EFFECTS OF DAIRY PRODUCTS ON BLOOD PRESSURE, ENDOTHELIAL FUNCTION, LIPIDS AND LIPOPROTEINS, AND INTRACELLULAR ION DYNAMICS IN CAUCASIAN ADULTS WITH STAGE 1 HYPERTENSION.

A Thesis in
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by
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ABSTRACT

Background: Hypertension (≥140/90 mm Hg) is responsible for more deaths than any other risk factor for CVD and is predicted to become the leading cause of death and disability worldwide by 2020. It is a common and powerful independent predisposing factor for development of coronary heart disease, stroke, peripheral arterial disease, and heart failure. Approximately 65 million Americans (29%) are at increased risk for morbidity and premature death due to elevated blood pressure. Diet and lifestyle modification are the first lines of defense against hypertension; yet, the optimal diet to maximize blood pressure-lowering is uncertain due to limited and conflicting studies. The high prevalence of hypertension, its powerful impact on the incidence of CVD, and the potential impact of control justify high priority efforts to detect and treat elevated blood pressure. Evidence suggests that dietary patterns that include increased calcium and dairy consumption may lower blood pressure or limit its rate of increase with age, however, findings from these studies demonstrate considerable heterogeneity in the blood pressure response to increased calcium.

Objective: The aim of the present study was to clarify the role of dairy products in blood pressure regulation via favorable alterations in intracellular ion balance, hemodynamics, hormone systems, lipids and lipoproteins, and endothelial function.

Design: Using a randomized, 3-period, cross-over design, 23 volunteers (16 male and 7 female) with stage 1 hypertension were fed in random order three experimental diets for 5
weeks each: an average American diet (AAD) serving as a control diet, a diet high in fruits and vegetables but low in dairy (F&V), and a diet similar to the F&V diet but rich in dairy products (D-F&V). All three experimental diets were compositionally matched for sodium (3500 mg), protein (18% kcal), monounsaturated fat (14% kcal), and dietary cholesterol (300 mg) content. The AAD was low in fruits, vegetables, and dairy foods and contained 36% of kcal as total fat, 15% of kcal as saturated fat, 7% of kcal as polyunsaturated fat, 46% of kcal as carbohydrate and 10.5 g dietary fiber. The design of the F&V and D-F&V diets was compositionally similar but differed from the AAD with respect to macronutrients (total fat 30% kcal, saturated fat 7% kcal, polyunsaturated fat 9% kcal, and carbohydrate 52% kcal, and dietary fiber 27 g). In addition, the amount and kind of fruits and vegetables were identical across these two experimental diets. To specifically test the effects of dairy, the D-F&V diet provided 3.4 servings/d of dairy products compared with 0.4 servings/d in the F&V and AAD diets. The D-F&V diet was higher in calcium (1200 mg), potassium (4600 mg), magnesium (420 mg) and phosphorus (1700 mg) compared with the F&V and AAD diets. The calcium content of the F&V and AAD diets was matched (400 mg), but the F&V diet vs. the AAD was higher in potassium (3900 vs. 1750 mg), magnesium (370 vs. 190 mg), and phosphorus (1300 vs. 1150 mg). This innovative diet design allowed us to examine whether a diet rich in dairy products could reduce blood pressure in adults with essential hypertension to a greater extent than a diet high in fruits and vegetables but low in dairy foods and a diet typical of the American diet. At the end of each diet period, blood pressure, lipids and lipoproteins, calcium regulatory and renin-angiotensin systems, erythrocyte cations,
intraplatelet free ionic calcium mobilization, cell adhesion molecules, and endothelial function were measured.

**Results:** Compared to the low calcium AAD and F&V diets, the D-F&V diet significantly lowered intraerythrocyte calcium by ~4.4% (p < 0.0007) and significantly increased intraerythrocyte magnesium by ~17.4% (p < 0.004). The ratio of intraerythrocyte calcium to magnesium also was significantly lower on the D-F&V compared with the F&V and AAD diets (p < 0.0002). Intraerythrocyte sodium tended to be lower on the D-F&V diet vs. the F&V diet (p < 0.06) and intraerythrocyte potassium was unaffected by diet type. The diet-related changes (i.e. experimental diet – AAD control diet) in RBC Ca were negatively correlated with age (r = -0.52, p = 0.0406) and positively correlated with BMI (r = 0.50, p = 0.0508).

The D-F&V diet also lowered the active metabolite of vitamin D, 1,25-dihydroxyvitamin D$_3$ (p = 0.0004) by 19.0% compared to the F&V diet and by 28.4% compared to the AAD. The levels of serum calcium and the other calcium-regulating hormones, PTH and calcitonin, did not change across diets. Intraerythrocyte calcium was significantly correlated with 1,25-dihydroxyvitamin D$_3$ (r = 0.51, p = 0.0229), suggesting that intracellular calcium was reduced via suppression of this hormone.

Angiotensin II levels were 42.6% lower on the D-F&V diet compared with the AAD (p < 0.0044). Angiotensin II levels on the F&V diet were intermediary and not significantly different from either the D-F&V diet (p = 0.1855) or the AAD diet (p = 0.2422). However, measures of renin activity and ACE activity did not significantly differ between diets.
Flow mediated dilation of the brachial artery was similar across all three experimental diets. However, the D-F&V diet produced a significantly larger basal arterial diameter than the F&V (p = 0.05) and control (p = 0.0042) diets. Similarly, the largest peak diameter followed the D-F&V diet, which was 6.7% greater than the control diet (p = 0.0232). Furthermore, the changes in intraerythrocyte magnesium were positively correlated with the changes in FMD (D-F&V r = 0.35, p = 0.1788; F&V r = 0.68, p = 0.0018), such that participants with the greatest increase in RBC Mg exhibited the greatest improvements in FMD.

The D-F&V diet reduced VCAM-1 by about 18-19% compared with the F&V diet (p < 0.0541) and the AAD diet (p < 0.0543). Compared with the AAD diet, ICAM-1 was significantly reduced (~6.7%) by both the D-F&V (p < 0.0550) and F&V diets (p < 0.0571). E-selectin was significantly reduced following the D-F&V diet vs. the control diet (p = 0.0036) and marginally reduced vs. the F&V diet (p = 0.0677). P-selectin followed a similar pattern, with the D-F&V diet eliciting the lowest levels (D-F&V vs. AAD, p = 0.017; D-F&V vs. F&V, p = 0.1393).

The D-F&V and F&V diets elicited significantly greater reductions in total and LDL cholesterol than AAD (p’s < 0.05); the magnitude of the decreases (-6% and 4%, respectively) was comparable in these two lower saturated fat diets. There was a main effect of diet for the change in HDL cholesterol (p = 0.0049); both the D-F&V and F&V diets lowered HDL cholesterol by approximately 5.7% compared with the AAD. The ratios of LDL:HDL cholesterol and Total:HDL cholesterol remained unchanged. Triglycerides were reduced by each diet, however; only the changes from screening
following the AAD were significant ($p = 0.0432$), probably due to the higher fat content of the AAD diet.

In the complete sample ($n=23$), all three experimental diets significantly decreased systolic (SBP) and diastolic (DBP) blood pressure compared to screening (SBP: -7.0%, -8.7%, -8.5%; DBP: -5.7%, -7.7%, -7.6% for AAD, F&V, and D-F&V diets, respectively, $p < 0.0004$). Furthermore, the F&V and D-F&V diets elicited significantly greater decreases in SBP and DBP compared to the AAD ($p \leq 0.05$); however, the two intervention diets did not differ. Although not measured at screening, mean arterial pressure, cardiac output, total peripheral resistance, and stroke volume were similar across the three experimental diets. Diet-related changes in intraerythrocyte calcium were positively correlated with changes in DBP (D-F&V $r = 0.55$, $p = 0.0259$; F&V $r = 0.46$, $p = 0.0539$). Changes in SBP were also positively associated with changes in intraerythrocyte calcium, but the correlations were nonsignificant (D-F&V $r = 0.37$, $p = 0.1593$; F&V $r = 0.35$, $p = 0.1558$).

Because changes in intracellular calcium are postulated to influence blood pressure regulation, a post hoc analysis was conducted to determine possible treatment effects based on each subject’s diet-related change in intraerythrocyte calcium. Subgroup analysis ($n=18$) revealed that only subjects who responded to the dairy-rich diet by significantly reducing intracellular calcium, termed the (Ca)i Change group, exhibited significantly greater decreases in DBP on the D-F&V ($-13.7 \pm 3.4$) compared with the AAD ($-8.1 \pm 3.4$, $p < 0.0001$) and the F&V diet ($-10.1 \pm 3.4$, $p = 0.0135$). The magnitude of the change in DBP on the F&V diet was also significantly greater than the control diet ($p = 0.0181$). Furthermore, the subjects in the (Ca)i Change group had significantly
lower DBP while on the D-F&V diet compared with their counterparts (p = 0.03). SBP followed a similar pattern. In the (Ca)i Change group, the D-F&V diet elicited a significantly greater reduction in SBP compared with the AAD (-18.6 ± 3.9 vs. -13.7 ± 3.9, p < 0.0141). The change in SBP on the F&V diet was intermediary and did not differ from the other two diets. Levels of DBP and SBP were similar across all three diets in subjects who did not exhibit substantial reductions in intraerythrocyte calcium.

In 16 subjects, arginine vasopressin-stimulated increases in cytosolic free ionic calcium in platelets after each diet period were measured. On all three diets, arginine vasopressin induced a rapid elevation in intracellular free Ca\(^{2+}\) that was significantly different from the baseline level (p < 0.0001). After this initial rise, Ca\(^{2+}\) incrementally declined until returning to baseline levels after about 120 seconds post-stimulation. In addition, the peak change in intracellular free Ca\(^{2+}\) was significantly higher following the AAD (492 ± 45\%) vs. the F&V diet (352 ± 44\%, p = 0.0159) and the D-F&V diet (372 ± 47\%, p = 0.0469).

Correlations existed between several study variables following the control AAD. As previously reported, age was correlated with SBP (r = 0.62, p = 0.0028) and FMD (r = -0.47, p = 0.0309). Intraerythrocyte calcium also was positively associated with age (r = 0.49, p = 0.0274), 1,25-dihydroxyvitaminD\(_3\) (r = 0.51, p = 0.0229), total peripheral resistance (r = 0.58, p = 0.0077), and SBP (r = 0.58, p = 0.0075) and negatively associated with cardiac output (r = -0.49, p = 0.0267). RBC Na was positively associated DBP (r = 0.70, p = 0.0006), while RBC K was negatively associated with DBP (r = -0.46, p = 0.0407). The ratio of 24-h Na urinary to urinary Ca was marginally associated with SBP (r = -0.24, p = 0.0610) and MAP (r = -0.38, p < 0.0930), such that as the ratio
increased the levels of SBP and MAP decreased. The relationship between urinary Na:Ca and SBP became significant when the correlation was adjusted for age ($r = -0.45$, $p < 0.0471$). As expected, there was a strong inverse association between total peripheral resistance and cardiac output ($r = -0.89$, $p < 0.0001$) and SBP was positively correlated with total peripheral resistance ($r = 0.51$, $p = 0.0170$) and negatively correlated with cardiac output ($r = -0.048$, $p = 0.0274$). In addition, renin activity and angiotensin II were directly related ($r = 0.43$, $p = 0.0506$).

**Conclusion:** The results of the present study confirm that a low fat diet rich in fruits, vegetables, and dairy products reduces blood pressure in Caucasian stage 1 hypertensive adults. Of particular interest, the addition of three servings of dairy foods to a diet already high in fruits and vegetables and low in total and saturated fat enhances the blood pressuring lowering response in some types of hypertension. Furthermore, our results suggest that the addition of dairy foods may alter vascular tone, via suppression of angiotensin II or normalization of intracellular ion balance; however, due to the small sample size, caution is warranted in forming generalized conclusions.
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<td>AAD</td>
<td>average American diet</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<tr>
<td>AI</td>
<td>adequate intake</td>
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<tr>
<td>ATP III</td>
<td>Third Adult Treatment Panel</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>Ca</td>
<td>calcium</td>
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<td>(Ca(^{2+}))(_i)</td>
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<td>CAM</td>
<td>cell adhesion molecule</td>
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<tr>
<td>CHD</td>
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<td>cardiac output</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<td>Dietary Approaches to Stop Hypertension</td>
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<td>DBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>D-F&amp;V</td>
<td>dairy-rich, fruits and vegetables diet</td>
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<tr>
<td>EAR</td>
<td>estimated average requirement</td>
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<td>FMD</td>
<td>flow mediated dilation</td>
</tr>
<tr>
<td>F&amp;V</td>
<td>fruits and vegetables diet</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>intracellular adhesion molecule-1</td>
</tr>
<tr>
<td>JNC 7</td>
<td>7th Report of the Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure</td>
</tr>
<tr>
<td>K</td>
<td>potassium</td>
</tr>
<tr>
<td>Keal</td>
<td>kilocalorie(s)</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>Mg</td>
<td>magnesium</td>
</tr>
<tr>
<td>(Mg(^{2+}))(_i)</td>
<td>intracellular magnesium</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>Na</td>
<td>sodium</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NTG</td>
<td>nitroglycerin</td>
</tr>
<tr>
<td>P</td>
<td>phosphorous</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cell or erythrocyte</td>
</tr>
<tr>
<td>RDA</td>
<td>recommended dietary allowance</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>TG</td>
<td>triglycerides</td>
</tr>
<tr>
<td>TPR</td>
<td>total peripheral resistance</td>
</tr>
<tr>
<td>V-CAM-1</td>
<td>vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>SVHS</td>
<td>super video home system</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I feel great responsibility to thank my co-advisors, Drs. Penny Kris-Etherton and Sheila West. Their guidance, support, and encouragement over the past five years were invaluable and will be forever remembered.

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Chapter 1

Introduction
1.1 Cardiovascular disease

Atherosclerotic cardiovascular disease (CVD) remains the leading cause of death in the United States and other developed countries. In 2002 over 900,000 Americans died from CVD, representing 38% of all deaths or 1 of every 2.6 deaths (1). Globally, CVD caused about 16.7 million deaths, which translates to approximately one-third of world-wide deaths (2). CVD is pervasive and represents several diseases of the heart including coronary heart disease (CHD), stroke, congestive heart failure, hypertension, diseases of the arteries, congenital cardiovascular defects, and rheumatic heart disease. Approximately 70 million Americans, or about 25% of the population, have some form of CVD (1). The economic burden of CVD is staggering, costing the United States about $393.5 billion this year alone.

CVD is regarded as a multifactorial process that starts in childhood and involves a variety of predisposing risk factors, each of which is best considered as an ingredient of a cardiovascular risk profile (3). A number of major risk factors for atherosclerotic CVD have been delineated and shown to contribute powerfully to CVD risk. These include nonmodifiable factors (i.e. age, sex, and family history) and modifiable risk factors (i.e. cigarette smoking, hypertension, dyslipidemia, obesity, diabetes, and physical inactivity). Identification of the modifiable risk factors through extensive epidemiologic research has stimulated interest in preventing CVD and encouraged public health initiatives against smoking, hypertension, and hypercholesterolemia.

Recent reports, including one comprised of more than 120,000 patients enrolled in clinical trials of CHD (4), indicated at least one major risk factor to be present in 85% of
men and 81% women, and another report involving nearly 400,000 individuals enrolled in three United States cohort studies showed that among those with fatal CHD, 87-100% had exhibited at least one clinically elevated major risk factor (hypercholesterolemia, hypertension, smoking) (5). Indeed, treatment of major CVD risk factors is critical in preventing heart disease. Furthermore, the Centers for Disease Control estimates that proper nutrition would save over $33 billion in medical costs and $9 billion in lost productivity due to heart disease, cancer, stroke and diabetes annually (1). Thus, lifestyle modification, especially dietary modification, will play a key role in squelching CVD in the future.

1.2 Hypertension

Hypertension is responsible for more deaths than any other risk factor for CVD and is predicted to become the leading cause of death and disability worldwide by 2020 (6-8). It is a major risk factor for coronary heart disease, stroke, congestive heart failure, and end-stage renal disease. To estimate its prevalence, hypertension is defined as a systolic blood pressure (SBP) ≥ 140 mm Hg, a diastolic blood pressure (DBP) ≥ 90 mm Hg, or if the person is taking antihypertensive medications. Although substantial reductions in the prevalence of hypertension were made from 1976-1980 to 1988-1994 (9), more recent data show a slight increase in hypertension. According to data from the National Health and Nutrition Examination Survey (NHANES) 1999-2000, the prevalence of hypertension among adults is approximately 29%, an increase of about 4.0% from the 1988-1994 NHANES data (10). This translates to approximately 65
million Americans (1 in 3) who are at increased risk for morbidity and premature death due to elevated blood pressure (≥140/90 mmHg) (1). More importantly, the CVD sequelae imposed by hypertension occur at a 2- to 4-fold increased rate compared with their normotensive counterparts (Table 1-1).

Elevated blood pressure is related to development of CVD in a continuous graded fashion with no indication of a critical value. Risk of CVD increases with each increment in blood pressure, even within the normal range (11-15). As compared with normal blood pressure, high-normal (130-139/85-89 mm Hg) pressure, a seemingly innocuous level, is associated with a 2.5- and 1.6-fold hazard of CVD in women and men, respectively (16). An even more definitive demonstration of the continuous, graded influence of blood pressure on CVD risk comes from a recent meta-analysis of data from 61 prospective studies involving almost one million participants and 56,000 vascular deaths (15). This Prospective Studies Collaboration found that blood pressure is related to vascular mortality, without any indication of a threshold down to 115/75 mm Hg. Persons aged 40 to 69 years had a doubling of risk of stroke or coronary mortality with every 20-mm Hg increment in SBP (or 10-mm Hg higher DBP) throughout the entire range of blood pressure.

Although somewhat arbitrary, classification of blood pressure into stages provides clinicians another basis to make treatment decisions. Accordingly, the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) issued its seventh report (17) which streamlined the classification of blood pressure and highlighted the benefits of lowering blood pressure to optimal goal levels. Table 1-2 presents the new classification of blood pressure. Stage 1 hypertension is a
substantial contributor to atherosclerotic CVD, because it describes about 70% of those diagnosed with hypertension and accounts for well more than half of the deaths and disability attributed to hypertension. The level of Stage 2 hypertension was chosen to reflect future CVD or renal risk. About 50% of adults who have a heart attack and two-thirds of who have a stroke have SBPs higher than 160 mm Hg and the diastolic reading of 100 mm Hg carries a similar level of risk. JNC 7 is the first report to recognize a category of “prehypertension” (SBP 120-139 mm Hg or DBP 80-89 mm Hg) to emphasize the role of increased risk of CVD associated with blood pressure elevated above 115/75 mm Hg. Approximately 90% of individuals 55-65 years of age with blood pressures of 120/80 mm Hg or higher develop hypertension in the next 25 years (18). Furthermore, NHANES 1999-2002 data estimate that about 28% of adults age 18 and older or about 59 million people fall in this category of increased risk (1).
Table 1-1. Risk of CVD events in subjects with Hypertension: 36-Year Follow-up in Framingham Heart Study participants 35-64 years old

<table>
<thead>
<tr>
<th>CVD events</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary disease</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stroke</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardiovascular events</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Different from nonhypertensives, P < 0.001

Table 1-2. JNC 7 Classification of blood pressure<sup>1</sup>

<table>
<thead>
<tr>
<th>Category</th>
<th>Systolic (mm Hg)</th>
<th>Diastolic (mm Hg)</th>
<th>Introduce Lifestyle Modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 120 and &lt; 80</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Prehypertension</td>
<td>120-130 or 80-89</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Stage 1 hypertension</td>
<td>140-159 or 90-99</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Stage 2 hypertension</td>
<td>≥ 160 or ≥ 100</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> JNC 7 = Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

Source: JNC 7 (17)
1.3 Lipids and lipoproteins

The importance of elevated total and low-density lipoprotein (LDL) cholesterol levels in the etiology of CVD and the value of their reduction in the prevention of CVD are well recognized (19). CVD mortality increases when the levels of total and LDL cholesterol are higher than 200 or 130 mg/dl, respectively, and increases considerably when levels exceed 240 or 160 mg/dl, respectively. In the United States, approximately 50.7% or 107 million adults aged 20 years and older have high total cholesterol (≥ 200 mg/dl) (1). Likewise, 45.8% of the population exhibits elevated LDL cholesterol (≥ 130 mg/dl). The prevalence of elevated total cholesterol is slightly higher in women (19.1%) than men (17.2%), whereas men have slightly higher rates of elevated LDL cholesterol (48.5%) than women (43.3%) (1). For every 1% increase in LDL cholesterol levels, there is a 2 - 3% increase in CVD risk. As a major risk factor for CVD, LDL cholesterol is the major target for treatment to reduce risk of CVD (19).

While LDL cholesterol is the atherogenic aspect of total cholesterol, high-density lipoprotein (HDL) cholesterol has been linked to protection against CVD. HDL cholesterol is involved in reverse cholesterol transport and may inhibit atherogenesis; hence, is considered a negative risk CVD risk factor (20). An elevated level of HDL cholesterol (≥ 60 mg/dl) is protective. For every 1 mg/dl decrease in HDL cholesterol is associated with a 2 - 3% increase in CVD mortality. Presently, 39.0% of American men and 14.9% of American women have low HDL cholesterol (< 40 mg/dl) (1).

In addition, an elevated fasting blood triglyceride (TG) concentration has recently been considered an independent risk factor for premature CHD (21). However, the
independence of this relationship from related risk factors such as HDL cholesterol remains controversial. A new case-control study (22) in 653 men who had survived a myocardial infarction or undergone coronary artery bypass surgery and 1029 control subjects found that fasting plasma TG levels $\geq 200$ mg/dl were significantly associated with premature familial coronary artery disease even after adjusting for HDL cholesterol levels and other risk factors. Further examination of different combinations of different values for TG and HDL cholesterol revealed that the risk of premature heart disease due to TG levels was increased at all levels of HDL cholesterol, even when HDL cholesterol levels exceeded 40 mg/dL.

Numerous dietary intervention studies illustrate the importance of cholesterol-lowering for CVD risk reduction. Dietary interventions that replace saturated fat with polyunsaturated fat elicit LDL cholesterol lowering of about 13-15%, which is associated with a 12-44% reduction in CVD endpoints (23-27). Predictive equations developed by Keys et al. (28) and Hegsted et al. (29) in the early 1960s and later confirmed by Mensink and Katan (30), Hegsted et al. (31), Yu et al. (32) and Clarke et al. (33), also indicate that decreasing dietary saturated fatty acids and increasing monounsaturated fatty acids and polyunsaturated fatty acids in the diet may decrease the risk of CVD by decreasing plasma cholesterol.

The Third Adult Treatment Panel (ATP III) of the National Cholesterol Education Program (NCEP) recommends a fasting lipid profile in all adults aged 20 and older as an initial screening for primary prevention of CVD. Classification of clinical cut-points for LDL, total, and HDL cholesterol and triglyceride levels are presented in Table 1-3.
Table 1-3. ATP III Classification of LDL, Total, and HDL cholesterol and Triglycerides

<table>
<thead>
<tr>
<th></th>
<th>LDL cholesterol: primary target of therapy (mg/dL)</th>
<th>Total cholesterol (mg/dL)</th>
<th>HDL cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 100</td>
<td>&lt; 200</td>
<td>&lt; 40</td>
<td>&lt; 150</td>
</tr>
<tr>
<td></td>
<td>Optimal</td>
<td>Desirable</td>
<td>Low</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>100-129</td>
<td>200-239</td>
<td>≥ 60</td>
<td>150-199</td>
</tr>
<tr>
<td></td>
<td>Near optimal/above optimal</td>
<td>Borderline high</td>
<td>High</td>
<td>Borderline high</td>
</tr>
<tr>
<td></td>
<td>130-159</td>
<td></td>
<td></td>
<td>200-499</td>
</tr>
<tr>
<td></td>
<td>Borderline high</td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>160-189</td>
<td></td>
<td></td>
<td>≥ 500</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td></td>
<td></td>
<td>Very high</td>
</tr>
<tr>
<td></td>
<td>≥ 190</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very high</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: ATP III (19)
1.4 Risk Factor Clustering

CVD risk factors tend to cluster within individuals. In the Framingham cohort, at baseline, clustering of 2 or 3 risk factors with hypertension occurred at twice the rate predicted by chance, while a clustering of 3 or more occurred at four times the expected rate (Fig. 1-1) (13). Adults with higher levels blood pressure tend to have higher serum concentrations of total cholesterol, triglycerides, glucose, apolipoprotein B, and lower HDL cholesterol levels. Among adults who exhibit two or more risk factors, the most common combination (23.0%) was hypertension and hypercholesterolemia (1). Data from the NHANES II show that 40% of adults < 55 years of age with blood pressures >140/90 mmHg have serum cholesterol concentrations > 240 mg/dl, whereas cholesterol was elevated to this level in only approximately 20% of normotensive age-matched control subjects (1). Coexistence of hyperlipidemia and hypertension further compounds the risk of major cardiovascular events (34). According to a report of cohort studies conducted in 366,599 young and middle-aged men and women, persons with a low CVD risk profile (total cholesterol <200 mg/dl, blood pressure <120/80 mmHg, and no cigarette smoking) have a 72%-85% lower mortality rate from CVD compared to those with one or more of these three risk factors (35). The recognition that CVD risk factors cluster within individuals dictates that any regimen for the prevention and treatment of hypertension should address multiple CVD risk factors.
Figure 1-1. Risk factor clustering with hypertension in adult men and women.

Source: Framingham Heart Study Offspring (13)
1.5 Endothelial dysfunction: an emerging CVD risk factor

Endothelial dysfunction is an early physiologic event in atherogenesis. A healthy endothelium is not a passive barrier between the blood and subendothelial cells, but an important player in numerous hemostatic mechanisms. The major functions of a healthy endothelium are to maintain blood fluidity, regulate vascular tone, modulate leukocyte and platelet adhesion, and control leukocyte migration. In response to local stimuli, the endothelium synthesizes several molecules that are crucial for vasomotor function. Vasoconstricting factors include thromboxane A₂, prostaglandin H₂, and endothelin-1, while nitric oxide and prostacyclin are endothelium-derived vasodilators. Injury to the endothelium can occur from hypertension, dyslipidemia, hyperhomocysteinemia, and smoking, glycosylated products of diabetes mellitus, and viral and bacterial agents. This injury predisposes to thrombosis, leukocyte adhesion, and proliferation of smooth muscle cells in the arterial wall.

Measures of endothelial dysfunction include markers of endothelial activation (cell adhesion molecules) and impaired endothelium-dependent vasodilation (as indexed by the measurement of flow mediated dilation of a conduit artery). Flow mediated dilation is noninvasive method that assesses endothelial function using high-resolution ultrasound imaging to measure changes in conduit arterial diameter in response to increased flow (36). Most studies examine the brachial artery because of its large size and accessibility. One powerful vasodilatory stimulus that is known to require an intact endothelium is the hyperemic response that follows a period of vascular ischemia.
Following four to five minutes of cuff occlusion of the brachial artery, the increased blood flow triggers the endothelial release of nitric oxide in individuals with a healthy endothelium, and is therefore termed endothelium-dependent vasodilation. This vasodilatory mechanism fails to operate in the presence of endothelial dysfunction. The change in diameter caused by flow-mediated dilation is expressed as a percent change in arterial diameter relative to resting scans. In contrast, administration of sublingual nitroglycerin is used to assess endothelial-independent vasodilation. Nitroglycerin causes vasodilation by direct action of the smooth muscle, and thus, is independent of the presence or the state of the endothelium.

Endothelial dysfunction has been established as an important determinant of coronary risk. Patients with coronary heart disease have impaired endothelium-dependent vasodilation in brachial and coronary arteries, indicating a systemic nature of endothelial dysfunction in atherosclerosis (37). Because abnormal endothelial function is an early marker of CVD, the endothelium appears to be an ideal target for preventive therapy (38). Evidence is now emerging that improvement in endothelial function via diet can potentially reduce the prevalence of CVD beyond that achieved by traditional cholesterol-lowering therapy alone. Dietary factors that have been shown to favorably affect flow mediated dilation include reduction in dietary fat intake and increases in omega-3 fatty acids (39), antioxidant vitamins (i.e. vitamins A, C, and E), folic acid and L-arginine (40,41). It appears that multiple mechanisms mediate the effects of these dietary factors on flow mediated dilation. For example, omega-3 fatty acids may act by decreasing adhesion molecules and triglyceride levels, and via anti-thrombotic effects. Antioxidant vitamins protect against oxidative damage, folic acid prevents
hyperhomocysteinemia and L-arginine increases nitric oxide production, all of which favorably affect endothelial function.

Although the effects of dairy foods on endothelial function have yet to be tested, there is considerable reason to suspect that a dairy rich diet will have significant, independent effects on vascular function. Recent studies show that angiotensin converting enzyme (ACE) inhibitors have direct effects on vascular physiology, and these effects may be mediated by ACE inhibition that occurs at the vessel wall interface (42). Given the interest in ACE-inhibiting properties of milk peptides, this could be important. Improvements in vascular reactivity in animal (43) and in vitro models (44) have also been demonstrated with calcium supplementation.

1.6 Treatment of CVD through diet intervention

A preventive approach to atherosclerotic CVD is crucial because, once clinically present, the disease is apt to progress with lethal consequences. Prevention is feasible because observational research has identified several modifiable predisposing lifestyles that, when corrected, have been shown to reduce the likelihood of the development of clinical atherosclerotic CVD (45,46). The predominant CVD risk factors responsive to diet are hyperlipidemia, hypertension, diabetes, overweight/obesity, and thrombosis. Despite the growing list of emerging CVD risk factors, intervention efforts that focus on markedly impacting these major risk factors can precipitously reduce CVD (4).

In support of this concept, Hu and Willett (47) have proposed that an “optimal diet” could prevent the majority of cardiovascular disease in Western populations. In
addition, Kromhout et al. (48) conclude that CHD can be eliminated to a large extent in individuals < 70 years of age by implementing healthy lifestyle practices, including diet. The question of great importance is: what is the “optimal diet” for maximally reducing CVD risk? Several large, recent epidemiologic studies with both men and women (49-54), as well as smaller studies with women (55,56) have defined healthful dietary patterns that are associated with a reduced CVD risk and all-cause mortality. Trichopoulou et al. (49) reported that greater adherence to a Mediterranean dietary pattern that is high in fruits, vegetables, legumes, nuts, olive oil, moderately high in fish, dairy products, and wine, and low in red meat, was associated with a reduction in all-cause (25%) and coronary mortality (33%). In a small study conducted in Spain, Lasheras et al. (50) found that consumption of the Mediterranean-style diet was associated with a significant reduction (31%) in overall mortality in elderly subjects. In the Health Professionals Follow-up Study and the Nurses’ Health Study (51), a healthful dietary pattern that was rich in fruits and vegetables, nuts, soy, and fiber was associated with a 39% and a 28% reduction in CVD risk in men and women, respectively. When combined with a healthy lifestyle (exercise and no smoking), risk of coronary death and non-fatal infarction was reduced in the Nurses’ Health Study by as much as 83% (52). Similar benefits were seen for all-cause mortality (-31%), coronary mortality (-33%) and stroke (-42%) in women in the Breast Cancer Detection Demonstration Project, who consumed a healthy diet based on national recommendations (53). Michels and Wolk (54) reported a lower all-cause and CVD mortality among women who consumed 16-17 healthy foods, including low-fat dairy products, compared with women who consumed far fewer (0-8 foods). Millen et al. (56) reported a higher prevalence (from 50% to approximately 300%) of carotid
atherosclerosis in women following different dietary patterns that were not considered to
be heart healthy versus a heart healthy dietary pattern, which was higher in fruits,
vegetables, low-fat dairy, calcium, and folate. In a small study by Fung et al. (55), a
Prudent Dietary Pattern was beneficially correlated with biomarker risk factors (plasma
folate, insulin and homocysteine) for CVD and obesity. Collectively, the epidemiologic
studies have consistently shown that a healthful dietary pattern is associated with a
reduction in CVD events and risk.

The common cardioprotective food-based dietary patterns include a wide variety
of fruits and vegetables, whole grains, cereal fiber, low-fat meats, poultry, seafood, and
low-fat dairy products. As would be expected, these dietary patterns are higher in
micronutrient and fiber density, and lower in saturated fat, trans fatty acids, and sugar.
Some have reported higher dietary calcium, folate and vitamin E. Common attributes of
dietary patterns associated with increased CVD risk feature foods that are high in sugar,
saturated fat, trans fat and are low in dietary fiber and micronutrients. Consequently,
these diets have a low nutrient density.

1.7 Current guidelines for dietary management of dyslipidemia and hypertension

Diet always has been a cornerstone in the prevention and treatment of CVD. As
early as 1957, the American Heart Association recommended modification of dietary fat
to reduce the incidence of coronary heart disease (1). Subsequent nutrition
recommendations have been made for the prevention of dyslipidemia that advise
decreasing total fat, saturated fat and cholesterol; partially replacing saturated fat with
unsaturated fats; increasing dietary fiber, especially soluble fiber, as well as plant stanols/sterols, and achieving and maintaining an ideal body weight (2). Currently, the ATP III report recommends the Therapeutic Lifestyle Changes diet, which incorporates several dietary manipulations to maximally lower LDL cholesterol (Table 1-4) (57). Dietary recommendations also have been made to prevent and treat elevated blood pressure which initially involved avoiding high salt intake, inadequate potassium, excess alcohol and overweight and have evolved to include high intake of fruits and vegetables and low-fat dairy products (Table 1-5) (3). Thus, there is a long-standing history of evolving dietary guidance that has targeted major modifiable risk factors by diet (i.e., dylipidemia and hypertension) to reduce risk of CVD.
Table 1-4. Components of the Therapeutic Lifestyle Changes diet

<table>
<thead>
<tr>
<th>Component</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>25-35% of total kcal</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>&lt; 7% of total kcal</td>
</tr>
<tr>
<td>Polyunsaturated fat</td>
<td>Up to 10% of total kcal</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>Up to 20% of total kcal</td>
</tr>
<tr>
<td>Trans fat</td>
<td>As low as possible</td>
</tr>
<tr>
<td>Dietary cholesterol</td>
<td>&lt; 200 mg/d</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>50-60% of total kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>~ 15% of total kcal</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>20-30 g/d</td>
</tr>
<tr>
<td>Viscous fiber</td>
<td>10-25 g/d</td>
</tr>
<tr>
<td>Plant stanols/sterols</td>
<td>2 g/d</td>
</tr>
<tr>
<td>Total calories</td>
<td>Adjust total caloric intake to maintain desirable</td>
</tr>
<tr>
<td></td>
<td>body weight or prevent weight gain.</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Include enough moderate exercise to expend at</td>
</tr>
<tr>
<td></td>
<td>least 200 kcal/d</td>
</tr>
</tbody>
</table>

Source: ATP III (58)
Table 1-5. JNC 7: Lifestyle modification recommendations\(^1\)

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Expected reduction in SBP (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight reduction if overweight (^3)</td>
<td>A 10-kg weight loss is associated with SBP lowering of 5-20 mm Hg.</td>
</tr>
<tr>
<td>Adoption of the DASH eating plan (^4)</td>
<td>Consumption of the DASH diet has been associated with 8-14 mm Hg reduction in SBP.</td>
</tr>
<tr>
<td>Limit sodium intake to no more than 2.4 g sodium (6 g sodium chloride).</td>
<td>Reduction in dietary sodium may lower SBP 2-8 mm Hg.</td>
</tr>
<tr>
<td>Regular aerobic physical activity for 30 minutes per day, most days of the week.</td>
<td>This type of activity may lower SBP 4-9 mm Hg.</td>
</tr>
<tr>
<td>Moderation of alcohol consumption for those who already drink alcohol. Men: ≤2 drinks(^5) per day Women: 1 drink(^5) per day</td>
<td>Moderate alcohol consumption has been shown to lower SBP 2-4 mm Hg.</td>
</tr>
</tbody>
</table>

\(^1\) JNC 7= Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, SBP = systolic blood pressure

\(^2\) Effects are dose and time dependent.

\(^3\) Maintain normal weight (body weight mass 18.5-24.9 kg/m\(^2\)).

\(^4\) Diet rich in fruits, vegetables, and low-fat dairy products with reduced content of saturated and total fat.

\(^5\) 1 drink = 0.5 oz. ethanol (e.g., 12 oz. beer, 5 oz. wine, or 1.5 oz. 80-proof whiskey)

Source: JNC 7 (17)
Current guidelines for the treatment of hypertension are based on the seminal Dietary Approaches to Stop Hypertension (DASH) trial (59). The DASH diet, which emphasizes fruits, vegetables and low-fat dairy products and is low in saturated and total fat, significantly lowered blood pressure by 11.4/5.5 mmHg in subjects with hypertension compared to the control (average American) diet. It was particularly effective in African Americans with hypertension, reducing BP by 13.2/6.1 mmHg (60). The comparison fruit and vegetable diet that was low in dairy products had less pronounced but significant reductions. The reductions in blood pressure, which rival current blood pressure lowering medications, occurred within 2 weeks of consuming the DASH diet. The researchers estimated that coronary heart disease and stroke could be reduced by 15% and 27%, respectively, if Americans followed the a dietary pattern similar to the DASH diet (59). Thus, the DASH trial reaffirms the importance of specific dietary patterns in the prevention and control of hypertension. However, the DASH trial was not designed to determine the principal nutrients or foods that may have antihypertensive effects. One conclusion that can be made is that adopting a diet high in fruits and vegetables results in reductions in blood pressure. Further reductions in blood pressure can be achieved by adopting diets rich in dairy, fish, and low in red meat and fat (59). Therefore, discovery of the mechanism(s) by which this dietary pattern lowers blood pressure starts with the identification of the components responsible for the diet-related effects.

While the impact of dairy foods in the context of the DASH diet is not clear, there is considerable evidence of dairy’s role in blood pressure lowering. Epidemiologic
evidence demonstrates that consumption of milk and other dairy products is associated with reduced blood pressure (61-64), stroke (65-67), type 2 diabetes (68), and metabolic syndrome (69). Furthermore, a diet containing adequate intakes of dairy foods, fruits, and vegetables (similar to what has been recently labeled the DASH diet (59)) is associated with the lowest blood pressures in Americans (70). Randomized trials have confirmed a blood pressure-lowering effect of increased consumption of milk products (71-75). It is, however, difficult to relate any specific component of dairy products to the reduction of blood pressure since milk products are rich in calcium, potassium and magnesium, and low in sodium. Age-related increases in blood pressure are attenuated when dietary calcium is high (76). In addition to electrolytes, milk is a good source of protein and high intake of dietary protein has been associated with reduced blood pressure levels (77-79). Moreover, dairy proteins can be enzymatically degraded to peptide fragments, which have been reported to lower blood pressure (80).

Although hypertension is usually treated as a uniform condition, underlying heterogeneity of the condition is clinically relevant. For example, dietary sodium restriction has been shown to lower, have no effect, or increase blood pressure in equally hypertensive individuals (81). Also a single drug of choice has not been identified to treat all hypertension cases, because the efficacy of drug therapy depends on whether or not the chosen drug affects the underlying cause of hypertension (82). Abnormal steady-state cellular ion activity is an attractive hypothesis in an integrative model (81) that includes hypertension as one manifestation of a more generalized cellular ionic deficit that also involves insulin resistance, hyperinsulinemia, left ventricular hypertrophy, increased arterial stiffness, abnormal platelet aggregation, and accelerated atherosclerotic
Each aspect of hypertensive disease appears to reflect a shared cellular ionic lesion defined at least in part by elevated intracellular ionic calcium and reciprocally suppressed intracellular ion magnesium. The importance of intracellular ion homeostasis is further demonstrated by hypotensive responses to dietary calcium, which normalizes this cellular defect via suppression of calcium-regulating hormones (83). Furthermore, the activity of ion-active hormone systems (i.e. calcium-regulating and renin-angiotensin systems) may identify individuals who may be especially responsive to diets rich in minerals (84). Therefore, studies of the effects of specific diets, particularly diets rich in dairy foods and minerals, on individual physiological mechanisms that regulate blood pressure may provide useful information for developing effective treatments.

The purpose of the present study was to investigate whether a diet rich in dairy products can reduce blood pressure in adults with essential hypertension. The global hypothesis, which guided this research, was that the consumption of healthy diets rich in dairy products would elicit beneficial vascular effects in persons at risk for cardiovascular disease compared to a diet high in fruits and vegetables but low in dairy foods and a diet typical of the Western diet. The effect of a dairy-rich diet on steady-state concentrations of intracellular cations including calcium, magnesium, sodium, and potassium was evaluated. These cations are key because they contribute to final common pathways mediating blood pressure and may help explain underlying pathology of clinical hypertension. This study aimed at clarifying the role of dairy products in the regulation of blood pressure and in ameliorating cellular ion imbalances.
Chapter 2

Literature review
2.1 Blood pressure and CVD risk

Several large, representative surveys of United States population demonstrate the strong, positive association between blood pressure and heart attack, heart failure, stroke, and other CVD events. Data from the well-known Framingham Heart Study and 60 other observational and epidemiologic databases (Fig. 2-1) have firmly established a strong, positive, and continuous relationship between initial blood pressure and future risk of death from ischemic heart disease (15). Within each decade of life, for each blood pressure increase of 20/10 mm Hg, beginning at 115/75 mm Hg, the risk of ischemic heart disease-related death doubles. A similar relationship was observed between initial blood pressure and risk of stroke or other vascular death. Furthermore, these data suggest that a 10 mm Hg reduction in usual SBP or 5 mm Hg reduction in usual DBP, which has been achieved in several randomized trials of long duration (85,86), would be associated with ~40% lower risk of stroke death and about 30% lower risk of death from ischemic heart disease or other vascular causes throughout middle age. Even a 2 mm Hg lower usual SBP would produce ~10% and 7% reduction in stroke mortality and ischemic heart disease mortality, respectively. Maintaining a lower blood pressure of just a few mm Hg is feasible via dietary changes (i.e. reducing sodium intake, increasing calcium, potassium, and magnesium intake (87,88)) and, according to this study, should prevent large numbers of premature deaths and disabling strokes.
Figure 2-1. Relationship of risk of death from ischemic heart disease (on logarithmic scale) and initial blood pressures measured at the beginning of each decade of life in nearly 1 million participants in 61 epidemiologic studies. Larger boxes indicate estimates with smaller 95% confidence intervals, which are indicated for smaller boxes by the vertical lines. The total number of fatal ischemic cardiac deaths was 30,143. The best-fit regression line for each decade of life ignores the point corresponding to the lowest blood pressure (<115/70 mm Hg), which includes some individuals with very low blood pressures. These age-specific regression lines demonstrate the strong, positive, and continuous relationship between blood pressure and risk of death from ischemic heart disease. Source: Lewington, et al. (15).
2.2 Dietary strategies for blood pressure lowering

Strategies to prevent and treat CVD in people with hypertension have been based on pharmacologic therapy. A typical reduction of 5 mm Hg in DBP from drug treatment is estimated to reduce the incidence of CHD events and cerebrovascular disease by 15% and 45%, respectively (89). Dependence upon pharmacologic therapy, however useful, is an inadequate resolution to the problem of hypertension-related CVD. It is well known that the risk of CVD increases with progressive elevations in blood pressure beginning at normal levels. An increment of 20 mm Hg in SBP or 10 mm Hg in DBP doubles the risk in people with a BP of 115/75 mm Hg or greater (17). Therefore, a large portion of the population, who do not meet the threshold for drug therapy, is still at increased risk due to elevated blood pressure. Furthermore, more than 30% of hypertensive Americans are unaware of their problem, more than 40% remain untreated, and approximately 40% of men and 50% of women with known hypertension have pressures that exceed recommended values (10). In consideration of these facts, national guidelines include nonpharmacologic therapies, also termed lifestyle modifications, as the first-line of therapy to prevent and treat hypertension. These include weight reduction if needed, dietary sodium restriction, increased physical activity, moderation of alcohol consumption, and adoption of the DASH eating plan, all of which have been shown to effectively lower blood pressure (Table 1-5).

Accumulating epidemiologic and clinical data suggest that increasing the consumption of dairy products is one dietary change that could have an important impact on improving cardiovascular health. New research suggests that certain populations may
especially benefit from dietary interventions rich in dairy foods (60,90-92). Thus, consumption of dairy products, which has significantly decreased over the past three decades, may be one dietary change that could have an important impact on improving cardiovascular health. The following sections of this chapter will review dietary interventions that control blood pressure and highlight dairy’s role in regulating blood pressure.

2.2.1 Weight reduction

Excess body weight has been associated with an increased risk for high blood pressure as well as adverse effects on other CVD risk factors. Modest weight loss of 5% to 10% can result in decreased blood pressure in hypertensive (93-95) and normotensive adults (96-98). Another advantage of weight loss, is that it can potentially decrease the need for antihypertensive drugs and/or the dosage of such drugs. The Trial of Antihypertensive Interventions and Management Study examined the effects of weight loss alone or in combination with antihypertensive drugs on DBP for six months in overweight hypertensive subjects (n=787) (93). A weight loss of 5% or greater was associated with an 11.6 mm Hg drop in DBP, which was equivalent to the decrease in response to drug therapy. Weight loss in combination with drug therapy (50 mg/d atenolol) further reduced DBP by 18.4 mm Hg. In the Hypertension Control Program, subjects who were receiving effective drug therapy were randomized into different groups while medication was discontinued (95). One group made dietary changes (decrease energy, salt, and alcohol consumption), while the control group did not. The
dietary changes group had a net weight loss of 4.7% compared to the control group. After 4 years, 39% of the group who made dietary changes remained normotensive without drug therapy. In the control group only 5% remained normotensive. A study of similar design found that in the weight loss group (4.1% of basal weight), about 60% of the subjects remained normotensive compared with 35% in the control group (99). In conclusion, in multicenter clinical trials involving overweight subjects, weight loss has been the most effective single modality to reduce blood pressure in the short term.

A recent report by the Framingham investigators (100) evaluated the effect of both the amount of weight lost and the persistence of the weight loss on the risk for incident hypertension. Weight loss was evaluated in two groups of overweight adults, 623 aged 30 to 49 years and 605 aged 50 to 65 years. Overweight was defined as BMI $\geq 25$ kg/m$^2$. The groups were classified both according to the amount of weight lost over four years (weight change $<1.8$ kg [considered stable weight]; lost 1.8 to $<3.6$ kg; lost 3.6 to $<6.8$; lost $\geq 6.8$ kg) and by whether or not the loss was sustained over the next four years. After adjustment for a variety of factors, including age, sex, baseline BMI, and alcohol intake, a weight loss of 6.8 kg or more was associated with a 21% to 29% reduction in the long-term risk of hypertension. After further adjustment for cancer and cardiovascular disease occurring during follow-up, the risk reduction was even stronger for both middle-aged and older adults. Sustained weight loss of 1.8 kg or more over four years also substantially reduced hypertension risk by about 24% compared with those who maintained a stable weight.
2.2.2 Sodium reduction

Multiple studies report that dietary sodium intake is positively associated with blood pressure (101,102). Reducing sodium intake to about 2000-2400 mg of sodium per day has been shown to reduce blood pressure. Furthermore, a 30-40% reduction in dietary sodium intake has been found to reduce blood pressure by 3-5 mm Hg SBP and 2-3 mm Hg DBP in hypertensive subjects, and 2 mm Hg SBP and 0.5-1 mm Hg DBP in normotensive individuals (103-105). A meta-analysis of 17 clinical trials reported a mean reduction of 6 mm Hg SBP and 2 mm Hg DBP associated with a reduction in urinary sodium excretion by 95 mmol/day (106). Law and colleagues (107) estimated that universal reduction in sodium intake by 50 mmol/day may result in a 22% reduction in stroke incidents and 16% reduction in coronary artery disease. Within the general population, large subgroups are reported to be especially sensitive to the blood pressure effects of changes in dietary salt intake. These include African Americans, the elderly, persons with diabetes, the obese, and those with renal impairment (108). Thus, nearly all consensus panels have recommended sodium restriction to 2400 mg per day or less.

2.2.3 Weight loss and sodium reduction

The combined effect of weight loss and sodium reduction has been studied in several populations. Trial of Hypertension Prevention, Phase II evaluated the effect of weight loss alone, sodium reduction alone, and the combination of the two in 2382 overweight normotensive adults (SBP < 140 mm Hg and DBP 83-89 mm Hg) (98). Blood pressure significantly decreased at 6 months in all three groups. The weight loss
group, which lost > 4.4 kg, had a blood pressure reduction of -3.7/-2.7 mm Hg. Sodium restriction of 1800 mg/d was associated with a -2.9/-1.6 mm Hg lower blood pressure. The combination group reduced blood pressure by -4.0/-2.8 mm Hg over 6 months. After 36 months, the effects on blood pressure declined, but were still significant for the weight loss group (-1.3/0.9 mm Hg) and the sodium reduction group (-1.2/0.7 mm Hg), but not for the combined group. Despite decreased compliance to the interventions over time, the incidence of hypertension decreased by about 20% in all three groups over a 3- to 4-year follow-up. In addition, a study in overweight older adults (n=975) on monotherapy to control blood pressure evaluated the effects of weight loss and sodium reduction (94). In the group that lost weight (~10 lbs.) and decreased sodium intake to 900 mg/d, about 50% were able to discontinue drug therapy compared to about one-third of the those in a single intervention group.

The Trial of Hypertension Prevention, Phase I Follow-up study was the first to demonstrate the long-term effects of two 18-month interventions, sodium reduction or weight loss of 3.5 kg (109). The weight loss group vs. the usual care control group experienced a significant drop in SBP and DBP (-5.8/-3.2 mm Hg, p < 0.05), while the sodium restriction group had a significant drop in SBP only (-3.3 mm Hg, p < 0.05). At the conclusion of the 19-month interventions, all contact with subjects was abolished. After 7 years, the short-term weight loss intervention was significantly associated with a 77% decrease in incidence of hypertension, while the sodium-reduction intervention was associated with a non-significant 35% reduction in the incidence. The proportion of the weight loss group on antihypertensive drugs was also significantly lower than the proportion of the control group at follow-up.
Because high blood pressure can be alleviated without achieving ideal body weight, a modest reduction in weight of 5 to 10% is an important and realistic goal for overweight individuals. Furthermore, weight loss has been shown to confer improvements on dyslipidemia (110), insulin resistance (78,111), abnormalities in hemostasis and fibrinolysis (112), and overall quality of life (113). On the other hand, recidivism can be a problem for both weight loss and sodium restriction, and long-term adherence to such programs is relatively uncommon (114).

2.2.4 Dietary patterns

Several clinical studies of dietary patterns, not just single nutrients, have been shown to beneficially affect blood pressure. These patterns share common traits that include diets rich in fruits and vegetables, dietary fiber, dairy foods, and low in sodium, total and saturated fat. **Table 2-2** summarizes these innovative studies.
Table 2-2. Clinical nutrition studies of dietary patterns that demonstrate a blood pressure lowering effect

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Study Design /Duration</th>
<th>Intervention</th>
<th>SBP mm Hg</th>
<th>Results¹</th>
<th>DBP mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASH</td>
<td>N=459</td>
<td>Parallel /8 wks</td>
<td>Combination diet vs. control diet</td>
<td>-5.5 all subjects</td>
<td>-3.0 all subjects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fruit and Vegetable diet vs. control diet</td>
<td>-11.6 in HTN</td>
<td>-5.3 in HTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Combination diet vs. Fruit and Vegetable diet</td>
<td>-3.5 in NTN</td>
<td>-2.2 in NTN</td>
<td></td>
</tr>
<tr>
<td>(58, 59, 91)</td>
<td></td>
<td></td>
<td></td>
<td>-2.8 all subjects</td>
<td>-1.1 all subjects (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-7.2 in HTN</td>
<td>-2.8 in HTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.7 all subjects</td>
<td>-1.9 all subjects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-4.1 in HTN</td>
<td>-2.6 in HTN</td>
<td></td>
</tr>
<tr>
<td>DASH-</td>
<td>N=412</td>
<td>Crossover within two</td>
<td>Combination diet with 1.5 g, 2.4 g, or 3.0 g Na</td>
<td>-3.0 from high to low Na; all subjects</td>
<td>-1.6 from high to low Na; all subjects</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td>parallel arms /4 wks</td>
<td>Control diet with 1.5 g, 2.4 g, or 3.0 g Na</td>
<td>-6.7 from high to low Na; all subjects</td>
<td>-3.5 from high to low Na; all subjects</td>
<td></td>
</tr>
<tr>
<td>(86, 115,</td>
<td></td>
<td></td>
<td>Combination diet vs. control diet at</td>
<td>-5.9 all subjects</td>
<td>-2.9 all subjects</td>
<td></td>
</tr>
<tr>
<td>117)</td>
<td></td>
<td></td>
<td>High Na</td>
<td>-5.0 all subjects</td>
<td>-2.5 all subjects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medium Na</td>
<td>-2.2 all subjects</td>
<td>-1.0 all subjects (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low Na</td>
<td>-5.9 all subjects</td>
<td>-2.9 all subjects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-11.5 in HTN</td>
<td>-5.7 in HTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-7.1 in NTN</td>
<td>-3.7 in NTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Combination-low Na diet vs. Control-high Na</td>
<td>-8.9 all subjects</td>
<td>-4.5 all subjects</td>
<td></td>
</tr>
<tr>
<td>PREMIER</td>
<td>N=810</td>
<td>Free-living, parallel/ 6</td>
<td>Established recommendations vs. Advice only</td>
<td>-3.7 all subjects</td>
<td>-1.7 all subjects</td>
<td></td>
</tr>
<tr>
<td>(117,118)</td>
<td></td>
<td>months</td>
<td>Established recommendations + DASH diet vs. Advice only</td>
<td>-4.6 in HTN</td>
<td>-2.0 in HTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-3.5 in ≥ 50 years</td>
<td>-0.8 in ≥ 50 years (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-4.3 all subjects</td>
<td>-2.6 all subjects</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-6.3 in HTN</td>
<td>-3.6 in HTN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-6.4 in ≥ 50 years</td>
<td>-2.6 in ≥ 50 years</td>
<td></td>
</tr>
</tbody>
</table>
Table 2-2. Clinical nutrition studies of dietary patterns that demonstrate a blood pressure lowering effect continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Study Design /Duration</th>
<th>Intervention</th>
<th>SBP mm Hg</th>
<th>DBP mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vanguard Study (119)</strong></td>
<td>N=158 HTN, HTHL, and NTN</td>
<td>Parallel /10 wks</td>
<td>Prepared meal plan (meets published recommendations) vs. self-selected diet with nutrition counseling</td>
<td>-6.2 all subjects</td>
<td>-4.2 all subjects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prepared meal plan vs. baseline</td>
<td>-7.0 in HTN</td>
<td>-6.0 in HTN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-10.0 in HTHL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Self-selected diet vs. baseline</td>
<td>-9.0 in HTN</td>
<td>-6.0 in HTN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-5.0 in HTHL</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nowson et al. (120)</strong></td>
<td>N=94 DBP &lt;90 mm Hg SBP &lt;160 mm Hg</td>
<td>Free-living, parallel/ 4 months</td>
<td>DASH-type diet vs. control</td>
<td>-1.8 all subjects</td>
<td>0.0 all subjects (NS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High K diet vs. control</td>
<td>-4.4 all subjects</td>
<td>-2.0 all subjects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High dairy vs. control</td>
<td>NS all subjects</td>
<td>NS all subjects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High K diet vs. DASH-type diet</td>
<td>-3.5 all subjects</td>
<td>-1.9 all subjects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High dairy vs. DASH-type diet</td>
<td>+3.1 all subjects</td>
<td>+0.8 all subjects (NS)</td>
</tr>
<tr>
<td><strong>Nowson et al. (121)</strong></td>
<td>N=54 men DBP &lt;90 mm Hg SBP &lt;160 mm Hg</td>
<td>Free-living, parallel /12 wks</td>
<td>Weight-loss, DASH-type diet vs. baseline</td>
<td>-7.6 all subjects(^1, 3)</td>
<td>-5.4 all subjects</td>
</tr>
<tr>
<td></td>
<td>Overweight</td>
<td></td>
<td>Weight-loss, low-fat diet vs. baseline</td>
<td>-2.1 all subjects(^1, 3)</td>
<td>-1.0 all subjects</td>
</tr>
</tbody>
</table>

\(^1\) all results are significant, p < 0.05, unless noted as NS=nonsignificant.
\(^2\) Weight decreased by ~ 5.0 kg in both groups at the same rate.
\(^3\) Difference between the two intervention groups, P < 0.001

DASH=Dietary Approaches to Stop Hypertension; DBP=diastolic blood pressure; SBP=systolic blood pressure; HTN=hypertensive; wks=weeks; NTN=normotensive; NS=nonsignificant; Na=sodium; HTHL=hypertensive hyperlipidemic; BP=blood pressure
2.2.4.1 DASH and DASH-Sodium Trials

Designed to prevent and treat hypertension, the DASH eating plan is based on the seminal Dietary Approaches to Stop Hypertension (DASH) trial (59) and DASH-Sodium trial (87)(Table 2-2). The DASH trials were unique in that they tested the effects of modifying dietary patterns in people with DBP of 80-95 mm Hg and SBP of < 160 mm Hg.

In the DASH trial (59), participants (n=459) were fed a control diet for a 3-week run-in period. The control diet paralleled the macronutrient content of the typical American diet that is low in fruits and vegetables, high in SFA and total fat. Dietary calcium, potassium, and magnesium levels fell in the 25th percentile of the US diet. In a parallel-arm design, participants were then randomly assigned to one of three intervention diets for 8 weeks: the control diet, the fruits and vegetables diet, or the combination diet. The fruits and vegetable diet provided about 10 servings of fruits and vegetables per day, was high in potassium, magnesium, and fiber but similar to the control diet in macronutrient content. The combination diet was high in fruits and vegetables, low-fat dairy products, whole grains, fish, poultry, and nuts, and was low in fat, red meat, and sweets. Compared with the other two diets, the combination diet was reduced in saturated fat, total fat, and cholesterol, and moderately high in protein, calcium, potassium, and magnesium. All three diets were matched for sodium (3000 mg/d) and body weight was maintained throughout the entire 8 weeks. Table 2-3 presents the nutrient composition of the three diets.
### Table 2-3. DASH Menu Assayed Nutrient Values— 2100 Kcal

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control assayed</th>
<th>F&amp;V assayed</th>
<th>DASH assayed</th>
<th>DASH - F&amp;V</th>
<th>DASH - AAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (%kcal)</td>
<td>50.5</td>
<td>49.2</td>
<td>56.5</td>
<td>7.3</td>
<td>6</td>
</tr>
<tr>
<td>Protein (%kcal)</td>
<td>13.8</td>
<td>15.1</td>
<td>17.9</td>
<td>2.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Fat (%kcal)</td>
<td>35.7</td>
<td>35.7</td>
<td>25.6</td>
<td>-10.1</td>
<td>-10.1</td>
</tr>
<tr>
<td>Saturated (%kcal)</td>
<td>14.1</td>
<td>12.7</td>
<td>7.0</td>
<td>-5.7</td>
<td>-7.1</td>
</tr>
<tr>
<td>Monounsaturated (%kcal)</td>
<td>12.4</td>
<td>13.9</td>
<td>9.9</td>
<td>-2.5</td>
<td>-4</td>
</tr>
<tr>
<td>Polyunsaturated (%kcal)</td>
<td>6.2</td>
<td>7.3</td>
<td>6.8</td>
<td>-0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>233</td>
<td>184</td>
<td>151</td>
<td>-33</td>
<td>-82</td>
</tr>
<tr>
<td>Fiber (g) *</td>
<td>9</td>
<td>31</td>
<td>31</td>
<td>-22</td>
<td>-22</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3028</td>
<td>2816</td>
<td>2859</td>
<td>43</td>
<td>-169</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1752</td>
<td>4101</td>
<td>4415</td>
<td>314</td>
<td>2663</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>443</td>
<td>534</td>
<td>1265</td>
<td>731</td>
<td>822</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>176</td>
<td>423</td>
<td>480</td>
<td>57</td>
<td>304</td>
</tr>
<tr>
<td>Fruit sv/d</td>
<td>1.6</td>
<td>3.3</td>
<td>4.4</td>
<td>1.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Vegetable sv/d</td>
<td>2.0</td>
<td>3.0</td>
<td>2.7</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Dairy sv/d</td>
<td>0.5</td>
<td>0.3</td>
<td>2.7</td>
<td>2.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* target value, not assayed  
sv/d = servings per day  
AAD = Average American Diet  
DASH = Dairy, Fruits, and Vegetables Diet  
NR = not reported  
Source: Appel et al. (58)

Compared to the control diet, both intervention diets reduced blood pressure. The combination diet significantly lowered systolic and diastolic blood pressure by 5.5 and 3.0 mm Hg, respectively, compared to the control diet (59). Although to a lesser degree, the fruits and vegetables diet also significantly lowered blood pressure by 2.8/1.1 mm Hg compared to the control diet. In subjects with stage 1 hypertension (140/90 to 159/95 mm Hg, 29% of total sample), the combination diet decreased SBP by 11.6 mm Hg and
DBP by 5.3 mm Hg (60). Blood pressure also significantly decreased in normotensive subjects but by a much lesser extent (3.5/2.2 mm Hg). Hypertensive African Americans had the most robust response to the combination diet reducing blood pressure by 13.2/6.1 mm Hg compared to the control diet (60). Importantly, the reductions in blood pressure rivaled current blood pressure lowering medications, occurred within 2 weeks of consuming the DASH diet, and controlled hypertension in approximately 70% of the hypertensive group (92). The DASH researchers estimated that CHD and stroke could be reduced by 15% and 27%, respectively, if Americans followed the DASH diet (59).

While the amount of sodium (3,000 mg/d) provided was held constant across all three diets, it exceeded the current recommendation of < 2,400 mg/d. To explore the additional effect of sodium reduction in the context of the combination diet, the DASH-Sodium trial was conducted (87). In this trial, 412 men and women with high normal blood pressure (120/80 – 139/89 mm Hg) or stage 1 hypertension (140/90 – 159/95 mm Hg, 41% of total sample) consumed either the combination diet with varying levels of sodium or the control diet with varying levels of sodium. There were 3 levels of sodium intake: ‘high’ level of 3,300 mg/d, which is similar to average US intake; ‘intermediate’ level of 2,400 mg/d, which is the upper level of current recommendations; ‘low’ level of 1,500 mg/d. In a crossover design, subjects consumed their assigned diet (combination or control) at every level of sodium intake (1,500, 2,400, and 3,300 mg/d) for 4 weeks.

Reduction in sodium intake in the context of both the control and combination diet reduced blood pressure (from high sodium to low sodium a decrease of 3.0/1.6 mm Hg on the combination diet and 6.7/3.5 mm Hg on the control diet) (87). Compared to the combination diet, the sodium-related decrease in blood pressure was greater in the
control diet. However, those consuming the combination diet at the lowest level of sodium experienced the lowest mean blood pressure. Although these effects were significant in all subgroups, hypertensive individuals, those older than 45 years of age, women, and African Americans appeared to especially benefit from the combined interventions (90,115). In an ancillary study, Akita et al. (116) suggests that the blood-pressure-lowering effect of the DASH diet may be achieved through a natriuretic effect, with the diet basically acting as a natural diuretic. These results indicate that the DASH diet is beneficial throughout a range of sodium intakes and that the combined effects of the DASH diet and sodium restriction may be greater.

2.2.4.2 PREMIER Trials

The PREMIER study (117) tested the effects of implementing the DASH diet in combination with other recommendations for lowering blood pressure in a free-living population (Table 2-2). This was a multicenter, randomized, parallel-arm, free-living trial in 810 adults with above-optimal blood pressure (120-159 mm Hg systolic and 80-95 mm Hg diastolic blood pressure) and not taking antihypertensive medication. Of the subjects, 62% were women, 34% were African American, and the average age was 50 years. Average blood pressure was 135/85 mm Hg, 38% were hypertensive, and 95% were overweight. Participants were randomly allocated to one of three intervention groups for 6 months, including, an advice only group, which included one 30-minute personalized session with a registered dietitian, an established recommendations group, which included 18 sessions promoting weight loss of at least 15 lbs. if needed, 180
min/wk of moderate intensity exercise, limited sodium intake (≤ 2300 mg/d) and moderate alcohol consumption (≤ 1 oz. for men, ≤ 0.5 oz. for women), or an established recommendations plus DASH diet group, which included the established recommendations in conjunction with information on the DASH dietary plan.

Although all groups showed some reduction in blood pressure, a gradient effect was seen across the groups, with the established-recommendations-plus-DASH-diet combination showing the greatest reductions. The advice only group experienced a substantial decrease in blood pressure from baseline (-6.6/-3.8 mm Hg), while the established recommendations group and the established-recommendations-plus-DASH group had significantly larger decreases of -10.5/-5.5 and -11.1/-6.4 mm Hg, respectively (both p’s < 0.001). The addition of the DASH diet did not significantly lower blood pressure compared with the established recommendation group (p < 0.43) in the sample as a whole. However, subgroup analyses revealed that individuals 50 years or older experienced significantly greater reductions following the DASH diet compared with the group following established recommendations only (- 2.9/-1.7 mm Hg, p = 0.0081) (118). Furthermore, the prevalence of hypertension at 6 months was lowest in the DASH plus group (12%), which was significantly lower than the advice only group (26%, p < 0.001) and marginally different than the established recommendations group (17%, p = 0.12).

Analysis of diet records revealed that the DASH plus group consumed significantly more fruits and vegetables (7.8 ± 3.2 servings/d, p < 0.001) and dairy foods (2.3 ± 1.2 servings/d, p < 0.001) compared with the established recommendations group (5.1 ± 2.5 and 1.5 ± 1.1 servings/d, respectively), but the number of servings of these
foods were still lower than in the original DASH studies (9.6 servings/d of fruits and vegetables and 2.7 servings/d of dairy) (59). Therefore, the free-living population may not have experienced the best possible blood pressure-lowering effects of the DASH diet. In addition, multiple behavioral changes may lower blood pressure through the same biological pathways, thus when used in combination they may not demonstrate an additive effect.

This study is important because its sample population represents about 50% of US adults. Furthermore, subgroup analyses indicate that certain populations may be particularly responsive to hypotensive components of the DASH dietary pattern. Therefore, inclusion of the DASH diet during counseling of established recommendations offers promise in achieving blood pressure control without the use of drug therapy.

2.2.4.3 Vanguard Study

The Vanguard study (119) compared the effects of two intervention strategies on blood pressure (Table 2-2). The interventions shared the common goals of weight loss and diet modifications to meet published recommendations by the American Diabetes Association, American Heart Association, and the National Academies of Science. One intervention strategy was a prepared nutritional program, which provided prepackaged foods to subjects, while the other strategy provided nutrition counseling only (a session with a dietitian every 2 weeks). As a result, subjects lost a similar amount of weight (4-5 kg) over 10 weeks on both interventions. Both interventions significantly lowered blood pressure in all subjects. In the group as a whole and in hypertensive hyperlipidemic
subjects, the prepared nutritional program produced a greater reduction in blood pressure (–6.2/4.2 mm Hg). As expected, weight loss was a strong determinant of the blood pressure response. Independently of weight changes, dietary changes reflecting increased dietary potassium, calcium, and magnesium caused a further decrease in blood pressure in subjects on the prepared nutritional program. These data are consistent with the DASH studies and suggest that a healthy mineral rich diet is essential for the treatment of hypertension.

2.2.4.4 Study of the effects of a low sodium, high potassium diet vs. DASH-type diets on blood pressure and weight: Nowson et al. (120,121)

Nowson et al. (120) conducted a study examining the effects of three dietary interventions on blood pressure in a free-living Australian population using dietary recommendations based on numbers of servings of foods (Table 2-2). The interventions included advice on a) a DASH-type diet, b) a low sodium high potassium diet, and c) a high dairy food diet. Table 2-4 presents daily nutrient intakes during the three dietary intervention periods and a control diet. Compared to the control diet, the DASH-type diet significantly reduced SBP by 1.8 ± 0.5 mm Hg (p < 0.01), but not DBP, while the low Na, high K diet significantly reduced both SBP and DBP (–4.4 ± 0.8 /–2.0 ± 0.6 mm Hg). In addition, compared to the DASH-type diet, the low Na, high K diet produced a significantly greater drop in SBP and DBP (–3.5 ± 1.0, p < 0.001 and –1.9 ± 0.7, p < 0.05, respectively), while the high dairy diet actually increased SBP (+3.1 ± 0.9, p < 0.01).

These findings may be partially attributed to simultaneous changes in weight. Body
weight significantly decreased on the low Na, high K (-0.4 ± 0.2 kg, p < 0.05), but did not significantly change on the DASH-type diet. Plus, weight significantly increased on the high dairy diet (+0.9 ± 0.1 kg, p < 0.001). Furthermore, the DASH-type diet produced a considerably smaller drop in blood pressure than the original DASH trial (-5.5/-3.0 mm Hg) (59). This may be due to fact that the contrast in dietary intake of macro- and micronutrients between control and experimental diets was not as great as in the original DASH study (59). The control diet of the present study was typical of the Australian diet and thus was substantially higher in potassium and magnesium.

Table 2-4. Nutrient composition of three dietary interventions and control period
(Nowson et al. 120)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DASH-type</th>
<th>Low Na, High K</th>
<th>High dairy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, % kcal</td>
<td>32</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Saturated, % kcal</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Protein, % kcal</td>
<td>17</td>
<td>18</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Carbohydrate, % kcal</td>
<td>49</td>
<td>51</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>23</td>
<td>36</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>Na, mg</td>
<td>2715</td>
<td>2115</td>
<td>1150</td>
<td>2898</td>
</tr>
<tr>
<td>K, mg</td>
<td>2652</td>
<td>5070</td>
<td>5772</td>
<td>4251</td>
</tr>
<tr>
<td>Ca, mg</td>
<td>672</td>
<td>1275</td>
<td>916</td>
<td>1778</td>
</tr>
<tr>
<td>Mg, mg</td>
<td>302</td>
<td>492</td>
<td>530</td>
<td>409</td>
</tr>
<tr>
<td>P, mg</td>
<td>1391</td>
<td>2013</td>
<td>1862</td>
<td>2222</td>
</tr>
</tbody>
</table>

Another study by the same researcher group investigated the effect of a DASH-type weight-loss diet vs. a standard low-fat weight-loss diet on blood pressure (121). Fifty-four, middle-aged men with above optimal blood pressure were instructed on one of the diet interventions for 12 weeks. Both interventions reduced body weight to a similar
extent (4.9 ± 0.6 kg on the DASH-type and 4.6 ± 0.6 kg on the standard low-fat), however, the DASH-type weight-loss diet produced a significantly greater decrease in SBP (-5.5 ± 1.9 mm Hg, p = 0.006) and DBP (-4.4 ± 1.2 mm Hg, p = 0.001). Thus, a diet rich in low-fat dairy foods, vegetables, and fruits had a more favorable effect on blood pressure even with simultaneous weight loss than a low-fat diet low in these foods.

2.2.4.5 Conclusion

Although, the DASH trials (59,87) reaffirm the importance of specific dietary patterns in the prevention and control of hypertension, the studies were short term and under artificial conditions. The PREMIER study (117,118), which assessed the effectiveness of the DASH dietary pattern and adherence to the diet among free-living persons, found smaller reductions in blood pressure, but identified that older adults (≥ 50 years) may especially benefit from a DASH-type diet. The studies by Akita et al. (116), Resnick et al. (119), and Nowson et al. (120,121) lend support to the hypothesis that a mineral rich diet that is moderate in sodium is useful in blood pressure management. Overall, the results of these dietary pattern trials are consistent with observational evidence and offer strong support for recommending the DASH diet, which is compatible with other dietary recommendations for the reduction of CVD, cancer, and osteoporosis.

Identifying particular foods that show benefit is important in defining the optimal diet to treat and/or prevent CVD. Because of multiple differences between experimental DASH-type diets and control diets, the nutrients or foods responsible for lowering of blood pressure cannot be identified. Although benefits of certain dietary patterns cannot
be attributed to a single dietary factor, certain foods and nutrients confer hypotensive effects. Some evidence exists that consumption of dairy products as well as calcium, magnesium, and potassium, which are also rich in dairy foods, is inversely associated with blood pressure.

### 2.3 Dairy foods and blood pressure/CVD Risk: Epidemiologic studies

A strong epidemiologic correlation has been shown between dairy food consumption and reduced blood pressure. Using the NHANES I database of over 10,000 individuals, McCarron et al. (70) reported that low consumption of dairy foods rather than other foods was predictive of increased blood pressure. A dietary calcium intake of greater than 1000 mg/d was associated with a 40% to 50% reduction in hypertension prevalence, consistent with findings of the DASH trial (92). The Coronary Artery Risk Development in Young Adults (CARDIA) study documented an approximate 65% reduction in the emergence of hypertension with a similar dairy intake over 10 years (69). Other large cross-sectional population studies in the United States (61) and Italy (64) also demonstrate that consumption of whole milk is significantly lower in hypertensive than normotensive persons and that a low dairy calcium intake is inversely associated with SBP. A cross-sectional study in Puerto-Rico (n=7932, men aged 45-64 years) found that men who consumed no milk vs. those who drank at least one liter of milk per day had two times the prevalence of hypertension (62). A similar study in 6496 men of Japanese decent living in Hawaii reported that intakes of milk, as well as, potassium, calcium, and
protein were all inversely associated with blood pressure levels while controlling for other risk factors (63).

A 22-year follow-up of over 3000 men in Honolulu Heart Program revealed that non-drinkers of milk had a 2-fold greater rate of thromboembolic stroke as compared with those who consumed half a liter of milk per day (65). Intake of calcium from non-dairy sources was not associated with the reduced stroke risk, indicating that other constituents of milk may be important. A new prospective study (67) found that men (n=655) who consumed at least 200 ml of milk per day were half as likely to experience ischemic stroke [odds ratio = 0.52 (0.27 to 0.99)] compared with those drinking less. The odds ratio for an ischemic heart disease event was also significantly less [odds ratio = 0.88 (0.56 to 1.40)]. An inverse association between the risk of ischemic stroke and dietary calcium intake has also been observed in the large prospective Nurses’ Health Study with over 85,000 middle-aged American women (66). The increase in risk of ischemic stroke was limited to women with low calcium intake (<600 mg/d). This association was stronger for dairy calcium than for non-dairy calcium. However, not all studies observe an inverse association between dietary calcium and blood pressure or stroke (122,123).

2.4 Dairy foods and blood pressure regulation: Clinical studies

While the impact of dairy foods in the context of the DASH diet is not clear, there is considerable evidence of dairy’s role in blood pressure lowering. Bierenbaum et al. (72) preformed a randomized cross-over study in which 50 free-living normotensive men
and women consumed several dairy products (yogurt, cottage cheese, and 1% milk) to equal 1,150 mg of dairy calcium per day for 8 weeks. The dairy rich diet significantly lowered systolic blood pressure by 5 mm Hg (p < 0.02), but did not significantly affect diastolic blood pressure compared to the dairy poor diet. A major limitation of the study is that the dairy poor diet replaced milk with orange juice (32 oz.), which has also been shown to decrease blood pressure. Another free-living study in 82 normotensive and hypertensive individuals found that daily consumption of 1 quart of skim milk for 8 weeks caused a significant decrease in both diastolic and systolic blood pressure (4.7 mm Hg and 4.5 mm Hg, respectively, p < 0.05) compared to a control group who did not alter their milk intake (71,75). An even bigger reduction in blood pressure (13/8 mm Hg) was reported in 27 hypertensive individuals who consumed about one liter of calcium-fortified skim milk (1,400 mg calcium/day) (15). Van Beresteijn and colleagues (74) related the hypotensive effect of milk to its mineral content in a Netherlands study of 60 young normotensive women, whose usual calcium intake was < 500 mg per day. In this randomized double-blinded parallel study, consumption of one liter of normal milk per day for 6 weeks reduced systolic blood pressure by 5 mm Hg (p < 0.03), but did not alter diastolic blood pressure significantly. Consumption of mineral poor milk with only one-tenth of the calcium, one-third of the potassium, and one-twentieth of the magnesium of the regular milk also significantly lowered systolic blood pressure by 2.3 mm Hg, suggesting that other factors in milk may have hypotensive effects. Not all studies demonstrate a hypotensive effect of dairy products however. After 4 weeks of increased dairy intake to equal 1,500 mg dietary calcium per day, ambulatory blood pressure did not change in 13 hypertensive men (124). Also, an increase in milk intake by 3 cups per
day for 12 weeks in 100 men and women aged 55-85 years did not affect blood pressure (125).

### 2.5 Components in dairy foods associated with blood pressure regulation

#### 2.5.1 Dietary calcium and blood pressure regulation

The nutrient receiving the majority of the recognition for dairy’s antihypertensive effect is calcium. Calcium, a potent mediator of cellular responsiveness to external stimuli, is a key element in the regulation of smooth muscle function, peripheral vascular tone, and thus blood pressure and volume homeostasis (126). A recent meta-analysis reviewing observational studies of dietary calcium and blood pressure showed a robust inverse correlation (127). Data from the Nurses’ Health Study showed that a 22% reduction in the risk of developing hypertension was correlated with a calcium intake above 800 mg/day compared to an intake below 400 mg/day (128). This result is consistent with previous work, which showed a 2 to 3 mm Hg increase in blood pressure in those who consume less than 300 mg/day compared to those consuming more than 800 mg/day (129).

Allender et al. (130) performed a meta-analysis on data pooled from 22 clinical trials (1231 persons) involving calcium supplements of 400 to 2160 mg/d. It showed a significant decrease in SBP of 0.5 mm Hg for trials involving normotensive persons and 1.7 mm Hg for trials involving hypertensive persons. A second meta-analysis by Bucher et al. (131) of 33 trials (2412 persons) involving calcium supplements of 1000 to 2000
mg/d showed a significant reduction in SBP of 1.3 mm Hg and 4.3 mm Hg in normotensive and hypertensive participants, respectively. Griffith and colleagues (132) analyzed 42 clinical trials (4560 persons) investigating the effects of calcium in various forms ranging from 500 to 2000 mg/d supplementation on systolic and diastolic blood pressure. They found that consumption of calcium was associated with small but statistically significant reductions in systolic (-1.44 mm Hg) and diastolic blood pressure (-0.84 mm Hg). Although these effects are modest in magnitude, they would be expected to have a meaningful effect on CVD risk at the population level. However, results of many clinical studies evaluating the effect of supplemental calcium on blood pressure are inconsistent (inverse effect (131,133-138) and null effect (139-143)).

2.5.2 Dietary magnesium and blood pressure regulation

Additional minerals rich in dairy foods have been shown to have independent inverse associations with blood pressure. As cited above, data from the Nurses’ Health Study revealed that the relative risk for hypertension for a magnesium intake of 300 mg/day or more was 0.77 (95% confidence interval, 0.67-0.80) compared with an intake of less than 200 mg/day (128). In a survey of 61 dietary variables in 615 men from the Honolulu Heart Program, dietary magnesium emerged as the strongest correlate of blood pressure, even stronger than calcium and potassium (144). A cross-sectional study of over 15,000 Americans found that serum and dietary magnesium were inversely associated with blood pressure, particularly in African Americans (145). However, recent NHANES III data do not show an association between magnesium intake and
blood pressure (146). Several randomized trials have tested whether magnesium supplementation reduces blood pressure, but the results have been inconsistent. Significant reductions in blood pressure have been shown in several studies (147-150), but not in others (88,151,152).

2.5.3 Dietary potassium and blood pressure regulation

Unlike magnesium, potassium intake was inversely associated with systolic and diastolic blood pressure using the NHANES III database of over 17,000 men and women (146). A meta-analysis of randomized clinical trials found that potassium supplementation reduced both systolic (5.9 mm Hg) and diastolic blood pressure (3.4 mm Hg) (153). Another meta-analysis of 33 randomized controlled trials in which potassium was the only difference between intervention and control groups found a significant but less robust decreases in systolic and diastolic blood pressure of 3.11 mm Hg and 1.97 mm Hg, respectively (154). The effect of potassium was enhanced in those with a high intake of sodium. In a large clinical trial, 300 normotensive women with low habitual intakes of potassium, calcium, and magnesium received 16 weeks of supplemental potassium (40 mmol), calcium (1200 mg), and magnesium (336 mg) or placebo (88). The changes in 24-hour ambulatory blood pressure between treatment and control group were only significant for potassium (-2.0, 95% confidence interval, -3.7 to –0.3), suggesting that potassium vs. calcium and magnesium may have the strongest influence on blood pressure.
2.5.4 Dietary patterns high in minerals and blood pressure regulation

Overall, studies utilizing dairy foods as sources of calcium have shown stronger and more consistent inverse associations than supplemental calcium (127,132). This suggests that the combination of naturally occurring nutrients in dairy foods contributes to its hypotensive effects. Using survey data from NHANES III and NHANES IV, Townsend et al. (155) confirmed that inadequate intake of the minerals calcium, magnesium, and potassium best predicted high blood pressure in persons with hypertension (isolated SBP hypertension). This dietary pattern was characterized by low intakes of dairy products, fruits, and vegetables. Another study evaluated the combined effect of dietary calcium, magnesium, and potassium on blood pressure in 20,921 Dutch men and women aged 20-59 years (156). Researchers found that those who consumed a diet with intakes in the upper tertiles of all three minerals had a significantly lower SBP and DBP compared to those who had intakes in the lower tertiles (men: SBP = -1.3 (95% CI: -2.6, -0.1), DBP = -1.9 (95% CI: -2.7, -1.0), women: SBP = -1.8 (95% CI: -3.1, -0.5), DBP = -1.5 (95% CI: -2.4, -0.7). Taken together, these results suggest that dietary patterns rich in calcium, potassium and magnesium may lower blood pressure, especially in hypertensive individuals.

2.5.5 Bioactive peptides and blood pressure regulation

Peptide fragments released from milk proteins by enzymatic proteolysis during gastrointestinal digestion, fermentation, and other food processing methods, have been shown to possess several biochemical and physiological properties (157). For example,
cheese contains certain peptides that during the ripening period are converted into angiotensin-converting enzyme (ACE) inhibitory peptides (158). Numerous animal studies report that a variety of casein-derived peptides are able to lower blood pressure (159-161); however the sample sizes of these studies were small and baseline blood pressure data were limited making interpretation difficult. The hypotensive effect of milk peptides has yet to be verified in humans. However, orally ingested peptides and intestinal peptide products, including ACE inhibitors have been shown to enter peripheral blood and have systemic effects, including decreasing angiotensin II. The most potent ACE inhibitory peptides derived from milk are Val-Pro-Pro and Ile-Pro-Pro. Recently, a placebo-controlled study showed that 95 ml/day of sour milk, which contained two ACE-inhibitory tripeptides, Val-Pro-Pro and Ile-Pro-Pro, resulted in a significant decrease in blood pressure compared to a placebo milk in hypertensive subjects (162). Furthermore, decreased ACE activity in the aorta and the presence of the two tripeptides in the aorta after consumption of the sour milk indicate that these tripeptides were responsible for the hypotensive effect. Although not as strong as pharmacologic ACE inhibitors, these peptides have been suggested to contribute to the blood pressure lowering effect of dairy products. Another class of milk-derived peptides is thought to interact with opioid receptors that are located throughout the cardiovascular system (163). Thus, opioid receptor stimulation at the level of the endothelium by milk-derived peptides has also been proposed to induce nitric oxide release and improve endothelial function.
2.6 Populations that may be particularly responsive to a dairy-rich diet

2.6.1 Threshold effect

Several researchers have suggested that a threshold for dietary calcium exists and that attaining this level of calcium intake is important in blood pressure control. Equally, increasing dietary calcium beyond the threshold may not affect blood pressure. Harlan and Harlan (129) found that individuals consuming < 300 mg/d calcium experienced 2-3 mm Hg higher blood pressures compared to those consuming > 800 mg/d calcium. McCarron et al. (70) observed that supplementation with calcium in hypertensive patients who have an intake of calcium that exceeds 600 mg/d may not benefit from supplementation. Therefore, individuals who consume low dairy/calcium diets may be particularly responsive to supplementation of calcium. Other characteristics identified by studies that may predict an individual’s response to a calcium rich diet include, adults aged 50 years or older, diabetic patients, African Americans, those who are lactose intolerant (i.e. low calcium intake).

2.6.2 Renin types of hypertension

Recent discoveries about the underlying heterogeneity of hypertension may partially account for discordant findings of calcium effects on blood pressure. Calcium works in several ways to decrease blood pressure, therefore the importance of any given mechanism depends on the underlying type of hypertension. Although it is well recognized that a spectrum of mechanisms contributes to the pathophysiology of
hypertension, antihypertensive treatment is rarely based on the underlying mechanism. Individuals with hypertension can be stratified according to plasma renin levels (82). Hypertensive individuals with predominantly sodium volume-dependent hypertension are identified by a low plasma renin activity level (< 0.65 mg/mL/hr). Conversely, high/normal renin hypertension is identified if plasma renin activity is ≥ 0.65 ng/mL/h.

In contrast to low renin hypertension, high renin hypertension is an angiotensin-mediated vasoconstrictor hypertension. Blood pressure lowering is optimized when pharmacologic treatment is based on whether a patient has low vs. high/normal renin hypertension (164).

Approximately 30% of hypertensive individuals have low renin activity, while another 10-15% have elevated renin activity (165). These renin subgroups of the hypertensive population follow certain hormonal profiles (Table 2-5) (165,166). Low renin hypertensive individuals often have low circulating levels of serum calcium and calcitonin, and high levels of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D3, while the opposite pattern is observed in high renin hypertensive patients. Overall, individuals with low renin, salt-sensitive hypertension may have more potential to be in a calcium deficient state and thus more responsive to supplemental calcium, whereas those with normal to high renin, salt-resistant hypertension may not respond to calcium.
Table 2-5. Characteristics of low renin and high renin hypertension

<table>
<thead>
<tr>
<th>Low Renin Hypertension</th>
<th>Normal/High Renin Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Renin activity</td>
<td>↑ or normal Renin activity</td>
</tr>
<tr>
<td>↑ PTH</td>
<td>normal PTH</td>
</tr>
<tr>
<td>↑ 1, 25-dihydroxyvitamin D3</td>
<td>normal 1, 25-dihydroxyvitamin D3</td>
</tr>
<tr>
<td>↓ or normal serum Ca^{2+}</td>
<td>normal serum Ca^{2+}</td>
</tr>
<tr>
<td>↓ calcitonin</td>
<td>normal calcitonin</td>
</tr>
<tr>
<td>↑ intracellular Ca^{2+}</td>
<td>normal intracellular Ca^{2+}</td>
</tr>
<tr>
<td>“Salt-sensitive”</td>
<td>“Salt-insensitive”</td>
</tr>
</tbody>
</table>

Source: Adapted from Resnick (81)

The heterogeneity of hypertension parallels the heterogeneity of calcium metabolism, which also is coupled to renin activity; however, the link between the renin-angiotensin system and calcium metabolism is not clear. Recently 1,25-dihydroxyvitamin D₃ was shown to suppress renin transcription by a VDR-mediated mechanism in vitro (167,168). On the other hand, a study in humans reported that renin levels remained unaltered after injection of 1,25-dihydroxyvitamin D₃ in the presence of constant plasma ionized calcium, while the total peripheral resistance increased (169). Interestingly, earlier research demonstrated that administration of 1,25-dihydroxyvitamin D₃ increased blood pressure in patients with low renin hypertension and decreased blood pressure in those with high renin hypertension (170,171). In addition, PTH is known to be a potent down-regulator of VDR which may cancel the effects of 1,25-
dihydroxyvitamin D₃ when both hormones are elevated (e.g. calcium deficiency) (172). This evidence suggests that alterations in calcium homeostasis and the renin-angiotensin system are not just coincidental, but contribute to the pathogenesis of hypertension. Moreover, the seemingly divergent roles 1,25-dihydroxyvitamin D₃ plays in blood pressure regulation demands further explanation.

2.7 Dairy foods and lipids and lipoproteins

In addition to beneficial hypotensive effects, the DASH diet that is low in total fat (27% of kilocalories) and saturated fat (7% of kilocalories) decreased total cholesterol [-13.7(95% confidence interval (CI), -18.8, -8.6) mg/dl] and LDL cholesterol [-10.7(95% CI, -15.4, -6.0) mg/dl] compared with both the fruits and vegetables diet (low in dairy) and the control diet (173). HDL cholesterol was also significantly reduced [-3.7(95%CI, -5.1, -2.2) mg/dl], but triglyceride levels were unchanged by the DASH diet. The fruits and vegetables and control diets elicited a similar effect on lipids and lipoproteins. It should be noted, however, that in the study by Obarzanek et al. (173) the macronutrient profile of the fruits and vegetables and control diets was similar, which would explain the comparable blood lipids and lipoproteins results.

The association of daily calcium intake and plasma lipids and lipoproteins as well as body composition was examined in a cross-sectional study in adults (235 men, 235 women) aged 20-65 years. After statistical adjustment for body fat mass and waist circumference, daily calcium intake was negatively correlated with plasma LDL cholesterol, total cholesterol, and total:HDL cholesterol in men and women (all p’s <
In women, those who consumed < 600 mg/d had a significantly greater ratio of total:HDL cholesterol than those who consumed > 1000 mg/d (p < 0.05). Most of the dietary calcium was derived from dairy products (62% and 60% of daily calcium intake was provided by dairy products for women and men, respectively). However, not all studies have demonstrated a hypolipidemic effect of calcium. Two fairly large intervention studies by Karanja et al. (174,175) found that hypertensive patients at baseline consumed less calcium, magnesium, and potassium than normotensive patients and had significantly higher LDL cholesterol and lower HDL cholesterol levels, but no changes occurred in plasma lipids or lipoproteins with either calcium supplementation or counseling that increased dietary calcium intake.

2.7.1 Calcium, magnesium, and lipids and lipoproteins

There is some evidence in the literature demonstrating a cholesterol-lowering effect of a high calcium diet. The binding of calcium to fatty acids and bile acids in the intestine results in increased fecal lipid excretion, which is associated with decreased circulating LDL cholesterol levels (176,177). Results from a short-term study in moderately hypercholesterolemic men showed that dietary fortification with 2200 mg/d calcium reduced total and LDL cholesterol by 6% and 11%, respectively, when compared to a low calcium diet (177). Reid et al. (178) conducted a randomized, placebo-controlled trial in which 223 healthy, postmenopausal women not on lipid-lowering therapy received calcium citrate (1 g elemental Ca/d, n=111) or placebo (n=112) for one year. HDL cholesterol significantly increased 7% (p < 0.01) and LDL cholesterol
significantly decreased 6% (p< 0.04) in the calcium-supplemented group compared to the changes in the placebo group. Thus the HDL to LDL cholesterol ratio increased by 16% (p< 0.0009). Triglyceride levels were not affected by treatment. In another trial (179), 145 postmenopausal received 1500 mg/d calcium (combination calcium carbonate and tricalcium phosphate) for 1.5 to 2 years. HDL cholesterol increased 4.3% (p< 0.001) and LDL cholesterol decreased 3.3% (p< 0.02). Consistent with these findings, Bell et al. (180) reported that 1.2 g/d of calcium as carbonate (6wk) decreased LDL cholesterol (4.4%, p< 0.05) and increased HDL cholesterol (4.1%, p< 0.05) in 56 hypercholesterolemic patients.

Research by Karanja and co-workers (174) suggests that calcium supplementation (1000 mg/d) has a hypocholesterolemic effect in normotensives with mild hyperlipidemia, but not hypertensives. A study by Bostick et al. (141) did not find an effect of calcium carbonate (1 and 2 g/d) on lipid levels (4 months) in 193 subjects with a history of sporadic colonic adenoma; however cholesterol levels were secondary endpoints to colon cell proliferation and all blood samples were not fasted, the majority of subjects did not have elevated cholesterol at baseline, and many were taking lipid-lowering medication and changes in use of these medications were not reported.

In animal models, dietary magnesium deficiency increases the blood levels of LDL and VLDL cholesterol and reduces HDL cholesterol. In a human study, concentrations of LDL cholesterol, VLDL cholesterol, and triglycerides were inversely correlated with ionic magnesium in the blood (181). In the Atherosclerosis Risk in Communities Study (n=15,248), serum magnesium was inversely related to serum triglycerides, while dietary magnesium was positively related to HDL cholesterol,
independent of age and body mass index (145). Experimental models of magnesium deficiency suggest that either reduced clearance of triglyceride or decreased lipoprotein-lipase activity may account for elevated levels of triglycerides (182,183). Magnesium supplementation has also been shown to improve the lipid profile by decreasing LDL cholesterol and triglycerides and increasing HDL cholesterol in type 1 (184) and type 2 (185) diabetic patients.

2.8 Intake gaps

Despite the strong evidence of the benefits of dairy intake, studies consistently show that dairy intake is inadequate across the US population. Milk consumption has substantially decreased over the last 3 decades (186-189). Adolescent milk consumption declined from 2.5 ± 0.07 servings per day in 1965 to 2.0 ± 0.05 servings per day in 1996 (p < 0.001) and was not compensated for by an increase in other dairy products (186). A recent report (190) of nationally representative data from the 1977-1978 Nationwide Food Consumption Survey, the 1989-1991 and 1994-1996 Continuing Surveys of Food Intake by Individuals (CSFII), and 1999-2001 NHANES found that for all age groups (2-60 years), sweetened beverage consumption increased by 135%, while milk consumption decreased by 38%, with a total calorie increase of 278. Therefore, it is not surprising that the intake of the minerals supplied by dairy foods, calcium and magnesium, are also lower than recommended.

Current dietary recommendations or adequate intakes (AI) for calcium are 500 mg for children aged 1 to 3 years, 800 mg for children aged 4 to 8 years, 1,300 mg for
adolescents aged 9 to 18 years, 1,000 mg for adults aged 19 to 50 years, and 1,200 mg for adults 51 years or older. According to data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96 (191,192), only 12% of adolescent females and 32% of adolescent males are meeting 100% of the AI for calcium. Only 16% of women ages 20 to 29, 14% of women ages 30 to 39, 11.5% of women ages 40 to 49, 5% of women ages 50 to 59, 4% of women 60 and older are meeting 100% of the AI for calcium. More men than women are meeting calcium recommendations, presumably because of their higher energy intake. Yet less than 15% of adult men aged 50 or more achieve the AI for calcium. Because of America’s low calcium intake, national organizations have deemed it a major public health problem (192).

Magnesium intake is also lower than national recommendations. The estimated average requirement (EAR) and recommended dietary allowance (RDA) for magnesium are 330 mg and 400 mg for men aged 19-30 years and 350 mg and 420 mg for men aged >31 years. Among women, the EAR and RDA are 255 mg and 310 mg aged 19-30 years and 265 mg and 320 mg aged > 31 years, respectively. Recent data from NHANES, 1999-2000, reveal that the median dietary magnesium intake of the US adult population was below both the EAR and RDA in all sex, ethnic, and age groups studied (except the EAR for Caucasian men aged 31-50 years) (193). Magnesium intakes decreased with increasing age across all ethnicities, men consumed significantly higher amounts than women, and African Americans consumed significantly lower amounts than Caucasians. CSFII, 1994-1995, data also report low magnesium intakes representing 83% of the RDA for women and 94% of the RDA for men (194). Excessive use of refined and processed foods along with reduced consumption of dairy foods partially account for low
magnesium intakes. Refining whole wheat and sugar cane removes approximately 80% to 100% of the magnesium.

### 2.9 Intracellular calcium as a determinant of blood pressure

The role of intracellular calcium in the regulation of vascular tone and blood pressure has been recently described, but is not fully understood. \((\text{Ca}^{2+})_i\) acts as a second messenger and is highly involved in modulating vascular tone (Fig. 2-2). First, the potent vasodilator nitric oxide is produced by a calcium-calmodulin dependent step where the enzyme constitutive nitric oxide synthase (cNOS) converts L-arginine to nitric oxide and citrulline. Nitric oxide acts on vascular smooth muscle and platelets through soluble guanylate cyclase, which converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP). cGMP causes smooth muscle relaxation by decreasing \((\text{Ca}^{2+})_i\), which subsequently leads to a decrease in the contractility of Ca-dependent apparatus. Muscle relaxation occurs when \((\text{Ca}^{2+})_i\) levels return to baseline as \((\text{Ca}^{2+})_i\) is taken up into intracellular stores or effluxed from the cell via the plasma membrane CaATPase and Na-Ca exchange mediated processes.

Conversely, increases in \((\text{Ca}^{2+})_i\) cause the opposite effect. Depolarization and/or opening of calcium channels in vascular smooth muscle cells cause extracellular ionic calcium to enter the intracellular cytosolic space. This increase in \((\text{Ca}^{2+})_i\) subsequently stimulates the release of stored calcium from the sarcoplasmic reticulum, thus increasing \((\text{Ca}^{2+})_i\) throughout the entire cytosol. A second mechanism involves circulating agonists (such as angiotensin II) that cause \((\text{Ca}^{2+})_i\) release from intracellular stores via receptor-
mediated action. No matter which mechanism is at work however, the net contribution of a high concentration of \((\text{Ca}^{2+})_i\) signals a cascade of events that results in vasoconstriction. Therefore, the balance of nitric oxide and \((\text{Ca}^{2+})_i\) is critical to maintain vascular tone.

The contribution of calcium in regulating vascular tone is dependent on magnesium. Magnesium modifies the processes that control calcium homeostasis on a cellular level by regulating calcium entry, exit, and other actions (like release from intracellular stores). These effects of magnesium maintain a steady state and buffer the cellular responsiveness to \(\text{Ca}^{2+}\)-driven stimulation. Therefore, low intracellular magnesium levels cannot offset rises in \((\text{Ca}^{2+})_i\) by either extracellular entry or intracellular release. Taken together, deficient intracellular ionic magnesium \([\text{Mg}^{2+}]_i\) would result in exaggerated Ca-induced stimulation and promote vasoconstriction. A recent study in 15 people with mild to moderate hypertension found that supplementation of 600 mg of magnesium for 3 weeks significantly reduced blood pressure by 7.6/3.8 mm Hg (195). The researchers suggested that the increase in intraerythrocyte magnesium levels and decrease in sodium levels after supplementation could partially explain the blood pressure reductions. Likewise, low concentrations of potassium have been shown to limit Na, K-ATPase activity, resulting in increased intracellular sodium (196). Elevated sodium in the cytosol would slow the Na-Ca exchange and thus lead to increased \((\text{Ca}^{2+})_i\).
Figure 2-2. Endothelial-derived vasoconstricting and vasodilating factors. ACE = angiotensin converting enzyme; cNOS = constitutive nitric oxide synthase; L-Arg = L-arginine; NO = nitric oxide; 1,25(OH)VD = 1,25-dihydroxyvitamin D3; R = receptor; G = G protein; PLC = phospholipase C; PI= phosphatidylinositol; IP3= inositol-1,4,5-triphosphate; SR= sacroplasmic reticulum; VGC = voltage gated channel; MLCK = myosin light chain kinase; MLC = myosin light chain; P = phosphate; GTP = guanosine triphosphate; cGMP = cyclic guanosine monophosphate

Source: Adapted from Resnick (81)
2.10 Ionic Hypothesis

A growing body of evidence suggests that hypertension, atherosclerosis, diabetes, metabolic syndrome, and obesity are all characterized by an underlying impairment in \( (Ca^{2+})_i \) (81). The ionic hypothesis, put forth by Resnick (197,198), explains the concurrent appearance of these metabolic abnormalities by a generalized defect in steady-state cell ion handling, resulting in high intracellular free calcium and low intracellular free magnesium (Fig. 2-3). Minor changes in calcium and magnesium levels have major effects on cellular excitability and response. Previous research (81) suggests that this ionic misbalance in vascular smooth muscle cells would result in vasoconstriction, arterial stiffness, and hypertension; in adipose and skeletal tissue it would result in insulin resistance; in pancreatic \( \beta \)-cells it would result in potentiated insulin secretion, in renal tubules it would result in increased sodium retention and calcium and magnesium excretion, in inflammatory cells it would result in increased release of reactive oxygen species and cytokines (199). High \( (Ca^{2+})_i \) and reciprocally low \( (Mg^{2+})_i \) levels in vascular smooth muscle cells result in vasoconstriction (200) and arterial stiffness (201). Individuals with essential hypertension typically have significantly higher basal levels of intracellular calcium (202-208) and sodium (208) and lower levels of intracellular magnesium (208-210) than normotensives. In addition, a strong positive correlation exits between blood pressure and intracellular calcium levels in a variety of cell types (202,203,205,211). However, the ionic hypothesis does not predict what factors contribute to this cellular ion defect or if the contributing factors can be modified.
A recent rat study highlights the possibility that reversal of elevated \((\text{Ca}^{2+})_i\) levels will prevent both the insulin resistance and hypertension (212). This innovative study measured insulin resistance via the euglycemic clamp in rats fed a high-fat diet and in spontaneously hypertensive rats. Injection of an \((\text{Ca}^{2+})_i\) chelator before the clamp restored insulin sensitivity and normalized blood pressure in both rat populations. These results not only demonstrate that sustained excessive \((\text{Ca}^{2+})_i\) plays a common role in these two disease states, but also suggest that treatments that lower \((\text{Ca}^{2+})_i\) may potentially prevent/attenuate these diseases.

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**Figure 2-3. The ionic hypothesis.** Excess intracellular Ca and Na with reciprocally low intracellular Mg is hypothesized to be an underlying cellular abnormality that manifests several tissue defects resulting in cardiovascular disease. LVH=left ventricular hypertrophy; CAM=cellular adhesion molecules
Source: adapted from Resnick (84)
2.11 Regulation of intracellular ions

Calcium-regulating hormones and the renin-angiotensin system coordinately mediate the blood pressure effects of differing dietary calcium and sodium intakes at the cellular level by altering cellular handling of mono- and divalent ions (Fig. 2-4). It has been postulated that abnormalities of these systems may lead to increased \((\text{Ca}^{2+})\), levels in vascular smooth muscle cells that consequently, increase systemic vascular resistance and blood pressure (84). In addition, diets high in sodium and low in calcium can perpetuate and enhance the dysfunction (73). It is tempting to speculate that nitric oxide production in individuals with essential hypertension is not sufficient to prevent the contraction of smooth muscle cells due to increased \((\text{Ca}^{2+})\) (213). I hypothesize that the imbalance in levels of intracellular ions not only stimulates vaso- and renal constriction, but also is associated with endothelial dysfunction (increase in adhesion molecules and decrease in flow mediated dilation).
Figure 2-4. Adverse effects of a High Na, Low Ca diet on cellular ion homeostasis leading to increased CVD risk.

VSMC=vascular smooth muscle cell; FMD=flow mediated dilation; UCa/UNa excretion=the ratio of urine calcium to urine sodium excretion

Source: adapted from (81)
2.11.1 Calcium-regulating hormones

The beneficial effect of dietary calcium on blood pressure regulation may seem paradoxical (increased intake of calcium results in decreased intracellular calcium) however; it can be partially explained by the effects of calcium-regulating hormones on the vasculature (83). The fact that both 1,25-dihydroxyvitamin D₃ and parathyroid hormone (PTH) are often elevated in low renin states of hypertension may be especially important. A review of epidemiologic studies which found no relation between dietary calcium and blood pressure, revealed that circulating PTH (136) and 1,25-dihydroxyvitamin D₃ (214) were directly related to blood pressure. This suggests that these hormones, which are triggered by a low intake of calcium, are responsible for the hypertensive response.

Inconsistent results have been reported in studies evaluating the association of both vitamin D metabolites and PTH and blood pressure regulation (214-218). Besides 1,25-dihydroxyvitamin D₃ role as a steroid hormone, it can also stimulate rapid calcium influx through a nongenomic pathway in a variety of cells including vascular smooth muscle cells (219-221). In models of rat artery-derived smooth muscle cells, 1,25-dihydroxyvitamin D₃ increased calcium channel current resulting in changes in cytosolic calcium (222,223). In this way, elevated levels of circulating 1,25-dihydroxyvitamin D₃ have been independently associated with increased peripheral vascular resistance (Fig. 2-5) (169,224). Finally, receptors for 1,25-dihydroxyvitamin D₃ are wide-spread and have been discovered in vascular smooth muscle (225) and myocardial tissue (226).
Figure 2-5. Proposed mechanism for the adverse effects of a low dairy diet on vascular health. A low calcium, high sodium diet causes an increase in intracellular calcium and reciprocal decrease in intracellular magnesium, via the increased activity of calcium-regulating hormones and the renin-angiotensin system. This results in increased blood pressure.

Source: adapted from Sowers et al. (227)
As with 1,25-dihydroxyvitamin D3, PTH has also been shown to have properties that can potentially increase peripheral vascular resistance (Fig. 2-5). PTH increases intracellular ionic calcium by enhancing calcium influx in erythrocytes (228) and vascular smooth muscle cells (229). Salt-loading diets have been demonstrated to increase PTH, resulting in increased ionic calcium in erythrocytes and blood pressure (73,230). Conversely, increased dietary calcium have been shown to block the rise in PTH and cause both erythrocyte calcium (73) and platelet calcium (231) and blood pressure to return to baseline levels (73). Further evidence for a hypertensive effect of PTH is the fact that PTH may inhibit Na, K-ATPase, resulting in an accumulation of intracellular sodium, which slows Na-Ca exchange (e.g. increased (Ca\(^{2+}\)). It also has been hypothesized that PTH may increase intracellular ionic calcium by depleting intracellular magnesium, however, more research is needed in this regard.

In contrast to the experimental and clinical data that suggest an association between chronic hyperparathyroidism and hypertension, acute infusion of PTH causes vasodilation and hypotension (232,233). Acute intravenous injection of PTH in rats caused a decrease in arterial blood pressure by a direct vasodilatory effect (234). Interestingly, the acute vasodilatory action of PTH infusions have been shown to be more pronounce in rats fed a calcium sufficient diet compared to rats consuming a low calcium diet (235). In contrast, chronic PTH infusions in canines (236) and healthy adults (237) cause increases in blood pressure. These observations imply that chronic and acute parathyroid states affect blood pressure through different mechanisms yet to be elucidated. Recently, Schleiffer et al. (238) used an animal model to demonstrate that the hypotensive properties of PTH are not nitric oxide dependent.
I postulate that increases in 1,25-dihydroxyvitamin D₃ and PTH secondary to low calcium diets stimulate ionic calcium influx in vascular smooth muscle cells, thereby causing increased vascular resistance (Fig. 2-6). If this is true, then increasing dietary calcium should suppress calcitropic hormones and reduce intracellular ionic calcium and systemic vascular resistance. Therefore, the additional hypotensive effect of the calcium-rich diet over a fruit and vegetable diet may be mediated by reductions in these hormones. Indeed, reductions in PTH and 1,25-dihydroxyvitamin D₃ in response to calcium-rich diets have been correlated with decreased blood pressure as well as decreased intracellular calcium (84).
Figure 2-6. Hypotheses: The beneficial effects of dairy products on vascular health.

Dairy calcium normalized intracellular ion levels, via the suppression of calcium-regulating hormones. Bioactive peptides in dairy foods may inhibit ACE activity and lower angiotensin II production.
2.11.2 Renin-angiotensin system

Activation of the renin-angiotensin system is critically involved in the pathogenesis of hypertension as well as atherosclerosis. Renin is a proteolytic enzyme that is released primarily by the kidney, in response to decreases in circulating blood volume and in response to sympathetic nervous system stimulation. Renin acts upon its substrate to produce angiotensin I, a peptide with no known biological activity. The powerful vasoconstrictor angiotensin II is produced when angiotensin I is cleaved by the angiotensin converting enzyme (ACE). Interestingly, a tissue ACE system has been found in endothelial cells throughout the vascular system (239). As the vasoactive component of the system, angiotensin II is involved in the regulation of blood pressure, vascular tone, cell growth and migration, and stimulates production of vasoconstrictors (240).

A major mechanism whereby angiotensin II may contribute to vascular pathology is by increasing \((\text{Ca}^{2+})_i\), levels in vascular smooth muscle cells. Through binding its membrane receptor, angiotensin II activates protein kinase C and the production of inositol triphosphate, causing subsequent mobilization of stored calcium ions to the cytoplasm and reciprocal sequestration of free magnesium ions into storage (Fig. 2-5). Therefore, elevated levels of angiotensin II as seen in high renin hypertension can lead to elevated intracellular free calcium and low magnesium levels resulting in vasoconstriction.

The genotype that leads to an active renin-angiotensin system and thus high blood pressure has been found to be responsive to the DASH diet, suggesting that one
mechanism of action of the DASH diet is to affect renin-angiotensin system (91). Dairy products may be the key components of the DASH diet that alter this system. Several bioactive peptides derived from dairy products have hormone-like ACE-inhibitory properties (241-243). These potential ACE inhibitory peptides may ultimately lower circulating levels of angiotensin II and thereby limit the mobilization of calcium in vascular cells. Another benefit of ACE inhibitors is that they can stop the inactivation of bradykinin, which exhibits vasodilatory action via increased release of nitric oxide.

I hypothesize that bioactive peptides in the dairy-rich diet will contribute to decreased vascular resistance by decreasing the efficiency of ACE, resulting in lower levels of circulating angiotensin II (Fig. 2-6). Due to its fundamental role in mediating endothelial integrity, angiotensin II is also important in other pathological processes underlying vascular diseases.

2.11.3 Circulating lipids

Along with calcium-regulating hormones, circulating lipids influence the regulation of intracellular ions. Increased circulation of free fatty acids exacerbates insulin resistance and vascular resistance. These effects are associated with the action of free fatty acids to increase intracellular calcium concentrations and lower magnesium levels (244). Delva et al. (245) found that intralymphocyte free magnesium is inversely correlated to plasma triglycerides and that low intracellular magnesium characterized a group of hypertensive patients with high triglycerides. A secondary study also confirmed that intralymphocyte free magnesium is low in normotensive subjects with
hypertriglyceridemia (183). Similar ionic effects have been associated with oxidized LDL particles in a variety of tissues (246). These observations may partially explain the parallel accumulation of cholesterol and calcium in human arteries with age (247) as well as the promotion of atherosclerotic lesions by concurrent magnesium depletion in animal models (182). Moreover, supplemental magnesium has been shown to lower serum cholesterol and triglycerides and attenuate the atherosclerotic process substantially in these animal models (182). Taken together, these data indicate an association between lipoprotein metabolism and intracellular ion homeostasis in humans.

2.12 Endothelial health

It is known that the vascular endothelium plays a key role in circulatory homeostasis through its ability to regulate the vascular milieu via the synthesis and release of biologically active substances, such as nitric oxide. Measures of endothelial dysfunction include markers of endothelial activation (cell adhesion molecules) and impaired endothelium-dependent vasodilation (indexed by the measurement of flow mediated dilation of the brachial artery). Impaired endothelium-dependent vascular reactivity is an early characteristic of vascular disease and is strongly correlated with both coronary disease and its risk factors. Hypertension alters endothelial function as well as morphology. Platelets and monocytes adhere to endothelial cells to a greater extent than in normotensive control vessels, and endothelium-dependent vascular relaxation is impaired (248).
2.12.1 Cell adhesion molecules

The adhesion of circulating leukocytes to the endothelial cells plays an important role in the initiation of atherosclerosis (249). The cellular adhesion molecules (CAMs) mediate the adherence and subsequent migration of leukocytes across the vascular endothelium. CAMs are poorly expressed by a healthy endothelium, but are upregulated during inflammation and atherosclerosis. The selectins mediate the initial rolling of inflammatory cells along the endothelial cells (E-selectin) and platelets (P-selectin). Intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) are thought to regulate the attachment and transendothelial migration of leukocytes. After activation from cytokines, CAMs are shed from the surface of endothelial cells, leukocytes, and platelets and can be measured in serum. They are considered biomarkers of endothelial activation and ICAM-1 (250) and P-selectin (251) have been shown to be predictors of future CVD risk.

Patients with essential hypertension have been shown to have impaired flow mediated dilation (252) as well as elevated levels of E-selectin (253,254), ICAM-1 and VCAM-1 (255-257), and P-selectin (258,259). In addition, reductions in blood pressure with medication have been found to improve endothelial function. Therefore, a short-term dietary intervention that may lower blood pressure, like a dairy-rich diet, offers promise in improving endothelial function.

It has been postulated that early endothelial dysfunction and hypertension-induced chronic distension of arteries may upregulate the renin-angiotensin system (260). Angiotensin II is associated with numerous pathways implicated in the advent of
atherosclerosis, which include increased monocyte adhesiveness (261-263), LDL oxidation, VCAM-1 expression (264,265), ICAM-1 expression (255), decreased endothelial nitric oxide synthesis, decreased flow mediated dilation, and vascular smooth muscle cell replication (266). Moreover, ACE inhibitors have been suggested to achieve vascular protection through mechanisms that go beyond their primary therapeutic actions of blood pressure reduction. The Trial on Reversing ENdothelial Dysfunction (TREND) was one of the first studies that demonstrated an improvement in endothelial dysfunction with an ACE inhibitor (quinapril 40g daily) in 129 normotensive patients with coronary artery disease (267). Quinapril improved endothelial dysfunction without altering lipids or reducing blood pressure. In addition, soluble ICAM-1, VCAM-1, and E-selectin have recently been reported to decrease after ACE inhibition and these changes were independent of reductions in blood pressure (256,268). It also has been shown in vivo that LDL-induced intracellular calcium transients in endothelial cells play a role in the induction of VCAM-1 and E-selectin expression in human umbilical endothelial cells (269). Therefore, potential ACE inhibition with dairy peptides may attenuate the full activation of the renin-angiotensin system and prevent potential vascular damage by angiotensin II.

Another cell adhesion molecule, P-selectin, mediates interactions among platelets, leukocytes, and endothelial cells and may play a critical role in the thrombosis, inflammation, and atherosclerosis (270). Because it is contained in platelets and endothelial cells, P-selectin is considered a marker of platelet and endothelial activation (271,272). New research also indicates that P-selectin is not only a marker of platelet activation but a direct inducer of pro-coagulant activity (273). The translocation of P-
selectin from Weibel-Palade bodies in endothelial cells to the plasma membrane has been shown to be dependent upon a rise in intracellular ionic calcium (269). Because platelet activation is controlled by the concentration of intracellular ionic calcium similar to vascular smooth muscle cells, they are often used as a model to evaluate the relationship between calcium homeostasis and vascular disease. Therefore, a diet deficient in calcium may follow similar pathways that result in increased intracellular calcium and platelet activation (i.e. increased P-selectin). It also has been shown in vivo that LDL-induced intracellular calcium spikes in endothelial cells play a role in the induction of VCAM-1 and E-selectin expression (269). However, it is not known whether a chronic state of high intracellular calcium leads to elevated levels of soluble CAMs. If so, a dairy-rich diet may result in lower circulating levels of soluble P-selectin, VCAM-1, and E-selectin compared to a low calcium diet.

2.12.2 Flow mediated dilation

There are several reasons to suspect that intracellular calcium has an important impact on endothelial function. First, flow mediated dilation is a nitric oxide-mediated response that is controlled by changes in the levels of \((Ca^{2+})_{i}\). The acute increase in \((Ca^{2+})_{i}\) is a key event for the production and release of nitric oxide, a potent vasodilator. A transient increase in levels of \((Ca^{2+})_{i}\) in endothelial cells can result from certain antagonists as well as fluid shear stress (274). It has been postulated that fluid shear stress causes formation of inositol 1, 4, 5-triphosphate through the activation of phospholipase C, resulting in the release of \((Ca^{2+})_{i}\) from intracellular storage spaces.
However, the regulation of (Ca\(^{2+}\))\(_{i}\) is not clearly understood and it appears that the balance of nitric oxide and (Ca\(^{2+}\))\(_{i}\) is critical. It has been postulated that chronic exposure of endothelial cells to shear stress could alter calcium regulatory mechanisms that may result in accumulation of (Ca\(^{2+}\))\(_{i}\). It is tempting to speculate that nitric oxide production in individuals with essential hypertension is not sufficient to prevent the contraction of smooth muscle cells due to excess (Ca\(^{2+}\))\(_{i}\) (213). This hypothesis is supported by the demonstration that in essential hypertension (Ca\(^{2+}\))\(_{i}\) is elevated but nitric oxide-induced cyclic guanosine monophosphate is decreased (275,276). Also, a study in pregnant black women found that intraplatelet calcium response to arginine vasopressin was exaggerated in women who subsequently developed preeclampsia and that these women experienced increased vascular resistance (277). Furthermore, a study in lean Zuker rats found that calcium supplementation reduced vascular reactivity (278).

Several studies also show that supplementation with magnesium can promote vasorelaxation by an endothelium-dependent pathway (279,280). Using a dog model, Person et al. (281) demonstrated that low blood magnesium levels impaired nitric oxide release from the coronary endothelium. A study in 50 patients with stable coronary heart disease found that magnesium supplementation of 365 mg per day increased intracellular ionic magnesium and that (Mg\(^{2+}\))\(_{i}\) and flow mediated dilation were positively correlated at baseline (r = 0.48, \(p < 0.002\)) (282). In addition, magnesium supplementation significantly increased flow mediated dilation by 15.5% (\(p = 0.02\)) compared to baseline.

Evidence is emerging that an improvement in endothelial function via diet can potentially reduce the prevalence of CVD beyond that achieved by traditional cholesterol- and blood pressure lowering therapies. Yet, there has been little research to
assess the role of dairy products in improving endothelial function and in attenuating renin-angiotensin system activation. In addition, there is scarce research evaluating whether intracellular calcium and magnesium levels influence flow mediated dilation. Well-controlled human studies are necessary to evaluate whether a diet rich in dairy will elicit beneficial effects on endothelial function in a hypertensive population. I speculate that a dairy-rich diet will improve flow mediated dilation by decreasing intracellular calcium and increasing intracellular magnesium levels. The improvement in flow mediated dilation may even be independent of blood pressure changes.

2.13 Platelet activation

Blood platelets play a major role in CVD and thrombosis. Platelets are anucleated blood cells that originate from the cytoplasm of megakaryocytes in the bone marrow and circulate to discriminate between normal endothelial cell lining and areas with lesions. Under physiologic conditions, this function contributes to hemostasis, but in diseased arteries it may lead to thrombotic occlusion of the vessel lumen with obstruction of blood flow and subsequent tissue damage. Maintaining a balance between blood fluidity and rapid thrombus formation in response to injury is a key attribute of endothelial cells, which control vascular tone and synthesize inhibitors as well as activators of platelet function and blood clotting (283,284). The main trigger for the formation of a hemostatic thrombus after traumatic vascular injury is the loss of the endothelial cell barrier between extracellular matrix components and flowing blood. The response of platelets to this
event develops in three successive but closely integrated phases that involve adhesion, activation and aggregation (Fig. 2-7).

Figure 2-7. Process of platelet adhesion, activation, and aggregation. Platelets initiate thrombosis at the site of a ruptured plaque: the first step is platelet adhesion (a) via the glycoprotein Ib receptor in conjunction with von Willebrand factor. This is followed by platelet activation (b), which leads to a shape change in the platelet, degranulation of the alpha and dense granules, and expression of GP IIb/IIIa receptors on the platelet surface with activation of the receptor, such that it can bind fibrinogen. The final step is platelet aggregation (c), in which fibrinogen (or von Willebrand factor) binds to the activated GP IIb/IIIa receptors of two platelets.
Alterations in platelet activation have been associated with pathologic conditions leading to either thromboembolic or hemorrhage disorders (285). Platelet activation is controlled by the concentration of intracellular ionic calcium \([\text{Ca}^{2+}]_i\) similar to vascular smooth muscle cells. Levels of \((\text{Ca}^{2+})_i\) in unstimulated platelets are generally higher in hypertensive subjects than in normotensive subjects (203,204,207,286). Furthermore, there is a positive correlation between \((\text{Ca}^{2+})_i\) in platelets and blood pressure (202). Platelet stimulation results in a spike in \((\text{Ca}^{2+})_i\), which appears to be due to influx across plasma membrane and release from internal stores (287). This spike in \((\text{Ca}^{2+})_i\) is a very early event that precedes several activation responses, including shape change, aggregation, secretion, and expression of procoagulant activity (288).

Because platelets are important in the interaction with and expression of biological activities in both monocytes and endothelial cells, hyperactive platelets may play a role in the early event of atherosclerosis (285). It is hypothesized that hyperactive platelets may need weaker agonist stimuli than less active ones to secrete activation products and undergo aggregation. More importantly, it is unclear whether the small difference in resting \((\text{Ca}^{2+})_i\) levels in platelets from persons with hypertension vs. those without hypertension reflects a difference in the activated state associated with cell function. Therefore, research has focused on ways of modulating \((\text{Ca}^{2+})_i\) as a means to influence platelet function. A study in 30 essential hypertensive patients and 30 sex- and age-matched normotensive control subjects found that thrombin-evoked \((\text{Ca}^{2+})_i\) responses were significantly enhanced in the hypertensive group vs. their normotensive counterparts (204). Similar results were reported by Lechi et al. (289) after thrombin-stimulation and Touyz and Schiffrin (290) after angiotensin II-stimulation. A new study
in 16 sedentary but otherwise healthy men found that moderate exercise decreased platelet (Ca^{2+})_i elevation induced by ADP, decreased platelet adhesion and binding of von Willbrand factor to platelets, and decreased glyco-protein IIb/IIIa activation and P-selectin expression on platelets (291). Also, a study in pregnant black women found that (Ca^{2+})_i response to arginine vasopressin was exaggerated in women who subsequently developed preeclampsia and that these women experienced increased vascular resistance (277). Finally, calcium antagonists have been shown to reduce platelet aggregation via reduction in (Ca^{2+})_i levels in vitro (292).

### 2.14 Conclusion

Several lines of evidence indicate that diets rich in dairy and/or calcium are able to reduce blood pressure in individuals with mild hypertension. Significant positive correlations between blood pressure and PTH and 1, 25-dihydroxyvitamin D3 have been reported. These calcitropic hormones and angiotensin II, which may be elevated in hypertension, can influence intracellular calcium levels. 1,25-dihydroxyvitamin D3 is reported to alter intracellular calcium by increasing the flow of calcium into the cytosol from the blood. PTH has both hypotensive and hypertensive properties, however over the long term elevated PTH levels appear to increase intracellular calcium and blood pressure in humans. Along with many pressor and atherogenic effects, angiotensin II has also been shown to increase intracellular calcium via a receptor-mediated mechanism. Compared to normal, nitric oxide production in individuals with essential hypertension is low and thus may not be sufficient to prevent the contraction of smooth muscle cells due
to increased (Ca^{2+})_i. Therefore, dairy foods rich in calcium may decrease blood pressure and improve flow mediated dilation by blocking rises in calcitropic hormones, resulting in decreased (Ca^{2+})_i and enhanced production of nitric oxide. Increases in calcium and dairy consumption have been shown to lower intracellular erythrocyte calcium, PTH, 1,25-dihydroxyvitamin D3 (73,293) and erythrocyte sodium (73,294) in both normotensive (294) and hypertensive (73,293) individuals. In addition, calcium has been shown to improve vascular reactivity in animal (43) and in vitro studies (44). Furthermore, bioactive peptides in dairy have the potential to alter the hypertensive effects of angiotensin II by attenuating the ability of ACE to form angiotensin II. In addition to calcium, dairy products are rich in potassium and magnesium, which have been shown to be inversely associated with blood pressure. Both cations are important in regulating intracellular calcium and deficiency in either may result in exaggerated calcium-driven vasoconstriction. Furthermore, supplemental magnesium has been shown to improve nitric oxide production and flow mediated dilation in heart disease patients. Finally, there is some evidence, although not consistent, that dietary calcium also beneficially affects circulating lipid levels. Taken together, this data suggests that components in dairy foods may decrease blood pressure and improve endothelial health by mediating intracellular calcium and nitric oxide levels.
Chapter 3

Effects of dairy products on blood pressure, endothelial function, lipids and lipoproteins, and intracellular ion dynamics in Caucasian adults with stage 1 hypertension.
3.1 Introduction

CVD remains the leading cause of death in Western society, affecting one in every four Americans (1). Approximately 65 million Americans are at increased risk for morbidity and premature death due to elevated blood pressure (>140/90 mmHg) (1). Hypertension is a major risk factor for coronary heart disease, stroke, and congestive heart failure. Coexistence of hyperlipidemia and hypertension further compounds the risk of major cardiovascular events. Moreover, recent data report that CVD incidence is on the rebound after marked decline between 1970-1990 due to improved blood cholesterol levels, improved health care, and other factors (295,296). Thus, the need to identify new strategies as well as improve existing approaches for preventing CVD is of great public health importance.

Hypertension is a clinical condition that reflects a diverse spectrum of underlying causes that are affected by genetic and environmental factors. The cornerstone for the prevention and treatment of hypertension is through diet and lifestyle modification (297). Nonpharmacologic treatment for hypertension includes weight loss if overweight, increased physical activity, decreased consumption of alcohol and sodium. Some evidence exists that consumption of dairy products as well as calcium, magnesium, and potassium, which are also rich in dairy foods, is inversely associated with blood pressure. There has been little insight on which dietary factors may serve to protect against hypertension. New research suggests that African Americans, individuals 50 years or older, diabetic patients, those who are lactose intolerant, and those who have a low habitual calcium intake may especially benefit from dietary interventions rich in dairy
foods. Thus, consumption of dairy products, which has significantly decreased over the past three decades (186-189), may be one dietary change that could have an important impact on improving cardiovascular health.

Although hypertension is usually treated as a uniform condition, underlying heterogeneity of the condition is clinically relevant. For example, dietary sodium restriction has been shown to lower, have no effect, or increase blood pressure in equally hypertensive individuals (81). Also a drug of choice has not been identified to treat all hypertension cases, because the efficacy of drug therapy depends on whether or not the chosen drug affects the underlying cause of hypertension. Abnormal steady-state cellular ion activity is an attractive hypothesis in an integrative model that includes hypertension as one manifestation of a more generalized cellular ionic deficit that also involves insulin resistance, hyperinsulinemia, left ventricular hypertrophy, increased arterial stiffness, abnormal platelet aggregation, and accelerated atherosclerotic disease (81). Each aspect of hypertensive disease appears to reflect a shared cellular ionic lesion defined at least in part by excess intracellular ionic calcium and reciprocally suppressed intracellular ion magnesium. The importance of intracellular ion homeostasis is further demonstrated by hypotensive responses to dietary calcium, which normalizes this cellular defect via suppression of calcium-regulating hormones. Furthermore, the activity of ion-active hormone systems (i.e. calcium-regulating and renin-angiotensin systems) may identify individuals who may be especially responsive to diets rich in minerals. Therefore, studies of the effects of specific diets, particularly diets rich in dairy foods and minerals, on individual physiological mechanisms that regulate blood pressure may provide useful information for developing effective treatments.
The present study focused on the relationship between hypertension and the steady-state concentration of intracellular cations such as calcium, sodium, and magnesium. These cations are key because they contribute to final common pathways mediating blood pressure. This study aimed to clarify the role dairy foods in the regulation of blood pressure and in ameliorating cellular ion imbalances. The information obtained from this study may help explain underlying pathology of clinical hypertension and thereby aid in predicting blood pressure responsiveness to dietary modification.

3.2 Rationale for the study

The DASH trial tested the effects of three dietary patterns, rather than individual nutrients, on blood pressure. Therefore, the DASH trial was not designed to determine the principle nutrients or foods that may have antihypertensive effects. The only conclusion that can be made is that adopting a diet high in fruits and vegetables results in reductions in blood pressure and that further reductions in blood pressure can be achieved by adopting diets rich in dairy, fish, and low in red meat and total fat (59). Therefore, discovery of the mechanism(s) by which this dietary pattern lowers blood pressure starts with the identification of the components responsible for the diet-related effects. Accumulating epidemiologic and clinical data suggest that increasing the consumption of dairy products is one dietary change that could have an important impact on improving cardiovascular health. The goals of the present study are 3 fold; (1) replicate the DASH Study findings while controlling macro- and micronutrient contents of experimental diets,
(2) isolate potential hypotensive effect of dairy products, and (3) identify potential mechanisms of action of a DASH-type dairy-rich diet by examining changes in a number of variables related to blood pressure regulation, including systemic hemodynamics, hormone levels, intracellular calcium dynamic, lipids and lipoproteins, and endothelial function. (Fig. 3-1).

Figure 3-1. Potential mechanism for the cardioprotective effect of a dairy rich diet.

VSMC=vascular smooth muscle cell; FMD=flow mediated dilation; CAM=cellular adhesion molecules
3.3 Objectives and Hypotheses

The primary goal of the present study was to determine the extent to which a dairy-rich diet alters the intracellular content of calcium, sodium, magnesium, and potassium in subjects with essential hypertension compared to a diet high in fruits and vegetables and low in dairy products and an average American control diet. A secondary goal was to assess the effects of a healthy diet, containing dairy foods on blood pressure, calcium regulatory and renin-angiotensin systems, lipids and lipoproteins, cell adhesion molecules, and endothelial function.

**Objective 1:** To determine the effect of a diet high in dairy, fruits, and vegetables on intracellular ion homeostasis (i.e., calcium, magnesium, sodium, and potassium), ion-activity hormone systems (i.e., calcium-regulating and renin-angiotensin systems), and hemodynamics (i.e., systolic and diastolic blood pressure, cardiac output, and total peripheral resistance) compared to a diet high in fruits and vegetables and an average American diet.

**Hypothesis:** A diet high in dairy products would result in lower intraerythrocyte calcium and sodium and higher magnesium levels than the fruit and vegetable diet and the control diet. Given its higher calcium content, the dairy-rich diet was predicted to increase/normalize serum calcium concentrations and lower PTH and 1,25-dihydroxyvitaminD₃, causing lower intracellular calcium levels and therefore lower vascular resistance and blood pressure compared to the fruits and vegetables.
diet and the control diet. In addition, bioactive peptides in dairy products would limit ACE activity and consequently lower angiotensin II levels in the blood, which also may contribute to lower intracellular calcium and lower blood pressure.

**Objective 2:** To assess the effects of a diet rich in dairy on blood lipids and endothelial function (cell adhesion molecules and flow mediated dilation) compared to a diet high in fruits and vegetables and low in dairy and an average American diet.

**Hypothesis:** Compared to the average American diet, the dairy-rich and fruit and vegetable diets, which are lower in total and saturated fat, were expected to reduce total and LDL cholesterol, VCAM-1, ICAM-1, E-selectin, and P-selectin. Additionally, lower angiotensin II and intracellular calcium levels, in response to the dairy-rich diet, would further decrease the expression of cellular adhesion molecules compared to the fruits and vegetable diet. Flow mediated dilation was predicted to be improved following the dairy-rich diet compared to the other two experimental diets due to the normalization of intracellular ion levels (decreased intracellular calcium and increased intracellular magnesium).

**Objective 3:** To determine the relationship between levels and diet-related changes between intracellular ions (i.e., calcium, magnesium, sodium, and potassium) and blood pressure, total peripheral resistance, and flow mediated dilation.
**Hypothesis:** Lower levels of intracellular calcium elicited by the dairy-rich diet would be associated with reductions in blood pressure and total peripheral resistance as well as improved flow mediated dilation. Specifically, levels of blood pressure and total peripheral resistance would be positively associated with intracellular calcium and sodium and negatively associated with intracellular magnesium. The opposite would be expected for flow mediated dilation.

**Objective 4:** To investigate the effects of three experimental diets, including (1) a diet rich in dairy foods, fruits and vegetables, (2) a diet rich in fruits and vegetables but low in dairy, and (3) a control diet typical of Western consumption, on the arginine vasopressin-stimulated increase in cytosolic free calcium in platelets.

**Hypothesis:** The dairy rich diet will significantly curtail the arginine-vasopressin-evoked increase in intraplatelet free Ca\(^{2+}\) compared with the control and the fruits and vegetables diets via lower resting levels of cytosolic Ca\(^{2+}\).
3.4 Methods and Subjects

3.4.1 Study design

A multicenter controlled feeding study was conducted to evaluate the effects of three isocaloric diets on intracellular ions, hemodynamics, lipids and lipoproteins, ion-active hormone systems, and endothelial function. The two study sites were located at the Penn State University (PSU) Study Center at University Park, PA, and the Pennington Biomedical Research Center (PBRC) at Baton Rouge, LA. A total of 23 subjects were studied, 17 from the PSU site and 6 from the PBRC site.

A randomized, three-diet, three-period crossover design was employed. Subjects were randomly allocated to one of three groups: 1) a dairy-rich fruits and vegetables (D-F&V) diet, 2) a dairy-poor fruits and vegetables (F&V) diet, or 3) an average American control diet (AAD) (~35% fat, 15% saturated fat). Subjects consumed each diet for 5 weeks with at least a two-week compliance break in between before crossing over to the other diets.

During diet periods, subjects were required to maintain their usual activities and exercise levels, and their body weight was controlled by adjusting total energy intake. At the end of each diet period, 12-hour fasting blood samples were taken by venipuncture on two consecutive days and the samples were processed according to the protocols for each endpoint assay. The study design is shown in Fig. 3-2.
3.4.2 Subjects

Eligibility criteria

Men and women, 22-70 years of age, body mass index (BMI) below 35 kg/m², and Stage 1 hypertension as defined as systolic blood pressure = 140-159 and/or diastolic blood pressure = 90-99 mmHg were eligible for the study. Subjects had normal or moderately elevated blood cholesterol (LDL cholesterol less than 190mg/dL (about the 95th percentile of NHANES III), HDL-cholesterol in the 15-95th percentile of NHANES III, and triglycerides less than 350 mg/dL). Women taking hormone replacement therapy, oral contraceptives, and premenopausal women also were included.

Individuals were excluded if they smoked, took blood pressure-lowering, lipid-lowering, MAOI, Viagra and/or dihydroergotamine medications, consumed nutritional...
supplements, had food allergies, latex allergies and/or allergies to glycerol trinitrate. Anyone diagnosed with osteoporosis, Reynaud’s disease, a peripheral arterial disease, angina, and intermittent claudication and/or anyone who had undergone a heart bypass or angioplasty were excluded. Individuals taking any medication known to have an effect on blood pressure, blood lipids, or blood calcium were excluded (i.e., Effexor and Fosamax). All medications taken by a potential subject were checked in the Physicians Desk Reference for food/drug interactions and effects on blood pressure and blood lipids. People with low blood pressure, SBP below 140 and/or DBP below 90 mm Hg or people with Stage 2 hypertension (SBP above 160 and/or DBP above 100 mm Hg) did not qualify.

Identification of exclusions was based on data obtained from medical questionnaires, psychological evaluation, blood chemistry (i.e., blood glucose, lipids and lipoproteins, leukocyte count, thyroid-stimulating hormone, minerals and liver enzymes), and a physical examination.

As an option for treated hypertensive individuals, who were currently taking only one blood pressure lowering drug, we allowed a medication wash-out period. This required permission of their physician and a labor-intensive monitoring period after they discontinued the drug. Appendix A details the protocol for discontinuation of blood pressure medication. After the wash-out period, subjects started the screening process.
Subject recruitment

According to the inclusion and exclusion criteria listed above, recruiting strategies at both sites, employed throughout this study, included:

- Ads in four local newspapers (averaged 3-day ad sequence every month throughout the study)
- Flyers and brochures in local businesses (300+)
- Flyers sent to Penn State Employees via direct campus mail (1500+)
- Community blood pressure screenings (5 at PSU, every 2 weeks at PBRC)
- Radio Public Service Announcements (over 6 months at Hershey area)
- Radio ads (run over 6 months in Hershey area)
- Meetings with four physician groups in State College, PA, to encourage referrals
- Visits at two PSU retirement communities to discuss the study and conduct blood pressure screenings
- Offer of delivery of food to participants at home during study
- Letters to local churches and other organizations encouraging members to volunteer
- Overall, 400 prospective subjects were screened by phone and over 100 subjects were screened in the clinic.

Subject screening

The screening procedures started with an initial telephone interview (Appendix B). Potential subjects who met the criteria in the telephone interview were invited to
schedule the first of three screening visit at the study clinic (Appendix C-E). During the first screening visit, prior to any of the following activities, potential subjects were required to read and sign the Informed Consent Form (Appendix F). Then, anthropometric data (age, height and weight) and blood pressure were collected from each subject. Baseline blood samples (at least 12-hour fasting) were drawn for blood chemistry screening (measuring blood glucose, lipids and lipoproteins, leukocyte count, thyroid-stimulating hormone, plasma minerals and liver enzymes). A second clinic visit was scheduled if all study eligibility criteria were met and if he/she was willing to continue with the process. The second screening included blood pressure measurement, postural hypotension determination, and urinalysis. If the participant met eligibility requirements, a final screening visit was scheduled and included blood pressure measurement and clinician physical. Overall, subjects had to meet all eligibility criteria and have Stage 1 hypertension at all three screening visits, which was most common reason for exclusion.

3.4.3 Experimental diet design

The experimental diets were designed to evaluate the unique role of dairy foods in blood pressure regulation (refer to Table 3-1). One control diet and two experimental diets were formulated using menus developed in the original DASH Study and modified according to the experimental diet criteria proposed herein using Nutritionist V software (N-Squared Computing, First DataBank Division, San Bruno, CA). The dietary macronutrient compositions of the three diets were measured by chemical analyses and then adjusted appropriately to meet the target levels for nutrients. The experimental diets
included an average American diet (AAD) serving as a control diet, a diet high in fruits and vegetables but low in dairy foods (F&V), and a diet similar to the F&V diet by rich in dairy products (D-F&V). The F&V and D-F&V diet designs were compositionally similar with respect to macronutrients (total fat 30% kcal, saturated fat 7% kcal, monounsaturated 14% kcal, polyunsaturated fat 9% kcal, protein 18% kcal, and carbohydrate 52% kcal), as well as cholesterol (300 mg/d), sodium (3500 mg/d), and fiber (27 g/d). In addition, the amount and kind of fruits and vegetables were identical across these two experimental diets. To specifically test the effects of dairy, the D-F&V diet provided 3.4 servings/d of dairy products compared with 0.4 servings/d in the F&V and AAD diet. The dairy products used in all three diets were a combination of milk, yogurt and full-fat cheese. The F&V and the D-F&V diets included low-fat and non-fat milk and yogurt products and full-fat cheese, while the control diet include whole milk and full-fat dairy products. The calcium content of the F&V and AAD diets was matched and the milligram quantity of calcium did not surpass 600 mg/d at the highest kcal level (i.e., at 3100 kcals the calcium will be at 550 mg, at a more typical kcal level, 2400 kcal, the diet will provide 450 mg of calcium). The level of sodium in all three diets is what is typically consumed in our society.

Overall, the D-F&V diet was higher in calcium, potassium, magnesium and phosphorus compared with the F&V diet, which primarily reflected the increase in dairy products provided by the D-F&V diet. The control diet was the typical American diet, which is low in fruits, vegetables, and dairy foods, and thus differs in macronutrient profile (36% fat, 15% saturated fat, 300 mg cholesterol). Since the diet high in fruits and vegetables and low in dairy products was compositionally similar to the DASH diet with
respect to macronutrients and differed from the dairy rich diet in micronutrients provided from dairy products, this diet design allowed us to evaluate specific effects of dairy products on cardiovascular health.

For each experimental diet, eight different caloric levels (1800, 2100, 2400, 2700, 3000, 3300, 3600, 3900 kcal) were developed in order to maintain subjects’ body weight at the entry levels. A 6-day cycle menu for each caloric level was developed (Appendix G). Energy levels were adjusted by proportionally adjusting the weight of each menu item. Unit foods (100 Kcals each) that were compositionally identical to the experimental diets were used to adjust calorie levels so that subjects maintained body weight.

### Table 3-1. Sample menu nutrient analysis (based on 2100 kcal per day)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>AAD</th>
<th>F&amp;V</th>
<th>D-F&amp;V</th>
<th>D-F&amp;V - F&amp;V</th>
<th>D-F&amp;V - AAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (%kcal)</td>
<td>46</td>
<td>52</td>
<td>52</td>
<td>None</td>
<td>6</td>
</tr>
<tr>
<td>Protein (%kcal)</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Fat (%kcal)</td>
<td>36</td>
<td>30</td>
<td>30</td>
<td>None</td>
<td>6</td>
</tr>
<tr>
<td>Saturated (%kcal)</td>
<td>15</td>
<td>7</td>
<td>7</td>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>Monounsaturated (%kcal)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Polyunsaturated (%kcal)</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>None</td>
<td>125</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>10.5</td>
<td>27</td>
<td>27</td>
<td>None</td>
<td>16.5</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>3500</td>
<td>3500</td>
<td>3500</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1750</td>
<td>3900</td>
<td>4600</td>
<td>700</td>
<td>2850</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>400</td>
<td>400</td>
<td>1200</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1150</td>
<td>1300</td>
<td>1700</td>
<td>400</td>
<td>550</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>190</td>
<td>370</td>
<td>420</td>
<td>50</td>
<td>230</td>
</tr>
<tr>
<td>Fruit (serving/d)</td>
<td>1.6</td>
<td>5.2</td>
<td>5.2</td>
<td>None</td>
<td>3.6</td>
</tr>
<tr>
<td>Vegetable (serving/d)</td>
<td>2.0</td>
<td>4.4</td>
<td>4.4</td>
<td>None</td>
<td>2.4</td>
</tr>
<tr>
<td>Dairy (serving/d)</td>
<td>0.4</td>
<td>0.4</td>
<td>3.4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
3.4.4 Study protocol

Subjects were required to eat all food provided by the study research kitchens and not eat any other food. On weekdays, subjects consumed at least one meal per day in the feeding center. Other weekday meals, snacks, and weekend meals were packaged for takeout in coolers. Diet compliance was monitored according to procedures routinely used in our research centers (Appendix H and I).

Since blinding of the diets was not possible, specific measures were taken to insure that blinding is accomplished for the collection of quality data. All kitchen, menu, compliance and related data were collected and entered only by kitchen staff, whereas all clinic and laboratory data were collected and entered only by clinic staff. Those staff taking endpoint measurements (flow mediated dilation, blood pressures) had no knowledge of the diet assignments of the participants. At the start of the study and throughout, participants were cautioned not to discuss dietary issues with clinic staff and clinic issues with the kitchen staff.

For safety considerations, blood pressure was monitored throughout the entire study. Blood pressure was measured during the first five days of each diet period and once a week thereafter using an automated device (Dinamap Pro 100 Monitor, Critikon, Inc) (Appendix J). The results were not given to the participant or used as outcome variables.
3.4.5 Materials

Fluo-4-acetoxyethyl ester (Fluo-4 AM) was obtained from Molecular Probes (Eugene, OR). HEPES, bovine serum albumin (BSA), dimethylsulfoxide (DMSO), NaCl, KCl, MgCl₂, NaHCO₃, Na₂HPO₄, glucose, choline chloride, hydrogen peroxide, nitric acid, arginine vasopressin, and reduced glutathione were obtained from Sigma Chemical Co. (St. Louis, MO). The monoclonal antibody specific for GPIIb/IIIa complex conjugated with phycoerytrin (CD41-PE) was from Beckman Coulter (Fullerton, CA).

3.4.6 Biological sample collection

Endpoint collection occurred during the last week of each diet period. The following assays were performed at the Core Endocrinology Laboratory at Hershey Medical Center: lipids, lipoproteins, 24-hour urinary electrolytes, serum calcium, calcium-regulating hormones, renin activity, ACE activity, and angiotensin II. I conducted assays to evaluate ionic calcium transients in stimulated platelets using flow cytometry and diet-related changes in cell adhesion molecules using ELISA kits. Erythrocyte electrolytes were determined by inductively coupled plasma-mass spectrometry (ICP-MS) at the Penn State Materials Characterization laboratory. Table 3-2 provides a brief description of how and where the endpoints were determined.
Table 3-2. Overview of study endpoints.

<table>
<thead>
<tr>
<th>Blood and Urine Endpoints</th>
<th>Sample</th>
<th>Method</th>
<th>Where analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td>--</td>
<td>mercury manometer</td>
<td>PBRC, PSU</td>
</tr>
<tr>
<td>FMD</td>
<td>--</td>
<td>ultrasound</td>
<td>PBRC, PSU</td>
</tr>
<tr>
<td>Total Intracellular Ca, Na, Mg, K</td>
<td>erythrocytes</td>
<td>ICP-MS</td>
<td>PSU</td>
</tr>
<tr>
<td>Free ionic calcium changes</td>
<td>platelets</td>
<td>Flow cytometry</td>
<td>PSU</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium regulating:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>Serum</td>
<td>IRA</td>
<td>Hershey</td>
</tr>
<tr>
<td>1,25-dihydroxyvitamin D₃</td>
<td>Serum</td>
<td>RIA</td>
<td>Hershey</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Serum</td>
<td>RIA</td>
<td>Hershey</td>
</tr>
<tr>
<td>Renin-angiotensin system:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin activity</td>
<td>Plasma</td>
<td>RIA</td>
<td>Hershey</td>
</tr>
<tr>
<td>ACE activity</td>
<td>Serum</td>
<td>Enzymatic assay</td>
<td>Hershey</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>Plasma</td>
<td>RIA</td>
<td>Hershey</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>Serum</td>
<td>Enzymatic assay</td>
<td>Hershey</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>Serum</td>
<td>Freidewald equation</td>
<td>Hershey</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Serum</td>
<td>Enzymatic assay</td>
<td>Hershey</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Serum</td>
<td>Enzymatic assay</td>
<td>Hershey</td>
</tr>
<tr>
<td><strong>Cell adhesion molecules:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Serum</td>
<td>ELISA</td>
<td>PSU</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Serum</td>
<td>ELISA</td>
<td>PSU</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Serum</td>
<td>ELISA</td>
<td>PSU</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Serum</td>
<td>ELISA</td>
<td>PSU</td>
</tr>
<tr>
<td><strong>Supportive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>Serum</td>
<td>colormetric assay</td>
<td>Hershey</td>
</tr>
<tr>
<td>24-hr Ca</td>
<td>urine</td>
<td>colormetric assay</td>
<td>Hershey</td>
</tr>
<tr>
<td>24-hr Na</td>
<td>urine</td>
<td>ion sensitive electrode</td>
<td>Hershey</td>
</tr>
<tr>
<td>24-hr K</td>
<td>urine</td>
<td>ion sensitive electrode</td>
<td>Hershey</td>
</tr>
</tbody>
</table>

When bolded, PSU represents assays I performed.

PBRC = Pennington Biomedical Research Center, PSU = Penn State University, ICP-MS = inductively coupled plasma-mass spectrometry, Ca = calcium, Na = sodium, Mg = magnesium, K = potassium, PTH = parathyroid hormone, IRA = immunochemiluminometric assay, RIA = radioimmunoassay, ELISA = enzyme-linked immunosorbent assay.
3.4.6.1 Preparation of serum and plasma

Blood samples were taken after a 12-hour fast at the end of each of the three dietary interventions. Because of reported variability in lipid endpoints, two samples on consecutive days were drawn for lipid analysis at the end of each diet. Samples were drawn into appropriately treated Vacutainer blood collection tubes. Serum samples were allowed to clot at room temperature and the serum was collected and stored in cryovials at –80°C until analysis. Blood to be processed for plasma was immediately spun in a refrigerated centrifuge, after which the plasma was placed into cryovials and stored in the same freezer.

3.4.6.2 Preparation of erythrocytes

At the end of each diet period, 5 ml of blood were collected in heparinized Vacutainer tubes. All samples, including those from the PBRC site, were prepared for ICP-MS at the Penn State campus as follows. The samples were centrifuged at 1,500 x g for 10 minutes at 4°C after which the plasma and buffy coat were aspirated and discarded. The remaining erythrocytes were washed three times by suspension in 140 mM choline chloride (ice cold), with 10 min centrifugations at 4°C and aspiration of the supernatant. After the last washing, a microhematocrit capillary sample was taken for determination of hematocrit (80-90%). Aliquots of 500 µl of erythrocytes were placed in three eppendorff tubes each containing 1 ml of 140 mM choline chloride. The aliquots were prepared for determination of cation levels by lysing in one volume of distilled-deionized water and frozen at –80°C for subsequent analysis.
3.4.6.3 Preparation of platelets

To avoid artificial platelet activation, blood for platelet study was drawn after all other samples into a 4.5 ml sodium citrate Vacutainer tube with a 19-gauge needle. Anticoagulated blood was diluted 1:10 in modified Tyrode’s buffer (137 mM NaCl, 2.8 mM KCl, 1 mM MgCl₂, 12 mM NaHCO₃, 0.4 mM Na₂HPO₄, 0.35% BSA, 10 mM HEPES, 5.5 mM glucose, pH 7.4) and incubated at 37°C for 15 minutes with 5 μM Fluo-4 AM from a 1-mM stock solution in DMSO. Fluo-4 AM is a calcium sensitive dye that is converted intracellularly to the membrane impermeable Fluo-4 AM free acid. Fluo-4 AM has a negligible fluorescence as a free acid, which is enhance upon binding to Ca²⁺. After loading the blood with Fluo-4 AM, 25 μL of this sample was directly labeled with 5 μL of CD41-PE, a monoclonal antibody specific for GPIIb/IIIa complex conjugated with phycoerytrin, to identify the platelet population. After 15 minutes of incubation at room temperature in the dark, 1 ml of modified Tyrode’s buffer was added.

3.4.6.4 Preparation of urine specimens

Twenty-four hour urine samples were treated with reduced glutathione and kept cool in a cooler with ice packs until they were processed by staff, who measured total urine volume and stored aliquots at -80 °C until analysis. Participants were asked to note the time that the collection began and ended and any times they failed to collect the sample as directed. Samples with less than 22 hours collection time were discarded and participants were instructed to collect them again in the next 24-hour period if possible.
3.4.7 Physiologic measurements

3.4.7.1 Determination of blood pressure

During the last week of each diet period, four sets of blood pressure measurements were taken on separate days. All blood pressure measurements were performed using standard, commercially available mercury column manometers and stethoscopes following the protocols outlined in the Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (17). Blood pressure readings were taken in a quiet room where no other activity was occurring and where temperature fluctuations were minimal. Participants were instructed not to engage in vigorous exercise or ingest food or caffeine within ½ hour of the measurements. After a five minute rest period in the seated position, three stethoscopic measurements were taken, separated by one minute. Briefly, with cuff connected to the manometer, the first and fifth phases were recorded, by reading the pressure to the nearest 2 mm Hg. The first sound heard in a series of at least two sounds was recorded for systolic blood pressure (the point of appearance of Korotkoff sounds). For diastolic blood pressure, the first silence in a series of at least two silences was recorded (the point of disappearance). After waiting 30 seconds with the subject sitting quietly, the measurements were repeated two more times. The means of all available endpoint measures were used for analysis. All staff collecting blood pressure data were trained and certified in the use of a standard protocol and were kept blinded to participant treatment assignments.
3.4.7.2 Determination of total peripheral resistance, cardiac output, and stroke volume

Echocardiographic studies were performed at the same visit at which brachial artery flow mediated dilation was assessed (once at the end of each diet). Sonographers at each site were carefully trained according to a protocol that was validated by Hinderliter and colleagues (298) against an invasive technique (cardiac dye dilution).

The sonographer used a 2.5 MHz phased array transducer with the subject in the left lateral decubitus position. Blood pressure was simultaneously measured using an automated device (Omron HEM-705CP (Vernon Hills, IL). Two-dimensional images from the parasternal long axis view were recorded on sVHS tape for measurement of the left ventricle outflow tract diameter. Pulsed Doppler tracings of left ventricle outflow velocity were obtained from the apical five-chamber view with the sample volume placed just proximal to the aortic valve, recorded on SVHS videotape. Doppler curves were excluded if systolic flow velocities were not well defined. A single experienced investigator, Dr. Hinderliter at the University of North Carolina, interpreted all studies blindly. Measurements were averaged over three cardiac cycles. Stroke volume was determined as follows. Diameter of the left ventricle outflow tract was measured during systole at the aortic annulus, and cross-sectional area (CSA) was calculated assuming a circular shape. The time-velocity integral (TVI) of the outflow tract Doppler spectral display was determined by tracing the black/white interface of the flow profile. Stroke volume (the product of CSA and TVI), heart rate and blood pressure were used to calculate cardiac output and total peripheral resistance using standard formulae.
3.4.7.3 Determination of lipids and lipoproteins

Serum total and HDL cholesterol and triglycerides were determined by standard enzymatic assays with commercially available kits. HDL cholesterol was determined after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium. LDL cholesterol was calculated using the Friedewald equation: LDL cholesterol = total – [HDL cholesterol + (triglycerides/5)].

3.4.7.4 Determination of basal arterial diameter, peak diameter, and flow mediated dilation

During the final week of each diet period flow mediated dilation of the brachial artery, an indicator of endothelial health, was measured. This measurement occurred at the same time of day and after a 12-hour fast for each diet period for each subject. This assessment was completed under controlled conditions by trained and blinded sonographers.

Our method is adopted from Sorensen and colleagues (36) and we use identical techniques and scoring software as that used by the Framingham investigators. The brachial artery above the elbow of the dominant arm was scanned in longitudinal sections, and scans were taken at rest, during reactive hyperemia, again at rest. Changes in diameter were assessed by external B mode ultrasound imaging (Acuson Aspen equipped with a 10 mHz linear array transducer; Acuson, Mountain View California). This technique is capable of reliably detecting changes in vascular diameter as small as 0.1 mm. Participants wore a standard blood pressure cuff on the left arm and
measurements were taken every other minute during each rest period. All blood flow velocity measurements were collected using pulsed Doppler flow with the probe at a 70° angle to the vessel.

Baseline measurements of blood flow, blood pressure, and heart rate were obtained after 15 minutes of supine rest. A pneumatic tourniquet was placed around the right forearm, distal to the target artery, and the upper arm and hand were supported by pillows. The sonographer recorded an image of the brachial artery under resting conditions for one minute. Next, the cuff was inflated to a pressure of 200 mmHg (or 50 mmHg above systolic pressure, 250 mmHg maximum) for five minutes. The sonographer recorded images throughout this occlusion period. Increased flow was induced by sudden cuff deflation. A second scan was video taped beginning 15 seconds before cuff deflation and continuing for 120 seconds afterwards. After 15 minutes of quiet rest, a second resting scan was recorded for one minute. Ultrasound images were stored on high quality SVHS tape and analyzed by a pair of trained observers in the Vascular Health Interventions Laboratory at Penn State, both of who were blinded to the treatment conditions. Arterial diameters (baseline and peak) were measured using commercially available software designed exclusively for this purpose (Brachial Tools, Medical Imaging Applications, Iowa City, IA).

3.4.7.5 Determination of brachial arterial diameter pre- and post-nitroglycerin administration

As done in several previous dietary intervention studies (39,298), nitroglycerin (glycerol trinitrate (NTG), 400 μg) was administered under the tongue to induce
vasodilation that was independent of the vascular endothelium. The purpose of this second assessment was to show whether the dietary treatment alters vascular sensitivity to a nitric oxide donor (the drug, NTG), termed endothelium-independent dilation. This test was considered a control procedure and was not expected to change due to dietary treatments.

3.4.7.6 Determination of cell adhesion molecules

Serum soluble cell adhesion molecules ICAM-1, VCAM-1 and E- and P-selectin were measured with commercially available ELISA kits (R&D Systems, Minneapolis, MN).

3.4.7.7 Determination of serum calcium and calcium-regulating hormones

Serum intact PTH was measured by immunochemiluminometric assay (Nichols Institute Diagnostics, San Clemente, CA), and radioimmunoassays (RIA) were used to determine serum 1,25-dihydroxyvitaminD₃ (Immundiagnostic System Inc., Fountain Hills, AZ) and serum calcitonin (Diagnostic Systems Laboratories, Inc., Webster, TX). Serum calcium was measured by colormetric assay.

3.4.7.8 Determination of plasma renin activity, serum ACE activity, and plasma angiotensin II

Plasma renin activity and angiotensin II were measured by RIA and ACE activity was measured with an enzymatic assay.
3.4.7.9 Determination of erythrocyte cations

Erythrocyte calcium, magnesium, sodium, and potassium were assayed using ICP-MS (Finnigan Element High Resolution) at the Materials Characterization laboratory on the Penn State campus. The erythrocyte suspension was digested at 100ºC for 2 hours with 0.6ml nitric acid and 0.6ml hydrogen peroxide in a Teflon screw top container. Then an Indium internal standard was added, and digests were diluted to 6ml final volume with distilled-deionized water. Instrument calibration was performed using standards purchased from High Purity Standards (Analytical West, Santa Rosa, CA).

3.4.7.10 Determination of 24-hr urinary electrolytes

These measures were collected both to ensure compliance with the dietary protocol and to provide explanatory mechanisms for any blood pressure reductions that we observe. Urine Ca was measured via colorimetric assay, while urine Na and urine K were measured with the ion sensitive electrode method.

3.4.7.11 Determination of intraplatelet ionic calcium mobilization

The determination of intraplatelet ionic calcium [(Ca²⁺)i] was carried out in 17 subjects according to Monteiro and colleagues (299) at the Penn State site within 2 hours of blood draw. Immediately following the preparation of the platelets, (Ca²⁺)i was determined using EPICS XL Flow Cytometer. Each day the machine was assessed for quality control using Flow Check alignment beads (Beckman Coulter). These beads
consistently fell in the same channels with a coefficient of variation of < 1.0%. The machine had a 15-m argon laser tuned at 488 nm and was set up to measure forward angle scattered light, side angle scattered light, Fluo-4 AM, and phycoerytrin fluorescence intensities. Fluorescence was collected through a 488-nm blocking filter via a 550-nm long-pass dichroic through a 525-nm bandpass (Fluo-4 AM) and via a 600-nm long-pass dichroic through a 575-nm bandpass (phycoerytrin). Platelets were identified using analytical gates based on forward and side scatter signals and on the CD41+ events.

After determination for 30 seconds of baseline Fluo-4 AM fluorescence from the platelet population, cell aspiration into the flow cytometer was paused and 25 μL of arginine vasopressin (33.3 uM) was added to the sample. The acquisition was resumed and recorded for three minutes. Rectangular analysis regions were defined over time (the X-axis) and covered the whole length of the fluorescence axis (Y-axis). The cytometer software automatically displayed the statistical mean and median for each time slice. These time regions (one at baseline and 12 others) were used as gating windows to analyze kinetic changes in intraplatelet free calcium.

3.4.8 Data analysis

This was a randomized, three-period, three-diet, cross-over study conducted at two locations. All data analyses were performed with the mixed models procedure in SAS (version 9.1; SAS Institute, Inc, Cary, NC). Data are expressed as least squares means ± SE. Tests of normality (Shapiro-Wilk) were performed on residuals and any severe departures from normality were corrected with the appropriate transformation.
Because of skewed distributions, triglycerides were log-transformed prior to analysis, and unadjusted means are reported in the text and tables. The preliminary model included type of diet (AAD, D-F&V, and F&V), diet period, and potential carry over effects (sequence of diet presentation) as fixed effects and subject as a random effect. Site of data collection (PSU vs. PBRC) and age were included as covariates. For the blood pressure and lipid data, the model was simultaneously adjusted for fasting values at screening. The degrees of freedom were adjusted for unequal group variance by Satterthwaite’s approximation. To determine the source of any significant main effects or interactions, the conservative Tukey post hoc test was used.

To replicate analyses performed in the DASH trial and to confirm the above analyses, change was calculated as the difference between screening and end-of-diet levels for SBP and DBP. The model included diet, sequence of diet presentation, period, and their interactions with simultaneous adjustment for screening values, study site, and age. This strategy did not change the pattern or significance of the diet effects, and both sets of results are reported herein.

Pearson’s correlation analyses were conducted to evaluate the association of blood pressure, hemodynamic variables, and endothelial function with intraerthrocyte cations, as well as PTH, 1,25-dihydroxyvitamin D3, and angiotensin II.

In addition, a post hoc two-way analysis of covariance (subgroups, average change in RBC calcium ≥ 4.9 uM vs. following the D-F&V diet vs. average change in RBC calcium < 4.9 uM) was carried out for blood pressure and RBC cations. The model was identical to the one previously described with the addition of this grouping variable.
and its interaction with diet. Since, RBC calcium levels following the D-F&V diet were measured on 18 subjects only, the sample size was reduced from 23 to 18 subjects.

### 3.4.8.1 Sample size

In this study, all subjects consumed each of the three experimental diets and therefore acted as their own control in comparisons between diets. This type of repeated-measures design increases the statistical power to detect differences between diets over the between-subjects design in which each diet group is comprised of different individuals. The completed sample size of 23 subjects was substantially less than originally planned due to challenges in recruitment. The proposed sample size of 40 was based on attempting to detect a difference in systolic blood pressure of 2.1 mmHg between the F&V diet and D-F&V diet, using a two-sided, 0.025-significance level test that had 85% power.

The sample size calculated using our present data was consistent with what was proposed because the variance of the present data was similar to the estimation of variance that we used previously. In the present study, the standard deviation of the paired difference after consuming the D-F&V and F&V diets was 4.67 mm Hg. In order to have 85% power to detect an effect of 2.1 mm Hg, we would have needed a sample size of 48 when alpha equals 0.025 (two tailed test). Presently (n=23) the difference between the intervention diets (F&V vs. D-F&V) is only 0.34 mmHg (not 2.1 mm Hg). Therefore, we were not adequately powered to show that the diets are equal or significantly different (n=23, SD =4.67, effect size of 0.34, Power = 6%). However, we
are adequately powered to evaluate differences in intracellular calcium between the D-F&V and F&V diets. The sample size of 23 was based on attempting to detect a difference in intracellular calcium of 5.7 μM between the F&V diet and D-F&V diet, using a two-sided, 0.05-significance level test that had 90% power.

3.5 Results of primary analyses (n=23)

3.5.1 Subject characteristics at study entry

Subjects who completed at least two diet periods were included in the data analysis, for a total sample size of 23 subjects. Sixteen participants from the PSU site completed study in its entirety and one participant completed two of three diet periods (missing D-F&V diet due to time constraints of the participant). At the PBRC site, four subjects completed the study, two subjects completed two diet periods (both missed the AAD due to early termination of the study at the PBRC site), and two subjects completed only one diet period. The study included 16 men and 7 women, one of which was premenopausal. The premenopausal woman was tested during the follicular phase of her menstrual cycle.

Table 3-3 shows demographic and cardiovascular risk characteristics of the subjects at study entry. Women weighed significantly less the men (p = 0.0022) and BMI tended to be lower in women (p = 0.0664). Based on BMI, men were classified as obese and women were overweight. Men and women were comparable in age, blood pressure,
and lipid levels. Subjects at PSU and PBRC sites did not significantly differ on any variable at study entry (data not shown).

Table 3-3. Subject characteristics at study entry stratified by sex

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Men</th>
<th>Women</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n=23)</td>
<td>(n=16)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.3 ± 2.0</td>
<td>42.9 ± 3.1</td>
<td>50.1 ± 3.6</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>196.3 ± 7.7</td>
<td>214.6 ± 9.6</td>
<td>161.0 ± 11.3 a</td>
</tr>
<tr>
<td>BMI</td>
<td>28.8 ± 0.9</td>
<td>30.3 ± 1.3</td>
<td>26.2 ± 1.6</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>140.5 ± 1.9</td>
<td>140.1 ± 3.0</td>
<td>143.1 ± 3.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>91.1 ± 1.3</td>
<td>91.0 ± 2.0</td>
<td>88.7 ± 2.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.4 ± 6.4</td>
<td>204.5 ± 9.9</td>
<td>186.6 ± 11.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>122.9 ± 6.1</td>
<td>135.8 ± 9.1</td>
<td>108.4 ± 10.8</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49.1 ± 2.4</td>
<td>48.4 ± 3.6</td>
<td>54.7 ± 4.3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>112.3 ± 12.4</td>
<td>102.4 ± 19.9</td>
<td>117.6 ± 23.5</td>
</tr>
</tbody>
</table>

1 Data presented as least squares mean ± SE.
2 Subjects at PSU and PBRC sites did not significantly differ on any variable.
3 Different from Men, p = 0.0022

3.5.2 Treatment effects on blood pressure

The blood pressure results are presented in Table 3-4. There was a main effect of diet for SBP (p = 0.0149). The D-F&V and the F&V diets significantly lowered SBP compared with the AAD (Tukey p = 0.05 and Tukey p = 0.02, respectively). However, the two intervention diets elicited similar effects. We also observed a period effect (p = 0.0291) for SBP. SBP was significantly lower during diet period 3 compared with diet period 1 (Tukey p = 0.0245), suggesting that SBP continued to decrease throughout the study. Like SBP, there was a main effect of diet for DBP (p = 0.0047). The D-F&V and
the F&V diets significantly lowered DBP compared to the AAD (Tukey p = 0.0166 and Tukey p = 0.0087, respectively).

Analyses of change scores revealed that all three experimental diets significantly lowered SBP and DBP vs. screening (all p’s < 0.0004) (Fig. 3-3 A). Confirming the levels analyses, the magnitude of change for SBP and DBP was significantly greater following the D-F&V and F&V diets compared with the AAD. As with the levels analysis, an effect of period was observed for SBP (p = 0.0291).

Similar to SBP and DBP, mean arterial pressure (MAP) was significantly lowered by all three diets compared with screening levels (AAD –6.9 ± 1.2, p < 0.0001; F&V –8.8 ± 1.2, p < 0.0001; D-F&V –8.5 ± 1.2, p < 0.0001) (Fig. 3-3 B). Furthermore, the reduction in MAP was significantly greater following the D-F&V and F&V diets compared with the AAD (p = 0.0016) (Table 3-4).

### 3.5.3 Treatment effects on total peripheral resistance, cardiac output, and stroke volume

Table 3-4 presents levels of total peripheral resistance, cardiac output, and stroke volume at the end of each diet. There were no significant diet-related changes in these hemodynamic variables.
Table 3-4. Effects of diet on blood pressure and hemodynamic variables\(^1\)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>140.5 ± 1.9</td>
<td>130.7 ± 1.5 (^a)</td>
<td>128.3 ± 1.5 (^b)</td>
<td>128.6 ± 1.5 (^b)</td>
<td>0.0149</td>
<td>-2.4 ± 0.9 (0.0193)</td>
<td>-2.0 ± 0.9 (0.0525)</td>
<td>0.3 ± 0.9 (0.9183)</td>
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<tr>
<td>DBP (mm Hg)</td>
<td>91.1 ± 1.3</td>
<td>85.9 ± 1.2 (^a)</td>
<td>84.1 ± 1.2 (^b)</td>
<td>84.2 ± 1.2 (^b)</td>
<td>0.0047</td>
<td>-1.8 ± 0.6 (0.0087)</td>
<td>-1.7 ± 0.6 (0.0166)</td>
<td>0.1 ± 0.6 (0.9715)</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>107.6 ± 1.0</td>
<td>100.9 ± 1.2 (^a)</td>
<td>98.9 ± 1.2 (^b)</td>
<td>99.1 ± 1.2 (^b)</td>
<td>0.0016</td>
<td>-2.0 ± 0.6 (0.0030)</td>
<td>-1.8 ± 0.6 (0.0087)</td>
<td>0.2 ± 0.6 (0.9373)</td>
</tr>
<tr>
<td>Cardiac Output (L/min)</td>
<td>NM</td>
<td>4.6 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>NS</td>
<td>0.6 ± 0.2</td>
<td>-0.1 ± 0.2</td>
<td>-0.2 ± 0.2</td>
</tr>
<tr>
<td>Total Peripheral Resistance</td>
<td>NM</td>
<td>1643 ± 94</td>
<td>1554 ± 95</td>
<td>1637 ± 97</td>
<td>NS</td>
<td>-89 ± 83</td>
<td>-6 ± 82</td>
<td>83 ± 85</td>
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<tr>
<td>(dynasec*cm(^{-5}))</td>
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<tr>
<td>Stroke Volume (ml/beat)</td>
<td>NM</td>
<td>68.6 ± 3.1</td>
<td>70.3 ± 3.2</td>
<td>68.4 ± 3.2</td>
<td>NS</td>
<td>1.7 ± 2.8</td>
<td>-0.2 ± 2.6</td>
<td>-1.9 ± 2.8</td>
</tr>
</tbody>
</table>

\(^1\) Data are least squares means ± SE, n=23. Within rows, values with different letter subscripts are different, Tukey P ≤ 0.05. The exact Tukey adjusted P-values are listed also. Different from screening: * P < 0.0004.

\(^2\) Period effect, P < 0.0291 (Period 3 is lower than Period 1, Tukey P < 0.0245).

NS = nonsignificant, NM = not measured
Figure 3-3. A) Diet-related changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from study entry, n=23. AAD = average American diet, F&V = fruits and vegetables diet, D-F&V = dairy-rich fruits and vegetables. * Different from screening, $p < 0.0004$ † Different from AAD, Tukey $p < 0.05$. B) Diet-related changes in mean arterial pressure (MAP) from study entry, n=23. * Different from screening, $p < 0.0001$ † Different from AAD, Tukey $p < 0.009$. 
3.5.4 Treatment effects on lipids and lipoproteins

Table 3-5 presents the lipid and lipoprotein results. There were main effects of diet for LDL and HDL cholesterol (p < 0.0001 and p = 0.0385, respectively). Multiple comparison analyses revealed that the lipid levels did not differ statistically between the D-F&V and the F&V diets. Compared with the AAD, LDL cholesterol was significantly lower following the F&V diet (Tukey p < 0.0001) and the D-F&V diet (Tukey p = 0.0002). In contrast, the AAD had the most favorable effects on HDL cholesterol. HDL cholesterol was significantly higher on the AAD compared to both the F&V and D-F&V diets (all Tukey p’s < 0.001). We also observed an effect of time for LDL and HDL cholesterol (both p’s < 0.04). LDL and HDL cholesterol were incrementally increased throughout the study, such that levels at period three were significantly higher than levels at period one (Tukey p’s < 0.05). Furthermore, there was a diet by period interaction for total cholesterol (p = 0.0305). Total cholesterol was significantly higher on the AAD during period 3, when compared to all periods of D-F&V and F&V diets. Triglyceride levels were not significantly different among the three diets.

Change score analyses confirmed the levels analyses and revealed that both the F&V and D-F&V diets significantly reduced total and LDL cholesterol from screening (p’s ≤ 0.05), while the AAD did not (Fig. 3-4). Furthermore, the D-F&V and F&V diets elicited significantly greater reductions in total and LDL cholesterol than AAD (Tukey p’s < 0.05). There was a main effect of diet for the change in HDL cholesterol (p = 0.0049); both the D-F&V and F&V diets lowered HDL cholesterol to a greater extent
The ratios of LDL:HDL cholesterol and Total:HDL cholesterol remained unchanged. Triglycerides were reduced by each diet, however; only the changes following the AAD were significant ($p = 0.0432$), probably due to the higher fat content of the AAD diet. Due to this decrease in triglycerides in conjunction with an increase in HDL cholesterol, the AAD diet elicited the lowest ratio of triglycerides to HDL cholesterol (AAD vs. D-F&V $p = 0.0300$; AAD vs. F&V $p = 0.0986$).
Table 3-5. Effects of diet on lipids and lipoproteins

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<tbody>
<tr>
<td>Total-C ²</td>
<td>194.4 ± 6.4</td>
<td>189.4 ± 3.7</td>
<td>177.8 ± 3.6 *</td>
<td>182.1 ± 3.7 *</td>
<td>0.0305 ²</td>
<td>-11.7 ± 2.6</td>
<td>-7.3 ± 2.6</td>
<td>4.3 ± 2.5</td>
</tr>
<tr>
<td>LDL-C ³</td>
<td>122.9 ± 6.1</td>
<td>123.3 ± 3.9 a</td>
<td>112.2 ± 3.9 b</td>
<td>116.1 ± 3.9 b</td>
<td>0.0001</td>
<td>-11.0 ± 2.3 (0.0001)</td>
<td>-7.2 ± 2.3 (0.0066)</td>
<td>3.9 ± 2.2 (0.2032)</td>
</tr>
<tr>
<td>HDL-C ⁴</td>
<td>49.1 ± 2.4</td>
<td>48.6 ± 1.3 a</td>
<td>45.8 ± 1.3 b</td>
<td>45.9 ± 1.3 b</td>
<td>0.0385</td>
<td>-2.8 ± 0.7 (0.0001)</td>
<td>-2.7 ± 0.7 (0.0002)</td>
<td>0.1 ± 0.6 (0.9948)</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>112.3 ± 12.4</td>
<td>93.5 ± 6.9 *</td>
<td>97.8 ± 6.9</td>
<td>102.1 ± 6.9</td>
<td>NS</td>
<td>4.3 ± 4.4</td>
<td>8.7 ± 4.4</td>
<td>4.3 ± 4.3</td>
</tr>
<tr>
<td>Total:HDL-C</td>
<td>4.1 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>NS</td>
<td>0.0 ± 0.6</td>
<td>0.1 ± 0.6</td>
<td>0.0 ± 0.6</td>
</tr>
<tr>
<td>LDL:HDL-C</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>NS</td>
<td>0.0 ± 0.6</td>
<td>0.0 ± 0.6</td>
<td>0.0 ± 0.6</td>
</tr>
<tr>
<td>TG:HDL-C</td>
<td>2.5 ± 0.4</td>
<td>2.1 ± 0.2 a</td>
<td>2.4 ± 0.2 a,c</td>
<td>2.5 ± 0.2 b,c</td>
<td>0.0283</td>
<td>0.3 ± 0.1 (0.0986)</td>
<td>0.3 ± 0.1 (0.0300)</td>
<td>0.1 ± 0.1 (0.8710)</td>
</tr>
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</table>

¹ Data are least squares means ± SE, n=23. Within rows, values with different letter subscripts are different, Tukey P ≤ 0.05. The exact Tukey adjusted P-values are listed also. Different from screening: * P < 0.05.

² Diet by period interaction, P < 0.0305.

³ Period effect, P < 0.0061 (Period 1 is lower than Period 3, Tukey P’s < 0.0201; Period 2 is lower than Period 3, Tukey P < 0.0096).

⁴ Period effect, P < 0.0385 (Period 1 is lower than Period 3, Tukey P’s < 0.0403).

C = cholesterol, TG = triglycerides, NS = nonsignificant
Figure 3-4. Diet-related changes in total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and triglycerides (TG) from study entry, n=23. AAD = average American diet, F&V = fruits and vegetables diet, D-F&V = dairy-rich fruits and vegetables.

* Different from screening, p ≤ 0.05
† Different from AAD, Tukey p ≤ 0.05
3.5.5 Treatment effects on basal arterial diameter, peak deflation diameter, and FMD

There was a main effect of diet for basal arterial diameter and peak diameter (p = 0.0300 and p = 0.0113, respectively) (Table 3-6). The D-F&V diet produced a significantly larger basal arterial diameter than the F&V (Tukey p = 0.05) and control (Tukey p = 0.0042) diets. Similarly, the largest peak diameter followed the D-F&V diet, which was significantly greater than the control diet (Tukey p = 0.0232). The peak diameter following the F&V diet did not significantly differ from the D-F&V or the control diets. However, there was no effect of diet for percent change in FMD, such that the percent change in the diameter of the brachial artery after cuff deflation was similar in magnitude across the three diets (Fig. 3-5).
Table 3-6. Effects of diet on endothelial function

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<tr>
<td>Basal arterial diameter (mm)</td>
<td>4.3 ± 0.2  a</td>
<td>4.4 ± 0.2  a</td>
<td>4.6 ± 0.2  b</td>
<td>0.0300</td>
<td>0.1 ± 0.1 (0.5028)</td>
<td>0.4 ± 0.1 (0.0042)</td>
<td>0.3 ± 0.1 (0.0523)</td>
</tr>
<tr>
<td>Peak deflation diameter (mm)</td>
<td>4.5 ± 0.2  a</td>
<td>4.6 ± 0.2  a,b</td>
<td>4.8 ± 0.2  b</td>
<td>0.0113</td>
<td>0.1 ± 0.1 (0.4584)</td>
<td>0.3 ± 0.1 (0.0232)</td>
<td>0.2 ± 0.1 (0.2158)</td>
</tr>
<tr>
<td>FMD (%Δ)</td>
<td>4.9 ± 0.6  a</td>
<td>5.1 ± 0.6  a,b</td>
<td>4.7 ± 0.6  b</td>
<td>NS</td>
<td>0.2 ± 0.7 -0.3 ± 0.7</td>
<td>-0.4 ± 0.7</td>
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AFTER NTG

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<tr>
<td>Basal arterial diameter (mm)</td>
<td>4.1 ± 0.2  a</td>
<td>4.3 ± 0.2  a,b</td>
<td>4.4 ± 0.2  b</td>
<td>0.0146</td>
<td>0.2 ± 0.1 (0.1422)</td>
<td>0.3 ± 0.1 (0.0346)</td>
<td>0.1 ± 0.1 (0.6816)</td>
</tr>
<tr>
<td>Peak diameter (mm) 3</td>
<td>4.81 ± 0.23 a</td>
<td>4.98 ± 0.23 a,b</td>
<td>5.01 ± 0.23 b</td>
<td>0.0018</td>
<td>0.17 ± 0.09 (0.1477)</td>
<td>0.21 ± 0.08 (0.0503)</td>
<td>0.03 ± 0.09 (0.9196)</td>
</tr>
<tr>
<td>NTG dilation (%Δ) 3</td>
<td>17.3 ± 1.4 a</td>
<td>16.3 ± 1.5 a</td>
<td>18.1 ± 1.4 b</td>
<td>NS</td>
<td>-1.0 ± 1.5</td>
<td>0.7 ± 1.5</td>
<td>1.7 ± 1.5</td>
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<tr>
<td>VCAM-1 (ng/ml)</td>
<td>650.1 ± 33.9 a</td>
<td>648.6 ± 32.9 a</td>
<td>547.6 ± 33.1 b</td>
<td>0.0290</td>
<td>-1.5 ± 43.0 (0.9993)</td>
<td>-102.5 ± 42.6 (0.0543)</td>
<td>-101.0 ± 41.9 (0.0541)</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>241.6 ± 14.0 a</td>
<td>225.3 ± 14.0 b</td>
<td>225.3 ± 14.0 b</td>
<td>0.0329</td>
<td>-16.3 ± 6.8 (0.0571)</td>
<td>-16.3 ± 6.8 (0.0550)</td>
<td>0.0 ± 6.7 (1.0000)</td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>141.8 ± 9.4 a</td>
<td>132.3 ± 9.3 a,b</td>
<td>112.9 ± 9.2  b</td>
<td>0.0198</td>
<td>-9.5 ± 10.3 (0.6281)</td>
<td>-28.8 ± 10.0 (0.0179)</td>
<td>-19.3 ± 9.9 (0.1393)</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>31.1 ± 2.4 a</td>
<td>29.8 ± 2.4 a,b</td>
<td>27.6 ± 2.4  b</td>
<td>0.0043</td>
<td>-1.3 ± 1.0 (0.4307)</td>
<td>-3.5 ± 1.0 (0.0036)</td>
<td>-2.2 ± 1.0 (0.0677)</td>
</tr>
</tbody>
</table>

1 Data are least squares means ± SE, n=23. Within rows, values with different subscripts are different, Tukey P ≤ 0.05.

The exact Tukey adjusted P-values are listed also.

2 n=20

3 n=21

NTG=nitroglycerin administration, NS = nonsignificant.
Figure 3-5. Effects of diet on basal arterial diameter, peak deflation diameter, and FMD, n=23.
3.5.6 Treatment effects on basal arterial diameter, peak deflation diameter, and FMD after nitroglycerin administration

As expected, nitroglycerin administration produced greater dilation of the brachial artery than cuff occlusion (Table 3-6). As observed during the FMD test, the D-F&V diet was associated with a significantly larger basal arterial diameter and peak diameter after nitroglycerin administration compared with the control diet (Tukey p = 0.0346 and Tukey p = 0.05, respectively). However, the basal and peak diameters did not differ on the D-F&V diet vs. the F&V diet (Tukey p = 0.6816 and Tukey p 0.9196, respectively). Likewise, the percent change in artery diameter was not affected by diet (Fig. 3-6).

Figure 3-6. Effects of diet on basal and arterial diameter and percent change after nitroglycerin administration, n = 21.
3.5.7 Treatment effects on cell adhesion molecules

There was a main effect of diet for VCAM-1 ($p = 0.0290$), ICAM-1 ($p = 0.039$), P-selectin ($p = 0.0198$), and E-selectin ($p = 0.0043$) (Table 3-6). The D-F&V diet reduced VCAM-1 by about 18-19% compared with the F&V diet (Tukey $p < 0.0541$) and the AAD diet (Tukey $p < 0.0543$). Compared with the AAD diet, ICAM-1 was significantly reduced by both the D-F&V (Tukey $p < 0.0550$) and F&V diets (Tukey $p < 0.0571$). E-selectin was significantly reduced following the D-F&V diet vs. the control diet (Tukey $p = 0.0036$) and marginally reduced vs. the F&V diet (Tukey $p = 0.0677$). P-selectin followed a similar pattern, with the D-F&V diet eliciting the lowest levels (D-F&V vs. AAD, Tukey $p = 0.017$; D-F&V vs. F&V, Tukey $p = 0.1393$).

3.5.8 Treatment effects on serum calcium and calcium-regulating hormones

The results for serum calcium and calcium regulating hormones are presented in Table 3-7. Serum calcium remained constant throughout the three diet periods. However, there was a significant effect of diet on the active metabolite of vitamin D, 1,25-dihydroxyvitamin D$_3$ ($p = 0.0004$). 1,25-dihydroxyvitamin D$_3$ was 19.0% lower following the D-F&V diet compared with the F&V diet (Tukey $p = 0.0162$) and 28.4% lower than the AAD diet (Tukey $p = 0.0004$). The levels of the other hormones, PTH and calcitonin, did not change across diets.
3.5.9 Treatment effects on components of the renin-angiotensin system

Angiotensin II levels were significantly lower on the D-F&V diet compared with the AAD (p < 0.0044) (Table 3-7). Angiotensin II levels on the F&V diet were intermediary and not significantly different from either the D-F&V diet (Tukey p = 0.1855) or the AAD diet (Tukey p = 0.2422). However, measures of renin activity and ACE activity did not significantly differ between diets.
Table 3-7. Effects of diet on calcium regulating and renin-angiotensin systems

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<tbody>
<tr>
<td>Serum Ca (mg/dl)</td>
<td>9.3 ±0.1</td>
<td>9.4 ±0.1</td>
<td>9.4 ±0.1</td>
<td>NS</td>
<td>0.1 ±0.1</td>
<td>0.0 ±0.1</td>
<td>-0.1 ±0.1</td>
</tr>
<tr>
<td>1,25-dihydroxyvitaminD₃(pmol/l)</td>
<td>112.2 ±7.0 a</td>
<td>104.0 ±7.0 a</td>
<td>87.4 ±7.0 b</td>
<td>0.0004</td>
<td>-8.2 ±5.8 (0.3476)</td>
<td>-24.8 ±5.8 (0.0004)</td>
<td>-16.6 ±5.7 (0.0162)</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>44.7 ±4.5</td>
<td>44.8 ±4.4</td>
<td>42.8 ±4.5</td>
<td>NS</td>
<td>0.1 ±3.1</td>
<td>-1.9 ±3.1</td>
<td>-2.0 ±3.0</td>
</tr>
<tr>
<td>Calcitonin (pg/ml)</td>
<td>9.6 ±0.7</td>
<td>9.6 ±0.7</td>
<td>9.4 ±0.7</td>
<td>NS</td>
<td>0.0 ±0.3</td>
<td>-0.2 ±0.3</td>
<td>-0.2 ±0.3</td>
</tr>
<tr>
<td>Renin activity (ng/ml/hr)</td>
<td>0.6 ±0.2</td>
<td>0.8 ±0.2</td>
<td>0.9 ±0.2</td>
<td>NS</td>
<td>0.2 ±0.1</td>
<td>0.3 ±0.1</td>
<td>0.1 ±0.1</td>
</tr>
<tr>
<td>ACE activity (U/l)</td>
<td>36.0 ±1.4</td>
<td>37.7 ±1.5</td>
<td>36.1 ±1.4</td>
<td>NS</td>
<td>1.7 ±2.0</td>
<td>0.1 ±2.0</td>
<td>-1.6 ±2.0</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>9.4 ±1.2 a</td>
<td>7.5 ±1.2 a,c</td>
<td>5.4 ±1.3 b,c</td>
<td>0.0063</td>
<td>-1.9 ±1.2 (0.2422)</td>
<td>-4.0 ±1.2 (0.0044)</td>
<td>-2.1 ±1.1 (0.1855)</td>
</tr>
</tbody>
</table>

1 Data are least squares means ± SE, n=23. Within rows, values with different subscripts are different, Tukey P ≤ 0.05.

The exact Tukey adjusted P-values are listed also.

NS = nonsignificant
3.5.10 Treatment effects on RBC cations

There was a significant effect of diet for RBC Ca (p = 0.0001), RBC Mg (p = 0.0010), and RBC Na (p = 0.0518) (Table 3-8). RBC Ca was significantly lower following the D-F&V diet compared with both the F&V (Tukey p = 0.0006) and AAD (Tukey p = 0.0002) diets. Conversely, RBC Mg was significantly increased following the D-F&V relative to the other two diets (Tukey both p’s < 0.004). Compared with the F&V diet, the D-F&V diet tended to lower RBC Na (Tukey p = 0.0567), but was not different from the control diet (Tukey p = 0.8229). RBC K was not significantly altered by any of the treatments.

In addition, I calculated the ratio of RBC Ca to other RBC electrolytes. As expected, RBC Ca:Mg followed the same pattern as RBC Ca such that the ratio was significantly lower on the D-F&V vs. the other two (Tukey both p’s = 0.0001). The RBC Ca:K ratio also was significantly lower following the D-F&V diet (Tukey both p’s = 0.006). The Ca:Na ratio was lowest following the D-F&V diet, and significantly different from the control diet (Tukey p = 0.0033) and marginally different from the F&V diet (Tukey p = 0.0591). The RBC Na:K ratio was not altered by the diets.
Table 3-8. Effects of diet on red blood cell (RBC) cations

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<tbody>
<tr>
<td>RBC Ca (μM)</td>
<td>12.6 ± 1.0 a</td>
<td>12.1 ± 1.1 a</td>
<td>6.9 ± 1.1 b</td>
<td>0.0001</td>
<td>-0.5 ± 1.15 (0.9077)</td>
<td>-5.7 ± 1.2 (0.0002)</td>
<td>-5.2 ± 1.2 (0.0006)</td>
</tr>
<tr>
<td>RBC Mg (mM)</td>
<td>2.3 ± 0.1 a</td>
<td>2.3 ± 0.1 a</td>
<td>2.7 ± 0.1 b</td>
<td>0.0001</td>
<td>-0.0 ± 0.1 (0.9654)</td>
<td>0.4 ± 0.1 (0.0034)</td>
<td>0.4 ± 0.1 (0.0021)</td>
</tr>
<tr>
<td>RBC Na (mM)</td>
<td>6.6 ± 0.6</td>
<td>8.0 ± 0.6</td>
<td>6.2 ± 0.6</td>
<td>0.0518</td>
<td>1.4 ± 0.7 (0.1481)</td>
<td>-0.4 ± 0.7 (0.8229)</td>
<td>-1.8 ± 0.7 (0.0567)</td>
</tr>
<tr>
<td>RBC K (mM)</td>
<td>86.1 ± 2.6</td>
<td>89.0 ± 2.6</td>
<td>88.0 ± 2.8</td>
<td>NS</td>
<td>2.9 ± 3.3</td>
<td>1.9 ± 3.5</td>
<td>-1.1 ± 3.5</td>
</tr>
<tr>
<td>RBC Ca:Mg</td>
<td>5.6 ± 0.5 a</td>
<td>5.5 ± 0.5 a</td>
<td>2.9 ± 0.5 b</td>
<td>0.0001</td>
<td>-0.1 ± 0.5 (0.9833)</td>
<td>-2.7 ± 0.5 (0.0001)</td>
<td>-2.7 ± 0.5 (0.0001)</td>
</tr>
<tr>
<td>RBC Ca:K</td>
<td>0.15 ± 0.01 a</td>
<td>0.14 ± 0.01 a</td>
<td>0.09 ± 0.02 b</td>
<td>0.0004</td>
<td>-0.01 ± 0.01 (0.5967)</td>
<td>-0.07 ± 0.02 (0.0004)</td>
<td>-0.05 ± 0.02 (0.0051)</td>
</tr>
<tr>
<td>RBC Ca:Na</td>
<td>2.0 ± 0.2 a</td>
<td>1.8 ± 0.2 a,c</td>
<td>1.2 ± 0.2 b,c</td>
<td>0.0044</td>
<td>-0.3 ± 0.2 (0.4496)</td>
<td>-0.8 ± 0.2 (0.0033)</td>
<td>-0.5 ± 0.2 (0.0591)</td>
</tr>
<tr>
<td>RBC Na:K</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>NS</td>
<td>0.01 ± 0.01 (0.4027)</td>
<td>-0.01 ± 0.01 (0.7946)</td>
<td>-0.02 ± 0.01 (0.1641)</td>
</tr>
</tbody>
</table>

Data are least squares means ± SE. AAD n=20, F&V n=21, D-F&V n=18.

NS = nonsignificant
3.5.11 Treatment effects on urinary electrolytes

As expected, there were significant main effects of diet for urinary Ca (p = 0.0214) and urinary K (p = 0.0001) (Table 3-9). Urinary calcium excretion was greatest on the D-F&V diet, and was significantly different from the F&V diet (Tukey p = 0.0158). However, urinary Ca levels following the D-F&V diet and control diet did not differ (Tukey p = 0.4078). Urinary potassium was significantly elevated on the D-F&V diet compared with the F&V diet (Tukey p = 0.0051) and the AAD diet (Tukey p = 0.0001). Although the level of urinary Na did not differ across the diets, the ratio of urinary Na to urinary K was significantly increased during the AAD diet vs. the other two diets (both Tukey p’s < 0.0004). The same was true for the ratio of urinary Ca to urinary K; the AAD was significantly higher vs. the other two diets (both Tukey p’s < 0.04). Interestingly, the ratio of urinary Na:Ca was highest following the F&V diet and significantly different from AAD and D-F&V diets (both Tukey p’s < 0.004).
Table 3-9. Effects of diet on urinary electrolytes

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<tbody>
<tr>
<td>Urinary Ca (mg/24h)</td>
<td>107.2 ± 17.3 a,c</td>
<td>87.8 ± 16.0 a</td>
<td>126.5 ± 16.1 b,c</td>
<td>0.0214</td>
<td>-19.4 ± 15.0 (0.4078)</td>
<td>19.3 ± 15.1 (0.4139)</td>
<td>38.7 ± 13.2 (0.0158)</td>
</tr>
<tr>
<td>Urinary Na (mg/24h)</td>
<td>3605 ± 322</td>
<td>3570 ± 280</td>
<td>3871 ± 281</td>
<td>NS</td>
<td>-35 ± 391</td>
<td>266 ± 392</td>
<td>301 ± 351</td>
</tr>
<tr>
<td>Urinary K (mg/24h)</td>
<td>1983 ± 265 a</td>
<td>2510 ± 237 a</td>
<td>3334 ± 238 b</td>
<td>0.0001</td>
<td>526 ± 277 (0.1522)</td>
<td>1351 ± 278 (0.0001)</td>
<td>824 ± 246 (0.0051)</td>
</tr>
<tr>
<td>Urinary Na:K</td>
<td>2.3 ± 0.2 a</td>
<td>1.4 ± 0.2 b</td>
<td>1.2 ± 0.2 b</td>
<td>0.0001</td>
<td>-1.0 ± 0.2 (0.0003)</td>
<td>-1.1 ± 0.2 (0.0001)</td>
<td>-0.2 ± 0.2 (0.3595)</td>
</tr>
<tr>
<td>Urinary Ca:K</td>
<td>0.07 ± 0.01 a</td>
<td>0.03 ± 0.01 b</td>
<td>0.04 ± 0.01 b</td>
<td>0.0040</td>
<td>-0.03 ± 0.01 (0.0031)</td>
<td>-0.03 ± 0.01 (0.0332)</td>
<td>0.01 ± 0.01 (0.5599)</td>
</tr>
<tr>
<td>Urinary Na:Ca</td>
<td>31.9 ± 8.4 a</td>
<td>57.5 ± 8.0 b</td>
<td>43.7 ± 8.0 a</td>
<td>0.0005</td>
<td>25.6 ± 6.0 (0.0004)</td>
<td>11.8 ± 6.0 (0.1389)</td>
<td>-13.8 ± 5.3 (0.0337)</td>
</tr>
</tbody>
</table>

1 Data are least squares means ± SE, n=23. Within rows, values with different subscripts are different, Tukey P ≤ 0.05. The exact Tukey adjusted P-values are listed also.
3.5.12 Treatment effects on intraplatelet ionic calcium mobilization

Testing of intracellular free calcium mobilization was performed in 16 subjects at the PSU site by adding a platelet agonist (arginine vasopressin) to whole blood stained with Fluo-4 AM and CD41-PE monoclonal antibody. Variations in fluorescence of the CD41+ platelet population were recorded over time. Fig. 3-7 displays the time course and the defined time regions of a typical experiment. Percent change in fluorescence from baseline of unstimulated platelets was used to investigate diet-related and time-related changes in intracellular free calcium. There was a main effect of time for percent change in intracellular free Ca$^{2+}$ from baseline ($p < 0.0001$). Under these experimental conditions, arginine vasopressin induced a rapid elevation in intracellular free Ca$^{2+}$ that was significantly different from the baseline level ($p < 0.0001$) (Fig. 3-8). After this initial rise, Ca$^{2+}$ incrementally declined until returning to baseline levels after about 120 seconds post-stimulation. In addition, there was a main effect of diet ($p < 0.0001$) when collapsing across all time points, with the control diet producing significantly higher levels of intracellular free Ca$^{2+}$ than the other two diets (both Tukey p’s $< 0.007$). Further investigation confirmed that the peak change in intracellular free Ca$^{2+}$ was significantly higher following the control diet vs. the F&V diet (Tukey $p = 0.0159$) and the D-F&V diet (Tukey $p = 0.0469$) (Table 3-10).
Figure 3-7. Procedure and time course of a typical kinetic assay of intraplatelet free ionic calcium mobilization. The data correspond to the platelet population tagged with CD-41 in whole blood. This figure illustrates the collection of baseline fluorescence for 30 seconds, with a pause for the addition of arginine vasopressin, after which acquisition of data was resumed. The vertical lines are analysis regions on the time axis (sec) and cover the entire length of fluorescences.
Figure 3-8. Percent change in cytosolic free Ca\textsuperscript{2+} in Fluo-4-labeled platelets activated with arginine vasopressin, n = 16. Different letters represent significant differences in fluorescence after stimulation and across time, collapsing across diets (time effect, p < 0.0001). Cytosolic free Ca\textsuperscript{2+} returned to resting levels around 120 seconds post-stimulation. The AAD control diet elicited significantly greater levels of cytosolic free Ca\textsuperscript{2+} vs. the intervention diets, collapsing across time (diet effect, p < 0.0001).

Table 3-10. Peak change in intracellular free Ca\textsuperscript{2+} from resting levels according to diet, n = 16.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Peak change in intracellular free Ca\textsuperscript{2+} (% Δ from baseline ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAD</td>
<td>492 ± 45%</td>
</tr>
<tr>
<td>F&amp;V</td>
<td>352 ± 44%*</td>
</tr>
<tr>
<td>D-F&amp;V</td>
<td>372 ± 47%*</td>
</tr>
</tbody>
</table>

* Different from AAD, Tukey p < 0.04
3.6 Results of correlation analyses

Because of proposed relationships between intracellular ions, blood pressure, hormonal systems, and endothelial function, Pearson correlation analyses were conducted to test possible associations between study variables.

3.6.1 Correlation between variables following the control (AAD) diet

Table 3-11 presents correlations between study variables following the control (AAD) diet. Age was positively correlated with SBP ($r = 0.62, p = 0.0028$) and negatively correlated with FMD ($r = -0.47, p = 0.0309$), both of which are consistent with our previous work (Fig. 3-9 A, B). Furthermore, a significant positive correlation was observed between age and RBC Ca ($r = 0.49, p = 0.0274$; Fig. 3-9 C) and age and 1,25-dihydroxyvitaminD$_3$ ($r = 0.44, p = 0.0452$).

RBC Ca also was positively associated with 1,25-dihydroxyvitaminD$_3$ ($r = 0.51, p = 0.0229$; Fig. 3-9 D), total peripheral resistance ($r = 0.58, p = 0.0077$; Fig. 3-9 E), and SBP ($r = 0.58, p = 0.0075$; Fig. 3-9 F) and negatively associated with cardiac output ($r = -0.49, p = 0.0267$). RBC Na was positively associated DBP ($r = 0.70, p = 0.0006$), while RBC K was negatively associated with DBP ($r = -0.46, p = 0.0407$). 1,25-dihydroxyvitaminD$_3$ was also negatively correlated with BMI ($r = -0.46, p = 0.0380$) and cardiac output ($r = -0.45, p = 0.0399$) and tended to be positively correlated with total peripheral resistance ($r = 0.36, p = 0.1050$).

The ratio of 24-h Na urinary to urinary Ca was marginally associated with SBP ($r = -0.24, p = 0.0610$) and MAP ($r = -0.38, p = 0.0930$), such that as the ratio increased the
levels of SBP and MAP decreased. The relationship between urinary Na:Ca and SBP became significant when the correlation was adjusted for age (r = -0.45, p = 0.0471; Fig. 3-9 G).

As expected, there was a strong inverse association between total peripheral resistance and cardiac output (r = -0.89, p < 0.0001), and SBP was positively correlated with total peripheral resistance (r = 0.51, p = 0.0170) and negatively correlated with cardiac output (r = -0.48, p = 0.0274). In addition, renin activity and angiotensin II were directly related (r = 0.43, p = 0.0506).

Table 3-11. Correlations between study variables during the control diet.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>BMI</th>
<th>1,25(OH)2D</th>
<th>TPR</th>
<th>CO</th>
<th>SBP</th>
<th>DBP</th>
<th>FMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Ca</td>
<td>0.49 (0.03)</td>
<td>-0.26 (0.26)</td>
<td><strong>0.51 (0.02)</strong></td>
<td><strong>0.58 (0.01)</strong></td>
<td><strong>-0.49 (0.03)</strong></td>
<td><strong>0.58 (0.01)</strong></td>
<td>0.06 (0.80)</td>
<td>-0.22 (0.36)</td>
</tr>
<tr>
<td>RBC Mg</td>
<td>0.05 (0.85)</td>
<td>0.02 (0.92)</td>
<td>0.19 (0.43)</td>
<td>-0.21 (0.37)</td>
<td>0.19 (0.42)</td>
<td>0.09 (0.70)</td>
<td>0.29 (0.21)</td>
<td>0.25 (0.29)</td>
</tr>
<tr>
<td>RBC Na</td>
<td>0.42 (0.07)</td>
<td>-0.04 (0.86)</td>
<td>-0.04 (0.86)</td>
<td>0.21 (0.37)</td>
<td>-0.13 (0.60)</td>
<td>0.39 (0.09)</td>
<td><strong>0.70 (0.01)</strong></td>
<td>-0.27 (0.24)</td>
</tr>
<tr>
<td>RBC K</td>
<td>0.13 (0.58)</td>
<td>-0.10 (0.67)</td>
<td>0.12 (0.61)</td>
<td>-0.32 (0.17)</td>
<td>0.29 (0.22)</td>
<td>-0.18 (0.45)</td>
<td><strong>-0.46 (0.04)</strong></td>
<td>-0.07 (0.75)</td>
</tr>
<tr>
<td>1,25(OH)2D</td>
<td>0.44 (0.05)</td>
<td><strong>-0.46 (0.04)</strong></td>
<td>--</td>
<td>0.36 (0.11)</td>
<td><strong>-0.45 (0.04)</strong></td>
<td>0.35 (0.12)</td>
<td>-0.11 (0.64)</td>
<td>-0.02 (0.94)</td>
</tr>
<tr>
<td>TPR</td>
<td>0.23 (0.31)</td>
<td><strong>-0.58 (0.01)</strong></td>
<td>0.36 (0.11)</td>
<td>--</td>
<td><strong>-0.89 (0.00)</strong></td>
<td><strong>0.51 (0.02)</strong></td>
<td>0.28 (0.21)</td>
<td>-0.26 (0.25)</td>
</tr>
<tr>
<td>CO</td>
<td>-0.10 (0.68)</td>
<td><strong>0.43 (0.05)</strong></td>
<td><strong>-0.45 (0.04)</strong></td>
<td><strong>-0.89 (0.00)</strong></td>
<td>--</td>
<td><strong>-0.48 (0.03)</strong></td>
<td>--</td>
<td>0.38 (0.09)</td>
</tr>
<tr>
<td>SBP</td>
<td><strong>0.62 (0.01)</strong></td>
<td>-0.31 (0.18)</td>
<td>0.35 (0.12)</td>
<td><strong>0.51 (0.02)</strong></td>
<td><strong>-0.48 (0.03)</strong></td>
<td>--</td>
<td>0.38 (0.09)</td>
<td>-0.21 (0.36)</td>
</tr>
<tr>
<td>DBP</td>
<td>0.25 (0.27)</td>
<td>-0.12 (0.62)</td>
<td>-0.11 (0.64)</td>
<td>0.28 (0.21)</td>
<td>-0.15 (0.51)</td>
<td>0.38 (0.09)</td>
<td>--</td>
<td><strong>-0.03 (0.91)</strong></td>
</tr>
<tr>
<td>FMD</td>
<td><strong>-0.47 (0.03)</strong></td>
<td>0.37 (0.10)</td>
<td>-0.02 (0.94)</td>
<td>-0.26 (0.25)</td>
<td>0.09 (0.71)</td>
<td>-0.21 (0.36)</td>
<td><strong>-0.03 (0.91)</strong></td>
<td>--</td>
</tr>
</tbody>
</table>

1 correlations (p-value), bold indicates significance, p < 0.05. TPR = total peripheral resistance, CO = cardiac output, FMD = flow mediated dilation, 1,25(OH)2VD = 1,25-dihydroxyvitamin D3, SBP = systolic blood pressure, DBP = diastolic blood pressure.
A

Age (years)

SBP on AAD
(mm Hg)

\[ r = 0.62 \]

\[ p = 0.0028 \]

B

Age (years)

FMD on AAD
(% Δ)

\[ r = -0.47, \ p = 0.0309 \]

C

Age (years)

RBC Calcium on AAD
(μM)

\[ r = 0.49, \ p = 0.0274 \]
**D**

1,25(OH)₂VitaminD₃ on AAD (pmol/l)

\[ r = 0.51, \ p = 0.0229 \]

**E**

TPR on AAD (dyne-sec*cm⁻⁵)

\[ r = 0.58, \ p = 0.0077 \]

**F**

SBP on AAD (mm Hg)

\[ r = 0.58, \ p = 0.0075 \]
Figure 3-9. Correlations between study variables during the control AAD diet. A) age and systolic blood pressure (SBP) n=21, B) age and flow mediated dilation (FMD) n=21, C) age and red blood cell (RBC) calcium n=20, D) 1,25-dihydroxvitamin D₃ and RBC calcium n=20, E) total peripheral resistance (TPR) and RBC calcium n=20, F) SBP and RBC calcium n=20, G) SBP and 24-hr urinary sodium to calcium ratio n=21.
3.6.2 Predictors of diet-related changes in RBC cation levels (n=16)

The diet-related changes (i.e. experimental diet – AAD control diet) in RBC Ca were predicted by age and BMI (Fig. 3-10 A, B). The change in RBC Ca following the D-F&V diet was negatively correlated with age (r = -0.52, p = 0.0406), such that the largest reductions in RBC Ca were experienced in the oldest participants. Furthermore, BMI was positively correlated with the diet-related change in RBC Ca (r = 0.50, p = 0.0508). A higher BMI was associated with a smaller change in RBC Ca following the D-F&V diet.

In addition, the absolute levels of RBC cations during the control diet predicted the diet-related changes in these cations. Individuals with the highest levels of RBC Ca following the control diet had the largest decreases in RBC Ca following the D-F&V diet (r = -0.78, p = 0.0004) as well as the F&V diet (r = -0.60, p = 0.0089) (Fig 3-10 C). Furthermore, the diet-related change in RBC Mg was negatively correlated with level of RBC Mg at the control diet, such that those with the lowest levels of RBC Mg experienced the greatest diet-related increases in RBC Mg (D-F&V r = -0.57, p = 0.0170; and F&V r = -0.42, p = 0.0839). Similar patterns were observed for RBC Na and RBC K (D-F&V r = -0.51, p = 0.0413; F&V r = -0.16, p = 0.5295 and D-F&V r = -0.82, p < 0.0001; F&V r = -0.60, p = 0.0087, respectively)
Δ in RBC Calcium from AAD (μM)

A

Age (years)

Δ in RBC Calcium from AAD (μM)

B

BMI (kg/m²)

Δ in RBC Calcium from AAD (μM)

C

RBC Calcium on AAD (μM)

r = -0.16, p = 0.54

r = -0.52, p = 0.0406

r = -0.16, p = 0.54

r = 0.50, p = 0.0508

r = -0.60, p = 0.0089

r = -0.78, p = 0.0004

r = -0.52, p = 0.0406

r = 0.17, p = 0.50
Figure 3-10. Correlations between changes in RBC calcium and study variables.

A) change in RBC calcium from AAD and age n=18, B) change in RBC calcium from AAD and body mass index (BMI) n=18, C) change in RBC calcium from AAD and levels of RBC calcium on AAD control diet n=18.

Diet type:  

---
3.6.3 Predictors of diet-related changes in blood pressure

Levels of SBP following the control diet were negatively correlated with the diet-related changes in SBP (D-F&V r = -0.52, p = 0.0190; F&V r = -0.57, p = 0.0087). While DBP followed a similar pattern, the correlations did not reach statistical significance (D-F&V r = -0.21, p = 0.3790; F&V r = -0.30, p = 0.1860).

Diet-related changes in RBC Ca predicted changes in DBP. Participants who experienced the greatest drops in RBC Ca also experienced the greatest reductions in DBP (D-F&V r = 0.55, p = 0.0259; F&V r = 0.46, p = 0.0539; Fig. 3-11 A). Changes in SBP were also positively associated with changes in RBC Ca, but the correlations were nonsignificant (D-F&V r = 0.37, p = 0.1593; F&V r = 0.35, p = 0.1558; Fig. 3-11 B), probably due to the small sample size of 18.
Figure 3-11. Correlations between changes in RBC calcium and changes in blood pressure. A) change in RBC calcium and diastolic blood pressure (DBP) from AAD n=18, B) change in RBC calcium and systolic blood pressure (SBP) from AAD n=18.
3.6.4 Predictors of diet-related changes in FMD

FMD following the control diet was a significant predictor of the diet-related changes in FMD. The changes in FMD following the D-F&V and F&V diets were negatively correlated with the percentage FMD at the control diet \((r = -0.71, p = 0.0022\) and \(r = -0.59, p = 0.0094\), respectively). This means that subjects with the highest FMD on the control diet exhibited the smallest improvements on the intervention diets.

Although FMD was not significantly correlated with RBC Mg levels following the control diet, FMD was significantly and positively associated with RBC Mg following the D-F&V diet \((r = 0.77, p = 0.0003)\) and F&V diet \((r = 0.51, p = 0.0175)\) (Fig. 3-12 A). Furthermore, the diet-related changes in RBC Mg were positively correlated with the diet-related changes in FMD \((D\text{-}F\&V r = 0.35, p = 0.1788; F\&V r = 0.68, p = 0.0018)\), such that participants with the greatest increase in RBC Mg exhibited the greatest improvements in FMD (Fig. 3-12 B).
Figure 3-12. Correlations between RBC magnesium and flow mediated dilation (FMD). A) levels of RBC magnesium and FMD on all diets, AAD n=20, D-F&V n=18, F&V n=21, B) change in RBC magnesium and FMD from AAD n=20.

Diet type:  

A

B

Δ in RBC Magnesium from AAD (μM)

Δ in FMD from AAD (%Δ)

Δ in FMD from AAD (%Δ)

Δ in FMD from AAD (%Δ)
3.6.5 Predictors of diet-related changes in VCAM-1

The diet-related changes in VCAM-1 and the diet-related changes in angiotensin II were positively correlated following the D-F&V diet ($r = 0.53$, $p = 0.0173$) and the F&V diet ($r = 0.52$, $p = 0.0153$) (Fig. 3-13). This association remained significant when adjusted for diet-related changes in lipids and lipoproteins as well as blood pressure. This suggests that angiotensin II induced the expression of VCAM-1, which has been demonstrated in other studies.

![Correlation graph showing the relationship between changes in VCAM-1 and angiotensin II](image)

**Figure 3-13. Correlations between the changes in VCAM-1 and angiotensin II from AAD, $n=21$.** Diet type: □ D-F&V  ▮ F&V
3.6.6 Predictors of diet-related changes in peak intraplatelet free Ca$^{2+}$ (n=13)

The peak intraplatelet free Ca$^{2+}$ following the control diet was significantly correlated to the diet-related changes in peak intraplatelet free Ca$^{2+}$ (D-F&V $r = -0.71$, $p = 0.0063$; F&V $r = 0.72$, $p = 0.0056$). Following the D-F&V diet, changes in peak intraplatelet free Ca$^{2+}$ were marginally correlated with changes in RBC Ca ($r = 0.52$, $p = 0.0697$) such that the greatest decreases in the change in peak intraplatelet free Ca$^{2+}$ were associated with the greatest decreases in RBC Ca (Fig. 3-14).

Figure 3-14. Correlations between the changes in peak free ionic intraplatelet calcium and RBC calcium from AAD, n=12. Diet type: D-F&V, F&V.
3.7 Results of secondary analyses (n=23)

Effects of three diets on blood pressure in hypertensive subjects who exhibited large decreases in RBC Ca after consuming a dairy-rich diet vs. those who did not.

Because changes in intracellular calcium are postulated to influence blood pressure regulation, I conducted a post hoc analysis to evaluate possible treatment effects based on each subject’s diet-related change in RBC Ca. To calculate the diet-related change in RBC Ca, I first pooled the RBC Ca data from the control and F&V diet (since they did not statistically differ) and then subtracted the level of RBC Ca following the D-F&V diet. However, since I determined RBC Ca levels on only 18 of the 23 subjects following the D-F&V diet, the total sample size was reduced by 5. While all subjects (n=18) experienced decreases in intracellular calcium [(Ca)\textsubscript{i}] following the D-F&V diet, some had large decreases in RBC Ca, while others only had modest decreases (Fig. 3-15). The mean decrease was -4.9 \mu M. In addition, there appeared to be a natural break between -3.3 \mu M and -6.1 \mu M. Therefore, I grouped subjects as those who responded to the dairy rich diet by decreasing RBC Ca by -3.4 \mu M or more as the (Ca)\textsubscript{i} Change Group (n=8). Those who did not exhibit this decrease were labeled the (Ca)\textsubscript{i} Stable Group (n=10).
Figure 3-15. Change in RBC calcium following the D-F&V diet by subject.

The (Ca)_i Change Group exhibited a decrease in RBC Ca by 3.4 μM or more (n=8), whereas the (Ca)_i Stable Group exhibited a decrease in RBC less than 3.3 μM (n=10).

To verify that the results of the post hoc analysis, which included this new grouping variable, were not an artifact of the change in sample size from 23 to 18, I first applied the model previously described from the primary analysis to the sample of 18. This strategy (i.e., n=18 vs. n=23) did not change the pattern or significance of the diet effects (data not shown). Therefore, the decrease in sample size did not appear to affect the conclusions inferred from the post hoc analysis. The results for RBC cations and SBP and DBP are presented below. Results for other study variables were tangential and presented in Appendix K in table form.
3.7.1 Subject characteristics at study entry according to group (n=18)

Eight subjects were categorized into the (Ca)$_i$ Change group with an average change in RBC Ca following the D-F&V diet of $-7.5 \pm 1.1$. The remaining ten subjects fell into the (Ca)$_i$ Stable group and had an average change in RBC Ca of $-2.6 \pm 0.7$, which was significantly less than the (Ca)$_i$ Change group ($p = 0.0064$). Subject characteristics at study entry stratified by group are presented in Table 3-12. The groups did not significantly differ in age, weight, blood pressure, or lipids at study entry.

Table 3-12. Subject characteristics at study entry, least squares mean $\pm$ SE $^1$

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=23)</th>
<th>(Ca)$_i$Change group (n=8)</th>
<th>(Ca)$_i$Stable group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.3 $\pm$ 2.0</td>
<td>49.7 $\pm$ 3.4</td>
<td>44.2 $\pm$ 3.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.2 $\pm$ 1.9</td>
<td>175.3 $\pm$ 3.6</td>
<td>178.3 $\pm$ 3.2</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>196.3 $\pm$ 7.7</td>
<td>180.6 $\pm$ 13.1</td>
<td>209.0 $\pm$ 11.7</td>
</tr>
<tr>
<td>BMI</td>
<td>28.8 $\pm$ 0.9</td>
<td>26.7 $\pm$ 1.5</td>
<td>30.0 $\pm$ 1.4</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>140.5 $\pm$ 1.9</td>
<td>143.5 $\pm$ 3.0</td>
<td>139.4 $\pm$ 2.7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>91.1 $\pm$ 1.3</td>
<td>92.4 $\pm$ 2.2</td>
<td>89.3 $\pm$ 1.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194.4 $\pm$ 6.4</td>
<td>197.7 $\pm$ 10.0</td>
<td>204.5 $\pm$ 8.9</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>122.9 $\pm$ 6.1</td>
<td>128.0 $\pm$ 8.8</td>
<td>130.2 $\pm$ 7.9</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49.1 $\pm$ 2.4</td>
<td>46.3 $\pm$ 4.2</td>
<td>49.0 $\pm$ 3.8</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>112.3 $\pm$ 12.4</td>
<td>116.9 $\pm$ 24.0</td>
<td>126.5 $\pm$ 21.4</td>
</tr>
</tbody>
</table>

$^1$ Subjects at PSU and PBRC sites did not significantly differ on any variable at screening.

$^2$ RBC calcium levels following the D-F&V diet were measured in 18 subjects only.

3.7.2 Subject characteristics at end of AAD diet according to group (n=18)

Further examination of the groups following the control diet suggests that the (Ca)$_i$ Change group may display characteristics typical of low renin hypertension (Table
The intracellular ionic environment is influenced by extracellular environmental factors that affect ion-active hormone systems. Low renin hypertensive patients exhibit significantly lower serum calcium and calcitonin and reciprocally higher PTH, 1,25-dihydroxyvitaminD, and (Ca). High renin hypertensive patients exhibit oppositely skewed values. When comparing the two groups of subjects based on these factors following the AAD, we found that the (Ca)_i Change group followed the low renin hypertension profile when compared (Ca)_i Stable group. These deviations suggest that subjects in the (Ca)_i Change group will be more responsive to a diet rich in calcium, as has been shown in low renin hypertension.

Table 3-13. Clinical characteristics of low renin and high renin hypertension based on the work of Resnick (81), LS mean ± SE

<table>
<thead>
<tr>
<th>Characteristics of Low Renin HTN</th>
<th>High Renin HTN</th>
<th>(Ca)_i Change group (n=8)</th>
<th>(Ca)_i Stable group (n=10)</th>
<th>P-value Change vs. Stable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>↓</td>
<td>↑ or ↔</td>
<td>0.45 ± 0.22</td>
<td>0.94 ± 0.17</td>
</tr>
<tr>
<td>PTH</td>
<td>↑</td>
<td>↔</td>
<td>60.25 ± 10.83</td>
<td>39.17 ± 8.10</td>
</tr>
<tr>
<td>1,25(OH)<em>{2}VD</em>{3}</td>
<td>↑</td>
<td>↔</td>
<td>115.16 ± 16.63</td>
<td>114.08 ± 12.23</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td>↓ or ↔</td>
<td>↔</td>
<td>8.91 ± 0.17</td>
<td>9.50 ± 0.13</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>↓</td>
<td>↔</td>
<td>8.51 ± 1.86</td>
<td>11.46 ± 1.39</td>
</tr>
<tr>
<td>RBC Calcium</td>
<td>↑</td>
<td>↔</td>
<td>14.13 ± 1.22</td>
<td>11.59 ± 2.36</td>
</tr>
</tbody>
</table>

1 Values for (Ca)_i Change group and (Ca)_i Stable group were determined following the AAD.

HTN hypertension, ↔ normal, ↑ elevated, ↓ depressed
3.7.3 Treatment by group effects on RBC cations (n=18)

A significant diet by group interaction was observed for RBC Ca ($p = 0.0062$) (Table 3-14 and Fig. 3-16). As expected, RBC Ca was significantly lower following the D-F&V diet compared the other two diets in the (Ca)i Change group only (Fig. 3.x). RBC Ca did not significantly change in the (Ca)i Stable group. Furthermore, the two groups significantly differed in levels of RBC Ca following the D-F&V diet ($p = 0.0003$). There was a main effect of diet for RBC Mg ($p = 0.0042$) and no differences between groups ($p = 0.8888$). RBC Mg was significantly elevated following the D-F&V diet compared the other two diets (both Tukey p’s < 0.02) in the sample as a whole. There was a significant diet by group interaction for RBC Na ($p = 0.0473$), with only the (Ca)i Change Group exhibiting decreased RBC Na following the D-F&V diet vs. the F&V (Tukey $p = 0.0263$). There was no significant effect of group on RBC K and no diet-related change in RBC K, as observed in the sample as a whole. The ratios of RBC Ca to RBC Mg and RBC Ca to RBC K follow the same pattern as RBC Ca.
Table 3-14. Effects of diet on RBC cations by group¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>AAD</th>
<th>F&amp;V</th>
<th>D-F&amp;V</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Ca²</td>
<td>Stable Change</td>
<td>11.59 ± 1.22</td>
<td>11.34 ± 1.23</td>
<td>9.13 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>14.13 ± 2.36ᵃ</td>
<td>10.27 ± 2.48ᵃ</td>
<td>4.68 ± 2.38ᵇ†</td>
</tr>
<tr>
<td>RBC Mg³</td>
<td>Stable Change</td>
<td>2.31 ± 0.12ᵃ</td>
<td>2.39 ± 0.12ᵃ</td>
<td>2.69 ± 0.12ᵇ</td>
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<tr>
<td></td>
<td>Change</td>
<td>2.43 ± 0.16ᵃ</td>
<td>2.32 ± 0.17ᵃ</td>
<td>2.71 ± 0.16ᵇ</td>
</tr>
<tr>
<td>RBC Na²</td>
<td>Stable Change</td>
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<td>6.89 ± 0.94</td>
<td>6.68 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>5.94 ± 1.27ᵃ</td>
<td>9.85 ± 1.31ᵃᵇ</td>
<td>5.25 ± 1.26ᵃᶜ</td>
</tr>
<tr>
<td>RBC K</td>
<td>Stable Change</td>
<td>85.8 ± 4.4</td>
<td>89.9 ± 4.5</td>
<td>88.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>91.6 ± 5.9</td>
<td>90.6 ± 6.2</td>
<td>90.0 ± 5.9</td>
</tr>
<tr>
<td>RBC Ca:Mg²</td>
<td>Stable Change</td>
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<td>4.84 ± 0.55</td>
<td>3.44 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>5.06 ± 0.74ᵃ</td>
<td>4.2 ± 0.77ᵃ</td>
<td>1.75 ± 0.92ᵇ†</td>
</tr>
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<td>0.10 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Change</td>
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<td>0.11 ± 0.03ᵃᵇ</td>
<td>0.02 ± 0.02ᵇ†</td>
</tr>
<tr>
<td>RBC Na:K</td>
<td>Stable Change</td>
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<td>Change</td>
<td>0.07 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.06 ± 0.02</td>
</tr>
</tbody>
</table>

¹ Data are least squares means ± SE, n=18. Within rows, values with different superscripts are different, p < 0.05. Within columns, values with † are significantly different from the Stable group, p < 0.004.
² Diet by group interaction, p < 0.05
³ Diet effect, p = 0.0042
Figure 3-16. Treatment effects on red blood cell (RBC) cations according to group, n=18. (Ca)$_i$ Stable Group (n=10) is in red and (Ca)$_i$ Change Group (n=8) is in blue.
3.7.4 Treatment by group effects on blood pressure (n=18)

There was a significant interaction of diet and group (p = 0.0539) for the change in SBP from screening (Fig. 3-17 A). In the (Ca)i Change group, the D-F&V diet elicited a significantly greater reduction in SBP compared with the AAD (Tukey p = 0.0141). The change in SBP on the F&V diet was intermediary and did not differ from the other two diets. In addition, all three experimental diets significantly reduced SBP compared to screening (all p’s < 0.01).

There was a significant interaction of diet and group (p < 0.0001) for DBP (Fig. 3-17 B). In the (Ca)i Change group, DBP was incrementally lowered by the three experimental diets (D-F&V < F&V < AAD). In the (Ca)i Change group, the D-F&V diet elicited significantly greater decreases in DBP (-13.7 ± 3.4) compared with the AAD (-8.1 ± 3.4, p < 0.0001) and the F&V diet (-10.1 ± 3.4, p = 0.0135). The magnitude of the change in DBP on the F&V diet also was significantly greater than the control diet (p = 0.0181). Furthermore, the subjects in the (Ca)i Change group had significantly lower DBP while on the D-F&V diet compared with their counterparts (p = 0.03). The (Ca)i Stable group did not experience this. Compared to screening, DBP was significantly lowered in the by all three experimental diets in the (Ca)i Change group, while only the F&V diet significantly lowered DBP in the (Ca)i Stable group (p < 0.01).
**Figure 3-17. Treatment effects on blood pressure according to group, n=18.**

A) systolic blood pressure, B) diastolic blood pressure. (Ca), Stable Group (n=10) is in red and (Ca), Change Group (n=8) is in blue.
3.8 Discussion

Hypertension is an established risk factor for all clinical manifestations of atherosclerosis. It is a common and powerful independent predisposing factor for development of CHD, stroke, peripheral arterial disease, and heart failure. The high prevalence of hypertension, its significant impact on the incidence of CVD, and the potential impact of control justify high priority efforts to detect and treat elevated blood pressure. Diet and lifestyle modification are recommended as the first lines of defense against hypertension; yet, the optimal diet to achieve maximal blood pressure lowering remains uncertain. The aim of the present study was to clarify the role of dairy products in blood pressure regulation. The innovative diet design provided an opportunity to examine whether a diet rich in dairy products could reduce blood pressure in adults with essential hypertension to a greater extent than a diet high in fruits and vegetables but low in dairy foods and a diet typical of the Western diet. Unlike the DASH trial, this study provided a stage to isolate the contribution of dairy products to the hypotensive effect associated with a DASH-type diet. The results of this study support a variety of previous observations related to diet and blood pressure regulation. In addition to calorie balance and consumption of sodium and alcohol, we confirm that multiple nutrients influence blood pressure. Furthermore, this study provides critical new insights regarding the calcium-blood pressure relationship by identifying a subgroup especially responsive to a dairy-rich diet.
Goal 1: Replication of DASH study

In the sample as a whole, we observed a modest reduction in SBP and DBP of about 2 mm Hg during both the F&V and D-F&V diets compared to the control diet. Our reduction in blood pressure was considerably less than that reported by the original DASH study (59). The DASH diet produced a blood pressure reduction that averaged 11/6 mm Hg among those with hypertension and 4/2 mm Hg among those without hypertension, with the greatest reduction in black hypertensive persons. There are many possible reasons why our blood pressure results were less robust. First, our three experimental diets did not differ as greatly in nutrient composition as the diets in the DASH trial. Our control diet and the two intervention diets were matched for sodium, protein, and cholesterol content, whereas sodium was the only nutrient held constant across the three diets of the DASH study. In fact, the control diet for the DASH study was 29% lower in protein and 44% higher in cholesterol than the DASH combination diet, which may partially account for the greater drops in blood pressure and lipid levels than that of our study. The difference in magnesium content between the control and DASH combination diets was 24% (74 mg/d) greater than the difference between the control and the D-F&V diets in our study. The DASH study also had a 22 mg/d (3%) greater difference in calcium content between the control and combination diets than our control vs. the D-F&V diets. Thus, it is possible that the magnitude of the blood pressure reduction in the present study was related to the magnitude of the difference in specific nutrient intakes between the control and interventions diets.
More importantly, in the DASH trial, there was a greater contrast between the combination diet and the fruit and vegetable diet, with the combination diet providing substantially less total (-36%) and saturated fat (-47%), cholesterol (-17%), and fiber (-13%) and more protein (+20%). The present study employed a diet design to specifically test the effects of adding 3 servings of dairy foods to a similar diet that was low in dairy, and thus, eliminated any macronutrient differences between the two intervention diets, allowing only calcium, magnesium, potassium, and phosphorus to be increased with the increase in servings of dairy foods. In addition, the amount and kind of fruits and vegetables were identical across these two experimental diets, unlike the DASH trial. The DASH combination diet also included fish and nuts, which have also demonstrated independent hypotensive effects in other studies. Therefore, it is not surprising that the DASH trial found that the combination diet elicited greater decreases in blood pressure compared with the its fruit and vegetable counterpart, where we did not. In addition, the fruit and vegetable diet in the DASH trial vs. our F&V diet was higher in potassium (4101 mg vs. 3900 mg), calcium (534 mg vs. 400 mg), magnesium (423 mg vs. 380 mg), and lower in sodium (2816 mg vs. 3500 mg), which may contribute to the greater decrease in blood pressure with the DASH fruit and vegetable diet vs. our F&V diet.

It is likely that the beneficial effects of our intervention diets were masked by parallel reductions in blood pressure during the control diet. Compared to screening levels, both the D-F&V and the F&V diets produced similar drops in blood pressure (-12/-7 mm Hg), however, our control diet also significantly reduced blood pressure (-10/-5 mm Hg). In the original DASH trial, blood pressure remained unchanged during the control diet (59). This may be because our control diet was higher in magnesium and
protein and was associated with a higher urinary potassium excretion (of 452 mg/24hr) compared with the control diet of the DASH trial. Like the present study, the PREMIER trial reported a similar occurrence in which the control group experienced significant drops in blood pressure in the control group (117). The advice only control group experienced a decrease of 7/4 mm Hg in blood pressure, while blood pressure in the other two intervention groups dropped approximately 11/6 mm Hg.

Another reason the present study did not elicit the same blood pressure lowering effect as the DASH trial may have to do with the lack of diversity of the study sample. Like the hypertensive subgroup in the DASH study (92), our subjects were overweight, middle-aged men and women who had stage 1 hypertension. However, we did not include large numbers of subjects from different ethnic groups. Of the hypertensives in the DASH trial (n=133), 60% were women and 65% were African American (vs. 30% and 0%, respectively, in our study). African-Americans experience approximately twice the risk of hypertension experienced by Caucasians, with a greater fraction of African-Americans suffering uncontrolled hypertension compared to the overall US hypertensive population (17). This group is particularly vulnerable to the complications of untreated hypertension and are more sensitive to diets that promote calcium and potassium intake (300). Therefore, our small study of 23 Caucasian hypertensive adults may have limited the potential to observe a beneficial effect of a dairy-rich diet.

Finally, the impressive blood pressure-lowering results of the original DASH trial have not been replicated completely. In the DASH-Sodium trial in which the DASH diet was evaluated in conjunction with varying sodium levels, the blood pressure lowering response of the DASH diet vs. the control diet was of lesser magnitude than in the
original DASH study. Compared to the control diet, the DASH diet lowered blood pressure -5.9/-5.0 mm Hg on the high sodium level (150 mmol or 3450 mg), -5.0/-2.5 mm Hg on the intermediate sodium level (100 mmol or 2300 mg), and -2.2/-1.0 on the low level of sodium (50 mmol or 1150 mg). All reductions in SBP and DBP were significant (p < 0.05), except for DBP on the lowest level of sodium. Our results tend to be more in line with the degree of blood pressure reduction in the DASH-Sodium trial.

**Goal 2: Isolate the hypotensive effect of dairy foods in the context of the DASH diet**

There are several potential ways dairy foods can influence blood pressure, therefore, the importance of any given mechanism is dependent upon the underlying type of hypertension. For example, hypertension may occur due to hyperactivity of the sympathetic nervous system, upregulation of the renin-angiotensin system, or be sodium volume-dependent. The ionic hypothesis, put forth by Resnick (81), suggests that each aspect of hypertensive disease (i.e. elevated blood pressure, insulin resistance, hyperinsulinemia, left ventricular hypertrophy, increased arterial stiffness, abnormal platelet aggregation, and accelerated atherosclerotic disease) is a manifestation of an underlying cellular ionic defect, reflecting an elevated intracellular ionic calcium level and reciprocally suppressed intracellular ion magnesium level.

Several studies have highlighted the importance of the heterogeneity of hypertension by identifying certain groups that may be particularly responsive to a dairy-rich diet. As noted above, the DASH diet showed marked differences in its effectiveness between those with and without hypertension and between African Americans and non-African Americans. The DASH diet reduced blood pressure more in the participants with
hypertension than those without, and more in African Americans than Caucasians (60). Furthermore, the DASH diet has been shown to lower blood pressure more in individuals with a specific genotype that leads to the activation of the renin-angiotensin system (91). Subgroup analysis of the PREMIER study also revealed that adults aged 50 or more were especially responsive to the DASH diet (118). Although epidemiologic studies of the relationship between dietary calcium and blood pressure reveal that those with a low intake of calcium (<300-600 mg/d) exhibit higher blood pressures (70,129), intervention studies using calcium supplementation have shown inconsistent results. It is important to note that this lack of effect of dietary calcium may be influenced by the heterogeneity of the hypertensive population. Clinical studies showing a hypotensive effect of dietary calcium in salt-sensitive (73) and/or low renin hypertensive (166) subjects lend support to the theory that calcium and/or dairy foods may play a key role in blood pressure regulation in patients thought to be in a state of calcium deficiency.

Although the addition of 3 servings of dairy foods to a diet already high in fruits and vegetables did not elicit further reductions in blood pressure in the group as a whole, subgroup analysis revealed a unique role for dairy foods in the treatment of hypertension in some subjects. Only subjects who responded to the dairy-rich diet by significantly reducing intracellular calcium exhibited significant decreases in systolic and diastolic blood pressure on the D-F&V vs. the control and the F&V diets. Blood pressure remained unchanged across all three diets in subjects who did not exhibit substantial reductions in RBC calcium. Furthermore, the change in intracellular calcium was positively associated with the change in DBP, such that those who experienced the greatest drop in intracellular calcium also had the greatest reduction in DBP. Therefore,
categorizing subjects based on individual changes in intracellular calcium clearly affirms the importance of cellular ion homeostasis in blood pressure regulation in some types of hypertension and gives credence to the ionic hypothesis. In addition, when comparing the two groups of subjects following the control diet, we found that those who experienced significantly greater drops in RBC calcium also had lower serum calcium and calcitonin and reciprocally higher PTH, and 1,25-dihydroxyvitamin D₃. Although these group differences were not significant, except for serum calcium, they parallel Resnick’s hypothesis that low renin hypertensives are in a state of calcium deficiency and may be especially responsive to diets rich in dairy calcium (84). In keeping with his hypothesis, the subjects in the (Ca)ᵢ Change group were very responsive to a diet rich in calcium, whereas those in the (Ca)ᵢ Stable group were not. This reaffirms that the activity of ion-active hormone systems (i.e. calcium-regulating and renin-angiotensin systems) may identify individuals who may be especially responsive to diets rich in dairy calcium (84).

Goal 3: Identify cardioprotective mechanisms (including blood pressure lowering) unique to dairy products

Although not fully understood, the mechanisms by which dairy foods reduce blood pressure may include decreased vasoconstriction via normalization of intracellular ion levels (84), ACE inhibitory action of dairy-derived bioactive peptides (80), a natural diuretic action (116), and the synergistic effect of dairy minerals (155). Our study corroborates some of these mechanisms and refutes others.
Elevations in intracellular calcium, resulting in vasoconstriction, have been considered to be an important mechanism in the pathogenesis of essential hypertension. Individuals with essential hypertension typically have significantly higher basal levels of intracellular calcium (202-208) and sodium (208) and lower levels of intracellular magnesium (208-210) than normotensive individuals. In addition, a strong positive correlation has been repeatedly noted between blood pressure and intracellular calcium levels in a variety of cell types (202,203,205,211). As in our study, ameliorating defective ion homeostasis with a calcium rich diet has been related to improved blood pressure in previous studies. Zemel et al. (73) conducted a clinical feeding trial in 11 salt-sensitive hypertensive African American men and women to examine the effects of dietary manipulations of sodium and calcium on RBC cation metabolism and blood pressure. They found that the low calcium (356 mg/d), high sodium (4000 mg/d) diet elicited significant increases in PTH, which were associated with significant increases in RBC calcium (5.4 ± 0.7 to 11.1 ± 3.7 μM) and RBC sodium (9.6 ± 0.3 to 11.4 ± 0.4 mM) and significant decreases in RBC magnesium (2.2 ± 0.1 to 1.8 ± 0.1 mM). The addition of calcium (to equal 934 mg/d) to this high sodium (4000 mg/d) diet reversed these effects (73) and was associated with lower systolic and diastolic blood pressure (301,302). Interestingly, the finding that calcium supplementation reduced intracellular calcium and sodium in erythrocytes also has been reported in normotensive men (231,294). This latter study also noted significant decreases in ionic platelet calcium and sodium, and 1,25-dihydroxyvitamin D₃, and a significant –7.8 mm Hg drop in SBP with calcium supplementation (1000 mg twice daily for 16 weeks) (231,294). In the present study, we also observed a reduction in RBC calcium and RBC sodium and an increase in
RBC magnesium during the dairy-rich diet. Our results extend these findings to suggest that the addition of dairy foods, as the prime source of calcium, to a moderately high sodium diet typical of the American diet can lower RBC calcium and increase RBC magnesium by a similar degree in Caucasians. Furthermore, our data indicate that Caucasian subjects who respond to a dairy rich diet by exhibiting substantial decreases in RBC calcium may also experience significant reductions in blood pressure.

Another study by Oshima et al. (230) examining the relationship between salt intake, blood pressure, and intracellular calcium in lymphocytes mirrors our results. In this study (230), 16 hypertensive and 13 normotensive subjects received a diet containing 3g of salt for a week and then a 20g salt diet for the following week. Salt loading significantly increased blood pressure and lymphocyte calcium in hypertensive, but not normotensive, subjects. The change in blood pressure was significantly correlated with the change in lymphocyte calcium (r = 0.70, p < 0.01), but not to changes in serum calcium or urinary calcium excretion. Our study also found that the change in DBP was associated with the change in RBC calcium, suggesting that a variety of cell types are prone to the same intracellular ion misbalances, which can be manipulated by diet. Likewise, a study in hypertensive subjects (n=20) found that calcium supplementation (1500 mg/d) lowered intraplatelet free calcium by 25% compared with placebo in 8 weeks (293). Interestingly, fasting plasma insulin levels and insulin sensitivity (assessed by the euglycemic clamp) were significantly improved with calcium supplementation. However, blood pressure was not changed. Based on our findings, it is plausible that individuals with the greatest changes in intraplatelet free calcium may also have experienced significant decreases in blood pressure; however, this is speculative.
Overall, our results are in agreement with those previously reported. We found that stage 1 hypertensive patients consuming a dairy- and calcium-rich diet decreased intraerythrocyte calcium concentration by about 44% compared to the other two low dairy/calcium diets.

In addition, we observed a significantly greater drop in the calcium-regulating hormone, 1,25-dihydroxyvitamin D₃, on the dairy-rich diet vs. the low calcium control and fruits and vegetables diets. This is consistent with previous studies showing that increases in dietary calcium reduce 1,25-dihydroxyvitamin D₃ (73,124,142,293). Moreover 1,25-dihydroxyvitamin D₃ has been shown to stimulate rapid calcium influx through increasing calcium channel currents in a variety of cells including vascular smooth muscle cells (219-223). The present evidence corroborates this relationship by showing that the level of RBC calcium was positively associated with 1,25-dihydroxyvitamin D₃ during the two low dairy/calcium diets (AAD r = 0.51, p = 0.0229; F&V r = 0.53, p = 0.0139), but not the D-F&V (r = 0.25, p = 0.3165). Taken together, it is likely that the dairy-rich diet normalized intracellular calcium levels via suppression of 1,25-dihydroxyvitamin D₃.

Elevated levels of circulating 1,25-dihydroxyvitamin D₃ also have been independently associated with increased vascular resistance (169,224), and in the present study TPR tended to be positively associated with this hormone (r = 0.36, p = 0.1050). However, TPR was unchanged across all three diets. Nevertheless, there was a significant increase in brachial artery diameter on the dairy-rich diet, suggesting that the reductions in intracellular calcium were associated with vasodilation and confirming studies noting that a decrease in intracellular free ionic calcium concentrations induces
vasorelaxation (302-304). The reason for this discrepancy between the two measures of vascular tone may relate to the greater variability in measurements of TPR vs. artery diameter. For example, we have shown that the coefficient of variation for artery diameter is four fold lower than the variation for repeated measurements of TPR (305). However, our study was adequately powered to detect changes in TPR $\geq 10\%$ if one were present and this suggests that greater variability is not the most important reason we failed to find an effect of the diets on TPR. Perhaps more importantly, TPR is a measure of systemic vascular resistance and is influenced by a number of overlapping systems including sympathetic activity, adrenergic hormones, paracrine substances, and myogenic responses. In contrast, brachial artery diameter reflects the diameter of a single artery, and we speculate that changes in RBC calcium may be closely associated with calcium dynamics in the vascular smooth muscle cells. In addition, differences in methodologies for measuring intracellular calcium may also explain why TPR was unchanged across all three diets, even in subjects who experienced the greatest decreases in RBC calcium.

Located in the cytosol of a cell, free ionic calcium is the biologically active form of calcium, which participates in a second messenger system determining cellular responses to a variety of stimuli. On the other hand, determination of RBC calcium includes both free ionic calcium as well as calcium that is bound or stored. Therefore, the measurement of total calcium in RBC may miss subtle changes in free ionic calcium that relate to TPR. Although TPR was not affected by the diets, we did observe a strong positive correlation between RBC calcium and TPR on the control diet, which suggests that intracellular calcium may have some influence on systemic vascular resistance. Moreover, the biggest drops in blood pressure were from screening and since TPR was not measured at
screening, we cannot comment on whether or not the experimental diets decreased TPR from screening.

Bioactive peptides in dairy products have been shown to possess hypotensive actions in vitro and in animal models. In humans, the antihypertensive effect of milk protein-derived peptides has yet to be demonstrated. The most often studied mechanism underlying the blood pressure-lowering effect of milk protein-derived peptides is inhibition of the activity of ACE. ACE is an enzyme that catalyses the conversion of angiotensin I into angiotensin II, the effector compound of the renin-angiotensin system. Because angiotensin II has a central role in the regulation of blood pressure and vascular structure, reducing its levels via ACE inhibition is an effective way to lower elevated blood pressure (306). The present study in hypertensive adults did not find that the dairy-rich diet lowered ACE activity compared to the other two low dairy diets. On a similar topic, plasma renin activity also was unchanged in our study. Previous studies of the effects of diet on plasma renin activity have been inconsistent with some observing a decrease (301), an increase (294), or no change (307) with calcium enrichment of the diet. Our study supports those that did not observe a change in renin activity. Even though ACE activity and plasma renin activity did not appear to change, angiotensin II, the downstream product of ACE, was significantly lower on the D-F&V compared with the control diet. The level of angiotensin II on the F&V diet was intermediary. In addition to the well-known circulating renin-angiotensin system, components of the system are also located in several tissues including the heart, vascular wall, kidney, and brain (308). ACE in the endothelium is activated in hypertension. Okamura and colleagues (309) reported that during renovascular hypertension in the rat, vascular ACE
activity increased 3-fold secondary to the increase blood pressure. Angiotensin II was also increased due to the rapid conversion of angiotensin I by ACE. These results are strong evidence for a local ACE effect on angiotensin II production. Therefore, it is possible that the dairy-rich diet altered ACE activity at the tissue level, which manifested changes in circulating levels of angiotensin II, but not ACE activity. Other properties of milk protein-derived peptides include mineral binding, antithrombotic, immunomodulatory and opioid-like activity (80), however, the present study did not evaluate these specific bioactive peptide-related effects.

Due to the high content of potassium and calcium, the dairy-rich diet in the present study may have acted like a natural diuretic. Urinary potassium and calcium excretion were significantly higher on this diet compared to the fruits and vegetables diet. Potassium supplements are well-known to cause natriuresis and lower blood pressure (310,311). Initiation of calcium supplementation also enhances urinary sodium excretion during the first several days (73,301,302,312,313). In an ancillary study of the DASH-Sodium trial, Akita et al. (116) calculated the pressure-natriuresis curve to assess the potential diuretic effect of the DASH diet as one mechanism of its blood pressure-lowering action. The pressure-natriuresis curve was derived by plotting mean arterial pressure on the x-axis and urinary sodium on the y-axis. The DASH diet increased the slope of the curve without shifting the curve along the blood pressure axis, indicating that the diet enhanced sodium excretion at each blood pressure level. This represents a natriuretic action, which has been observed with other diuretics (314). During the control diet of the DASH-Sodium trial, mean arterial pressure was positively associated with urinary sodium excretion. This is similar to our finding that as the ratio of sodium to
calcium excretion increased, mean arterial blood pressure decreased. The D-F&V diet also significantly increased urinary potassium excretion compared with the other two diets. Urinary sodium remained constant across the three diets reflecting the controlled amount dietary sodium. Taken together, these findings suggest that the blood-pressure-lowering effect of the DASH diet and our dairy-rich diet may be achieved through a natriuretic effect, with the diets acting as natural diuretics.

Dyslipidemia is a major independent risk factor for CVD and improving the lipid profile has been consistently shown to effectively reduce CVD risk. It is well recognized that diets low saturated fat reduce blood cholesterol compared to higher saturated fat diets. The DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) Study (315) was a multi-center study designed to rigorously evaluate the effects of type and amount of dietary fat on lipids, lipoproteins and thrombogenic activity in different population groups. One arm of this study evaluated the effects of a step-wise reduction in total and saturated fat (replaced isocalorically with carbohydrate) in healthy subjects (total fat decreased from 37% to 30%, and further to 26%; SFA decreased from 16% to 9%, and further to 5%). The step-wise reductions in saturated fat (from 16% to 9% to 5%) resulted in a corresponding decrease in LDL cholesterol by 6.9% and 10.7%, respectively (315). However, the reduction in saturated fat was associated with a decrease in HDL cholesterol (7% and 12%, respectively) and an increase in TG levels (8% and 9%, respectively), both of which could potentially increase risk of CVD. This hypertriglyceridemic response to a diet low in total fat and high in carbohydrate has been documented over the past fifty years (316). Therefore, prevention of
hypertriglyceridemia through diet manipulations has become an important concern regarding public health. Evidence is emerging that fiber, particularly the soluble type, may play a therapeutic role in preventing the hypertriglyceridemic response to a high-carbohydrate diet (317).

In the present, the F&V and D-F&V diets were matched for macronutrients (total fat, saturated fat, and dietary fiber; 30% kcal, 7% kcal, and 27 g, respectively) and differed from the control diet (36% kcal, 15% kcal, 10.5 g, respectively). All three diets were matched for dietary cholesterol (300 mg). Our results replicate the findings of the DELTA study in that a lower fat diet reduced LDL and HDL cholesterol. In our study, total and LDL cholesterol levels significantly decreased on both the D-F&V diet (-3.9% and -5.8%, respectively) and fruits and vegetables diet (-6.1% and -9.0%, respectively) compared to the control diet. Levels HDL cholesterol were maintained by the control diet, while HDL cholesterol significantly decreased by approximately ~6% on both intervention diets. In contrast to the DELTA study, fasting triglyceride levels were similar across the three diets in our study, which may be due to the higher dietary fiber content of the intervention diets vs. the control diet.

The original DASH diet (27% kcal total fat, 7% kcal saturated fat) elicited similar lipid results, but they were greater in magnitude, presumably due to a greater contrast in the nutrient composition of its control diet (37% kcal total fat, 14% saturated fat, 246 mg dietary cholesterol, 10.8 g fiber) and combination diet (27% kcal total fat, 7% saturated fat, 188 mg/2100 kcal dietary cholesterol, 29.9 g/2100 kcal fiber) (173). Total cholesterol, LDL cholesterol, and HDL cholesterol were significantly reduced by 7.3%, 9.0%, and 7.6% compared to the control diet, but triglyceride levels were unchanged by
the DASH diet. In contrast to our results, the fruits and vegetables and control diets of
the DASH study elicited a similar effect on lipids and lipoproteins. This is likely because
the macronutrient profile of the fruits and vegetables and control diets was similar in the
DASH trial. The present study was designed to isolated the effects of adding 3 servings
of dairy to a diet already high in fruits and vegetables, therefore the two intervention diets
were matched for macronutrients and thus both differed from the control diet.

Unlike studies that report a decrease in cholesterol levels with calcium
supplementation (75,177,178), our calcium/dairy-rich diet did not elicit significantly
greater reductions in cholesterol levels compared with the F&V diet. Buoponame et al.
(75) investigated the effect of consuming one quart of skim milk a day for eight weeks on
blood cholesterol levels of 82 free-living adults. Total cholesterol levels decreased 6.6%
within four weeks in subjects who consumed the skim milk and whose initial blood
cholesterol levels were elevated (217 - 233 mg/dL). Therefore, the hypocholesterolemic
effect of skim milk may be greater in individuals with higher baseline cholesterol levels
than our study group (baseline total cholesterol = 194.4 ± 6.4). In a randomized single
blind study, 13 men aged 38 to 49 years with moderate hypercholesterolemia were fed a
metabolic diet approximating the typical American diet (34% kcal fat, 13% kcal saturated
fat, 240 mg/d dietary cholesterol) and either 400 or 2200 mg calcium (calcium citrate
malate) for ten days (177). When compared with the low calcium diet, the high calcium
diet lowered total and LDL cholesterol by 6% and 11%, respectively, while HDL
cholesterol was unchanged. Furthermore, fecal excretion of saturated fatty acids doubled
during the high calcium diet, suggesting that calcium’s beneficial effect may be explained
by the formation of calcium-saturated fatty acid complexes in the intestine. In the present
study the saturated fat content was very low and identical in the D-F&V and F&V diets, therefore, the additional calcium probably did not affect fat excretion.

Endothelial dysfunction underlies the development of overt coronary artery disease, correlates strongly with both coronary disease and its risk factors, and reverses in response to risk modification efforts. Impaired endothelial function, as assessed by FMD, has been consistently associated with advancing age (318). This was true in our sample as well. The mechanisms of why this occurs are unknown but may relate to increased oxidative stress (319). The parallel increase in intracellular calcium and age in our study may suggest another mechanism of lower FMD with increasing age.

We did not demonstrate a difference in FMD between the D-F&V, F&V, and the AAD diets. However, we did observe a positive correlation between the change in FMD and the change in RBC magnesium. This is not the first study to observe this relationship. Takase et al. (320) examined the effect of chronic stress on FMD of the brachial artery and RBC magnesium levels in 30 healthy male college students. Chronic stress was significantly associated with decreases in both FMD (from 7.4 ± 3.0 to 3.7 ± 2.3%, p < 0.05) and RBC magnesium (5.7 ± 0.4 to 5.5 ± 0.4 mM). As in our study, the change in FMD and RBC magnesium was significantly correlated (r = 0.43, p < 0.05). In addition, we observed a greater basal arterial diameter on the dairy-rich diet. While the significance of differences in basal arterial diameter is not yet known, new evidence highlights its potential importance. Recently, the Framingham Heart Study reported a strong association with CVD risk factors (sex, age, BMI, and hypertension medication and hormone replacement therapy usage) and basal brachial diameter (318). The diet-
related differences in basal arterial diameter suggest that some component of the dairy-rich diet has vasodilatory effects. This may be due to lower smooth muscle calcium with reciprocally higher magnesium, and/or decreased angiotensin II levels associated with this diet. Nonetheless, the significantly lower level of VCAM-1 and the consistent trends toward lower levels for adhesion molecules and P- and E-selectins on the dairy-rich diet suggest a beneficial effect of this diet on the endothelium. The mechanisms for these observations merit additional investigation.

Adhesion molecules such as VCAM-1, ICAM-1, P-selectin, and E-selectin are believed to have an important role in early atherogenesis by tethering leukocytes to the arterial wall and increasing the probability of transmigration to the intima. We found that the F&V and D-F&V diets were associated with lower levels of ICAM-1 vs. the control diet. P-selectin and E-selectin were significantly lower on the D-F&V vs. control diet and tended to be lower vs. the F&V diet. VCAM-1 was significantly lower on the D-F&V compared with the other two diets. A large body of evidence indicates that atherogenic lipoproteins upregulate the expression of adhesion molecules on endothelial cells. Native LDL binding to the LDL receptor in human coronary artery endothelial cells triggers a rise in intracellular calcium, increases the expression of VCAM-1 and E-selectin, and increases monocyte binding to the cells (321,322). Therefore, diet-related decreases in total and LDL associated with the D-F&V and F&V diets may have contributed to decreased levels of ICAM-1, VCAM-1, E-selectin, and P-selectin (which is also a marker of platelet activation) in the present study. Some previous data support this concept. In a recent study (323), intensive lifestyle modification (including decreased intake of saturated fat and dietary cholesterol) was associated with a significant
A decrease in E-selectin concentrations in the intervention group vs. a control group (from 53.0 ± 26.4 to 48.6 ± 24.9 ng/mL and from 51.8 ± 21.0 to 50.3 ± 20.8 ng/mL, respectively, p = 0.02). The change in E-selectin was positively associated with the change in total cholesterol (r = 0.17; p = 0.04) (323). In a controlled randomized study (324) in 56 women with moderately elevated cardiovascular risk found that a wholesome diet with increased consumption of fiber and decreased intake of saturated fat was associated with lower ICAM-1 and VCAM-1. Concentrations of P-selectin were 25% (p = 0.003) lower in hypercholesterolemic men who were given a Mediterranean and 17% (p = 0.068) lower on a low-fat diet compared with a diet high in saturated fat (39). Diets rich in alpha-linolenic acid have been shown to lower VCAM-1 and E-selectin by about 15% (p < 0.01) compared to a diet high in linoleic acid (325). Taken together, diet modifications consistent with recommendations for prevention of atherosclerosis are associated with a favorable profile of lipid and inflammatory parameters.

In addition, the dairy-rich diet significantly lowered VCAM-1 compared to the F&V diet. Although not statistically significant when compared to the F&V diet (p = 0.19), this may be attributed to the lower angiotensin II levels on the D-F&V. Angiotensin II is a major trigger of endothelial dysfunction in hypertensive individuals (326). It is responsible for generating reactive oxygen species, the breakdown of nitric oxide, and the upregulation of gene expression for adhesion molecules, endothelin, nuclear factor-kappa and other inflammatory mediators. Angiotensin II has been demonstrated to induce the expression of VCAM-1 (264,265). The fact that levels of angiotensin II and VCAM-1 were positively correlated during each diet lends further support to this theory.
The importance of diet in regulating intracellular ion homeostasis is further demonstrated in the present study by the investigation of calcium mobilization in stimulated platelets. Platelets play a key role in CVD. Platelet activation is controlled by the concentration of intracellular ionic calcium, which is generally higher in hypertensive subjects than in normotensive subjects (203,204,207,286). Hyperactive platelets are suggested to play a role in the early event of atherosclerosis (285). Platelets from hypertensive subjects are prone to a disproportionate increase in \((\text{Ca}^{2+})_i\) after stimulation with an agonist compared to normotensive subjects (204) (289) (290). Moderate exercise in sedentary normotensive men (291) and preeclampsia (277) have also been shown to exaggerate the intracellular free ionic calcium response to an agonist. The present study is the first to suggest that the average American diet may promote hyperactive platelets in hypertensive individuals. The average American diet elicited about a 130% greater increase in free ionic calcium in platelets stimulated with arginine vasopressin compared to the D-F&V and F&V diets. There are several differences between the two intervention diets and the AAD that may account for this finding. First, the AAD was higher in total and saturated fat than the two intervention diets, which may alter platelet function. Second, the AAD diet may have increased intracellular calcium in platelets at baseline (paralleling the changes we observed in RBCs), which may have lead to the higher spike in intraplatelet free \(\text{Ca}^{2+}\). However, this is probably not the case, because the F&V diet also had significantly higher RBC calcium levels vs. the D-F&V diet, but did not differ from the D-F&V diet in the magnitude of increase in intraplatelet free \(\text{Ca}^{2+}\) after stimulation. Finally, compared to the AAD, the two intervention diets were associated with significantly higher intracellular magnesium levels (measured in RBC). Thus, it is
possible that the lack of intracellular magnesium may have lead to this exaggerated platelet response during the AAD. The latter possibility is supported by studies that demonstrate that intracellular magnesium buffers the cellular responsiveness to Ca\(^{2+}\)-driven stimulation (195).

However, potential inferences to be made from the study of platelet stimulation are limited, since we did not measure other markers of platelet activation (i.e. platelet aggregation or platelet expression of activated conformation of GPIIb-IIIa, activation antigen CD62, and membrane-bound P-selectin). We did measure circulating levels of soluble P-selectin and observed a similar pattern. The AAD diet elicited the highest levels of P-selectin, which were significantly higher than the D-F&V diet. The F&V diet was in the middle and not significantly differing from either diet. Furthermore, the two intervention diets are presumably higher in antioxidants (327), which have been shown to protect against CVD by inhibiting platelet activation and aggregation (328). Taken together, the AAD appears to detrimentally affect platelet properties compared with the other diets high in fruits and vegetables, and dairy foods had no additional effects.

In addition, flow cytometry was a convenient way to evaluate platelet (Ca\(^{2+}\)) mobilization in whole blood. Although we could not quantify the absolute level of intraplatelet free Ca\(^{2+}\), one major advantage to this method is that sample manipulation is minimal in contrast to other methods involving separation procedures that cause artificial platelet activation. Also, whole blood platelets were analyzed in a physiologic milieu, where erythrocytes and white blood cells were included and could affect platelet responses to activation. More advanced studies of platelet reactivity using this technology will be advantageous in future research projects.
The results of the present study confirm that a low fat diet rich in fruits, vegetables, and dairy products reduces blood pressure in Caucasian stage 1 hypertensive adults. Of particular interest, the addition of three servings of dairy foods to a diet already high in fruits and vegetables and low in total and saturated fat enhances the blood pressuring lowering response in some types of stage 1 hypertension. This finding supports others that have identified certain hypertensive populations that are especially responsive to the DASH diet or calcium-rich diets. Therefore, a portion of the hypertensive population is extremely sensitive to low calcium moderate sodium diets, and thus may be particularly responsive to a diet rich in dairy foods. Epidemiologic studies also indicate that diets high in calcium blunt the rise in blood pressure that normally occurs with age (76). Even if diet modification does not produce a sufficient reduction of blood pressure to avoid drug therapy, the number of medications or dosages required for blood pressure control may be reduced. It has been shown that implementation of the DASH dietary pattern in addition to antihypertensive medication elicits greater decreases in blood pressure than drugs alone (329). Implementation of lifestyles that most favorably impact blood pressure has implications for the prevention and treatment of hypertension and for population-based strategies to shift the overall distribution of risk downward. On a population level, even a small decrease in DBP of 2 mm Hg has been estimated to reduce the prevalence of hypertension, the risk of coronary heart disease, and the risk of stroke by 17%, 6%, and 15%, respectively (330). More importantly, the present study showed that healthy diets, which include dairy products, have additional positive effects on other markers of cardiovascular risk. These include lipids and
lipoproteins, cell adhesion markers, and angiotensin II. In conclusion, our results provide support for including dairy foods in a healthy diet for the treatment of CVD.
Chapter 4

Summary and Conclusions
The results presented in this thesis have provided critical insights about how a DASH-type diet lowers blood pressure and, in particular, how the addition of dairy products to a healthy diet affects novel CVD risk factors in hypertension. In addition, a better understanding of the role dairy products play in maintaining intracellular ion homeostasis was gained. Moreover, this study demonstrates that a dairy rich diet can normalize intracellular calcium and magnesium levels in stage 1 hypertension, which may result in improved blood pressure in sensitive hypertensive adults. The major conclusions of this study are as follows:

1. A diet rich in fruits and vegetables, with or without the addition of 3 servings of dairy foods, significantly lowers blood pressure compared with the average American diet. Although the decrease in blood pressure was only ~2 mm Hg, this magnitude of reduction has been estimated to reduce the prevalence of hypertension, the risk of coronary heart disease, and the risk of stroke by 17%, 6%, and 15%, respectively.

2. The addition of 3 servings of dairy to a diet already high in fruits and vegetables conferred significant reductions in intraerythrocyte calcium levels and significant increases in intraerythrocyte magnesium levels. This suggests that intracellular ion levels are responsive to diet manipulation and that a dairy-rich diet produces an ideal intracellular environment (significantly lower ratio of intracellular calcium to magnesium).
3. Our results confirm other reports of a direct relationship between intracellular calcium and the calcium-regulating hormone 1,25-dihydroxyvitamin D3. Our results suggest that the dairy-rich diet conferred significant reductions in intraerythrocyte calcium levels, via the suppression of this hormone.

4. The subgroup analysis, based on subjects who exhibited significant decreases in RBC calcium following the D-F&V vs. those who did not, showed that changes in intracellular calcium levels partially determined the magnitude of the hypotensive effect of adding dairy foods to a diet already high in fruits and vegetables. Only individuals who responded to the dairy-rich diet by substantially reducing RBC calcium, experienced significant drops in SBP and DBP on the D-F&V diet vs. the low calcium F&V and control diets. This indicates that there are Caucasian hypertensive adults who are especially responsive to a calcium/dairy rich diet, whereas others are not. These results mirror other reports suggesting that the effectiveness of dairy foods in blood pressure control is influenced by the heterogeneity of the hypertensive population.

5. The dairy-rich diet was associated with significantly lower angiotensin II levels than the control diet. However, the F&V diet did not differ from the other diets. This finding suggests that some component in the dairy-rich diet affected the renin-angiotensin system, however, renin and ACE activities were unchanged.
This discrepancy might be due to changes in the activity of the tissue-based renin-angiotensin system.

6. While the percent change in flow mediated dilation did not differ among diets, several other markers of endothelial function suggest that dairy foods improve endothelial function. Basal arterial diameter was significantly higher on the D-F&V diet compared to both the F&V and AAD diets. Furthermore, peak diameter was highest on the D-F&V and significantly different from the AAD diet. The clinical significance of these differences is not clear but demand further research in light of recent findings from Framingham (318).

7. Soluble cell adhesion molecules also serve as markers of endothelial function. VCAM-1 was significantly lower following the D-F&V diet compared with the other two diets. This may be attributed to the significantly lower levels of angiotensin II on the D-F&V diet, since angiotensin II can increase expression of VCAM-1 and the two were correlated. ICAM-1 was significantly lower following both the D-F&V and F&V diets compared with the AAD diet. This is probably due to the difference in macronutrient contents of the experimental diets; diets lower in total and saturated fat are known to decrease ICAM-1. Compared with the AAD, both P-selectin and E-selectin were significantly lower following the D-F&V diet. Furthermore, P- and E-selectin levels tended to be lower on the D-F&V diet compared with the F&V diet. Taken together, it
appears as though dairy foods may confer beneficial effects on endothelial function.

8. A direct relationship exists between erythrocyte magnesium and FMD. The biological significance of this association is not clear yet.

9. A diet low in total and saturated fat, regardless of its content of calcium, has significant total and LDL cholesterol-lowering effects. Although both the dairy-rich and F&V diets decreased HDL cholesterol levels compared with the higher fat control diet, the intervention diets did not increase triglyceride levels. The increased soluble fiber content of the intervention diets may have attenuated the well-known hypertriglyceridemic effect of low fat diets.

10. Our study found that the control diet was associated with a significantly greater increase in intraplatelet free ionic calcium after stimulation with an agonist compared with the two lower fat intervention diets. Furthermore, soluble P-selectin, another marker of platelet activity, was significantly higher on the control diet vs. the dairy-rich diet. These results indicated that the average American diet may cause platelets to be in a state of hyperactivity in hypertension.
Chapter 5

Future Directions
The study presented herein has examined the effects of three different dietary patterns on several CVD risk factors in hypertensive adults. Intracellular ion levels, hemodynamics, and hormone systems were assessed to better understand putative biologic mechanisms by which dairy foods influence blood pressure and endothelial function. From the results of the present study it may be concluded that a diet high in fruits, vegetables, and dairy foods and low in total and saturated fat lowers blood pressure, LDL cholesterol, angiotensin II, and normalizes intracellular ion homeostasis, thereby lowering overall CVD risk. Furthermore, our data reaffirm that certain hypertensive populations are especially sensitive to dairy calcium/foods. Understanding the mechanisms by which dairy products affect multiple CVD risk factors (i.e., blood pressure, serum lipids/lipoproteins, endothelial function, and platelet activity) may lead to more effective strategies through nutriceutical or pharmacological interventions to reduce CVD risk in hypertension, a population at high risk for CVD. In addition, understanding the heterogeneity of hypertension will be key in discovering new ways to prevent and treat hypertension in the future. The focus of future studies emanating from this research should be directed as follows:

1. Our results were in a small homogeneous group of subjects. Our sample consisted of middle-aged, white adults with stage 1 hypertension, but not taking antihypertensive drugs; therefore, the results cannot be generalized to other population groups. Future studies should replicate this study design to evaluate whether dairy foods enhance blood pressure control in other groups including African Americans, patients with stage 2 hypertension or prehypertension, and in
patients whose blood pressure is being controlled with medication. The latter group is especially important since the current standard of care typically includes drug therapy and diet has been shown to reduce the need and/or dosage of drugs.

2. Our study, like previous reports, identified a subgroup of hypertensive adults especially sensitive to dietary calcium. Blood pressure regulation is controlled by mechanisms that involve genetic and environmental factors. Epidemiologic studies have shown that approximately 30% of the variance of blood pressure is attributable to genetic heritability and 50% to environmental influences (192). Because the etiologic heterogeneity of the disease, a single genetic marker is not likely to be discovered. However, the discovery of a positive linkage between a specific gene and hypertension will promote studies of novel treatments and may even reveal an unexpected mechanism of blood pressure control. Therefore, future studies of the genes related to blood pressure regulation (i.e. the renin-angiotensin system) are important to treat the underlying cause of hypertension in an individual.

3. Do subjects with low renin hypertension vs. high renin hypertension experience significant reductions in intracellular calcium in response to diets rich in calcium? Our study suggests that this may be true, but future studies should be designed to test this directly. If it is true, renin status may be one way clinicians can easily identify which individuals will respond to increased dietary calcium.
4. Does intracellular ion homeostasis affect other aspects of hypertensive disease? Because hypertension is associated with numerous other CVD risks, including diabetes, hyperinsulinemia, and obesity, future research should determine the effectiveness of manipulating intracellular ion balance via dietary interventions on other CVD risk factors. Some evidence exists that aberrant intracellular calcium handling results in reduced insulin sensitivity and an exaggerated inflammatory response.

5. In the present study the D-F&V diet was often significantly different than the control diet regarding endothelial function biomarkers, but only marginally different from the F&V diet. This may be due to the small, homogenous sample; therefore, future studies should evaluate the effectiveness of adding dairy products to a healthy diet on endothelial function in a larger group that is more diverse.

6. Does magnesium supplementation increase intracellular magnesium content and does this improve flow mediated dilation? The direct relationship between intracellular magnesium and flow mediated dilation in our study is very interesting. Maybe the component of the dairy-rich diet responsible for increased basal artery diameter is its higher content of magnesium. Future studies should be designed around this concept.
7. Does the duration of the intervention, dose of calcium, or type of dietary calcium (dairy or supplementation) influence blood pressure responses? Some evidence says this is true (331). Our diet periods were five weeks long and McCarron and Morris (138) report that at least eight weeks of intervention is necessary for the hypotensive effect of calcium to be expressed. Thus, future studies should determine differences in responses to dairy foods vs. supplemental calcium and the treatment period should be extended past five weeks.

8. Do dairy foods attenuate the activity of the renin-angiotensin system in circulation or at the tissue level? The isolated finding of lower levels of angiotensin II with no changes in renin or ACE activity on the D-F&V may suggest reduced tissue ACE activity. Confirmation of this finding is needed.

9. Does a dairy-rich diet affect platelet reactivity? Our study suggests that the control diet detrimentally affected platelets; however, it is not clear what component of the intervention diets was protective. Furthermore, our study only measured two markers of platelet function. Therefore, future research should embark studies of the potential effect dietary calcium or dairy products have on platelet reactivity and, in doing so, measure several critical markers of platelet function.
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APPENDIX A

Sequence of Events for Washout of BP Medication Prior to Enrollment in Study

1) Referral from Personal Physician

2) Telephone Screening by Study Staff

3) DEV 1 (Drug Evaluation Visit – 1)
   a. This visits include Informed Consent, GCRC Med. History form, BP, EKG, Cholestec Screen, blood draw for Chem 24/CBC/lipid panel, DEV 1 visit form

4) At this point, both the GCRC physician and the personal physician will have a copy of the lab results to date and will need to OK the person’s participation

5) Person stops medication

6) 1 week passes - DEV 2 (includes BP check and questions about adverse symptoms)

7) 1 week passes - DEV 3 (""")

8) 1 week passes – DEV 4 (""")

9) if after 21 days off meds, the person’s BP meets the study range (SBP 140-159, DBP 90-99), they can be enrolled in the study and begin the diet period;

……..if at any point in the 21 days, the person’s BP is above our screening range (SBP 135-170, DBP 88-104), they will be referred to their physician and the study coordinator will call the doctor to inform them that the person should be seen by them again for treatment
Telephone Interview Form

Penn-BP Study

Interviewer: ____________________________

Date ____________________________

Before asking any questions, please read the following paragraph to obtain verbal consent to conduct the telephone interview:

“We received your message that you are interested in participating in the ‘Blood Pressure Diet Study’. I will first read a brief description of the study. In this study we will give you all foods and beverages for 15 weeks. There will be three, 5-week feeding periods with a 2-week break in-between for a total time commitment of 19 weeks. During each of these three, 5-week feeding periods we will ask you to eat your weekday breakfast (6:30-9:30 am) or dinner (4:00-6:30 pm), from Monday to Friday, in the dining room of our Study Center, located in Henderson Building on the Penn State campus. Your lunch and other two meals and a snack will be packed for take out on weekdays. On the weekends your food will be packed in a large cooler that you will pick up on Friday afternoon. All the foods provided will be typical foods available in local grocery stores. We will ask that you not consume any other foods or beverages other than those provided during each 5-week interval. We will draw blood from you 7 times during the study, once at screening and on 2 consecutive days at the end of week 5 of each feeding period to determine your blood fat levels. Please note that this is not a weight-loss study, so you will be weighed daily to ensure you are not gaining or losing weight. You should be aware that the compensation for this study ($400) is considered income. If you are a Penn State employee it will be taxed, if you are not, it is reportable income. Are you still interested in the study?”

YES _____ (continue with interview)
NO _____ (thank them for their time and interest)

“I will now ask you a series of questions about your past medical history and your current lifestyle. If you agree to answer these questions, and it is then determined that you meet the criteria for this study, we will schedule you for a screening visit. Are you willing to answer these questions which will take about 15 minutes?”

YES _____ (continue with interview)
NO _____ (thank them for their time and interest)

1. Please give us your:

   Name __________________________________________________________ Date of Birth __________________
   Home address __________________________________________________________________________
   Daytime Phone# __________________________ Evening Phone# __________________________

2. What is your age? ___________     Interviewer:

   Your Height (ft and in)__________ Age between 22 and 70 y ☐ Yes ☐ No
   Your Weight (lbs) _____________ BMI between 20 and 37 ☐ Yes ☐ No
3. Are you currently on a weight-loss diet or program?  
   If yes, please specify  
   __________________________________________________________________________ 
   How long have you been on this weight loss diet? ________
   How much weight have you lost? __________ 
4. Will you be in the area for the entire 19 weeks of the study?  
   □ Yes  □ No 
5. Do you have access to the following appliances at home: 
   Refrigerator  
   □ Yes  □ No 
   Freezer  
   □ Yes  □ No 
   Microwave, oven or toaster oven  
   □ Yes  □ No 
6. Do you have any of the following medical conditions: 
   a. heart disease (stroke, heart attack, by-pass surgery, angina, angioplasty)  
      □ Yes  □ No 
   b. diabetes  
      □ Yes  □ No 
   c. high blood pressure  
      □ Yes  □ No 
   d. renal or kidney disease  
      □ Yes  □ No 
   e. latex allergy  
      □ Yes  □ No 
   f. rheumatoid arthritis  
      □ Yes  □ No 
   g. gastrointestinal disease (such as Crohn’s disease, irritable bowel syndrome, ulcer or history of bowel surgery, lactose intolerance).  
      □ Yes  □ No 
   h. blood clotting disorder  
      □ Yes  □ No 
   i. liver disease or cirrhosis  
      □ Yes  □ No 
   j. any condition that requires the use of steroids  
      □ Yes  □ No 
   k. gout (requiring treatment)  
      □ Yes  □ No 
   l. anemia (or sickle cell anemia)  
      □ Yes  □ No 
   m. lung disease (such as bronchitis, emphysema, asthma)  
      □ Yes  □ No 
   n. cancer within the last 10 years  
      □ Yes  □ No 
   o. thyroid disease (or any thyroid medication)  
      □ Yes  □ No 
   p. Problems with immune system (hepatitis, AIDS)  
      □ Yes  □ No 
   q. Vascular disease such as Reynaud’s  
      □ Yes  □ No 
   r. any other medical condition not specified in this list  
      specify ________________________________ 
   Explain any “yes” answers: __________________________________________________
7. Do you take any medication prescribed by a doctor? (this includes medications for any diseases, any type of pain medicine, and any drugs for treatment of depression or other mental health problems.)

☐ Yes  ☐ No

If yes, please specify the type of medication used, duration of use and reason:
______________________________________________
________________________________________________________________________

8. Do you take a medication that is a MAO inhibitor?

example: Nardil, Parnate
☐ Yes  ☐ No

9. Do you take any medication not prescribed by a doctor? Or any type of nutritional supplement, herb or vitamin?

If yes, please specify the type of medication used, duration of use and reason:
______________________________________________
________________________________________________________________________

If yes, are you willing to discontinue use during the study?

☐ Yes  ☐ No

10. Do you donate blood on a regular basis?

If yes, when was the last time you donated blood?
If you are accepted into the study, you must realize you will not be allowed to donate blood for the duration of the study.

11. Do you have any food allergies?

If yes, please specify foods

12. Do you have an allergy or sensitivity to foods containing nitrite or nitrates such as hot dogs, processed meats such as salami?

☐ Yes  ☐ No

13. Do you have any food restrictions related to religious practices?

Or are there any foods you refuse to eat?

If yes, please specify

14. Are you on a special diet prescribed by a doctor or self-prescribed?

If yes, please specify (specifically ask about low sodium diet)

☐ Yes  ☐ No

15. Do you exercise more than 10 hours a week or play sports regularly?

If yes, please specify

16. Do you currently smoke?

If No, have you ever smoked before?

If Yes, when did you have your last cigarette?
SUBJECT IS CONSIDERED ELIGIBLE FOR THE STUDY IF NO BOLDED RESPONSES ARE CIRCLED ON THE TELEPHONE INTERVIEW FORM AND THE WOMEN’S HISTORY FORM

If subject is eligible, schedule visit to General Clinical Research Center for Clinic Visit 1.

☐ Yes, subject eligible Date of screening: __________________________

☐ No, subject is not eligible – Reason: ________________________________

Give the subject directions to the General Clinical Research Center in Noll Lab on the PSU campus. Please have them meet the study staff at the 2nd floor nurse’s station. Enter the GCRC through the door that faces Atherton Street. Instruct them to put on their flashers, go to the nurse’s station to get a parking permit and then return to their car and put the permit on their mirror and turn off their flashers. The first visit will take approximately 1 hour to complete all of the paperwork and testing. Tell the subjects that they have to fast for 12 hours (no food or beverages except for water) and no alcohol for 48 hours before their scheduled testing time.
APPENDIX C

Penn-BP Study

Clinic Visit 1 Form

Date: ___/___/___
Reviewer’s Initials: ___ ___ ___

1. Has it been more than 12 hours since you late ate or drank anything except water? **If no, reschedule subject.**
   □ Yes □ No

2. Has it been more than 48 hours since you last consumed alcohol? **If no, reschedule subject.**
   □ Yes □ No

3. Have you exercised vigorously in the past 2 hours? **If yes, reschedule subject.**
   □ Yes

4. Has the subject read and signed the informed consent form? **If no, have subject review and sign consent form.**
   □ Yes □ No

5. Date subject stopped vitamin, mineral or herbal supplement: ___/___/___
   □ NA

6. Height and Weight Measurements
   a. Height (without shoes) ___________ (ft, in) ___________ (cm)
   b. Weight (without shoes) ___________ (lbs) ___________ (kg)
   c. BMI ________________________________
   d. Is BMI ≤ 37 kg/m^2 □ Yes □ No

7. Seated Blood Pressure Measurement

   Has the subject consumed caffeine, used any medication, eaten any food and/or exercised in the past 2 hours? **If yes, subject needs to be rescheduled for a blood pressure measurement.**
   □ Yes □ No

Instructions: Measure the subject’s arm circumference and choose the appropriate cuff. After applying the cuff, the subject must be quiet and remain continuously seated without legs crossed for 5 minutes. Wait 1 minute after each reading before taking the next reading.
a. First blood pressure measurement: __ __ __ / __ __ __ (SBP/DBP)
b. Second blood pressure measurement: __ __ __ / __ __ __
c. Third blood pressure measurement: __ __ __ / __ __ __
d. Average (2nd and 3rd) blood pressure measurement: __ __ __ / __ __ __
e. Is the avg SBP 140-159 mmHg and/or DBP 90-99 mmHg
   Yes No

8. Eating Attitude Test (EAT) Form Score
   Is the EAT form score < 30?
   Yes No

9. Cholestech Blood Lipids
   LDL _________ HDL _________ TG’s ____________

   Values reviewed ____________, ok to proceed?
   Yes No

10. Have nurse draw blood on subject and send to AML (American Medical Labs).
    Has blood been sent to AML?
    Yes No
    Are all the other lab results within normal range?
    Yes No
    If no, list abnormal values: ________________________________

    Have abnormal values been checked with the GCRC clinician?
    Yes No

11. Is subject eligible?
    Yes No

   If yes, subject needs to be notified and Clinic Visit 2 needs to be scheduled. Clinic Visit
   2 will be a blood pressure measurement, a check for postural hypotension, an EKG, and a
   urine sample. Remind the subject to not eat, exercise, consume caffeine containing foods
   or beverages, or take any medication 2 hours before their appointment time.

   Date: ____________________ Time: ____________________

   If no, subject needs to be notified and a follow-up done, i.e., test results sent to the
   subject and/or their private physician if they authorize it.

   a. Has a follow-up been done?
      Yes No

Comments:
APPENDIX D

Penn-BP Study

Subject ID: B P __ __ __

Clinic Visit 2 Form

Date: __ __ / __ __ / __ __
Reviewer’s Initials: __ __ __

1. Seated Blood Pressure Measurement

Has the subject consumed caffeine, used any medication, eaten any food and/or exercised in the past 2 hours? □ Yes □ No

If yes, the subject must be rescheduled for a blood pressure measurement.

Instructions: Measure the subject’s arm circumference and choose the appropriate blood pressure cuff. After applying the cuff, the subject must be quiet and remain continuously seated without legs crossed for 5 minutes. Wait 1 minute after each reading before taking the next reading.

a. First blood pressure measurement: ___ ___ / __ __ __ (SBP/DBP)

b. Second blood pressure measurement: ___ ___ / __ __ __

c. Third blood pressure measurement: ___ ___ / __ __ __

d. Average (2nd and 3rd) blood pressure measurement: ___ ___ / __ __ __

e. Is the avg SBP 140-159 mmHg and/or DBP 90-99 mmHg? □ Yes □ No

2. Check for postural hypotension

Instructions: place subject in supine position. Wait 5 minutes, take BP, Supine BP ___ ___ / ___ ___ (SBP/DBP) have subject stand and immediately take blood pressure. ___ ___ / ___ ___ (SBP/DBP)

a. Is the SBP decreased from supine BP by 20 mm Hg or more? □ Yes □ No

b. If answer is yes, is subject feeling faint, dizzy, etc? □ Yes □ No

Comment: _______________________________________________________

If answer is yes to part a, the GCRC clinician must be consulted. Has GCRC clinician cleared subject? □ Yes □ No

3. EKG

Page 1 of 2
a. Has an EKG been done on the subject? □ Yes □ No

b. Has the EKG been reviewed by the GCRC clinician? □ Yes □ No

c. Is subject cleared to participate in the study? □ Yes □ No

4. Has a urine sample been collected for analysis? □ Yes □ No

Is subject still eligible? □ Yes □ No

If yes, subject needs to be scheduled for Clinic Visit 3 that will take place at the GCRC. Clinic Visit 3 will be an additional blood pressure measurement and a physical done by the GCRC clinician. Remind subject not to eat, exercise, consume caffeine containing foods or beverages, or take any medication 2 hours before their appointment time.

Date: ________________ Time: ________________

If no, subject needs to be informed and a follow-up done i.e., test results sent to subject and/or their private physician if they authorize it.

a. Has a follow-up been done? □ Yes □ No

Comments:
APPENDIX E

Penn-BP Study

Clinic Visit 3 Form

Subject ID: B P __ __ __

Date: __ __ / __ __ / __ __

Reviewer’s Initials: __ __ __

1. Seated Blood Pressure Measurement

Has the subject consumed caffeine, used any medication, eaten any food and/or exercised in the past 2 hours? □ Yes □ No

If yes, subject needs to be rescheduled for a blood pressure measurement.

Instructions: Measure the subject’s arm circumference and choose the appropriate blood pressure cuff. After applying the cuff, the subject must be quiet and remain continuously seated without legs crossed for 5 minutes. Wait 1 minute after each reading before taking the next reading.

a. First blood pressure measurement: __ __ __ / __ __ __ (SBP/DBP)

b. Second blood pressure measurement: __ __ __ / __ __ __

c. Third blood pressure measurement: __ __ __ / __ __ __

d. Average (2nd and 3rd) blood pressure measurement: __ __ __ / __ __ __

e. Is the avg SBP 140-159 mmHg and/or DBP 90-99 mmHg □ Yes □ No

2. Has a physical been done by the GCRC clinician? □ Yes □ No

Is subject eligible? □ Yes □ No

If no, subject needs to be informed and a follow-up done i.e., test results sent to subject and/or their private physician if the subject authorizes it.

Has a follow-up been done? □ Yes □ No

If yes, subject is eligible for the study.

Have they been informed of the start date for the study? □ Yes □ No

Comments:
INFORMED CONSENT FORM FOR CLINICAL RESEARCH STUDY

Title of Project: Effects of Dietary Pattern on Blood Pressure and Vascular Reactivity

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Email: njz105@psu.edu
Kirsten Clemmer, PhD Graduate Student
814-863-2786
Email: kfc107@psu.edu

This is to certify that I, ________________________________ (print your name), have been given the following information regarding my participation as a volunteer in a program of investigation under the supervision of Drs. Kris-Etherton and West.

This consent form may contain words you do not understand. Please ask the study personnel to explain any words or information you do not clearly understand.
Purpose of the Study

I have been invited to participate in a clinical research study to test the effects of different dietary patterns on blood pressure and blood vessel health. The researchers are trying to understand the unique contribution of various foods to the blood pressure reductions seen in the original DASH (Dietary Approaches to Stop Hypertension) study. This new study may provide important information about the health effects of low-fat dairy products, fruits and vegetables on blood pressure and blood vessels.

Procedures to be Followed

Screening

I understand if I agree to participate in this study my participation will last for 19 weeks total. There will be three, 5-week diet periods and two, 2-week breaks between diet periods. Each of the diet periods will have some different foods in them but the menus are basically the same in each diet period. I will be given all of my food to consume during the three, 5-week diet periods. If I decide to participate in the study and I am eligible after the telephone screening, I will be further screened during a visit to the General Clinical Research Center (GCRC) at Penn State to determine eligibility to participate. The first visit will consist of filling out forms (medical history, eating attitudes test, personal information); measuring height and weight so my body mass index (BMI) can be calculated; measuring blood pressure (BP); and prickling my finger to obtain a drop of blood to measure blood fats (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides). If after these measurements, it is determined I am still eligible, a blood sample will be taken from my arm for a blood test for a complete blood count, to check liver, kidney and thyroid gland function and a blood fat panel. I will be contacted within 3-5 days with the results of the screening blood sample. If the blood tests find that I am still eligible for the study, I will be scheduled for a blood pressure measurement, a check for postural hypotension (low blood pressure when you change from laying down to standing), an electrocardiogram (EKG) to check my heart and a urine sample will be collected for analysis. For the EKG, I will have 10 adhesive sensors placed on my chest, arms and legs to measure how my heart is working. This visit will take approximately 1 hour. If I meet all of the study criteria and still wish to participate in the study, I will be asked to come to the GCRC one more time for a blood pressure measurement and a physical to be done by the GCRC clinician. The third visit to the GCRC should take about ½ hour. If I am still eligible for the study, I will be told when to report for the beginning of the first feeding period. There will be no charge for the screening blood work, urine analysis, EKG and physical.

Feeding Study

I will agree to eat only those foods (3 meals and a snack every day) and beverages provided to me (some non-caloric beverages are allowed for free choice) during the feeding periods of the study. I will come to the PSU Metabolic Diet Study Center (MDSC) dining room (224 North Henderson) Monday through Friday for breakfast or dinner, where meals will be prepared and provided for me. My other two meals and a snack will be packed for me to take and eat at a place of convenience. On Friday evenings, I will be given a cooler that contains my Friday dinner and Saturday and Sunday meals and snacks. I will be required to appropriately refrigerate and store all foods provided to me for take-out (it may be possible to work out a schedule of food pick-up just 2-3 days per week at Henderson to eliminate the daily appointment).

I will be weighed regularly either at my meal time in the Diet Center on Campus or at my home when meals are delivered and I will provide the staff dietitian with information about any non-study foods I may have eaten, any study foods not eaten and caffeine (limited to five, caffeine-containing beverages/day) and alcohol consumption (limited to 2 drinks/week). I am supposed to eat only the foods given me and nothing else. I must eat all of the food given me. If for some reason I fail to do this, it is important that I tell the
study staff that I did not follow protocol so they can make a note of it in my records. The information I provide to the study coordinators will be collected on two separate forms; one to be completed daily and one to be completed weekly. It should only take about 5 minutes to complete these forms each day. My calorie intake may be adjusted over the course of the study in order to maintain my screening body weight. I understand that this is not a weight-loss study. The diets are designed to meet my calorie needs and keep my body weight constant. Calorie intake will be adjusted up or down as necessary to maintain my weight. Also, I understand that I must keep my exercise level constant throughout the whole study.

My blood pressure will be measured by taking the average of three measurements, taken 2 minutes apart after a 10-minute rest period. Before each blood pressure measurement session, I will complete a form (Pre-Session Questionnaire) that will help the researchers measure different factors that may affect my blood pressure. During the first week of each diet period, my blood pressure will be checked 5 times. My blood pressure will be measured once/week during weeks 2, 3 and 4 and 4 times during the last 7 days of each diet period. If, for any reason my blood pressure goes over 160 systolic blood pressure (upper blood pressure number) and/or 100 diastolic blood pressure (lower blood pressure number) on two successive measurement days, I will be dropped from the study and referred to my private physician for immediate follow-up. This is done for my own protection.

**Endpoint Testing**

**Blood sampling:**
At the end of each diet period, after a twelve hour fast (consumption of no food or drinks except water), a blood sample will be taken from my arm on two consecutive days before breakfast. This will be done at the General Clinical Research Center on PSU campus. I understand that I cannot drink alcohol during the 48 hours prior to having my blood taken and that I cannot engage in vigorous physical activity 24 hours prior to having my blood taken. Approximately 150 ml (about 10 tablespoons) of blood will be collected at the end of each diet period (75 mls on two consecutive days). Therefore, over the 19-week study, blood will be taken on six days. In addition, my finger will be pricked to obtain a drop of blood at the end of each diet period to measure blood fat. Blood samples will be frozen and analyzed at the end of the study (when all 25 subjects have completed the study). At the end of your participation, you will receive your blood fat results for the 3 diet periods and the results will be explained. The other results of the study will only be available at the end of the entire study (which may take up to 2 years). My blood will be tested for the following: renin, angiotensin, aldosterone, vaspressin and ACE activity (blood pressure regulating substances in blood), cell adhesion molecules (substances in the blood that make other things stick to the sides of the blood vessels), blood fats (total cholesterol, LDL-cholesterol, HDL-cholesterol), electrolytes (calcium, potassium, sodium, phosphorus), and calcium-regulating hormones.

**Blood pressure:**
At the end of each diet period my blood pressure will be measured three times at four visits for a total of 12 separate measurements. This will be done at the Henderson Building on campus. I cannot engage in vigorous exercise and cannot ingest food or caffeine within 2 hours of the blood pressure measurements. Each set of blood pressure measurements should take about 20 minutes.

**Blood vessel function:**
Flow mediated dilation (FMD) will be measured at the end of each diet period at the General Clinical Research Center. FMD is a noninvasive procedure designed to measure the size and blood flow in a blood vessel (in the arm) before and after a standardized stimulus known to produce dilation (opening up) in healthy arteries. The procedure is as follows:

1. I will lie quietly on a hospital bed in a quiet, darkened room.
2. My right arm will be extended straight out from my shoulder and held in position using styrofoam cushions. I will be asked to hold a beanbag in my outstretched hand to avoid movement of my hand and fingers. A blood pressure cuff will be put on my forearm.
3. A technician, trained in ultrasound techniques, will sit at the head of the bed. The technician will place some ultrasound gel on my right arm and will place an ultrasound probe (which
looks like a microphone) on that arm. An image of the blood vessels in my arm will be viewed on the ultrasound equipment next to the bed.

4. The technician will continue to produce the ultrasound image for about 30 seconds and may need to move the probe over a small area of my arm to obtain the clearest image. Then the blood pressure cuff on my right arm will be inflated to approximately 50 mmHg greater than my resting systolic blood pressure (upper blood pressure number) and will not be inflated to more than 210 mmHg. During the next five minutes, while the cuff remains inflated, the technician will continue to capture and record the image. At the end of 5 minutes, the cuff will be deflated and images will continue to be captured and recorded for an additional 2 minutes. It is very important through all of this that I keep my arm as still as possible.

5. For the second measurement, nitroglycerin will be given to me (a small, fast dissolving tablet placed under my tongue containing 0.4 mg of nitroglycerin) and the image of the artery will be taken before, during and after the administration of the nitroglycerin. I will be asked to remain lying down for an additional 10 minutes. I will not be allowed to leave until 30 minutes have passed since I was given the nitroglycerin. This is for my own protection. These procedures should take about 1 hour. I cannot consume alcohol 48 hours before the day I am tested for FMD. I will be asked to fill out forms prior to the FMD testing (Pretest Questionnaire for Ultrasound Visit, Profile of Mood States, Depression Questionnaire, Perceived Stress Scale) to help the researchers interpret the results of my tests.

Cardiac Output:
Cardiac output, a test frequently done in a doctor’s office, will be done with me lying down on my side in a hospital bed. This test will be done at the same time as the FMD (see above) ultrasound test. I will be dressed in a hospital gown that opens in the front to allow access by the ultrasound technician to my chest. If I am female, another female will always be present in the room while these measurements are being done. I will have 3 adhesive patches attached to my chest, arms and legs for an electrocardiogram (EKG). The EKG will record how my heart works during this test. Blood pressure will be measured every five minutes. The ultrasound technician will sit close to the bed and will apply gel to my chest and then place a probe on my chest wall and move it around until a clear image of my aorta (the large blood vessel leaving the heart) is visible. The ultrasound image of my heart will be taken at various angles to measure and calculate the amount of blood that my heart pumps in a given time. This procedure should take about 30 minutes.

24-Hour Urine Collection:
At the end of each diet period, I will collect my urine for an entire 24-hour period. To do this, I will get up in the morning, go to the bathroom and discard my first urine of the day but record this time as my starting time. I will collect my urine for the rest of the day and night until the next morning in a 3-liter bottle that I am given by the study staff. I will keep the urine cool in the cooler with ice packs that I am given. I will record my stop and start times on the instruction sheet I am given and return the sample on one of the mornings when I come in to have my blood taken. My urine will be tested for electrolytes (calcium, potassium, sodium, phosphorus) and stress hormones.

Successful completion of this study depends on the total cooperation of the participants. If during the study, I cannot eat the foods provided and/or eat other foods, I will be asked to leave the study. If I do not complete the study, for whatever reason, the free food and screening blood assays will be considered the compensation for my participation up to that point.
Discomforts and Risks

Feeding Study

The diets used in this study are nutritionally adequate, whole-food diets. Foods will be prepared according to accepted standards of sanitation and provisions are made to ensure the safety of foods provided for off-site consumption. However, it is possible that incorrect food handling during shipping, storage or preparation, if not detected, could result in food-borne illness. Every effort will be made to safeguard against this possibility. Feeding studies that require on-site eating of meals and strict adherence to the diets provided may interfere with social activities centered around eating such as dining in restaurants. While the menus will provide some variety in the diets, the number of food items will be more limited than that available in an average grocery store. The limited variety may become boring over the course of the study.

If I choose option #2 for having meals delivered to my home, I must be present and have a study staff person come into my home to deliver the food, answer questions, and check my weight and blood pressure. Having to be present at preset times 2-3 days per week at my home may be inconvenient to me and I will need to be comfortable having study staff come into my home. All staff people will be Penn State employees and will be part of the research team of Dr. Penny Kris-Etherton or Dr. Sheila West.

Blood Sampling

The risks involved with taking blood from me include some local pain and bruising where the blood is taken. Well-trained and experienced phlebotomists will be used to take my blood. Blood sampling can also cause light-headedness and dizziness. If this occurs, the symptoms will be alleviated by having me lie flat with my feet raised. As with any procedure involving taking blood, infection is possible. All precautions will be taken to avoid infection.

Blood Pressure

I understand that there may be some discomfort from the inflation of the blood pressure cuff on my arm. There is a possibility of petechiae (red blotching) occurring in the skin in the area of where the blood pressure cuff is placed and below the cuff. This condition looks like a small area of redness or bruising which will disappear within 2-3 days.

Electrocardiogram (EKG)

There is a risk of mild discomfort with this technique, primarily related to the removal of the adhesive sensors. Redness at the site where the sensors have been placed is common, but temporary. It is possible that I could be allergic to the adhesive used in the sensors.

Urine Collections

The collection of a 24-hour urine sample will require me to carry a small cooler equipped with ice packs and a 3-liter brown plastic bottle. This may cause embarrassment for me and every effort has been made by the study staff to have me do the urine collections on days that are most convenient for me.

Cardiac Output

There is no known risk or discomfort associated with ultrasound imaging at the frequency we are using. As with an EKG procedure, the sensors may cause some redness and irritation. This procedure may cause discomfort and embarrassment for some women while the technician moves the probe around the chest area in order to get an acceptable image. Every effort will be made to minimize the discomfort and embarrassment. A female will be present in the room when this procedure is being done to a female subject.
Flow Mediated Dilation (FMD)

There is a possibility of petechiae (red blotching) occurring in the skin in the area of where the blood pressure cuff is placed and below the cuff. It is also likely that my hand and arm below the blood pressure cuff will experience a “tingly” feeling when the blood pressure cuff is released (the feeling of my hand and lower arm waking up after “falling asleep”). During the five minutes that the blood pressure cuff is inflated, my arm could become numb. This might be moderately painful. Nitroglycerin may be associated with headache, flushing, the transient lowering of the blood pressure, vertigo (sensation of spinning), palpitation (irregular heart beat), weakness, nausea, vomiting, sweating and fainting. These effects are usually short-lived and will be minimized by having me lie down for ten minutes after receiving the nitroglycerin and being monitored by the study staff for an additional 20 minutes. As with any medication, nitroglycerin may cause an allergic reaction, such as a rash, in some individuals. The use of nitroglycerin for the FMD procedure is not an approved use of this drug by the Food and Drug Administration (FDA). However, nitroglycerin has been used in many other similar research studies without any problem. In addition, nitroglycerin is a common drug that is prescribed for heart patients who have, or are at risk for, angina (heart pain). In addition, women on hormone replacement therapy have an increased risk of developing blood clots and stopping of the blood flow in the arm may increase this risk. However, to our knowledge, there have been no actual cases of blood clots reported in studies involving stopping of the blood flow in the arm.

Benefits to Me

I will be fed a nutritionally adequate diet for the three, 5-week diet periods. During this time I will receive all my food free of cost. I will have a chance to learn the principles of good nutrition practices. I will also receive the results of my screening blood work and information about how my blood cholesterol and blood vessel reactivity changed in response to the experimental diets. At the end of the study it will be explained how the diets are manipulated and how I can implement these changes into my own diet should I wish to continue eating this type of diet. However, no benefit from participation in this study is guaranteed.

Potential Benefits to Society

It is hoped that the information gained from this study will increase our understanding of the effects of dietary pattern on blood pressure and heart disease risk factors.

Statement of Confidentiality

I understand my participation in this research is confidential. All records are coded with a unique ID number and no names are used. Records containing names or other identifying information are kept under lock at the Metabolic Diet Study Center and the Vascular Health Lab. Only the investigators and their assistants will have access to my identity and to information that can be associated with my identity. All records associated with my participation in the study will be subject to the usual confidentiality standards applicable to medical records. In the event of publication of this research, no personally identifying information will be disclosed. My blood specimens and ultrasound imaging videotapes will be coded with my unique ID number and will be maintained until three years after the date from when the study is published, and then destroyed. At the end of the study (after all 50 subjects have completed the study), I will be given my laboratory results without cost, informed of the study results, and advised of the implications for my future care.

Right to Ask Questions

I have been given an opportunity to ask any questions I may have, and all such questions have been answered to my satisfaction. I understand that Drs. Kris-Etherton and West are available to answer any questions that I have at the time of my participation in this study or if I have questions in the future. I will be informed of any new information that may affect my willingness to participate. I may call the Office for Research Protections (814-865-1775) if I need further information about my rights as a research participant.
Compensation
I will receive all of my food at no cost to me for the three, 5-week feeding periods. For my time and participation in the study I will receive monetary compensation of $400.00 which will be given to me after completing the entire study. I understand that if I am an employee of Penn State University the compensation I receive is treated as taxable income and therefore taxes will be taken from the total compensation amount. I understand that if I am not employed by Penn State University I am required, by law, to claim the compensation I receive for participation in this study as taxable income. If I do not complete the study, for whatever reason, my free food and screening blood results will be considered the compensation for my participation up to that point.

Injury Statement
I understand that medical care is available in the event of injury resulting from research but that neither financial compensation nor free medical treatment is provided. I also understand that I am not waiving any rights that I may have against the University for injury resulting from negligence of the University or the investigators. For further information about this, I may call the Office of Research Protections at 814-865-1775.

Voluntary Participation
I understand my participation in this study is voluntary, that I may decline to answer any questions during the screening process or during the study. I am aware that refusing to answer a question may keep me from being able to participate in the study. I understand that I may withdraw from this study at any time by notifying the investigators or other study personnel. My withdrawal from this study or my refusal to participate will in no way affect my care or access to medical services. I understand that I may be asked to leave the study at any time if I do not comply with the study protocol.

In the event that abnormal lab test results are obtained during the initial screening or subsequently throughout the study, I will be informed as quickly as possible of these results and instructed to contact my private physician for further assessment. The lab test results will be made available to my private physician at my request.

This is to certify that I consent to and give my permission for my participation as a volunteer in the study entitled “Effects of a Dietary Pattern on Blood Pressure and Vascular Reactivity”. I certify that I am 18 years of age or older. I understand I will receive a signed copy of this consent form. I have read this form and understand the contents of this consent form.

Signature of Volunteer  Date

Printed Name of Volunteer

I, the undersigned, have defined and explained the study involved to the above volunteer.

Signature of Investigator  Date
APPENDIX G

Sample menus for each diet – 2100 kcal

1. Average American Diet

<table>
<thead>
<tr>
<th></th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
</tr>
<tr>
<td>Plain Bagel, Lender’s</td>
<td>82.5</td>
</tr>
<tr>
<td>Cream Cheese-Regular, Philadelphia</td>
<td>16.8</td>
</tr>
<tr>
<td>Hawaiian Punch</td>
<td>225</td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td></td>
</tr>
<tr>
<td>Cooked Macaroni, Mueller’s</td>
<td>163</td>
</tr>
<tr>
<td>Shrimp, boiled</td>
<td>49</td>
</tr>
<tr>
<td>Celery, diced</td>
<td>35</td>
</tr>
<tr>
<td>Tomato, cherry</td>
<td>52</td>
</tr>
<tr>
<td>Broccoli, fresh</td>
<td>72</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>20</td>
</tr>
<tr>
<td>Table Salt</td>
<td>3</td>
</tr>
<tr>
<td>Egg Yolk, cooked</td>
<td>2.8</td>
</tr>
<tr>
<td>BP Study – Shrimp Pasta Salad Dressing</td>
<td>15</td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td></td>
</tr>
<tr>
<td>Chicken Breast, raw</td>
<td>153</td>
</tr>
<tr>
<td>Butterball Turkey Smoked Sausage-Lean</td>
<td>32.5</td>
</tr>
<tr>
<td>BP Study – Jambalaya</td>
<td>174</td>
</tr>
<tr>
<td>Butter</td>
<td>20</td>
</tr>
<tr>
<td>Table Salt</td>
<td>1</td>
</tr>
<tr>
<td>Dinner Roll, Pepperidge Farm</td>
<td>28</td>
</tr>
<tr>
<td>Butter</td>
<td>10</td>
</tr>
<tr>
<td>Strawberries, sliced</td>
<td>120</td>
</tr>
<tr>
<td>Pound Cake</td>
<td>78</td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
</tr>
<tr>
<td>Chocolate Chip Cookie, Keebler Minichips Deluxe</td>
<td>46.5</td>
</tr>
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</table>
2. Fruits and Vegetables Diet

<table>
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<tr>
<th>Breakfast</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Bagel, Lender’s</td>
<td>85</td>
</tr>
<tr>
<td>Cream Cheese-Lowfat, Philadelphia</td>
<td>16.8</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>220</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Wheat Macaroni</td>
<td>175</td>
</tr>
<tr>
<td>Shrimp, boiled</td>
<td>49</td>
</tr>
<tr>
<td>Celery, diced</td>
<td>70</td>
</tr>
<tr>
<td>Tomato, cherry</td>
<td>105</td>
</tr>
<tr>
<td>Broccoli, fresh</td>
<td>51</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>21.5</td>
</tr>
<tr>
<td>Table Salt</td>
<td>2.4</td>
</tr>
<tr>
<td>Egg Yolk, cooked</td>
<td>9.2</td>
</tr>
<tr>
<td>BP Study – Shrimp Pasta Salad Dressing</td>
<td>15</td>
</tr>
<tr>
<td>Baby Carrots</td>
<td>56</td>
</tr>
<tr>
<td>Dinner Roll, Pepperidge Farm</td>
<td>23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Breast, raw</td>
<td>148</td>
</tr>
<tr>
<td>Butterball Turkey Smoked Sausage-Lean</td>
<td>33</td>
</tr>
<tr>
<td>BP Study - Jambalaya</td>
<td>210</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>19.5</td>
</tr>
<tr>
<td>Table Salt</td>
<td>2.2</td>
</tr>
<tr>
<td>Dinner Roll, Pepperidge Farm</td>
<td>23</td>
</tr>
<tr>
<td>Strawberries, sliced</td>
<td>87</td>
</tr>
<tr>
<td>Blueberries</td>
<td>87</td>
</tr>
<tr>
<td>Pound Cake</td>
<td>39</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pears, canned in juice</td>
<td>232</td>
</tr>
<tr>
<td>Cracklin Oat Bran Cereal, Kellogg’s</td>
<td>23</td>
</tr>
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</table>
3. Dairy-Fruits and Vegetables Diet

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<thead>
<tr>
<th>Breakfast</th>
<th>Grams</th>
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</thead>
<tbody>
<tr>
<td>Plain Bagel, Lender’s</td>
<td>75</td>
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<tr>
<td>Cream Cheese-Lowfat, Philadelphia</td>
<td>16.8</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>245</td>
</tr>
<tr>
<td>Apple Juice</td>
<td>220</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Lunch</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Wheat Macaroni</td>
<td>138</td>
</tr>
<tr>
<td>Shrimp, boiled</td>
<td>33</td>
</tr>
<tr>
<td>Celery, diced</td>
<td>70</td>
</tr>
<tr>
<td>Tomato, cherry</td>
<td>105</td>
</tr>
<tr>
<td>Broccoli, fresh</td>
<td>51</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>25</td>
</tr>
<tr>
<td>Table Salt</td>
<td>1.6</td>
</tr>
<tr>
<td>Egg Yolk, cooked</td>
<td>15.7</td>
</tr>
<tr>
<td>BP Study – Shrimp Pasta Salad Dressing</td>
<td>15</td>
</tr>
<tr>
<td>Baby Carrots</td>
<td>56</td>
</tr>
<tr>
<td>Dinner Roll, Pepperidge Farm</td>
<td>23</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>245</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Breast, raw</td>
<td>68</td>
</tr>
<tr>
<td>Butterball Turkey Smoked Sausage-Lean</td>
<td>33</td>
</tr>
<tr>
<td>BP Study – Jambalaya</td>
<td>145</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>16</td>
</tr>
<tr>
<td>Table Salt</td>
<td>2.5</td>
</tr>
<tr>
<td>Dinner Roll, Pepperidge Farm</td>
<td>23</td>
</tr>
<tr>
<td>Strawberries, sliced</td>
<td>87</td>
</tr>
<tr>
<td>Blueberries</td>
<td>87</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>245</td>
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</table>

<table>
<thead>
<tr>
<th>Snack</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pears, canned in juice</td>
<td>232</td>
</tr>
<tr>
<td>Cracklin Oat Bran Cereal, Kellogg’s</td>
<td>45</td>
</tr>
</tbody>
</table>
APPENDIX H

PennBP Study
DAILY MONITORING FORM

Subject ID: BP ___ ___
Today’s Date: __/__/__
Reviewer’s Initials: ________

To be completed by study staff:

Week: DP1 – 1 2 3 4 5 DP2 - 1 2 3 4 5 DP3 – 1 2 3 4 5

1. Past 24-hour caloric intake: 1800 2100 2400 2700 3000
2. Past 24-hour unit food intake: __________________

Participants, please complete the remainder of this form at the end of each day, based on the past 24 hours (or based on the past weekend if today is Monday).

3. Did you consume any caffeine containing beverages? □ Yes □ No
   If Yes, specify: description (e.g. coffee)
   ____________________________________________________________________
   ____________________________________________________________________
   ____________________________________________________________________
   ____________________________________________________________________
   ____________________________________________________________________

4. Did you drink any alcoholic beverages? □ Yes □ No
   If Yes, specify type and amount: _____________________________ ____________oz
   ____________________________________________________________________

5. Did you fail to eat/drink any study foods/drinks? □ Yes □ No
   If Yes, specify: description amount (oz or part of portion)
   ____________________________________________________________________

6. Did you eat/drink any non-study foods/drinks? □ Yes □ No
   If Yes, specify: description amount (oz or part of portion)
   ____________________________________________________________________

Page 1 of 2
7. How many ounces of caffeine-free beverages (water, diet/caffeine free soda, etc.) did you drink?  __ __ oz

8. Did you consume any sodium-containing foods, beverages or condiments?  
Please list below. Description: amount (oz or portion size)

_________________________________________________

_________________________________________________

_________________________________________________

Total mg sodium ______________________

9. Did you take any medications?  □ Yes   □ No
a. If Yes, specify: Description (e.g. Aspirin) Amount Reason

________________________  ____________ ____________________

________________________  ____________ ____________________

________________________  ____________ ____________________

10. Comments?
APPENDIX I

PennBP Study
WEEKLY MONITORING FORM

Subject ID: BP __ __ __
Today’s Date: _ _/ _ _/__
Reviewer’s Initials: _______

To be completed by study staff:

<table>
<thead>
<tr>
<th>Week</th>
<th>DP1 – 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>DP2 – 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>DP3 – 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
</table>

1. In the past week has your exercise level changed? □ Yes □ No
   If Yes, was it:
   □ More Active
   □ Less Active
   □ No Exercise

Please remember to keep your exercise level constant throughout the study.

2. Have you taken any prescription or non-prescription drugs in the past week? □ Yes □ No
   If Yes, specify: description
   amount
   ___________________________________________________________

3. Have you taken any vitamins, minerals or other supplements in the past week? □ Yes □ No
   If Yes, specify: description
   amount
   ___________________________________________________________

4. Have you been ill in the past week? □ Yes □ No
   If Yes, describe illness: ________________________________________

5. If you were ill in the past week, did your eating change as a result? □ Yes □ No
   If Yes, describe: ______________________________________________
APPENDIX J

Penn-BP Study
Blood Pressure Measurement

Subject ID ______________
Diet period: DP1 DP2 DP3
Diet period starts on ____________
(day of the week)

SAFETY CHECK Measurements

Instructions: Measure the circumference of the subject’s right arm and chose the appropriate cuff. After applying the cuff, the subject must rest and remain continuously seated without legs crossed for 5 minutes. Take two blood pressure measurements with the Omron automated monitor.

Arm measurement _____ cm  Cuff Size: Adult (24-32cm)___  Large Size (33-41cm)___

<table>
<thead>
<tr>
<th>Week 1, Day 1</th>
<th>Date __ __/ __ __/ __ __</th>
<th>Equipment: Omron# ______</th>
<th>Technician Initials __ __ __</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time: ________/<strong><strong>/</strong></strong> AM PM</td>
<td>First blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg (SBP/DBP)</td>
<td>Second blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg</td>
<td>Comments: ________________________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 1, Day 2</th>
<th>Date __ __/ __ __/ __ __</th>
<th>Equipment: Omron# ______</th>
<th>Technician Initials __ __ __</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time: ________/<strong><strong>/</strong></strong> AM PM</td>
<td>First blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg (SBP/DBP)</td>
<td>Second blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg</td>
<td>Comments: ________________________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 1, Day 3</th>
<th>Date __ __/ __ __/ __ __</th>
<th>Equipment: Omron# ______</th>
<th>Technician Initials __ __ __</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time: ________/<strong><strong>/</strong></strong> AM PM</td>
<td>First blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg (SBP/DBP)</td>
<td>Second blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg</td>
<td>Comments: ________________________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 1, Day 4</th>
<th>Date __ __/ __ __/ __ __</th>
<th>Equipment: Omron# ______</th>
<th>Technician Initials __ __ __</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time: ________/<strong><strong>/</strong></strong> AM PM</td>
<td>First blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg (SBP/DBP)</td>
<td>Second blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg</td>
<td>Comments: ________________________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week 1, Day 5</th>
<th>Date __ __/ __ __/ __ __</th>
<th>Equipment: Omron# ______</th>
<th>Technician Initials __ __ __</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time: ________/<strong><strong>/</strong></strong> AM PM</td>
<td>First blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg (SBP/DBP)</td>
<td>Second blood pressure measurement: _<strong><strong>/</strong></strong> mm Hg</td>
<td>Comments: ________________________________________________</td>
</tr>
</tbody>
</table>
First blood pressure measurement: __ __ __/ __ __ __ mm Hg (SBP/DBP)
Second blood pressure measurement: __ __ __/ __ __ __ mm Hg

Comments: ____________________________________________________________

**Week 2** Day of the week? ______ Date __ __/ __ __/ __ __ Equipment: Omron#

Time: __ __/ __ __ AM PM 

Technician Initials __ __

First blood pressure measurement: __ __ __/ __ __ __ mm Hg (SBP/DBP)
Second blood pressure measurement: __ __ __/ __ __ __ mm Hg

Comments: ____________________________________________________________

**Week 3** Day of the week? ______ Date __ __/ __ __/ __ __ Equipment: Omron#

Time: __ __/ __ __ AM PM 

Technician Initials __ __

First blood pressure measurement: __ __ __/ __ __ __ mm Hg (SBP/DBP)
Second blood pressure measurement: __ __ __/ __ __ __ mm Hg

Comments: ____________________________________________________________

**Week 4** Day of the week? ______ Date __ __/ __ __/ __ __ Equipment: Omron#

Time: __ __/ __ __ AM PM 

Technician Initials __ __

First blood pressure measurement: __ __ __/ __ __ __ mm Hg (SBP/DBP)
Second blood pressure measurement: __ __ __/ __ __ __ mm Hg

Comments: ____________________________________________________________

**Alert Readings for BP**
If during any stethoscopic blood pressure reading, SBP is 161-179 or DBP is 101-109, please alert Dr. West. Subjects should come back within 4 days to be checked again.

If SBP is greater than or equal to 180, or DBP is greater than or equal to 110, the patient needs to be referred to the GCRC physician and Dr. West should be notified.
## APPENDIX K

Diet-related changes in additional study variables according to group, n=18

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>AAD</th>
<th>F&amp;V</th>
<th>D-F&amp;V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-C</td>
<td>Stable</td>
<td>196.9 ± 6.3</td>
<td>187.5 ± 6.3</td>
<td>191.7 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>195.8 ± 9.0</td>
<td>180.9 ± 9.2</td>
<td>181.4 ± 9.0</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Stable</td>
<td>128.1 ± 7.0</td>
<td>121.6 ± 7.0</td>
<td>124.3 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>124.4 ± 10.4</td>
<td>112.7 ± 10.5</td>
<td>112.2 ± 10.4</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Stable</td>
<td>46.4 ± 2.1</td>
<td>43.0 ± 2.1</td>
<td>42.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>47.8 ± 3.1</td>
<td>45.8 ± 3.1</td>
<td>47.1 ± 3.1</td>
</tr>
<tr>
<td>TG</td>
<td>Stable</td>
<td>108.6 ± 11.9</td>
<td>120.7 ± 11.8</td>
<td>128.5 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>97.8 ± 17.7</td>
<td>92.2 ± 17.9</td>
<td>92.6 ± 17.7</td>
</tr>
<tr>
<td>Total:HDL-C</td>
<td>Stable</td>
<td>4.5 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>4.2 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>LDL:HDL-C</td>
<td>Stable</td>
<td>2.9 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>TG:HDL-C</td>
<td>Stable</td>
<td>2.6 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>2.4 ± 0.4</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>FMD</td>
<td>Stable</td>
<td>4.8 ± 1.1</td>
<td>6.3 ± 1.1</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>4.7 ± 1.5</td>
<td>4.0 ± 1.6</td>
<td>4.9 ± 1.5</td>
</tr>
<tr>
<td>MAP</td>
<td>Stable</td>
<td>100.8 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.4 ± 2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.1 ± 2.6&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>101.4 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.8 ± 3.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>96.3 ± 3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TPR</td>
<td>Stable</td>
<td>1484.9 ± 166.7</td>
<td>1465.2 ± 167.0</td>
<td>1619.3 ± 168.5</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>1722.7 ± 234.6</td>
<td>1462.2 ± 240.0</td>
<td>1518.6 ± 240.0</td>
</tr>
<tr>
<td>CO</td>
<td>Stable</td>
<td>5.0 ± 0.3</td>
<td>4.8 ± 0.3</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>4.3 ± 0.4</td>
<td>5.0 ± 0.4</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Stable</td>
<td>610.3 ± 57.5</td>
<td>577.8 ± 57.3</td>
<td>586.0 ± 56.9</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>675.1 ± 76.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>738.9 ± 81.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>428.2 ± 77.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Stable</td>
<td>249.5 ± 21.3</td>
<td>226.4 ± 21.2</td>
<td>217.4 ± 21.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>227.5 ± 30.8</td>
<td>222.1 ± 31.1</td>
<td>215.4 ± 30.8</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Stable</td>
<td>144.0 ± 15.5</td>
<td>116.9 ± 15.5</td>
<td>94.9 ± 15.4</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>153.0 ± 21.5</td>
<td>147.8 ± 22.3</td>
<td>137.7 ± 21.6</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Stable</td>
<td>34.7 ± 4.2</td>
<td>32.3 ± 4.2</td>
<td>29.6 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>25.1 ± 6.1</td>
<td>23.8 ± 6.2</td>
<td>22.8 ± 6.1</td>
</tr>
<tr>
<td>Serum Ca</td>
<td>Stable</td>
<td>9.4 ± 0.1</td>
<td>9.5 ± 0.1</td>
<td>9.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>9.1 ± 0.2</td>
<td>9.3 ± 0.2</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>1,25(OH)₂VD³</td>
<td>Stable</td>
<td>Change 114.3 ± 9.7</td>
<td>107.8 ± 9.7</td>
<td>89.0 ± 9.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>109.6 ± 13.3</td>
<td>91.9 ± 13.9</td>
<td>83.6 ± 13.4</td>
</tr>
<tr>
<td>PTH</td>
<td>Stable</td>
<td>53.3 ± 11.1</td>
<td>47.2 ± 11.3</td>
<td>55.6 ± 11.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>42.1 ± 7.8</td>
<td>45.4 ± 7.8</td>
<td>38.7 ± 7.8</td>
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<tr>
<td>Calcitonin</td>
<td>Stable</td>
<td>11.4 ± 1.2</td>
<td>11.0 ± 1.2</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>7.8 ± 1.8</td>
<td>7.9 ± 1.8</td>
<td>7.7 ± 1.8</td>
</tr>
<tr>
<td>Renin activity</td>
<td>Stable</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>0.6 ± 0.4</td>
<td>0.9 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>ACE activity⁴</td>
<td>Stable</td>
<td>34.7 ± 2.7</td>
<td>37.2 ± 2.7</td>
<td>29.9 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>44.8 ± 3.8</td>
<td>45.1 ± 3.9</td>
<td>49.2 ± 3.8</td>
</tr>
<tr>
<td>Angiotensin II⁵</td>
<td>Stable</td>
<td>9.1 ± 2.2</td>
<td>8.1 ± 2.1</td>
<td>5.4 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>10.9 ± 3.0</td>
<td>8.5 ± 3.1</td>
<td>5.2 ± 3.0</td>
</tr>
<tr>
<td>24-hr urinary Ca</td>
<td>Stable</td>
<td>124.1 ± 22.0</td>
<td>116.4 ± 22.0</td>
<td>141.3 ± 21.9</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>97.2 ± 31.3</td>
<td>71.6 ± 32.0</td>
<td>91.9 ± 32.0</td>
</tr>
<tr>
<td>24-hr urinary Na</td>
<td>Stable</td>
<td>3620 ± 444</td>
<td>3751 ± 442</td>
<td>3648 ± 439</td>
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<td></td>
<td>Change</td>
<td>3669 ± 593.4</td>
<td>3202 ± 633</td>
<td>3666 ± 628</td>
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<tr>
<td>24-hr urinary K⁵</td>
<td>Stable</td>
<td>2282 ± 386</td>
<td>2644 ± 386</td>
<td>3143 ± 386</td>
</tr>
<tr>
<td></td>
<td>Change</td>
<td>2161 ± 542</td>
<td>2165 ± 562</td>
<td>3631 ± 558</td>
</tr>
</tbody>
</table>

¹ Diet effect, p < 0.004, AAD is higher than other two diets, when collapsed across groups.
² Diet by group interaction, p < 0.05. Within rows, letters with different superscripts are different. * Different from Stable group, p = 0.0167.
³ Diet effect, p < 0.04, D-F&V is lower than AAD, when collapsed across groups.
⁴ Group effect, p < 0.02, Change group is higher than Stable group, when collapsed across diets.
⁵ Diet effect, p < 0.001, D-F&V is higher than both the F&V and AAD diets, when collapsed across groups.
EDUCATION
Ph.D., Integrative Biosciences, Nutrition Science option, The Pennsylvania State University, University Park, PA, Anticipated completion date: December 2005

Research Assistant
Study of the effects of a dairy-rich diet on intracellular calcium dynamics in adults with hypertension.
Experience in design, implementation, and statistical interpretation of human feeding trials.

Teaching Assistant
Nutrition 453: Diet in Disease             Spring & Fall Semesters of 2003
Nutrition 251: Introduction to the Principles of Nutrition            Fall of 2001 & Spring of 2002

B.Sc., Dietetics with Chemistry minor, Indiana University of Pennsylvania, Indiana, PA, December 1999, summa cum laude

AWARDS
ADA Foundation’s Irene J. Jones Memorial Scholarship, 2005
Life Sciences Consortium Fellow, the Pennsylvania State University, 2000-2002
Indiana University of Pennsylvania-APSCUF scholarship and Provost scholar, 1998
ARAMARK scholarship, 1997

SELECTED PUBLICATIONS


