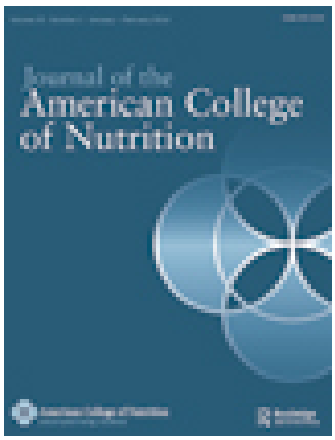

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# Effects of Dairy Products on Intracellular Calcium and Blood Pressure in Adults with Essential Hypertension

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Original Research

# Effects of Dairy Products on Intracellular Calcium and Blood Pressure in Adults with Essential Hypertension

**Kirsten F. Hilpert, PhD, Sheila G. West, PhD, Deborah M. Bagshaw, BS, Valerie Fishell, BS, Linda Barnhart, DO, Michael Lefevre, PhD, Marlene M. Most, PhD, Michael B. Zemel, PhD, Mosuk Chow, PhD, Alan L. Hinderliter, MD, and Penny M. Kris-Etherton, PhD**

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**Key words: dairy, milk, calcium, intracellular calcium, intracellular magnesium, vitamin D, hypertension**

**Background:** Consumption of dairy foods has been associated with lower blood pressure in certain populations.

**Objective:** This study examined the effects of dairy foods on blood pressure (BP) and intracellular calcium ($(Ca)_i$) and the dependence of BP changes on changes in $(Ca)_i$.

**Design:** Twenty-three stage 1 hypertensive adults were fed the following 3 experimental diets (5 wk each) in a randomized cross-over design study; a dairy-rich, high fruits and vegetables diet (D-F&V; 30% fat, 7% saturated fat (SFA), 3.4 servings/d dairy), a high fruits and vegetables diet (F&V; 30% fat, 7% SFA, 0.4 servings/d dairy), and an average Western diet (control; 36% fat, 15% SFA, 0.4 servings/d dairy). Systolic (SBP) and diastolic (DBP) BP, calcium regulatory hormones, and erythrocyte $(Ca)_i$ were determined.

**Results:** SBP and DBP were significantly reduced by ~2 mm Hg following both D-F&V and F&V diets vs. the control ($P < 0.05$). The D-F&V diet significantly lowered 1,25-dihydroxyvitaminD compared with the F&V and control diets ($P < 0.01$). Serum calcium, parathyroid hormone, calcitonin, and renin activity were unchanged. The D-F&V diet lowered $(Ca)_i$ vs. the other two diets ($P < 0.01$), and this change correlated with the fall in DBP ($r = 0.52$, $P < 0.05$). Subjects who responded to the D-F&V diet by significantly reducing $(Ca)_i$ exhibited significantly greater net decreases in DBP on the D-F&V vs. the F&V ($-2.8 \pm 1.0$ mm Hg) and control diets ($-5.4 \pm 1.0$ mm Hg; diet $\times$ group interaction, $P < 0.02$).

**Conclusion:** Consumption of dairy foods beneficially affects $(Ca)_i$, resulting in improved BP in a subgroup defined by $(Ca)_i$ response.

## INTRODUCTION

The Dietary Approaches to Stop Hypertension (DASH) study [1] showed that a diet high in fruits and vegetables significantly reduced systolic (SBP) and diastolic (DBP) blood pressure by 2.8 and 1.1 mm Hg, respectively, compared with a typical Western diet. The DASH diet, which emphasized fruits, vegetables and low-fat dairy products and lower saturated fat, elicited even greater reductions in blood pressure (BP) of 5.5 mm Hg SBP and 3.0 mm Hg DBP (5.5/3.0 mm Hg). The BP reductions on the DASH diet were similar to those achieved by medication, and the diet controlled hypertension in 70% of subjects with hypertension [2]. Although it suggested a unique hypotensive effect of dairy foods, the DASH study was not

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specifically designed to identify principal nutrients/foods that accounted for its BP effects. Furthermore, the biological mechanism(s) for this effect are not well understood.

Epidemiologic studies have shown that consumption of dairy products is associated with reductions in BP [3–6] and lower risks for stroke [7,8], type 2 diabetes [9–11], and metabolic syndrome [12,13]. The present study was designed to assess the contribution of dairy foods to the hypotensive effect of a DASH-type diet and to examine changes in intracellular calcium [$(Ca)_i$] as a potential mediator of this effect. $(Ca)_i$, a potent mediator of cellular response, is key in the regulation of smooth muscle function, peripheral vascular tone, BP and volume homeostasis [14]. Resnick and others [15,16] have proposed that hypertension and associated disorders result from elevated $(Ca)_i$ concentrations and reciprocally suppressed intracellular magnesium levels. Hypertension is associated with significantly higher intracellular calcium and sodium concentrations [17–20] and lower intracellular magnesium levels [21–23]. A direct correlation between BP and $(Ca)_i$ levels has been shown in a variety of cell types [17,19,24]. Several clinical studies indicate that dietary calcium normalizes intracellular ion levels, via the suppression of calcium-regulating hormones [25–28]. However, not all studies show a benefit of dietary calcium on BP [28–30]. Therefore, this study was conducted to clarify the effect of dairy foods as part of a DASH-type diet on BP, intracellular ions, and calcium-regulating hormones in individuals with stage 1 hypertension.

# METHODS

## Subjects

Participants had untreated stage 1 hypertension (SBP = 140–159 and/or DBP = 90–99 mm Hg), based on the mean of 2 measurements collected during 3 screening visits. All participants were Caucasian and included 16 men and 7 women 22–70 years of age, with normal or moderately elevated cholesterol (LDL cholesterol $<$ 4.91 mmol/L, HDL-cholesterol in the 15–95$^{th}$ percentile of NHANES III, and triacyglycerols $<$ 3.95 mmol/L). Exclusion criteria included: body mass index $>$ 35 kg/m$^2$; smoking; history of congestive heart failure, peripheral artery disease, myocardial infarction, stroke, angina, intermittent claudication, kidney stones, or renal disease; allergies to food, latex, or nitroglycerin; presence of any disease known to affect calcium balance; and use of nutritional supplements, hormone replacement therapy, or any drug known to alter calcium balance, BP, or cholesterol metabolism. Table 1 shows characteristics of the subjects at study entry.

## Study Design

A randomized, three-period, crossover controlled feeding study was conducted at two sites (Pennsylvania State University and Pennington Biomedical Research Center). Twenty-three subjects were studied, 17 at Penn State and 6 at Pennington. Twenty completed all diet periods and 3 completed two diet periods. The protocol was approved by the Biomedical Committee of the Institutional Review Board at both sites and written informed consent was obtained.

Endpoints were assessed at the end of each 5-week diet period, and a 2-week compliance break separated diets. Food was prepared in research kitchens according to study protocol. On weekdays, subjects ate one meal in the feeding center and body weight was recorded. Other meals and snacks were packaged for takeout. Diet compliance was monitored according to procedures used in our research centers. Subjects were instructed to maintain their usual lifestyle/exercise habits, to consume all foods provided, to abstain from consuming other foods, and to limit beverages containing caffeine ($<$ 5 servings/d) and alcohol ($<$ 2 servings/wk). Weight was maintained by adjusting calories.

**Table 1.** Subject Characteristics at Study Entry Stratified by Intracellular Calcium Response[1,2]

| | All subjects (n = 23) | $(Ca)_i$ Change group (n = 8) | $(Ca)_i$ Stable group (n = 10) |
|---|---|---|---|
| Age (years) | 45.3 ± 2.0 | 49.7 ± 3.4 | 44.2 ± 3.0 |
| Weight (kg)[3] | 89.3 ± 4.1 | 82.1 ± 6.0 | 95.0 ± 5.3 |
| Percent Male | 70 | 75 | 80 |
| Body mass index (kg/m$^2$) | 28.8 ± 0.9 | 26.7 ± 1.5 | 30.0 ± 1.4 |
| Systolic blood pressure (mm Hg) | 140.5 ± 1.9 | 143.5 ± 3.0 | 139.4 ± 2.7 |
| Diastolic blood pressure (mm Hg) | 91.1 ± 1.3 | 92.4 ± 2.2 | 89.3 ± 1.9 |
| Total cholesterol (mmol/L) | 5.2 ± 0.2 | 5.1 ± 0.3 | 5.3 ± 0.2 |
| LDL cholesterol (mmol/L) | 3.3 ± 0.2 | 3.3 ± 0.2 | 3.4 ± 0.2 |
| HDL cholesterol (mmol/L) | 1.2 ± 0.1 | 1.2 ± 0.1 | 1.3 ± 0.1 |
| Triacyglycerols (mmol/L) | 1.4 ± 0.2 | 1.3 ± 0.3 | 1.4 ± 0.2 |

[1] All values are LS means ± SE. $(Ca)_i$ Change Group, decrease in RBC calcium $\geq$ 3.4 $\mu$mol/L and $(Ca)_i$ Stable Group, decrease in RBC calcium $<$ 3.4 $\mu$mol/L (Change in RBC calcium calculated by D-F&V diet - mean F&V and AWD diets). The Change and Stable groups, n = 18; five samples were lost during the study.

[2] No significant differences between groups at study entry (PROC MIXED model and Tukey's least-squares-means test).

[3] No significant differences between men and women, except women weighed significantly less than men, $P < 0.003$. (PROC MIXED model and Tukey's least-squares-means test).

## Experimental Diets

Three experimental diets were designed to evaluate the unique role of dairy foods in BP regulation (Table 2). Diets included an average Western diet (AWD) as a control, a diet high in fruits and vegetables (F&V), and a similar diet containing 3.4 servings/d of dairy products (D-F&V). The F&V and D-F&V diets were matched for macronutrients, cholesterol, and fiber, and included the same fruits and vegetables in similar proportions. The F&V and AWD diets contained 0.4 servings/d of dairy products. The D-F&V diet included low-fat and non-fat milk and yogurt and full-fat cheese. The AWD diet included full-fat dairy products, while the F&V diet included lower fat dairy products. Sodium was matched across all 3 diets, and calcium was matched on the F&V and AWD diets and did not exceed 600 mg/d at the highest kilocalorie level.

Diets were formulated using menus from the DASH Study [1] and were modified to meet the experimental criteria using Nutritionist V (N-Squared Computing, San Bruno, CA.). A 6-day menu cycle with 8 kilocalorie levels (1800–3900 kcal) was developed for each diet. Nutrient composition of diets prepared at both study sites was determined by chemical analyses. Protein content was analyzed with a Perkin Elmer nitrogen analyzer. Total fat content was extracted with chloroform and methanol and fatty acid content was measured by gas chromatography [31]. Minerals (Ca, Na, Mg, K, and P) were determined by inductively coupled plasma-mass spectrometry.

## Blood Pressure

During the last week of each diet period, participants made four visits for BP measurements. SBP and DBP were measured in a controlled environment using a calibrated mercury manometer, stethoscope, and appropriately sized cuffs, following the JNC-VII guidelines (17). Participants were instructed not to exercise or ingest food/caffeine 30 minutes before the visit. After a 5 minute rest period, three stethoscopic measurements were taken, separated by 1 minute. Participants were seated with feet flat on the floor and arm held at heart level. SBP was recorded at appearance of Korotkoff sounds (phase I) and DBP was recorded at disappearance (phase V). Means of all endpoint measures were used for analyses. Technicians were certified in the use of a standard protocol and were blind to treatment assignment.

## Biochemical Assays

At study entry, serum total and HDL cholesterol and triacyglycerols were measured by 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.

Fasting blood samples and 24-hour urine samples were collected and stored at −80°C until analysis. Serum intact parathyroid hormone (iPTH) was measured by immunochemiluminometric assay (Nichols Institute Diagnostics, San Clemente, CA). Radioimmunoassays were used to determine serum 1,25-dihydroxyvitaminD (Immundiagnostic System Inc., Fountain Hills, AZ), serum calcitonin (Diagnostic Systems Laboratories, Inc., Webster, TX), and plasma renin activity. Serum and urine calcium were measured by colorimetric assay. Urine sodium and potassium were measured with the ion-sensitive electrode method.

Heparinized blood was collected in chilled tubes for determination of total intracellular calcium, magnesium, sodium, and potassium in red blood cell (RBC), according to the method

**Table 2.** Assayed Composition of Experiment Diets (Based on 2100 kcal per Day)[1]

| Nutrient | AWD | F&V | D-F&V | D-F&V minus F&V | D-F&V minus AWD |
|---|---|---|---|---|---|
| Carbohydrate (%en) | 46 | 52 | 52 | None | 6 |
| Protein (%en) | 18 | 18 | 18 | None | None |
| Fat (%en) | 36 | 30 | 30 | None | −6 |
| Saturated (%en) | 15 | 7 | 7 | None | −8 |
| Monounsaturated (%en) | 14 | 14 | 14 | None | None |
| Polyunsaturated (%en) | 7 | 9 | 9 | None | 2 |
| Cholesterol (mg) | 300 | 175 | 175 | None | −125 |
| Fiber (g) | 10.5 | 27 | 27 | None | 16.5 |
| Sodium (mg) | 3500 | 3500 | 3500 | None | None |
| Potassium (mg) | 1750 | 3900 | 4600 | 700 | 2850 |
| Calcium (mg) | 400 | 400 | 1200 | 800 | 800 |
| Phosphorus (mg) | 1150 | 1300 | 1700 | 400 | 550 |
| Magnesium (mg) | 190 | 370 | 420 | 50 | 230 |
| Fruit (serving/day) | 1.6 | 5.2 | 5.2 | None | 3.6 |
| Vegetable (serving/day) | 2.0 | 4.4 | 4.4 | None | 2.4 |
| Dairy (serving/day) | 0.4 | 0.4 | 3.4 | 3 | 3 |

[1] Nutrient values were calculated using Nutritionist V software (N-Squared Computing, San Bruno, CA) and subsequently measured by chemical analyses at both study sites. AWD, average Western diet; F&V, fruits and vegetables diet; D-F&V, dairy-rich fruits and vegetables diet; %en, percent of energy. To convert values to millimoles, multiply by 0.043498 for sodium, 0.025577 for potassium, 0.024951 for calcium, 0.032285 for phosphorus, and 0.041144 for magnesium.

described by Zemel et al. [26] using inductively coupled plasma mass spectrometry (Finnigan Element High Resolution). Erythrocytes were chosen as a cell model to represent global changes in intracellular calcium status and due to the ease with which they can be isolated.

### Statistical Analysis

The specific aims of the study were to test the effects of the D-F&V diet compared with the control diet; the effects of the F&V diet compared with the control diet; and the effects of the D-F&V diet compared with the F&V diet. The key outcome variables were change in SBP and change in DBP, defined as the difference between screening and end-of-diet levels. Statistical analyses were performed with SAS (version 9.1; SAS Institute Inc, Cary, NC). Results are expressed as least squares means ± SE. Data for serum triacyglycerols were logarithmically transformed (base 10) before conducting analyses because of skewed distributions. The mixed procedure (PROC MIXED) was used to test for effects of diet, order (the sequence of the 3 diets given to each subject), diet period and their interactive effects on each outcome variable. All models were adjusted for study site and age; models for BP change were also adjusted for screening values. When significant diet effects were detected, a Tukey's least-significant difference test was performed to determine the source of significant main effects or interactions ($P \leq 0.05$).

We also tested whether treatment effects differed according to magnitude of (Ca)i response in a subset of participants (n = 18). To determine diet-related changes in $(Ca)_i$, levels of RBC calcium on the F&V and control diets, which were not significantly different (12.1 ± 1.1 and 12.6 ± 1.0 μmol/L, P = 0.9), were pooled and subtracted from the D-F&V diet. The mean decrease in $(Ca)_i$ was −4.9 μmol/L, with a natural break between −3.3 and −6.1 μmol/L (Fig. 1). Subjects who responded to the D-F&V diet by decreasing $(Ca)_i \geq 3.4$ μmol/L were defined as the *$(Ca)_i$ Change Group* (n = 8). Others were labeled the *$(Ca)_i$ Stable Group* (n = 10) and calcium status was added to the models. Within-subject correlation analyses were used to test associations between RBC calcium and BP.

After a small reduction in weight after the first period (−0.85 ± 0.35 kg, $P < 0.03$), weight remained stable for the duration of the study. Changes in weight were equal in magnitude among diets (P = 1.0) and groups (P = 0.4). Adjustment for weight had no effect on the results, and unadjusted results are presented.

## RESULTS

### Diet Effects on BP Change, Calcium Hormones, RBC Ions, and 24-Hour Urine Electrolytes

All three experimental diets significantly decreased SBP and DBP from screening (SBP: −9.9 ± 1.5, −12.3 ± 1.5, −12.0 ± 1.5 mm Hg; DBP: −5.3 ± 1.2, −7.2 ± 1.2, −7.0 ± 1.2 mm Hg for AWD, F&V, and D-F&V diets, respectively, $P < 0.01$ for all). The reductions in SBP and DBP following the D-F&V and F&V diets were significantly greater compared with AWD (Table 3). The changes in SBP or DBP did not differ between the two intervention diets. For SBP, we observed a significant period effect. SBP was significantly lower during period 3 vs. period 1 (P = 0.02), suggesting that SBP continued to decrease throughout the study.

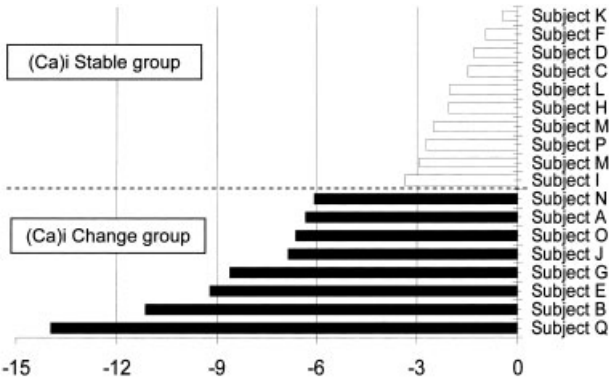


**Fig. 1.** Distribution of changes in RBC calcium on the dairy-rich diet. The $(Ca)_i$ Change Group decreased RBC calcium ≥ 3.4 μmol/L (black bars), whereas the $(Ca)_i$ Stable Group decreased it < 3.3 μmol/L (white bars). Change in RBC calcium was calculated by subtracting the D-F&V diet from the mean of F&V and AWD diets (no significant difference between F&V and AWD diets). *$(Ca)_i$ Change group* had a significantly greater reduction in RBC calcium following the D-F&V diet than the *$(Ca)_i$ Stable group* (−7.5 ± 1.1 μmol/L vs. −2.6 ± 0.7 μmol/L, $P < 0.01$). PROC MIXED model and Tukey's least-squares-means test.

While serum calcium remained constant, we observed the expected lower concentration of 1,25-dihydroxyvitaminD on the D-F&V diet vs. the F&V and control diets (Table 4). Measures of iPTH, calcitonin, and renin activity did not differ significantly between diets.

The concentration of RBC calcium was significantly lower and the concentration of RBC magnesium was significantly higher after the D-F&V diet vs. both the F&V and control diets (Table 4). Consequently, the ratio of RBC calcium:magnesium was significantly lower on the D-F&V diet. RBC potassium and RBC sodium did not differ significantly between diets.

There were significant main effects of diet for urinary excretion of calcium and potassium (Table 4). Urine calcium was greatest on the D-F&V diet, and was significantly different from the F&V diet. Urine potassium was significantly elevated on the D-F&V diet compared with the F&V diet and the AWD diet. Urine sodium did not differ across diets.

### Group Effects on BP Response by Diet (n = 18)

By design, the *$(Ca)_i$ Change group* had a significantly greater reduction in RBC calcium following the D-F&V diet than the *$(Ca)_i$ Stable group* (−7.5 ± 1.1 μmol/L vs. −2.6 ±

**Table 3.** Mean Changes in Blood Pressure according to Diet in All Subjects and Intracellular Calcium Response[1]

| | Effect of diet P-value | Interaction of diet and group P-value | Change in F&V minus change in AWD | | Change in D-F&V minus change in AWD | | Change in D-F&V minus change in F&V | |
|---|---|---|---|---|---|---|---|---|
| | | | mmHg | Tukey P | mm Hg | Tukey P | mm Hg | Tukey P |
| SBP | | | | | | | | |
| All subjects[2] | 0.015 | - | −2.4 ± 0.9 | <0.02 | −2.0 ± 0.9 | <0.05 | 0.3 ± 0.9 | <0.9 |
| (Ca)i Group | - | 0.05 | | | | | | |
| Stable | | | −2.7 ± 1.4 | <0.2 | −1.5 ± 1.4 | <0.5 | 1.2 ± 1.4 | <0.6 |
| Change | | | −2.1 ± 1.5 | <0.3 | −4.2 ± 1.5 | <0.02 | −2.1 ± 1.5 | <0.3 |
| DBP | | | | | | | | |
| All subjects | 0.005 | - | −1.8 ± 0.6 | <0.01 | −1.7 ± 0.6 | <0.02 | 0.1 ± 0.6 | <1.0 |
| (Ca)i Group | - | <0.0001 | | | | | | |
| Stable | | | −1.9 ± 1.0 | <0.2 | −0.4 ± 1.0 | <0.9 | 1.5 ± 1.0 | <0.3 |
| Change | | | −2.6 ± 1.0 | <0.02 | −5.4 ± 1.0 | <0.01 | −2.8 ± 1.0 | <0.02 |

[1] Data are least squares means ± SE, n = 23. Mean values have been adjusted for study site, age, and screening levels (refer to Table 1). The Ca Stable group, n = 10 and the Ca Change group, n = 8. AWD, average Western diet; F&V, fruits and vegetables diet; D-F&V, dairy-rich fruits and vegetables diet; SBP, systolic blood pressure; DBP, diastolic blood pressure

[2] Period effect, P = 0.03. PROC MIXED model and Tukey's least-squares-means test.

**Table 4.** Effect of Three Experimental Diets on Calcium Related Variables, Red Blood Cell Ions, and Urinary Electrolytes[1]

| Variable | AWD | F&V | D-F&V | P-value |
|---|---|---|---|---|
| Serum Calcium (mmol/L) | 2.33 ± 0.03 | 2.35 ± 0.03 | 2.35 ± 0.03 | NS |
| $1,25(OH)_2D$(pmol/L) | 112.2 ± 7.0[a] | 104.0 ± 7.0[a] | 87.4 ± 7.0[b] | 0.0004 |
| iPTH (pg/mL) | 44.7 ± 4.5 | 44.8 ± 4.4 | 42.8 ± 4.5 | NS |
| Calcitonin (pg/mL) | 9.6 ± 0.7 | 9.6 ± 0.7 | 9.4 ± 0.7 | NS |
| Renin activity (ng/mL/hr) | 0.6 ± 0.2 | 0.8 ± 0.2 | 0.9 ± 0.2 | NS |
| Red Blood Cell[2] | | | | |
| Calcium (μmol/L) | 12.6 ± 1.0[a] | 12.1 ± 1.1[a] | 6.9 ± 1.1[b] | 0.0001 |
| Magnesium (mmol/L) | 2.3 ± 0.1[a] | 2.3 ± 0.1[a] | 2.7 ± 0.1[b] | 0.0010 |
| Sodium (mmol/L) | 6.6 ± 0.6 | 8.0 ± 0.6 | 6.2 ± 0.6 | 0.0518 |
| Potassium (mmol/L) | 86.1 ± 2.6 | 89.0 ± 2.6 | 88.0 ± 2.8 | NS |
| Calcium:magnesium | 5.6 ± 0.5[a] | 5.5 ± 0.5[a] | 2.9 ± 0.5[b] | 0.0001 |
| 24-hour Urine[3] | | | | |
| Calcium (mg) | 107.2 ± 17.3[a,b] | 87.8 ± 16.0[b] | 126.5 ± 16.1[a] | 0.0214 |
| Sodium (mg) | 3605 ± 322 | 3570 ± 280 | 3871 ± 281 | NS |
| Potassium (mg) | 1983 ± 265[a] | 2510 ± 237[a] | 3334 ± 238[b] | 0.0001 |

[1] All values are least squares means ± SE; n = 23. AWD, average Western diet; F&V, fruits and vegetables diet; D-F&V, dairy-rich fruits and vegetables diet; 1,25(OH)2VD,1,25-dihydroxyvitaminD; iPTH, intact parathyroid hormone; NS, nonsignificant. Values in a row with different superscript letters are significantly different, Tukey P ≤ 0.02 (PROC MIXED model and Tukey's least-squares-means test).

[2] n = 18.

[3] To convert values to millimoles per day, multiply by 0.024951 for calcium, 0.043498 for sodium, and 0.025577 for potassium.



0.7 μmol/L, P < 0.01). The groups did not differ significantly in study entry characteristics (Table 1).

There was a significant diet by group interaction for change in SBP (Table 3). In the $(Ca)_i$ *Change group,* the D-F&V diet elicited a significantly greater reduction in SBP compared with the AWD, while the F&V diet did not differ from the other diets.

There was a significant diet by group interaction for DBP (Table 3). In the $(Ca)_i$ *Change group,* the D-F&V diet elicited significantly greater decreases in DBP compared with the F&V and AWD diets. The magnitude of the change in DBP on the F&V diet also was significantly greater than the control. This pattern in BP response was not evident in the $(Ca)_i$ *Stable group.*

Within-subject correlations between RBC calcium and BP support the subgroup analyses. The reduction in RBC calcium associated with the D-F&V diet correlated with the decrease in DBP (r = 0.52, P < 0.05) and 1,25-dihydroxyvitaminD (r = 0.59, P < 0.03). Participants who exhibited the greatest decreases in RBC calcium also experienced the greatest reductions in 1,25-dihydroxyvitaminD and DBP. Changes in SBP were not significantly correlated with changes in RBC calcium.

## DISCUSSION

In the present study, we observed a modest additional reduction in SBP and DBP (about 2 mm Hg) after the F&V and

D-F&V diets versus the control diet. The addition of 3 servings of dairy to a diet high in fruits and vegetables, however, had no effect on mean blood pressure changes, except in the subgroup characterized by appreciable decreases in intracellular calcium. The D-F&V diet significantly reduced (Ca)i levels and significantly increased intracellular magnesium levels, and reductions in (Ca)i were correlated to improved BP. Thus, a hypotensive effect of dairy products was evident only in subjects most responsive to changes in intracellular ion levels.

Our findings agree with previous studies showing that a calcium-rich diet favorably affects (i.e., decreases) intracellular ion levels. A clinical feeding trial [26] in 11 salt-sensitive hypertensive African Americans showed that a low calcium (8.9 mmol/d), high sodium (174 mmol/d) diet significantly increased PTH, which was associated with significant increases in RBC calcium (5.4 $\pm$ 0.7 to 11.1 $\pm$ 3.7 $\mu$mol/L) and RBC sodium (9.6 $\pm$ 0.3 to 11.4 $\pm$ 0.4 mmol/L) and significant decreases in RBC magnesium (2.2 $\pm$ 0.1 to 1.8 $\pm$ 0.1 mmol/L). The addition of calcium (to equal 23.4 mmol/d) to the high sodium diet reversed these effects [26] and was associated with lower SBP and DBP [25,32]. Calcium supplementation (50 mmol/d for 16 wk) in normotensive men also has been shown to reduce intracellular calcium and sodium, 1,25-dihydroxyvitaminD, and SBP [27,33]. In the present study, we observed a similar reduction in RBC calcium, RBC sodium and 1,25-dihydroxyvitaminD, and an increase in RBC magnesium during the dairy-rich diet. Collectively, these results suggest that intracellular ion levels are responsive to diet manipulation and that consumption of dairy foods produces a favorable intracellular environment (lower ratio of intracellular calcium to magnesium). It is important to note that while we focused on calcium and its role in decreasing blood pressure in the current study, it is possible that changes in other micronutrients contributed to the response. The D-F&V diet was higher in calcium, potassium, magnesium, and phosphorous, and RBC levels of calcium were lower and magnesium was higher. Thus, it may be that the blood pressure response noted was the result of changes in micronutrients beyond calcium or due to other components of dairy products.

In agreement with several studies [26,28–30], we observed significantly greater decreases in 1,25-dihydroxyvitaminD on the dairy-supplemented diet vs. the F&V and control diets, which contained lower levels of dietary calcium. The present study found a direct relationship between the changes in 1,25-dihydroxyvitaminD and RBC calcium. 1,25-dihydroxyvitaminD has been shown to stimulate rapid calcium influx through increasing calcium-channel currents in a variety of cells including vascular smooth muscle cells [34–36]. Thus, we propose that the calcium-rich D-F&V diet favorably affected (decreased) $(Ca)_i$ levels via suppression of 1,25-dihydroxyvitaminD.

Our reduction in blood pressure was notably less than that reported in all subjects (4.2/5.4) by the original DASH study [1]. However, in Caucasian subjects the DASH diet produced a less robust blood pressure reduction of 3.3/2.4 mm Hg, which is comparable to the results of our small sample of 23 Caucasians [37]. There are other possible reasons why our BP changes were smaller in magnitude than in the original DASH study. 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 ($-12$ mm Hg SBP and $-7$ mm Hg DBP), however, unlike the original DASH study, our control diet also significantly reduced blood pressure ($-10$ mm Hg SBP and $-5$ mm Hg DBP). This effect of our control diet was unexpected; nonetheless, this response partially accounts for the smaller net decreases in BP observed in our intervention diets. We speculate that our control diet was healthier than the participants habitual diet, a finding we note repeatedly in our studies. In addition, inclusion of a run-in period in our study (as was done in the DASH study) may have prevented the BP decrease on the control diet.

Another reason the present study did not elicit the same BP-lowering effect as the DASH trial may relate to the lack of diversity of our small sample. Like the hypertensive subgroup in the DASH study [2], our subjects were overweight, middle-aged adults with stage 1 hypertension. In the DASH trial, 60% of the subjects were African American, the group showing the most robust dietary response [37]. Therefore, our study of 23 Caucasian hypertensive adults may have underestimated the magnitude of BP change that is possible with dairy foods, as African Americans are particularly vulnerable to the complications of untreated hypertension and are more sensitive to changes in calcium and potassium intake [38].

In the DASH trial, there was a greater contrast in the nutrient composition of the experimental diets than in the present study. 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 DASH study had greater differences in protein, cholesterol, fiber, and magnesium content between the control and DASH diet than our control vs. the D-F&V diet. Furthermore, the present study was specifically designed to isolate the contribution of 3 servings of dairy foods by comparing the D-F&V diet to a similar diet that was low in dairy. Therefore, the two intervention diets (D-F&V and the F&V) were carefully matched to eliminate the macronutrient differences present in the DASH trial, while allowing calcium, magnesium, potassium, and phosphorus to increase with the increased consumption of dairy foods (refer to Table 2). In the DASH trial, the DASH diet provided less total fat (27% vs 37% of total kcal), less saturated fat (6% vs. 16% of total kcal), less cholesterol (150 vs. 300 mg/day), and more protein (18% vs. 15% of total kcal) than its fruit and vegetable counterpart. Plus, the amount and type of fruits and vegetables were identical across our two intervention diets, and this differs from the original DASH diet design. 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, and we did not demonstrate such large differences as in the DASH study.

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 [5,39], 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 [26] and/or low renin hypertensive [40] subjects lend support to the theory that calcium and/or dairy foods may play a key role in blood pressure regulation in specific patient groups (i.e. those thought to be in a state of calcium deficiency). In our study, we investigated intracellular calcium changes and observed that those who had the greatest drop in intracellular calcium also had the greatest BP-lowering response to the D-F&V diet. The (Ca)i Change group exhibited a significantly greater reduction in BP on the D-F&V vs. control diet (− 4.2/−5.4 mm Hg) and F&V diet (−2.1/−2.8 mm Hg; nonsignificant for SBP). Although addition of 3 servings of dairy foods to a diet high in fruits and vegetables did not elicit further reductions in BP in the sample as a whole, subgroup analysis revealed a unique role for dairy foods in the treatment of hypertension. Future studies should examine whether the $(Ca)_i$ response is dose dependent and determine the basis for this phenotypic response in order to facilitate *a priori* discrimination between responders and non-responders to this dietary intervention.

In conclusion, 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. This magnitude of reduction has been estimated to reduce the prevalence of hypertension, the risk of coronary heart disease, and the risk of stroke in the population by 17%, 6%, and 15%, respectively [41]. The addition of 3 servings of dairy products to a diet already high in fruits and vegetables conferred significant reductions in (Ca)i and significant increases in intracellular magnesium levels. Furthermore, the hypotensive effect of dairy products was evident only in subjects most responsive to changes in intracellular calcium, which lends support to other studies that have identified specific populations that are particularly responsive to calcium-rich diets. Our findings suggest that the BP responses to increasing dietary calcium are associated with attenuation of the widely reported elevation in intracellular calcium in hypertensive subjects.

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