of an apple = 138 g.

$$\frac{138 \text{ g}}{\text{apple}} \times \frac{0.8 \text{ units}}{\text{g wet wt.}} = \frac{110 \text{ units}}{\text{apple}}$$

3. Frequency Factor^d = 20.

$$\frac{110 \text{ units}}{\text{apple}} \times 20 = 2200 \text{ (Inhibition Score)}$$

^aHarlan and Mann (1982).

^bAdams (1975) and calculation by Gaskin.

^cSee Appendix I.

^dSee Table 1, page 45; factor for once a week consumption.

II. Calculation of Vanadium Index.

A. Determine Vanadium Content Score.^a

B. Determine Serving Size.^b

C. Calculate Vanadium Index of the food item based on the frequency of consumption by the subject.

$$\text{Vanadium Content Score}^a \times \text{Serving Size}^b \times \text{Food Frequency Factor}^c = \text{Vanadium Index.}$$

Example of a Vanadium Index calculation for a subject who consumed a dill pickle once a week:

1. Vanadium Content Score^a of a dill pickle = 3.

2. Serving Size^b of a dill pickle = 65 g.

3. Frequency Factor^c = 20.

4. Vanadium Index for a subject who consumed a dill pickle once a week = $3 \times 65 \times 20 = 3900$.

^aSee Table 4, page 49, proportional to vanadium concentration (ppb, wet weight).

^bSee Appendix I.

^cSee Table 1, page 45, factor for once a week consumption.

APPENDIX I

· SERVING SIZES FOR FOODS AND BEVERAGES CONTAINING
IN VITRO Na^+, K^+ -ATPase INHIBITORS
AND/OR VANADIUM

SERVING SIZES FOR FOODS AND BEVERAGES CONTAINING IN VITRO

Na⁺,K⁺-ATPase INHIBITORS AND/OR VANADIUM^a

<u>Beverages</u>	<u>Serving Size</u>	<u>Grams</u>
Tea	1 c	240
Cocoa	1 c	240
Sanka	1 c	240
Coffee	1 c	240
Whole Milk	1 c	244
Skim Milk, Dried	1 c	23 ^b
Grape Juice	1 c	253
Cola, Canned	12 oz	369
Beer, Canned	12 oz	360
White Wine	1/2 c	120
Rose Wine	1/2 c	120
Red Wine	1/2 c	120
<u>Fruits</u>		
Apple	1	138
Banana	1	120
Cantaloupe	1/2	272
Peach	1	100
Pear	1	165
Pineapple	1/2 c	78
Orange	1	130
Watermelon	1 c	160
<u>Vegetables</u>		
Cabbage	1/4 head	170
Carrot	1	72
Cauliflower	1/2 c	63
Corn	1/2 c	83
Cucumber	6 slices	28
Eggplant	1/2 c	100
Lettuce	1/4 head	135
Potato	1	156
Radish	5	23
Sweet Potato	1	115

	<u>Serving Size</u>	<u>Grams</u>
Tomato	1	90
Turnip Greens	1/2 c	73
Yellow Squash	1/2 c	90
Navy Beans	1/2 c	95
Field Peas	1/2 c	80
<u>Breads and Cereals</u>		
Oatmeal	1 c	240
Breakfast Cereal, Natural	1 c	30
Brown Rice	1 c	195
White Rice	1 c	205
White Bread	2 slices	56
Whole Wheat Bread	2 slices	56
<u>Meats</u>		
Bacon	2 slices	15
Bologna	1 slice	13
Beef Liver	1 slice	85
Calf Liver	1 slice	85
Pork Liver	1 slice	85
Chicken (Light)	3 oz	85
Chicken (Dark)	3 oz	85
Ground Beef	3 oz	85
Pork Chop	3 oz	85
<u>Seafood</u>		
Codfish	1	65
Lobster	1 c	145
Mackerel, Fresh	1 Fillet	105
Trout, Fresh	1 Fillet	100
Sardines	4	48
Scallops	6	60
Tuna, Canned	1/2 c	80
<u>Fats and Oils</u>		
Margarine	1 T	14
Butter	1 T	14
Lard	1 T	13
Corn Oil	1 T	14
Soybean Oil	1 T	14

	<u>Serving Size</u>	<u>Grams</u>
<u>Miscellaneous</u>		
Dill Pickle	1	65
Peanut Butter	2 T	32
Milkshake, Chocolate	1	291
Cornmeal Muffin	1	40
Dill Seed	Dash	0.2
Dill	Dash	0.1
Black Pepper	Dash	0.2
Egg Yolk	1	15

^aUsed in the calculation of Inhibitor Scores and Vanadium Indices. See Appendix F.

^bAmount of dried skim milk added to water to yield 1 cup of fluid skim milk.

APPENDIX J

SUBJECT RESPONSIBILITIES

Responsibilities of Subjects Participating
in the Human Erythrocyte Na⁺,K⁺-ATPase,
Hypertension, and Diet Study

1. Call on the first day of your menstrual period to schedule the day and time of blood sampling. Call Gloria at 974-6252 or leave a message at 974-3491 or at 974-6256.
2. Fast, consuming no food or beverages, other than water, for at least 10 hours prior to blood sampling.
3. Come to give two blood samples on each of two separate occasions approximately one month apart, between 6 a.m. and 9 a.m. on the scheduled day. Blood pressures and weights will be taken during blood sampling sessions.
4. Keep a 7-day food intake record following the donation of the first blood sample. Remember! Accuracy is very important when recording your food intake. You will be required to measure food intake using measuring cups, measuring spoons, and by measuring dimensions with a ruler. Remember during the study period: DO NOT CHANGE YOUR EATING HABITS!
5. Return 7-day record once your 7-day recording period is completed.

REMINDERS

1. RECORD EVERYTHING CONSUMED!
2. Record immediately after every meal.
3. Amounts or Dimensions!
4. Level (L)? Rounded (R)? Heaping (H)?
5. Recipes, Labels, Brand names!
6. Preparation - Baked? Broiled? Boiled? Fried? Raw?
7. Vitamin Supplement? Need label or nutrients and amounts!
8. Seasonings used - Spices? Salt? Margarine? Butter? Oil? Etc.
9. Alcoholic Beverages - Ingredients? Proof? Brand?
10. Fortified? Enriched?
11. Fruits and Vegetables - Raw? Cooked? Canned? Frozen?

APPENDIX K

VITAMIN SUPPLEMENT COMPOSITIONS

VITAMIN SUPPLEMENT COMPOSITIONS

Subject Number	Calcium (mg)	Iron (mg)	Potassium (mg)	Ascorbic Acid (mg)	Thiamin (mg)	Ribo- flavin (mg)	Niacin (mg)	Vitamin A (IU)	Days ^a
495	336	18	0	600	15	10	100	5000	7
588	0	18	0	60	1.5	1.7	20	5000	7
643	0	0	0	60	1.5	1.7	20	5000	7
869	100	18	0	50	2.0	2.5	20	5000	2
839	0	0	1560 ^b	0	0	0	0	0	7
839	0	0	0	500	0	0	0	0	1

^aNumber of days supplement was taken during 7 days of dietary record-keeping.

^bAs a component in Klorvess, a potassium-chloride supplement; see Table 5, page 59.

VITA

Gloria Annette Gaskin, born June 21, 1960, is the daughter of Dr. and Mrs. Lewis J. Gaskin. She is originally from Atlanta, Georgia, but grew up in Raleigh, North Carolina. The author attended E. C. Brooks Elementary School, Josephus Daniels Junior High School, and Ravenscroft Upper School, where she graduated in June of 1978.

In May of 1982 the author received a Bachelor of Science degree in Home Economics with a concentration in Foods and Nutrition and a minor in Business Administration from Meredith College in Raleigh, North Carolina.

The author began graduate studies in Food Science at The University of Tennessee, Knoxville, in September of 1982. In the fall of 1982 she worked as a laboratory assistant in the Department of Nutrition and Food Sciences. The author accepted an Agricultural Experiment Station Graduate Research Assistantship in January of 1983, under the direction of Dr. Nina L. Marable. In March of 1985, the author completed a Master of Science degree in Food Science from The University of Tennessee, Knoxville.