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Effects of Whole Milk and Full-Fat Dairy Products on Blood Pressure and Vascular Function in Adults with Elevated Blood Pressure

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by

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Dedication

To my wife, Jenna Roy, for always being a constant source of encouragement and love throughout my training process.

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Effects of Whole Milk and Full-Fat Dairy Products on Blood Pressure and Vascular Function in Adults with Elevated Blood Pressure

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Addition of skim milk and non-fat dairy intake to the normal routine diet has been demonstrated to reduce blood pressure (BP) and improve vascular function in adults with elevated BP. High consumption of whole milk and full-fat dairy products is accompanied by elevated saturated fat intake and an increase in plasma cholesterol concentration. The solitary effects of full-fat dairy products added to the normal diet in adults with elevated BP is not known. Therefore, the primary aim of the present study was to determine the efficacy of reducing elevated BP and improving vascular function when adding whole milk and full-fat dairy products to the normal diet.

Sixty participants with elevated BP underwent a randomized controlled crossover dietary intervention consisting of high dairy and control conditions. Within the high dairy condition, participants underwent an increase in 4 daily servings of whole milk and full-fat dairy products for 4 weeks in addition to their normal diets. Dairy consumption was eliminated during the control condition for another four weeks while consuming a counterbalanced diet. A 2-week washout period separated the two conditions to remove residual effects from the previous condition.

In study 1, we sought out to determine the effects of whole milk and full-fat dairy products on arterial BP in adults with elevated BP. We utilized two different but complimentary

assessments incorporating seated and ambulatory BP measurements. There were no changes in systolic or diastolic BP measures for seated BP or ambulatory (e.g. 24-hour, daytime, and night time) measures. In study 2, we studied the effects of added consumption of whole milk and full-fat dairy to the normal routine diet on vascular function in adults with elevated BP. No significant changes were seen in arterial stiffness, endothelial function, or cardiovagal baroreflex sensitivity.

Taken together, the findings of this dissertation study demonstrate that unlike skim milk and non-fat dairy products, whole milk and full-fat dairy products do not exert hypotensive effects or improvements in vascular function.

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Chapter 1: General Introduction

Hypertension has been defined as a persistently elevated systolic (SBP) (≥140 mmHg) and/or diastolic BP (DBP) (≥90 mmHg) [1-3]. Approximately 1 in 3 adults over the age of 18 years are affected by hypertension, representing an estimated 68 million adults within the United States [3]. The most common forms of cardiovascular disease (CVD) resulting from sustained hypertension include left ventricular hypertrophy and subsequent heart failure, coronary arterial disease, and ischemic stroke [4]. Most hypertension cases stem from unknown etiologies, and about 70% receive treatment with less than half being controlled [3]. Although the initial events leading to hypertension are unclear, vascular dysfunction seems to play a significant role in propagating its development.

Hypertension can be modulated through a variety of behavioral modifications [1, 5-7]. In particular, high consumption of non- and low-fat dairy products has emerged as an effective strategy against hypertension [5, 8, 9]. We reported that non-fat dairy products not only exerted hypotensive effects [10] but also improved key vascular functions related to hypertension [11]. Reduced central arterial stiffness associated with increased non-fat dairy intake is a critical finding as increased arterial stiffness has been viewed as a causal mechanism linked to elevated systolic BP as well as an independent contributor to CVD risk [12-14].

Compared with non-fat dairy products, the effects of consuming high amounts of full-fat dairy products are not clear and remain controversial with data demonstrating beneficial effects [15, 16] or no effects on BP reduction [17, 18]. Moreover, unlike the traditional notion, recent meta-analytical studies have demonstrated that the overall effects of dietary saturated fatty acids contained in whole milk and full-fat dairy products are not associated with CVD risks [19, 20]. A

recent intervention study modified the original eating plan of the Dietary Approaches to Stop Hypertension (DASH) trial by increasing saturated fat content of the diet through replacement of non- and low-fat dairy for full-fat dairy products [16]. The higher-fat DASH study revealed similar hypotensive effects to the original DASH trial. However, the study was unable to isolate the effects of the full-fat dairy products from other food items responsible for reducing BP (i.e. fruits and vegetables). Currently, no studies have established a causal link of whole milk and full-fat dairy products on BP, or vascular function, in adults with elevated BP when added to the normal routine diet.

The overall goal of the study was to determine if whole milk and full-fat dairy products exert hypotensive effects as seen with our previous investigation of skim milk and non-fat dairy products. In addition, we evaluated changes in the arterial dynamics corresponding to changes in arterial BP through central thoracic arterial stiffness and endothelial function and how such arterial dynamics consequentially affected neurogenic factors such as baroreceptor sensitivity.

PURPOSES AND HYPOTHESES

Study #1: The aim of study 1 was to determine if a dietary intervention that includes whole milk and full-fat dairy products, reduces arterial BP in adults with elevated BP. We hypothesized that the solitary addition of conventional whole milk and full-fat dairy products would induce significant decreases in seated and ambulatory BP measures in this population.

Study #2: The aim of study 2 was to determine whether the changes in arterial stiffness and/or endothelial vasodilatory function were key mechanisms by which dairy products may reduce BP. We hypothesized that the hypotensive effects of dairy products would be significantly associated

with the corresponding reductions in arterial stiffness and/or increases in endothelial function and subsequent improvements in baroreceptor sensitivity.

Chapter 2: Study 1 - Addition of Whole Milk and Full-Fat Dairy Products to the Normal Routine Diet on Blood Pressure

ABSTRACT

Regular consumption of low- and non-fat dairy products reduces blood pressure (BP) in adults with elevated BP. Currently, it is unknown if conventional full-fat dairy products exert similar hypotensive effects. We aimed to determine if adding whole milk and full-fat dairy products to the normal routine diet would reduce BP in adults with elevated BP. Sixty adults (mean age±SEM; 58±2 years) with elevated systolic BP (systolic/diastolic BP: 120-159 / <99 mmHg) were randomized into a controlled crossover intervention trial. The trial consisted of 4 weeks of high dairy and control conditions separated by a 2-week washout period. The high dairy condition consisted of +4 servings/d of conventional full-fat dairy products to the normal routine diet, while the control condition (with +4 servings/d of fruit and plant-based products) eliminated dairy products from the diet. Seated systolic BP did not change significantly in either condition. When the analyses were divided into subgroups of men and women, there were no changes in systolic BP in either sex across either dietary period. Ambulatory (24-hour) systolic BP did not change significantly in the high dairy (133±2 vs. 131±1 mmHg) and control conditions (132±2 vs. 131±1 mmHg). No significant changes were observed for diastolic BP or pulse pressure during either condition for seated and ambulatory measures. The solitary addition of whole milk and full-fat dairy products to the normal routine diet does not exert hypotensive effects in adults with elevated BP.

INTRODUCTION

For most adults living in industrialized societies, systolic blood pressure increases progressively throughout the life span [21, 22]. The increases in systolic BP with aging have important clinical consequences as elevated systolic BP is one of the major independent risk factors for cardiovascular disease (CVD) [23, 24]. To accomplish a reduction in the incidence of systolic hypertension, we must identify effective strategies to prevent and treat elevated systolic BP in aging adults. Guidelines incorporating lifestyle modifications, including dietary changes, are universally the first-line approach used to prevent and treat elevated BP [25].

Observational studies suggest that high consumption of dairy products is associated with reduced risk of hypertension [15, 17, 26, 27]. Indeed, several interventional studies have specifically investigated the hypotensive effects of non- and low-fat dairy products on BP [9, 10, 28]. We have previously demonstrated that the solitary manipulation of conventional non-fat dairy added to the normal routine diet reduced both seated and ambulatory (24-hour) systolic BP in middle-aged and older adults with elevated BP [10]. Unlike research studies evaluating low and non-fat dairy products on BP, the available research studies examining the effect of whole milk and full-fat dairy products on BP are extremely limited and are highly controversial with some showing no relation [29, 30] while others demonstrating benefits [31] or even increased risk of hypertension [17]. Most investigators have attributed the inability of full-fat dairy products to modulate BP to milk fat that is rich in saturated fat (>60% of milk fat), which could in turn increase low density lipoprotein (LDL) cholesterol and elevate cardiovascular disease risk [32, 33]. However, the pathogenetic role of saturated fat has become controversial in recent years [20, 34, 35] as the intake of saturated fat also increases high density lipoprotein (HDL) cholesterol

offsetting the adverse effects of elevated LDL-cholesterol [36]. Moreover, there are studies indicating that milk and milk fat do not adversely affect plasma cholesterol concentrations [16, 37]. Recently, an abbreviated 3-week DASH study incorporating full-fat dairy products in place of low-fat dairy products revealed similar hypotensive effects to the original DASH diet [16]. However, the study was limited in isolating the effects of the full-fat dairy products thereby preventing reduced blood pressure changes to full-fat dairy alone.

Therefore, the purpose of the present randomized, controlled crossover dietary intervention was to determine the effects of adding whole milk and full-fat dairy products to the normal routine diet on BP in adults with elevated BP. We hypothesized that full-fat dairy products would be effective in lowering both seated and ambulatory BP.

METHODS

Subjects: A total of 60 subjects who were free of overt chronic diseases participated in the present study. During a 2-week run-in period, all eligible subjects went through screening and had to demonstrate a consistent resting systolic BP between 120-139 mmHg (prehypertension) or 140-159 mmHg (stage 1 systolic hypertension) with diastolic BP of less than 99 mmHg on two separate occasions during the run-in periods. The BP values were set in accordance with the pre-existing guidelines of high BP [25]. Exclusionary criteria included overt chronic disease, BMI > 45 kg/m^2, lactose intolerance, high baseline dairy intake (>3 servings/day), pregnancy or lactation, strenuous physical activity (>3 times/week), excessive alcohol consumption (>21 drinks/wk), or appearance of chronic diseases as assessed during the screening. A data safety monitoring board was established to monitor any adverse events. All procedures of the study were reviewed and

approved by the University of Texas at Austin's Institutional Review Board. Written informed consent was obtained from all candidates prior to participating in any procedures.

Experimental Protocol: With the exception of the dairy and non-dairy food items, the study design was identical to our previous study investigating effects of skim milk and non-fat dairy products on BP [10]. A randomized, controlled, crossover dietary intervention consisting of a 4-week high dairy or no dairy condition was conducted with a minimal 2-week washout period between dietary interventions, to remove residual effects associated with the previous intervention. The dietary intervention was 4 weeks long as BP changes very rapidly to intervention stimuli as early as 2-4 weeks as in our previous dietary intervention study [10]. The random allocation to the intervention sequence was conducted by using a coin-flip method. Measurements took place at the beginning and end of each dietary intervention period. Due to the nature of the experimental dietary conditions, it was not possible to blind subjects or investigators to the dietary condition, except during analyses. In the high dairy condition, subjects were asked to consume 4 servings of laboratory-provided dairy per day, in addition to their baseline dairy intake. Subjects could choose their 4 dairy servings from any combination of 8 fluid ounces of whole milk (Hill Country Dairies), 6 ounces of Brown Cow yogurt with cream on top (Stonyfield Farm), and/or 1.5 ounces of Swiss cheese (HEB Grocery) in addition to their normal routine diet. In the no dairy (control) condition, subjects eliminated all dairy consumption from their routine diet to maximally differentiate dietary dairy intake. In the control condition, subjects consumed 16-fluid ounces of Silk Coconut Milk (WhiteWave Services), 16-fluid ounces of HEB branded orange juice fortified with vitamin D and calcium (HEB Grocery), 2 ounces of single-serve Plantars salted peanuts (The Kraft Heinz Company), and 4-ounce single serve cups of Motts applesauce (Motts LLP) daily to

counterbalance total calories from the high dairy condition. All dairy and non-dairy products were provided by the investigators in daily packages. The subjects were free to consume the required servings spanned throughout the day or all at once. Dietary consultations were completed weekly by a licensed dietitian, with the exception of the 2-week washout period required to remove residual effects from the previous condition [10, 16]. Consultations involved dietary protocol instruction, compliance monitoring, and adjustment of caloric intake based on significant changes in body weight (±2 kg).

Measurements were taken at the beginning and end of each dietary condition at the same time of day to eliminate any diurnal effects. Subjects were asked to avoid exercise, caffeine consumption, and fast 12 hours prior to measurement. Premenopausal women were tested during self-reported early follicular phase of the menstrual cycle in both dietary conditions of the study. Throughout the entire experimental protocol, subjects were instructed to maintain their normal lifestyle (e.g., physical activity) aside from dietary changes prescribed by the dietitian.

Statistical Analysis: A priori testing for statistical power of 80% and sample size calculations were performed using nQuery Adviser computer software (Statistical Solutions, Boston, MA). Sample size calculations were based on estimated effect sizes for the dependent variables of previous lifestyle modification studies in adults [10, 38-40]. All statistical analyses were conducted using SPSS 23.0 statistical software (Chicago, IL). To test the effectiveness of the washout period, a 2-factor (sequence x visit) mixed-model repeated measures ANOVA was utilized. Prior to any statistical testing, assumptions of normality and variance were determined through each condition by utilizing the Shapiro-Wilk and Levene's test, respectively. To control random effects occurring due to condition and major dependent variables, mixed-effect modelling

was used to assess statistical significance. A 2-factor mixed-model ANOVA with repeated measures was used to evaluate differences between each dietary condition (condition x time) for subject characteristics, dietary changes, and BP measures. A 3-factor mixed-model ANOVA with repeated measures was also utilized to evaluate sex/gender differences (sex x condition x time) on BP measures. Further subgroup analyses were conducted to assess whether age and menopausal status could influence the outcome. Partial correlation analyses were conducted to detect associations attributed to separate dairy elements while controlling for the other dairy products given to participants. To follow significant interactions, post-hoc tests were performed using the Bonferroni Procedure. Per-protocol analysis was implemented for all subjects that have fully complied with the study [10]. The alpha value was set at $P<0.05$ for all analyses. Data are presented as means±SEM.

Detailed Methods:

Seated resting brachial BP

BP recordings were made under quiet, comfortable ambient (~24ºC) laboratory conditions. To avoid any possibility of investigator bias, measurements were made with a semi-automated device (HEM-907XL; Omron Healthcare, Vernon Hills, IL), which uses an oscillometric technique over the brachial artery of the right arm. Recordings were made in triplicate in the seated position. All measurements conformed strictly to the American Heart Association guidelines [41].

24-hour ambulatory BP

BP was recorded over a 24-hour period of normal daily activity utilizing a noninvasive ambulatory monitor (Model 90217; Spacelabs Healthcare, Snoqualmie, WA). The cuff was

programmed to inflate automatically every 15 minutes from 6:00 AM to 11:00 PM and every 20 minutes between 11:00 PM and 6:00 AM [10].

Blood Samples

Whole blood was collected by venous puncture. All plasma samples were centrifuged, decanted, and stored at -80ºC for future assay. Commercially available kits using enzymatic methods were used to determine whole blood concentrations of glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and glycated hemoglobin (DCA Systems; Siemens Healthcare Diagnostics, Los Angeles, CA). Quantification of plasma concentration of insulin were determined through enzyme-linked immunosorbent assays (Mercodia, Winston Salem, NC). Fasting concentrations of blood glucose and insulin were used to assess changes in insulin resistance utilizing homeostatic model assessment of insulin resistance (HOMA-IR) [42].

Dietary analyses

Under the supervision of a licensed dietitian, self-administered 3-day dietary recall forms were used to capture the subjects' diet at baseline and during the study conditions. A 3-day dietary recall form was also collected during the washout period. Dietary composition and caloric intake were analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX). Compliance was monitored through laboratory provided, self-administered compliance logs.

RESULTS

A total of 28 males and 32 females completed the study (Table 2.1). No adverse events were reported throughout the study period. There were no carryover effects during the 2-week washout period. Subject compliance for consuming laboratory-provided food items were 94% and 96% in the control and high dairy conditions. No changes in body weight or BMI were observed

throughout the duration of the study. There were slight but significant increases in total cholesterol concentrations during the high dairy condition ($P < 0.05$) but no changes were observed during the control condition. LDL-cholesterol was the main contributor to total cholesterol despite a lack of significance ($P = 0.074$) as HDL-cholesterol and triglycerides did not change. Blood glucose was also slightly elevated during the high dairy condition ($P < 0.05$). HOMA-IR did not change significantly and did not differ between the two dietary conditions.

There were no significant changes in total caloric intake after either condition (Table 2.2). In the high dairy condition, total dairy intake increased to 4.4±0.2 servings/day while it decreased to 0.0±0.1 servings/day in the control condition. As expected, dietary saturated fat and protein intake increased during the high dairy condition ($P < 0.001$ for both). Total carbohydrate intake was greater in the control than the high dairy condition ($P = 0.001$). There were significant reductions in sodium intake as a main effect of time ($P < 0.05$) but the interaction was not significant. There were no significant differences in sodium intake between the study conditions.

Seated BP data are presented in Figure 2.2. Seated systolic BP, diastolic BP, and pulse pressure did not change significantly in either condition. Despite no interaction effect, a main effect of time was present for the seated systolic BP ($P < 0.03$) (Figure 2.3) suggesting equivalent reductions in systolic BP for both control and high dairy conditions. No association in seated systolic or diastolic BP was found for whole milk (controlling for yogurt and cheese intake), yogurt (controlling for whole milk and cheese intake), or cheese (controlling for whole milk and yogurt intake).

Subgroup analyses revealed no changes in seated or ambulatory systolic and diastolic BP for men or women. When female subjects were divided into pre- and post-menopausal women,

premenopausal women did not exhibit different BP during baseline for seated or ambulatory measures from postmenopausal females and demonstrated no differential effects on any blood pressure with respect to dietary condition. With respect to age, no condition or time effect was found. However, as expected, the main effect of age did demonstrate significantly higher systolic BP values for both seated and ambulatory systolic BP values.

Ambulatory BP data are displayed in Figure 2.4**.** No significant changes in 24-hr systolic BP were observed in the control and high dairy conditions. The results were the same when the data were divided into daytime and nighttime systolic BP. Similarly, there were no significant changes in ambulatory diastolic BP or pulse pressure for 24-h, daytime, and night in either condition. When the data were stratified for sex, there were no significant differences in 24-hour, daytime, and night BP in either of the dietary interventions in both males and females. 24-hour and daytime ambulatory systolic BP were reduced to a similar extent over time ($P < 0.05$) (Figure 2.5). No partial correlations in 24-hour, daytime, or night time ambulatory systolic or diastolic BP were found for whole milk (controlling for yogurt and cheese intake), yogurt (controlling for whole milk and cheese intake), or cheese (controlling for whole milk and yogurt intake).

Table 2.1 Overall changes in selected subject characteristics and blood chemistry with no dairy and high dairy conditions.

	No Dairy		High Dairy	
Variables	Before	After	Before	After
Age (years)	58±2	-	58±2	-
Height (cm)	168±1	-	168±1	-
Body mass (kg)	85±2	85±2	84±2	85±2
BMI (kg/m^2)	29.2±0.8	29.3±0.8	29.1±0.8	29.2±0.8
Total cholesterol (mmol/L)	5.1±0.1	5.0±0.1	5.0±0.1	5.3±0.1*,†
HDL cholesterol (mmol/L)	1.30±0.07	1.26±0.06	1.32±0.06	1.31±0.06
LDL cholesterol (mmol/L)	3.04±0.12	3.02±0.12	3.02±0.12	3.28±0.12
Triglycerides (mmol/L)	1.46±0.12	1.41±0.12	1.41±0.12	1.42±0.12
Blood glucose (mmol/L)	5.29±0.10	5.27±0.10	5.27±0.10	5.46±0.10*,†
Glycated hemoglobin (%)	5.6±0.04	5.5±0.05	5.5±0.04	5.5±0.05
Insulin (pmol/L)	66±6	63±4	66±6	64±4
HOMA-IR	1.25±0.12	1.19±0.08	1.24±0.12	1.22±0.08

All values are means±SEMs. BMI=Body Mass Index, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, HOMA=Homeostasic Model Assessment. All significant variables were preceded by a significant interaction effect (time x condition). *$P < 0.05$ vs Before; †$P < 0.05$ vs After in the No Dairy condition.

Table 2.2 Overall changes in dietary intake with no dairy and high dairy conditions.

	No Dairy		High Dairy	
Variables	Before	After	Before	After
Calories (kcal/d)	1996±68	2061±60	1965±68	2106±58
Total Fat (g/d)	83±4	89±3	85±4	96±3
Saturated Fat (g/d)	27±1	28±1	27±1	39±1*,†
Monounsaturated Fat (g/d)	19±1	25±1*,†	20±1	20±1
Polyunsaturated Fat (g/d)	11±1	8±1*,†	10±1	10±1
Carbohydrate (g/d)	222±9	233±8	220±9	213±8†
Protein (g/d)	77±3	74±4	77±3	94±4*,†
Alcohol (g/d)	5±1	3±1	3±1	3±1
Sodium (mg/d)	3043±149	2558±160	3123±148	2820±157
Potassium (mg/d)	1901±106	2651±104*	1975±106	2226±102*,†
Calcium (mg/d)	892±56	1914±71*	817±56	1513±71*,†
Magnesium (mg/d)	210±14	209±14	210±13	214±13
Vitamin D (IU/d)	360±113	659±115*	369±112	449±115†
Dairy (servings/d)	0.7±0.2	0.0±0.1*	1.5±0.2†	4.6±0.1*,†

All values are means±SEMs. All significant variables were preceded by a significant interaction effect (time x condition). *$P < 0.05$ vs Before; †$P < 0.05$ vs After in the No Dairy condition

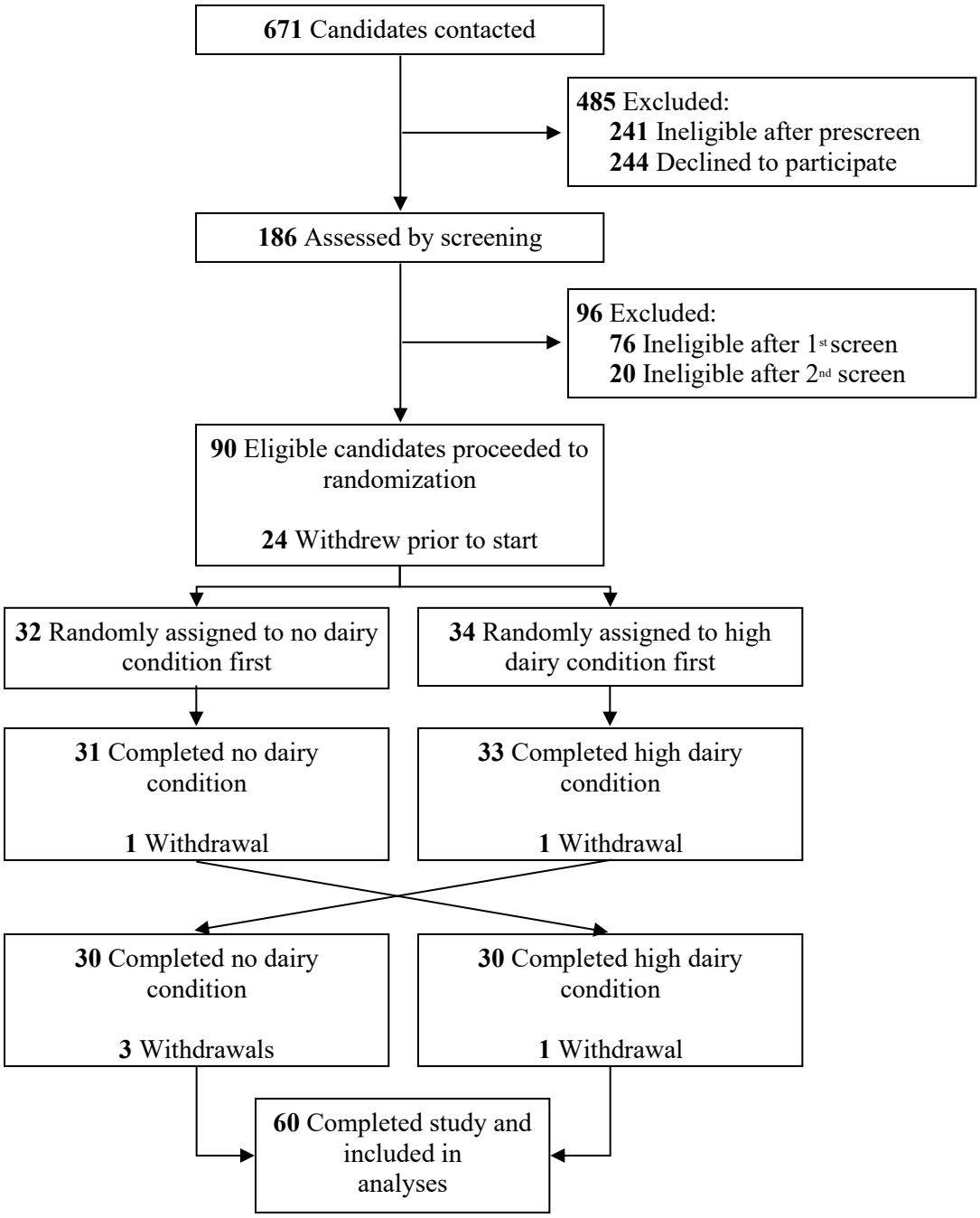

Figure 2.1 Participant flow chart through the dietary intervention trial.

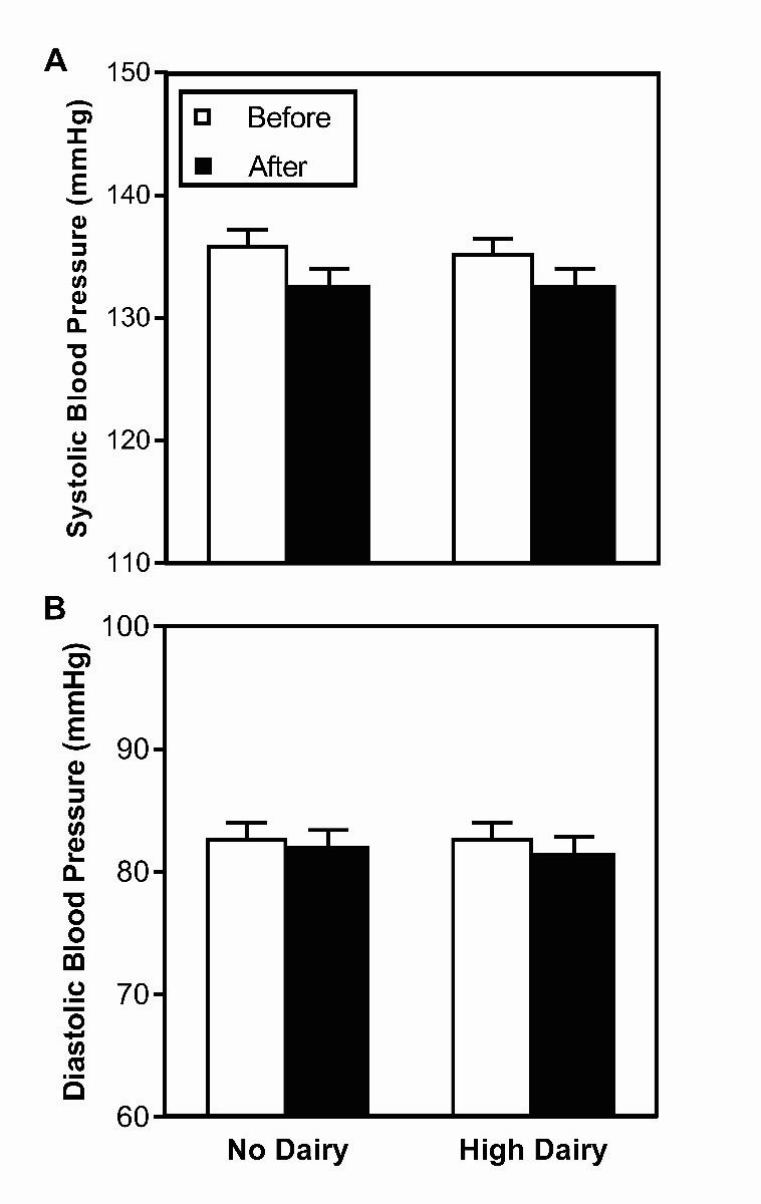

Figure 2.2 Seated brachial systolic (A) and diastolic (B) BP before and after no dairy and high dairy dietary conditions. There were no significant interaction effects.

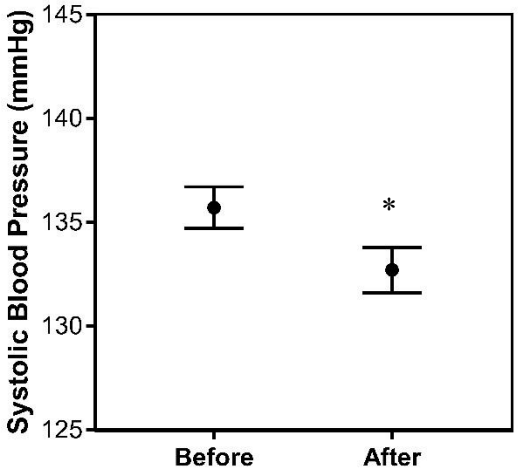

Figure 2.3 Main effect of time on seated brachial systolic BP. *Denotes significant main effect of time ($P < 0.05$).

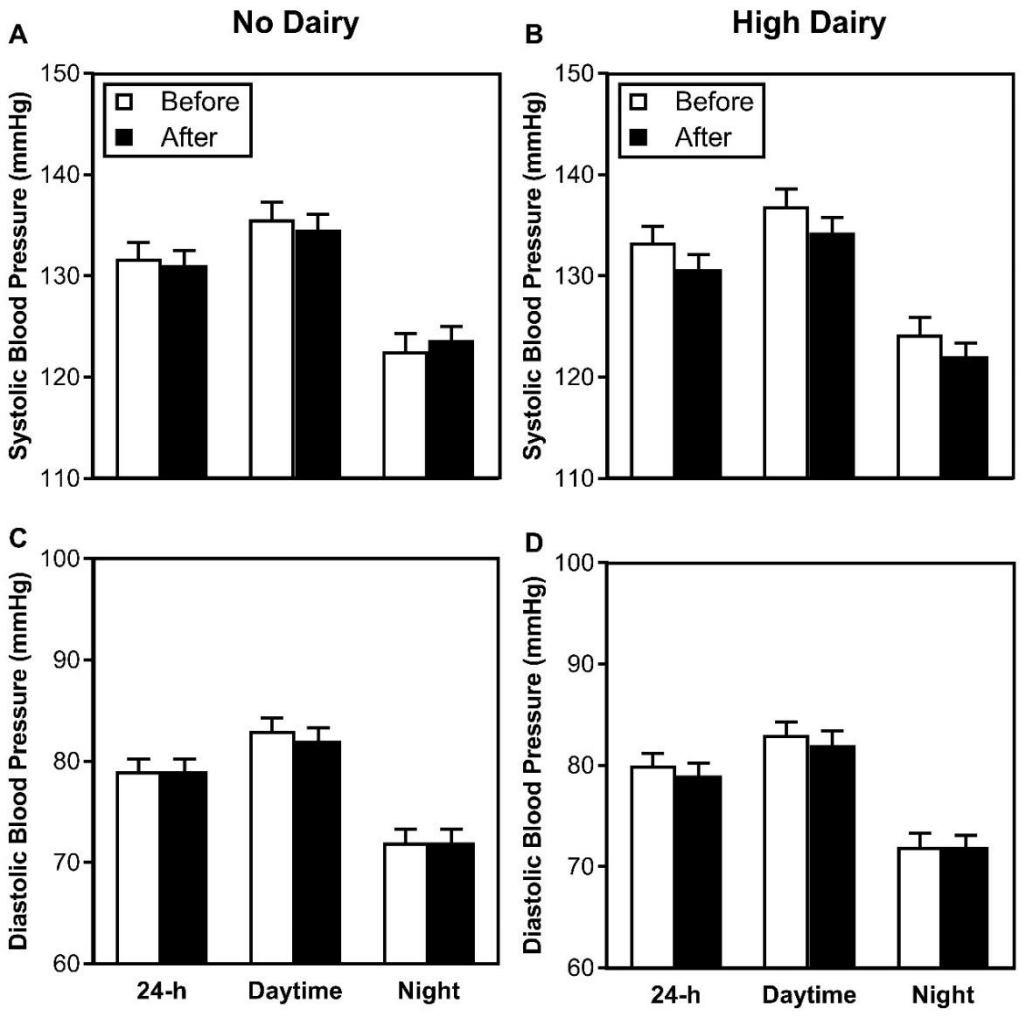

Figure 2.4 Ambulatory (24-hour), daytime, and night time systolic and diastolic BP before and after no dairy (A and C) and high dairy (B and D) conditions. There were no significant interaction effects.

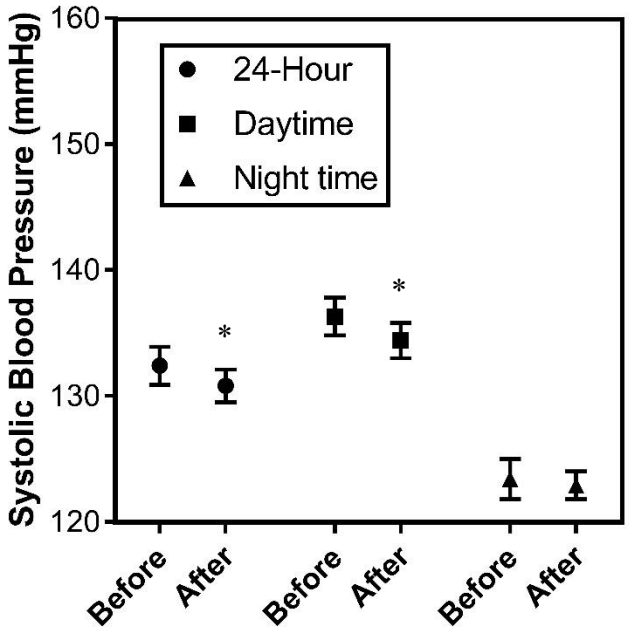

Figure 2.5 Main effects of time for ambulatory (24-hour), daytime, and night time BP. *Denotes significant main effect of time ($P < 0.05$)

DISCUSSION

The present study was the first to investigate the effects of conventional full-fat dairy products on seated and ambulatory BP in adults with elevated BP. We found that whole milk and full-fat dairy products did not lower BP significantly when consumed in addition to the normal routine diet. The results were consistent for both seated and ambulatory BP measures. These results suggest that unlike the hypotensive effects of skim milk and non-fat dairy products observed in our previous study [10], the solitary manipulation of conventional full-fat dairy products was not effective in reducing BP in adults with elevated BP.

We have previously demonstrated that solitary addition of skim milk and conventional non-fat dairy products to the routine diet reduced BP in middle-aged and older adults with elevated BP [10]. In the present study, we used the same interventional design, similar experimental protocol, and identical dependent measures that were successfully implemented in our previous study. However, the results of the present study were in marked contrast to the our previous investigation as the hypotensive effects of dairy products were not replicated with whole milk and conventional full-fat dairy products [10]. Only one other dietary intervention study has investigated the effect of full-fat dairy products on BP in humans when added to the normal diet [43]. A major limitation of that study was that the research subjects were young, healthy normotensive college students that had very low (i.e., normal) BP prior to the dietary interventions. In fact, BP was not reduced during the low-fat dairy condition, and the high-fat dairy condition increased BP significantly. The results of the present intervention study are consistent with some of the observational studies reporting no differences in BP between adults who consume high-fat dairy products and those who

do not (13-15). Taken together, these findings suggest that the regular intake of whole milk and full-fat dairy products added to the normal routine diet are not associated with reduced BP.

Similar to non- and low-fat dairy products, whole milk and full-fat dairy products contain similar or even greater amounts of constituents that have been implicated in the hypotensive effects. Lactotripeptides (LTPs), found in fermented dairy products, can exert BP-lowering effects that resemble ACE inhibitors [10, 38-40]. In addition, dairy proteins may also contribute to its hypotensive effects [44]. The hypotensive properties of dairy products have also been ascribed to micronutrients including potassium, calcium, and vitamin D [30, 45, 46]. These bioactive peptides are present in both whole milk and full-fat dairy products as in skim milk and non-fat dairy products. What then are the potential mechanisms underlying reduced BP that were not observed in the dietary intervention incorporating whole milk and full-fat dairy products? The lack of significant BP reduction in this study may be attributed to a greater amount of milk fat that is rich in saturated fat leading to vascular dysfunction and the interference with the hypotensive effects. Animal studies have demonstrated that high consumption of saturated fats results in the elevation of arterial BP [32, 33], presumably mediated through insulin resistance and/or oxidative stress [47]. The heightened inflammatory and oxidative response also promotes the renin angiotensin system (RAS) to produce angiotensin II (AngII) exerting chronic vasoconstrictive restraint on the arterial wall [48]. Clearly, further studies are needed to determine how constituents in full-fat dairy products, particularly saturated fat, interfere with the BP lowering effects.

Both pre and post measures of saturated fat consumption were lower in our previous investigation of non-fat dairy products [10] than in the current investigation. As carbohydrate and protein consumption were similar between the non-fat dairy study [10] and the current study,

elevated saturated fat intake may be a primary suspect for increased caloric content. Interestingly, monounsaturated fat content was significantly increased during the control condition while polyunsaturated fats were decreased. Increased consumption of monounsaturated fats by 8% of calories is enough to significantly reduce BP by approximately 9 mmHg in adults with elevated BP when saturated fat content was set to 6% of calories [49]. In the current study, monounsaturated fats were increased during the control while saturated fat content was maintained. Both protein and saturated fat content increased during the high dairy condition. The monounsaturated fat and protein content of the control and high dairy condition, respectively, may exert hypotensive effects, thereby explaining a potential time effect for seated and ambulatory systolic BP. However, the investigators were limited by nutritional labelling whereby food manufacturers are not required to disclose mono- and polyunsaturated fat content [50] leading to estimations derived from the USDA nutrient profile data bank [51]. Interestingly, several other studies examining the effects of engineered full-fat dairy products, incorporating elevated mono- and polyunsaturated fats based on altering bovine feed, have demonstrated reduced total and LDL-cholesterol [52-55]. Unfortunately, no studies have yet examined the implications surrounding BP in adults with elevated BP consuming mono- and polyunsaturated-enriched dairy-based products. Overall, our findings suggest that protein, and other dairy constituents within generic full-fat dairy products, were unable to overcome the negative effects of saturated fat.

Non- and low-fat dairy products have been widely recommended for the prevention of hypertension and CVD. However, a concern has been raised that full-fat dairy products may increase the risk of hypertension and CVD due to a high-fat content in dairy products. Indeed, increases in systolic BP have been reported with the consumption of saturated fats in experimental

studies [56, 57]. In the present study, total cholesterol concentration increased significantly in the high dairy condition. However, the duration of the present dietary interventions was relatively brief by design as BP responds robustly to dietary changes. It is not known if longer-term consumption of high-fat dairy products raises plasma cholesterol and BP thereby elevating the risks of CVD.

A major strength of the present study is the solitary addition of whole milk and full-fat dairy products to the normal routine diet. We reason that such conventional approach would be more applicable to the general population. A major limitation of the study may be varying degree of differences in macro- and micronutrients between the high dairy and non-dairy conditions. There were no differences in total calories, total fat, and sodium intake but carbohydrate, protein, and potassium intake were different between the dietary conditions. The no-dairy condition was created to be closely matched to the high dairy condition in terms of calories (e.g., whole milk vs. coconut milk). It should be noted that the primary intent of the control condition was to demonstrate that dependent measures (i.e. seated and ambulatory BP) would remain stable during the control condition. Another major limitation of the study was the different treatment for subgroup of subjects. Though premenopausal females were expected to get their baseline measures during their early follicular phase, the investigators did not validate the claims made by the participants. Some might further argue that subjects being non-compliant with the diet might confirm lack of significant findings. However, one could argue that the consumption of whole milk and full-fat dairy products from participants may be validated by increases in total cholesterol and blood glucose concentrations that would be expected with increases in saturated fat content, thereby validating.

Rising blood glucose and total cholesterol levels are to be expected with increasing saturated fat intake. In animal models, short-term responses to saturated fat intake have demonstrated reduced glucose clearance [47, 58]. Over a 24-hour period, saturated, monounsaturated, and polyunsaturated fats decrease glucose clearance by impairing insulin secretion and with saturated fat decreasing insulin sensitivity in overweight and obese non-diabetic humans [59]. In lean healthy volunteers, an increase in saturated fat consumption over a 4-week period did not induce changes in insulin resistance [60]. In contrast, a 3-month isoenergetic diet demonstrated reduced insulin resistance [61] when compared with a diets rich in monounsaturated fats. Therefore, it appears that elevated saturated fat consumption may exert greater effects over time in lean individuals. Within the current study, we did not observe increases in insulin resistance over a 4-week span based on HOMA calculations, but it may be likely that our dietary intervention might be too short. With respect to dairy protein, whey is effective in reducing insulin resistance in overweight and obese individuals over the span of 6 and 12 weeks [62]. Therefore, the dairy protein may offset the negative cardiometabolic effects of the saturated fats, but such effect may be dependent upon the adiposity of the individuals. One trend that is clear is that an increase in total cholesterol was seen in both shorter and longer-term studies with increase saturated fat intake with lean individuals [60, 61] thereby increasing CVD risk.

Further subgroup analyses did not provide any new further insights in the present study. In our first analysis, BP differences between males and females were assessed. When compared to males, females generally demonstrate a pronounced systolic BP at the seventh decade of life, as BP in males is typically higher until the sixth decade [21, 63]. However, sex differences were not seen in our study. A lack of sex differences was also demonstrated in our previous investigation

examining non-fat dairy products [10]. Studies demonstrating reduced BP with estrogen therapy in post-menopausal women suggest lack of estrogen is responsible for increasing BP in post-menopause [64, 65]. To further investigate potential impacts of estrogen, we assessed baseline and post-test BP measures between pre- and post-menopausal females. However, no significant differences were observed. Overall main effects of age were seen for both sexes with respect to elevated diastolic BP in the older (i.e. 6^{th} and 7^{th} decades) compared with the younger age groups (i.e. 2^{nd}, 4^{th}, and 5^{th} decades). Yet, such differences between age groups are to be expected as there is a decrease in diastolic BP after the 5^{th} decade of life [22].

Several unanswered questions have risen regarding the hypotensive properties of full-fat dairy products. What is the physiological mechanism as to how full-fat dairy products interfere with hypotensive effects of dairy products? Could elevated BP be attenuated when saturated fat in dairy is replaced with mono- and polyunsaturated fats? Could the increase in total cholesterol and glucose concentrations exert potential hypertensive effects if the duration of the dietary intervention was prolonged for 8-12 weeks?

In conclusion, the present dietary intervention study demonstrated that the incorporation of whole milk and full-fat dairy products into the normal routine diet did not reduce BP on adults with elevated BP. The present findings are in marked contrast to the dietary intervention study that we conducted using skim milk and non-fat dairy products with very similar experimental conditions. These findings are not consistent with the recommendations that all dairy products are beneficial in lowering BP.

Chapter 3: Study 1 - Effects of Whole Milk and Full-Fat Dairy Products on Subclinical Vascular Function in Adults with Elevated Blood Pressure

ABSTRACT

High consumption of low- and non-fat dairy products improves vascular dysfunction associated with elevated arterial blood pressure (BP). Currently, it is unknown if conventional full-fat dairy products mediate similar vascular responses. To determine if adding whole milk and full-fat dairy products to the normal routine diet improves vascular function in adults with elevated BP. Sixty adults (age±SEM; 58±2 years) with elevated blood pressure (systolic/diastolic; 120-159/<99 mmHg) were randomized into a controlled crossover intervention trial consisting of two 4-week dietary periods. The high dairy condition consisted of adding 4 daily servings of whole milk or full-fat dairy products to the normal diet and eliminated all dairy intake during the alternative condition. A 2-week washout period separated the dietary conditions. Carotid-femoral pulse wave velocity (cfPWV) did not change significantly in the high dairy (11.3±0.3 vs. 10.9±0.3 m/sec) and control conditions (11.2±0.3 vs. 11.0±0.3 m/sec). The results were consistent when ultrasound-derived vascular distension measures (arterial compliance, beta-stiffness index, and elastic modulus) were evaluated. Cardiovagal baroreceptor sensitivity (via Valsalva maneuver) demonstrated no significant change for either dietary condition. Brachial arterial flow-mediated dilation (FMD), a measure of endothelium-dependent vasodilation, did not change significantly during the high dairy (5.7±0.5 vs. 5.4±0.6%) and control conditions (6.5±0.5 vs. 5.6±0.6%). The solitary addition of whole milk and full-fat dairy products has no effect on subclinical vascular function in adults with elevated BP.

INTRODUCTION

The vasculature can be compartmentalized into a compliance function in the large elastic arteries and a conduit function residing in more downstream arteries. Accordingly, the elasticity/stiffness and the contractile state of the arterial wall are the primary components that determine vascular function. Inability of the vasculature to change its geometry in response to various stimuli (e.g., pulsatile flow, shear stress) is an indicator of declining arterial function. Age-associated increases in arterial stiffness contribute to the rise in arterial systolic blood pressure (BP) and overall cardiovascular disease (CVD) risk [12, 66]. Arterial stiffening, in turn, blunts the sensitivity of baroreceptors and attenuates beat-by-beat mechanisms for regulating arterial BP [38, 67, 68]. Additionally, the degree of endothelial dysfunction is closely associated with the severity of hypertension [69] although whether endothelial dysfunction initiates hypertension, or exists as a consequence thereof, remains highly controversial [70]. Therefore, strategies to improve vascular function should lead to better BP control and reduced CVD risks.

Dietary behavior is known to modulate vascular function associated with reduced arterial BP [71, 72]. In particular, regular dairy intake has been associated with reduced arterial stiffness and improved endothelial function [11, 71, 73]. Dietary interventional studies have documented that non- and low-fat dairy products would lead to improvements in subclinical vascular functions [11, 71, 74]. We have previously reported reduced arterial stiffness, increased endothelial function, and enhanced cardiovagal baroreflex sensitivity following the solitary addition of skim milk and non-fat dairy products to the normal routine diet [11]. However, whether whole milk and full-fat dairy products could exert similar effects on vascular function remain unclear. Due to the negative implications surrounding saturated fat contained in full-fat dairy products, some have

called for the replacement of full-fat with lower dairy fat alternatives. However, elevations in CVD-risk with saturated fat intake have not been supported by recent studies [20, 34, 35]. More importantly, no dietary interventional study has assessed the effects of adding whole milk and full-fat dairy products to the normal routine diet on vascular function.

Accordingly, the primary aim of the present study was to determine the effects of whole milk and full-fat dairy products on vascular function when added to the normal routine diet in adults with elevated BP. We hypothesized that dietary intervention consisting of full-fat dairy products would result in improved vascular function as determined by arterial stiffness, endothelial function, and cardiovagal baroreflex sensitivity.

METHODS

Subjects: A total of 60 adults (53% female) with elevated BP (137±1/83±1 mmHg) completed the study. Exclusion criteria were: overt chronic diseases, pregnancy or lactation, smoking, type I or II diabetes, milk allergy or lactose intolerance, high baseline dairy intake (>3 servings/d), BMI >45 kg/m^2, or strenuous physical activity (>3 times/wk). All eligible participants were screened during a 2-week run-in period to ensure a consistently elevated systolic BP at rest of 120-159 mmHg and diastolic BP of <99 mmHg on two separate occasions [25]. Prior to the participation, informed consent was obtained from all subjects in accordance with the University of Texas at Austin's Institutional Review Board.

Experimental Design: As depicted in Figure 3.1, participants were randomized into a high dairy or no dairy (control) condition for the first 4 weeks and performed the alternative condition for the final four weeks with a minimal 2-week washout period. For premenopausal women, a 4-week washout period was used to ensure that baseline measures were started in the

same early follicular phase for both dietary conditions. During the high dairy condition, subjects chose their 4 daily servings from a list of dairy items provided by investigators: 8 fluid ounces of generic Hill Country whole milk (Hill Country Dairies, Austin, TX), 6 ounces of single-serve container of Brown Cow yogurt with cream on top (Stonyfield Farm, Londonderry, NH) and 1.5 ounces of HEB branded Swiss cheese (HEB Grocers, San Antonio, TX). During the no-dairy condition, an isocaloric plant-based control for the dairy products were provided by investigators: 16 fluid ounces of HEB branded orange juice fortified with vitamin D and calcium (HEB Grocers, San Antonio, TX), 16 fluid ounces of Silk coconut milk (WhiteWave Services, Inc., Broomfield, CO), 2 ounces of Plantar salted peanuts (Kraft Heinz Company, Glenview, IL), and 4 ounces of Motts applesauce (Motts LLP, Plano, TX). In the control condition, subjects eliminated all the dairy consumptions from their diet in order to maximize the differential dairy intake between the two dietary conditions. All the dairy products were provided in daily packages by the investigators. Milk was provided by the gallon with measuring cups. Yogurt and cheese products were in single-serve packaging. The subjects were free to consume the required servings at any time of day as a single dose or all at once. Throughout the entire experimental period, the subjects were instructed to maintain their body weight, physical activity, as well as their usual diet other than those prescribed by the dietitian. All subjects met weekly with a licensed dietitian to measure body weight, receive weekly dietary food items, and ensure compliance. All measurements were taken before and after the dietary protocol following a 12-hour overnight fast (e.g., no caffeine, alcohol, food, or exercise).

Statistical Analyses: A priori testing was performed to determine sample size based on the effect size of previous interventional studies on similar vascular outcome measures (nQuery

Advisor, Statsols, Boston, MA) [11, 39, 40]. To control for random effects, two-factor (condition x time) mixed-model ANOVA with repeated measures were used assessed all subject characteristics and main dependent variables. Because there were no sex/gender-related differences, sex was dropped from one of the factors. To identify significant interactions, Tukey's Least Significant Difference (LSD) post hoc tests were performed. Pearson correlation coefficients were calculated for additional subgroup analysis. Partial correlation was utilized to assess associations in individual dairy elements while controlling for the other dairy products in the study for the main dependent variables. All statistical analyses were performed using SPSS 23.0 statistical software (SPSS, Inc., Chicago, IL). The alpha value was set at $P<0.05$ for all analyses. Data were presented as means±SEM.

Detailed Methods:

Blood Samples

All blood samples were taken from the antecubital fossa by venous acupuncture by a certified phlebotomist. Whole blood samples were collected, centrifuged, then the plasma was aliquoted and stored at -80ºC for future analyses. Plasma concentrations of endothelin-1 (R&D Systems, Minneapolis, MN), total nitric oxide (NOx) consisted of nitrite/nitrate (ENZO Life Sciences, Farmingdale, NY), and epinephrine/norepinephrine (Abnova, Taipei City, Taiwan) were determined via enzyme-linked immunosorbent assays to evaluate changes in circulating vasoactive factors in response to dietary interventions.

Pulse Wave Velocity and Blood Pressure

Participants were required to get their seated blood pressure readings weekly when they came in to receive their weekly portion of food. Measurements were taken in triplicate by an

automated device (HEM-907XL; Omron Healthcare, Vernon Hills, IL) in a dimly lit room. All blood pressure measurements were in accordance with the American Heart Association guidelines [41]. Supine brachial blood pressure was measured with the participants in the supine position using oscillometric automated sphygmomanometer (VP-1000 Plus, Omron Healthcare, Kyoto, Japan) to assess brachial-ankle index during the pre and post testing visits. Carotid-femoral pulse wave velocity, an index of arterial stiffness, and carotid artery pressure waveforms were obtained using an automatic vascular screening device (VP-1000 Plus, Omron Healthcare, Kyoto, Japan) as previously described [11].

Carotid Artery Compliance

The combination of ultrasound imaging of a common carotid with simultaneous tonometric-obtained arterial pressure waveforms from the contralateral artery permitted noninvasive determination of carotid artery compliance [75]. Arterial diameter was measured from the images derived from an ultrasound machine (iE33 Ultrasound System, Phillips, Bothel, WA) equipped with a high-resolution linear-array transducer as previously described [75]. A longitudinal image of the cephalic portion of the common carotid artery was acquired 1-2 cm proximal to the carotid bulb with the transducer placed at 90 degrees to the vessel. All image acquisitions were performed in duplicate, for data quality assurance, by a trained technician. All ultrasound-derived diameter data was analyzed utilizing automated image analysis software (Carotid Analyzer, Medical Imaging Applications, Coralville, IA) by the same investigator who was blinded to the dietary conditions. Arterial pressure waveforms were obtained using the arterial tonometry placed on the carotid artery and recorded on a data acquisition software (Windaq 2000,

Dataq Instruments, Akron, OH). Intima-media thickness was measured at end diastole as previously described [76].

To ensure comprehensibility, we characterized arterial mechanical properties of the carotid artery through several measures including compliance, arterial distension, elastic modulus, and beta-stiffness index. Arterial compliance is a measure of absolute change of cross-sectional area following blood pressure changes whereas arterial distension is a relative change of cross-sectional area following blood pressure changes [77, 78]. Beta-stiffness index controls for distension pressures brought about by changes in blood pressure [79]. Elastic modulus is a theoretical measure assessing the amount of pressure needed for a 100% stretch of the arterial wall [77].

Flow-Mediated Dilatation (FMD)

FMD is a non-invasive method to assess vascular endothelium-derived vasodilatory function as previously described [80, 81]. Brachial artery diameters and blood flow velocity was measured with an ultrasound machine equipped with a high-resolution linear array transducer (Phillips iE33 Ultrasound System, Bothel, WA). A longitudinal image of the brachial artery was acquired 5-10 cm proximal to the antecubital fossa. A BP cuff was placed on the forearm 3-5 cm distal to the antecubital fossa and inflated to 100 mmHg above resting systolic BP for 5 minutes using a rapid cuff inflator (E20, D.E. Hoakanson, Bellvue, WA). After cuff deflation, brachial artery diameters and blood velocity were measured for 3 minutes. All ultrasound-derived diameter data were analyzed by the same investigator who was blinded to dietary conditions, using automated image analysis software (Brachial Analyzer, Medical Imaging Applications, Coralville, IA). FMD was calculated as a percent change in brachial artery diameter from baseline to peak diameter.

Cardiovagal Baroreflex Sensitivity (BRS)

Cardiovagal BRS was determined using Valsava maneuver technique as previously described [82]. After deep inspiration, subject performed forced expiration though a mouthpiece with 1-inch diameter. An expiratory mouth pressure was maintained at 40 mmHg for 10 seconds using visual feedback (Windaq, Dataq Instruments). Measurements of R-R interval (ECG) and beat-to-beat arterial blood pressure (Portapres, Ohmeda) were collected throughout the testing period. Cardiovagal BRS was analyzed using the phase IV of the Valsalva maneuver. The R-R interval was regressed on the systolic blood pressure, the slope of this relation (ms/mmHg) represented the cardiovagal BRS if the linear regression coefficient (r) greater than 0.80.

RESULTS

Participants maintained body mass throughout the duration of the study. Dropout rate from the study after randomization and completing the first baseline visit was 10%. Overall, no sex-related differences between dietary conditions were found for any dependent variable. When females were divided into pre- and post-menopause, postmenopausal females exhibited greater central arterial stiffness than premenopausal females ($P<0.05$) despite no effects from time or treatment effects. Furthermore, BP medication had no impact on the dietary intervention.

During the intervention, total dairy intake was reduced from baseline to post testing visits during the control condition (0.7 ± 0.2 vs. 0.0 ± 0.1 servings/d; $P < 0.05$) and increased during the high dairy condition (1.5 ± 0.2 vs. 4.6 ± 0.1 servings/d; $P < 0.05$). The rise in dairy intake during the high dairy condition was significantly greater than during the control condition ($P < 0.05$). An in-depth analysis corresponding to macro- and micronutrient profiles for each of the dietary interventions has been published elsewhere.

Arterial stiffness parameters including brachial-ankle PWV and carotid augmentation index (Table 3.2) exhibited no changes. Central arterial distensibility measures encompassing carotid arterial compliance, distensibility, and elastic modulus remained consistent throughout the duration of the study. Interestingly, an interaction effect was seen approaching significant levels for beta stiffness index ($P = 0.052$), demonstrating elevated stiffness after the high dairy condition when compared to the control condition with respect to pairwise post hoc comparisons ($P = 0.049$). Central arterial stiffness did not exhibit changes between the dietary conditions (Figure 3.2A) as measured by cfPWV but did demonstrate significant reductions as a main effect of time ($P = 0.004$) (Figure 3.3). When divided by decade of age, a main effect of age was also present for cfPWV ($P < 0.001$). In addition, a positive relationship was demonstrated between cfPWV and age ($r = 0.46$; $P < 0.001$). Partial correlation did not reveal any associations between cfPWV and whole milk (controlling for yogurt and cheese intake), yogurt (controlling for whole milk and cheese intake), or cheese (controlling for whole milk and yogurt intake). *Due* to the poor quality of data, few participants were eliminated from analysis of the ultrasound-derived distensibility measures including arterial compliance, distension, beta-stiffness, and elastic modulus (total analyzed; n=58) and the arterial stiffness measure cfPWV (total analyzed; n=55).

As depicted in Figure 2.2B, endothelial function, as measured through FMD, did not change across both dietary conditions. Partial correlation between FMD and whole milk (controlling for yogurt and cheese intake), yogurt (controlling for whole milk and cheese intake), or cheese (controlling for whole milk and yogurt intake). In addition, consistent with the findings of central arterial stiffness and endothelial function, BRS also remained unchanged throughout the duration of the study (Table 3.2). However, a main effect of age was present for BRS ($P < 0.001$).

An inverse trend was also noted between age and BRS (r = 0.35; $P < 0.001$). Surprisingly, partial correlations revealed negative association between BRS and whole milk (controlling for yogurt and cheese intake)(r = -0.30; $P < 0.05$), yogurt (controlling for whole milk and cheese intake)(r = -0.29; $P < 0.05$), and cheese approaching significance (controlling for whole milk and yogurt intake)(r = -0.26; $P = 0.056$). *Endothelial*-derived factors, including endothelin-1 (vasoconstrictor) and total nitric oxide (vasodilator), remained unchanged across both dietary interventions (Table 3.3). Due to the inability to attain blood from every participant during each visit, a few participants were left out of the analysis (total analyzed; n=53).

Table 3.1 Selected subject characteristics.

Variables	No Dairy	High Dairy
Age (years)	58±2	58±2
Height (cm)	169±1	169±1
Body mass (kg)	85±2	85±2
BMI (kg/m^2)	29.3±0.8	29.2±0.8
Heart rate (bpm)	61±1	62±1

Values are means±SEM. There were no significant changes with either dietary condition.

Table 3.2 Changes in subject vascular profile.

	No Dairy		High Dairy	
Variables	Before	After	Before	After
Ankle-brachial index	1.13±0.01	1.13±0.01	1.14±0.01	1.13±0.01
Brachial-ankle PWV (m/sec)	14.9±0.3	14.6±0.3	14.9±0.3	14.5±0.3
Carotid augmentation index (%)	23±2	23±2	24±2	21±2
Baseline brachial artery diameter (mm)	3.97±0.10	4.00±0.10	4.00±0.10	3.98±0.10
Peak brachial artery diameter (mm)	4.21±0.10	4.21±0.10	4.21±0.10	4.21±0.10
Absolute FMD (mm)	0.25±0.02	0.21±0.02	0.23±0.02	0.21±0.02
Carotid arterial compliance ($mm^2/mmHg \times 10^{-1}$)	0.88±0.06	0.89±0.08	0.89±0.06	0.84±0.08
Arterial distensibility ($mmHg^{-1} \times 10^{-3}$)	3.43±0.17	3.57±0.22	3.36±0.17	3.37±0.22
β-stiffness index (U)	4.2±0.4	4.1±0.5	4.1±0.4	4.5±0.5
E_p (mmHg)	697±34	692±42	679±34	728±42
Carotid IMT (mm)	0.52±0.02	0.49±0.02	0.52±0.02	0.52±0.02
Cardiovagal BRS (ms/mmHg)	8.3±0.8	9.5±0.7	9.5±0.8	8.2±0.7

Values are means±SEM. There were no significant changes. PWV=pulse wave velocity, FMD=flow-mediated dilation, Ep=Peterson elastic modulus, IMT=intima media thickness, BRS=baroreflex sensitivity.

Table 3.3 Changes in plasma concentrations of vasoactive factors with the dietary interventions.

	No Dairy		High Dairy	
Variables	**Before**	**After**	**Before**	**After**
Endothelin-1 (pg/mL)	1.91±0.05	1.85±0.06	1.79±0.05	1.87±0.06
Nitric oxide (μmol/L)	29.3±1.9	30.1±2.6	25.3±2.0	32.1±2.7
Epinephrine (ng/mL)	21.9±5.3	25.5±4.0	30.4±5.3	28.4±4.0
Norepinephrine (ng/mL)	551±45	591±44	573±46	627±44

Values are means±SEM. There were no significant changes with either dietary condition.

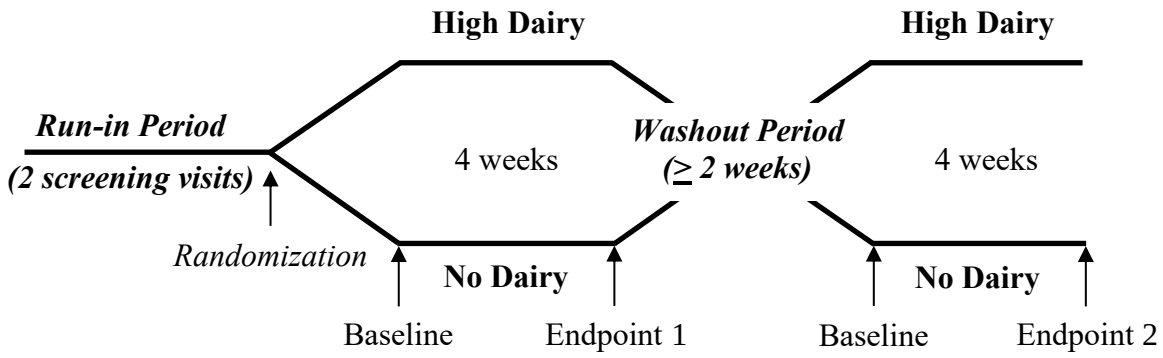

Figure 3.1 Overview of the randomized controlled crossover dietary intervention design.

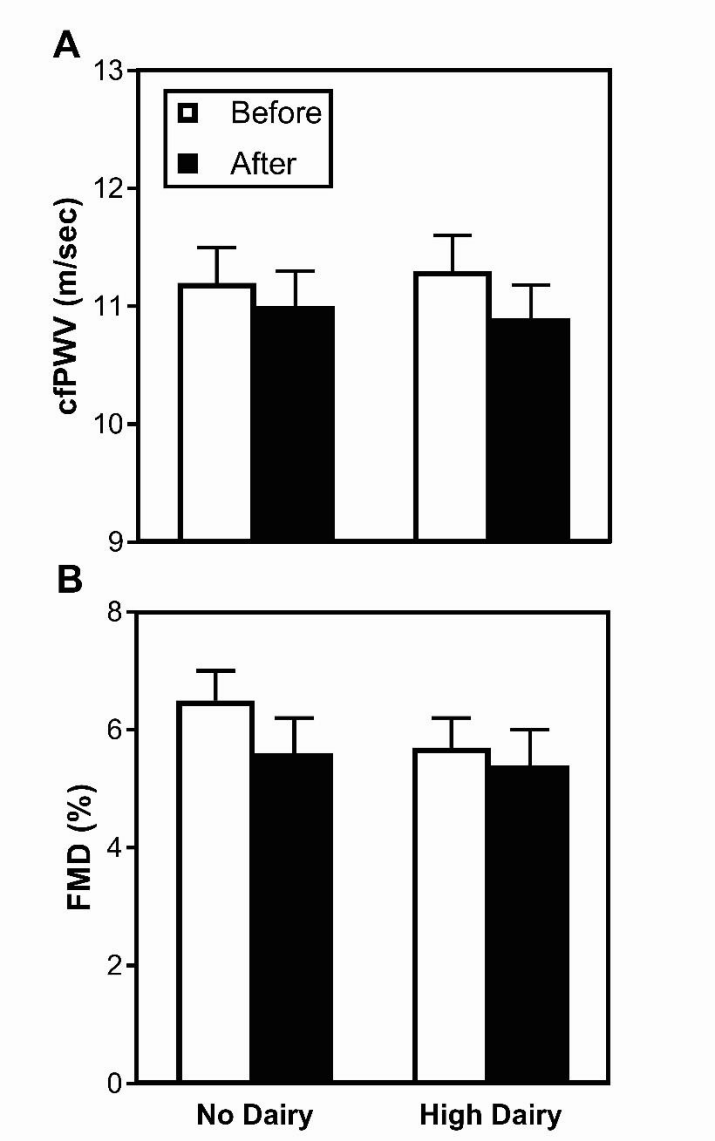

Figure 3.2 Carotid-femoral pulse wave velocity (cfPWV) (A) and brachial artery flow-mediated dilation (FMD) (B) before and after no dairy and high dairy conditions.

There were no significant interaction effects.

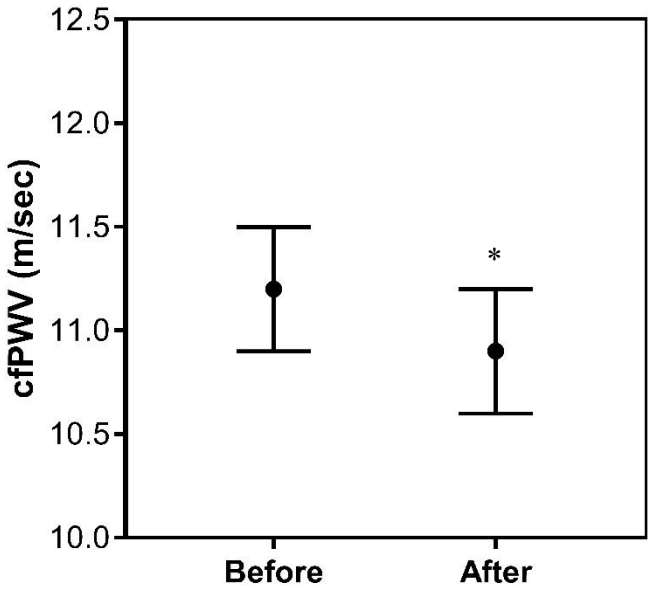

Figure 3.3 Main effect of time for carotid-femoral pulse wave velocity (cfPWV). *Denotes significant main effect of time ($P < 0.05$).

DISCUSSION

The findings of the present study indicate that whole milk and full-fat dairy products added to the normal routine diet do not improve key vascular functions implicated in elevating BP. Specifically, the vascular stiffness measures implemented in the present study did not change during the dietary intervention. No changes in endothelium-dependent vasodilation and cardiovagal baroreflex sensitivity were observed. A lack of dietary effects on vascular function may be perceived as negative findings but may also suggest that saturated fat contained in whole milk and full-fat dairy do not impair vascular function. The current investigation is the first dietary investigation study to determine the relationship between full-fat dairy products and arterial wall properties in adults with elevated BP.

An increase in the stiffness of the large elastic arteries located in the cardiothoracic circulation is thought to be the primary mechanism underlying the age-associated increase in systolic BP. In marked contrast to our previous investigation evaluating skim milk and non-fat dairy products [11], the present study utilizing the identical experimental protocol did not replicate the destiffening effects of dairy products as our whole milk and full-fat dairy products added to the normal routine diet did not induce changes in arterial stiffness. To assess arterial wall properties as comprehensively as possible, we implemented a variety of methods in the present study. Both propagation models (e.g., pulse wave velocity) and distention models (e.g., arterial compliance) consistently indicate that arterial stiffness did not change with either dietary intervention. Our findings corroborate with the findings of previous studies indicating no detrimental effects of dairy products on central arterial stiffness [73, 83]. Given these findings over the short time span of the investigation (i.e. 4 weeks), it remains unknown whether longer-

term full-fat dairy products might negatively impact the elastic mechanical properties of the arterial walls.

Arterial baroreceptor-mediated changes in BP parallel changes in heart rate as a way to control BP on a beat-to-beat basis [84]. The sensitivity of these cardiovagal baroreceptors are blunted in adults with elevated blood pressure [85]. In our previous investigation, regular intake of skim milk and non-fat dairy products improved the sensitivity of the baroreceptors, and such changes were correlated to reductions in central arterial stiffness [10]. However, full-fat dairy products did not induce changes in BRS in the present investigation. This finding may not be surprising given the lack of changes in arterial stiffness. The compliance of carotid arteries and aorta in which arterial baroreceptors are located is closely associated with cardiovagal BRS through the ability of these reflexogenic regions to transduce signals [38].

Reduced nitric oxide bioavailability appear to be responsible, at least in part, for the age-associated augmentation in the vasoconstrictor tone of large conduit arteries [86]. Regular non- and low-fat dairy intake can produce significant improvements in endothelial function [9, 11]. However, increased consumption of full-fat dairy products did not elicit similar improvements in endothelial function as estimated by FMD in the present study. Follow-up analyses of plasma endothelium-dependent biomarkers NOx are consistent with the results in FMD. Taken together, the regular consumption of whole milk and full-fat dairy products added to the normal routine diet does not appear to improve endothelial function in adults with elevated BP.

Dairy micronutrients (e.g., potassium) and small peptides (e.g., lactotripeptides) contained in the milk and dairy products have been shown to have bioactive properties that affect vascular function [39, 40, 87]. Lactotripeptides (LTPs), formed from fermented dairy proteins, improve

endothelial function and central arterial compliance [39, 40] and also exert greater hypotensive effects with hypertensive than pre-hypertensive patients [88]. Mechanistically, LTPs have been shown to upregulate endothelial nitric oxide synthase in the aorta of spontaneously hypertensive rats [89]. Considering the fact that both non-fat and full-fat dairy products include these bioactive elements, what explains the discrepancy between non-fat and full-fat dairy products on the influence on vascular function? We could only speculate on this but it is plausible that saturated fat in the full-fat dairy products may act to negate the beneficial effects of these bioactive compounds. Indeed, protein consumption in combination with saturated fats eliminate the negative vascular effects attributable to the saturated fats [48, 90, 91]. Clearly, more research is necessary to investigate this mechanism.

Even though we lack statistical power to properly conduct subgroup analyses, such analyses were undertaken. However, they did not provide any new insight into the research findings. Central arterial stiffness generally increases as a function of age [92], and arterial stiffening is known to reduce BRS as one advances in age [38]. Despite seeing no effects of sex, significant reductions in central arterial stiffness were seen among premenopausal females when compared with postmenopausal females. Premenopausal females generally have reduced arterial stiffness than their postmenopausal counterparts as estrogen is responsible for maintaining elasticity of the arterial walls [93]. With respect to partial correlational analyses, it was surprising to show significant negative associations between BRS and increased whole milk and yogurt intake. Such data suggests that less consumption of full-fat dairy products may protect baroreflex sensitivity.

A lack of significant change in vascular function may be considered a negative finding with respect to dietary intake of whole milk and full-fat dairy products. However, one may argue that no significant changes in vascular function may be perceived as a rather positive message given the negative ideology surrounding dairy fats and CVD risk. Consistent with the present study, acute and longer-term effects elevating intakes of full-fat fermented and non-fermented dairy products have demonstrated no significant increases in pro- inflammatory, oxidative, or atherogenic biomarkers [94, 95]. Since an increasing number of investigators have recently begun to question the validity of the dietary recommendation to replace whole milk with reduced-fat milk, it may be clinically relevant to understand the interaction between isolated dairy elements and their impact on CVD risk.

In summary, unlike nonfat dairy products demonstrating improvements in vascular function, whole milk and full-fat dairy products failed to improve vascular function in adults with elevated BP. However, our findings also suggest that there are no negative effects involved in consuming full-fat dairy products on key vascular functions that are involved in the pathogenesis of CVD. Based on our findings, four weeks of consuming full-fat dairy products do not contribute to vascular dysfunction. Such knowledge is beneficial for future investigations examining the effects of long-term studies assessing full-fat dairy products for other therapeutic modalities.

Chapter 4: Review of Literature

EPIDEMIOLOGY OF CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is responsible for 1 in every 4 deaths, amounting to an annual death rate of 600,000 persons within the United States alone [96]. CVD is the leading cause of death not only within the United States [96] but on a global scale [63, 97], most notably within economically developed countries [98]. Despite these trends, CVD-related mortality has been declining over the past half-century worldwide [99, 100]. Such progress is not surprising as the decline in mortality can be attributed to more effective diagnoses, treatment, and accessibility to healthcare. Developed countries are at the forefront of medicinal-based research affording therapeutic strategies to offset CVD risk. The U.S. has taken a leadership role in educating the public about effective strategies to reduce the pronouncement of disease within a clinical setting [101-103]. Adopting such strategies through educational reform and governmental regulations toward reducing risk factors has effectively lowered prevalence and incidence of CVD-related mortality within other countries [104, 105]. Currently, lifestyle modifications are the first approaches to lower risks of cardiovascular disease [2]. Behavioral modifications are inexpensive and have the potential to improve cardiovascular health and to help counteract disproportionate factors and/or predispositions to CVD.

Although a number of CVD risk factors can be reduced, several unmodifiable risk factors for progressing CVD exist: age, sex, race, and genetics. Chronological aging is strongly associated with advanced stroke and heart disease risk [106, 107]. However, referencing chronological age as a risk factor for CVD does not adequately portray biological parameters of the arterial vasculature that is a more accurate and more precise determinant of CVD risk [108]. In younger

ages, males demonstrate a greater death rate from CVD than females [106], whereas the death rate attributed to females surpasses males after the fifth decade [63, 109] as estrogen is believed to confer vascular protection during pre-menopause [110]. However, estrogen therapy to lessen CVD risk [111] is controversial for postmenopausal women as estrogen supplementation has been linked to development of breast cancer [112]. Family history of CVD further increases the risk of CVD-related events [113]. With respect to race, the Centers for Disease Control named CVD as the leading cause of death among non-Hispanic whites, non-Hispanic blacks, American Indians, and Alaskan Natives [96]. Although mortality rates from heart disease are similar among non-Hispanic whites and blacks, non-Hispanic blacks are more susceptible to stroke and diabetes [114]. A genetic predisposition may not entirely represent an underlying cause of CVD, as other disproportionate factors may play a role, particularly non-Hispanic blacks who have an overall lower socioeconomic status thereby limiting access to healthcare [115, 116].

HYPERTENSION AS A RISK FACTOR FOR CVD

Elevated BP is one of the leading risk factors for CVD-related mortality [23, 117] embodied as one of the many metrics to establish cardiovascular health [118]. Isolated systolic hypertension is the prevailing form of elevated BP when compared with systolic and diastolic hypertension or isolated diastolic hypertension [119]. Isolated systolic hypertension is accredited to consistently rising systolic BP with age and a concomitant reduction in diastolic BP after the fifth decade [22]. Furthermore, systolic BP is a greater predictor of CVD than diastolic BP [120]. A systolic goal of <140 mmHg has been linked to lower CVD events and mortality risks [121-123] and was a basis for the former cutoff value of stage I hypertension [1]. However, the most recent guidelines have established the cutoff for stage I hypertension as 130-139 mmHg in systolic

BP [124], which establishes the clinical relevance of controlling systolic BP to moderate CVD risk. For the purpose of this review, preexisting guidelines established at the onset of this study will be utilized [1].

Hypertension has been grouped into two etiological categories: primary and secondary hypertension. Primary, or essential, hypertension exists when its origin remains unknown whereas a preceding event characterizes secondary, or incidental, hypertension. Primary hypertension is often detectable in adolescents prior to being clinically diagnosed in young adulthood [125, 126]. Due to the progressing nature of untreated hypertension with time [126], adolescents will carryover formerly developed CVD risk factors associated with elevated brachial systolic BP [127, 128] into adulthood. Failure to diagnose a pronounced BP within children during the initial stages [129, 130] counters the ability to recognize appropriate origins. Though the etiology of primary hypertension may not be attributed to a single known cause, excessive levels of sympathetic nervous system activity, salt intake, and genetics are suspected to be some of the principal factors [131-134]. Lifestyle modifications in the form of diet and exercise are a first line approach in the management of elevated BP [1, 2]. Inability to regulate primary hypertension will further damage vascular and organ tissue augmenting BP in what earlier investigators phrased ‘the vicious circle of hypertension’ [135], or chronic hypertension. Sustained hypertension is a major contributable risk factor of hypertensive heart disease [4], a form of CVD resulting in left ventricular hypertrophy and eventual heart failure [136, 137].

In the recent two decades, the hypertension-related death rate has been steadily increasing within the U.S., while all-cause mortality has been declining [138]. Susceptible groups of hypertensive-related mortality include women over the age of 85 years, Hispanics, non-Hispanic

whites, and non-Hispanic blacks; despite a reducing trend in non-Hispanic blacks that nevertheless demonstrate a majority cause of death associated with hypertension [138]. Additionally, 67% of adults over the age of 60 years have been diagnosed with hypertension [63], and 90% of the normotensive population at the age of 55 years is expected to develop hypertension at some point in their lifetime [1]. Hypertension is projected to heighten its incidence from 972 million in 2000 to 1.56 billion in 2025, thereby increasing the global economic burden [139]. Every 20 or 10 mmHg increase in systolic BP or diastolic BP, respectively, doubles CVD risk [1]. Reducing the level of hypertension is of paramount clinical importance in moderating CVD risk.

LIFESTYLE MODIFICATIONS TO REDUCE HYPERTENSIVE RISK

In 2010, a statement from the American Heart Association (AHA) issued a national goal to further reduce CVD mortality by 20% by the year 2020 [118]. To achieve this goal, a follow-up study revealed a necessary shift in the population to meet "ideal cardiovascular health" qualifications, which appeared in approximately 0.1% of participants during the ARIC (Atherosclerosis Risk in Communities) study between 1987 and 1989 [140], demanding greater preventative efforts. The seven metrics assessing cardiovascular health include health measures (total cholesterol, BP, blood sugar) and behavioral factors (nonsmoking, BMI, physical activity, healthy diet score). A greater total number of ideal metrics corresponds to lower CVD risk [140]. For this review, dieting strategies and BP will be mainly emphasized.

The Mediterranean diet pattern was endorsed by the AHA, as part of the 2020 goals, to lower CVD risk – a dietary regimen rich in polyunsaturated fats, lean meats, and fiber-rich fruits, vegetables, nuts and whole-grains with diminished intake of saturated fats and sweetened beverages [118]. Although adherence to the Mediterranean diet is advantageous to reduce CVD-

related mortality [141, 142], higher risk individuals are less likely to comply with such a dietary regimen [143, 144]. The U.S. is the lowest ranked country practicing such dietary habits [145]. Yet, another study validated hypotensive effects of the DASH diet within the pre- and hypertensive community when compared against western and vegetarian diets [8]. Epidemiological studies have demonstrated associations with DASH dietary compliance and improved cardiovascular health [146]. The original DASH eating plan integrated fruits, vegetables, lean meats, whole-grains, low-fat dairy and less intake of sweets and saturated fats, encompassing some similarities to the Mediterranean diet without elevated dietary fats. Counseling patients within the primary care setting is a strong impetus for hypertensive patients to increase fruit, vegetable, low-fat dairy intake, and lower BP [147]. However, the effectiveness of reducing BP is limited when relying upon patient compliance opposed to clinical-based dietary provisions.

Variations of the DASH diet incorporating reduced levels of sodium intake [148, 149] are equivalently effective as monotherapeutic antihypertensive medication [150, 151]. The reported mean intake of sodium within the U.S. in 2010 was estimated around 3.6 g/d and a global consumption of approximately 3.95 g/d [152] contributing to 1.65 million annual CVD-related deaths [153]. Excessive dietary sodium intake acts in a dose-dependent manner in raising systolic BP and portrays hypersensitivity among older, hypertensive, and general black communities [153]. Individuals with sustained hypertension are affected more by a low-sodium DASH diet than those with lower BP levels [154]. Therefore, the AHA threshold for sodium consumption has been set to 1.5 g/d to lower BP within these sensitive groups and 2.3 g/d for unspecified groups [118] based on the DASH-sodium trial [148]. While there are multiple dietary sources containing sodium [155], roughly 77% has been attributed to processed foods [156] within the American diet.

Physiologically, abnormal kidney function, associated with advancing age and hypertension [157], causes inadequate processing of excessive sodium levels resulting in greater retention of water and subsequent volume overload heightening BP [132]. Consumption of unprocessed foods, ascertained by the low-sodium DASH eating plan [148], is appropriate and should be considered as a habitual practice among Americans' eating habits.

Aerobic exercise incorporated into a calorie-restricted DASH diet is also a practical approach in facilitating improved reductions of BP within an pre-, stage 1, and stage 2 hypertensive overweight population [71]. Body mass index (BMI), a surrogate measure of body fat, serves as a major cardiometabolic risk factor when classified as overweight [158, 159]. The AHA conceded to the BMI terms of the National Heart, Lung, and Blood Institute of 18.5-24.9 kg/m^2 despite disease events occurring the least when the BMI range is 18.5-22 kg/m^2 [118]. The prevalence of those meeting AHA's BMI requirements within the U.S. has been steadily declining for adults and adolescents [63] indicative of a proportional increase of overweight individuals [160]. Overweight children are at greater risk of developing other comorbidities, including hypertension, diabetes, and cholesterol, during their adolescence than normal weight children [161] carrying over into their adult years [162]. Regular aerobic exercise has been identified to reduce body fat in overweight children [163] and adults [164] when compared to their sedentary counterparts. Moreover, cardiorespiratory fitness has been established to decrease hypertensive risk [165], even among adults who exercised regularly during their former upbringing and have diminished physical activity later in adulthood [166, 167]. Due to varying responses of aerobic exercising, calorie-restricted dieting is a more favorable strategy to reduce body weight [168, 169]. The macronutrient profile of any calorie-restricted diet can be modified to effectively lose weight [170]

even to target traditional risk factors attributable to CVD risk [169]. Combining calorie-restricted diets and exercise strategies exerts additive effects to lower CVD risk and body weight [71, 169]. The efficacy of lifestyle modifications, through dieting and exercise to lessen CVD risk, has been advocated among several organizations [2, 5, 171].

Manipulation of the macronutrient profile, or routine dietary foods without calorie restriction, also effectively reduces CVD risk. The original DASH diet, rich in carbohydrates from natural sources [8], was responsible for cutting out refined sugars, which have been linked to hypertension [172, 173]. Multiple accommodative eating plans exist that similarly lower BP and CVD risk through enriched complex carbohydrate meals (i.e. DASH diet), or by partial replacement of carbohydrates with unsaturated fats (i.e. Mediterranean diet) or protein [49, 174]. However, other dieting strategies manipulate regular eating habits by adding specific food items to the regular diet to determine health benefits maintaining isocaloric conditions. The original DASH trial popularized low- and non-fat dairy consumption but ineffectively segregated its hypotensive features from the rest of the diet [8]. Observational studies have since demonstrated inverse associations of low-fat dairy intake and BP [17, 175]. Furthermore, randomized clinical trials have attributed hypotensive effects to 3-4 daily servings of low- [9] or non-fat dairy products [10] to improve vascular function associated with elevated BP [11] when added to the normal routine diet opposed to a diet where dairy was absent. Prior to these studies, the AHA cautiously refrained from including low-fat dairy as a dietary recommendation due to lacking causal evidence in 2010 [118], but have recently encouraged low-fat dairy consumption as part of a heart-healthy diet [176].

HYPOTENSIVE EFFECTS OF DAIRY CONSTITUENTS

The heart-healthy properties arising from non- and low-fat dairy products has been ascribed to the dairy proteins. The two major dairy proteins, casein and whey, compose 80% and 20% of total protein, respectively [177]. Compared to a normalized diet with varying protein sources, a three-month semi-isolated protein diet high in whey and casein, separately, revealed distinguishing elevations in glutathione, an antioxidant scavenger, within heart tissue of aged mice [178]. The effectiveness of whey protein to enhance intracellular glutathione is attributed to its enriched cysteine content [177], formerly identified as a rate-limiting substrate of glutathione synthesis [179]. Additionally, casein, rich in methionine [177], stimulates endogenous production of the intermediary byproduct homocysteine [180] before yielding cysteine and subsequent glutathione [44]. Excessive methionine intake [180], or reduced homocysteine flux, through deficient levels of choline [181], folate, B6 and B12 [182], are likely to result in hyperhomocysteinemia, an independent risk factor of CVD [183] responsible for oxidative damage [184] and vascular dysfunction [185]. Dairy products are a significant source of choline, folate, B6 and B12 [51] to negate the effects of copious levels of methionine alone. The DASH diet revealed significant reductions in serum homocysteine levels when contrasted against a western and vegetarian diet [186]. Therefore, dairy proteins assist expanding the glutathione pool demonstrating antioxidative characteristics and protection against vascular dysfunction.

Intriguingly, constituents of dairy proteins also accommodate antihypertensive and other advantageous vascular properties that lessen hypertensive risk. Casein-derived peptides, isoleucine-proline-proline and valine-proline-proline, or lactotripeptides, are the most common and potent peptides in dairy capable of reducing elevated systolic and diastolic BP [88, 187, 188].

Specifically, a LTP-based therapeutic intervention, analyzed against a placebo group, significantly lowered systolic BP, cardiac workload, and arterial vascular stiffening associated with CVD [189]. Improvements in arterial stiffness and endothelial function corresponding to LTP ingestion is associated with parallel reductions in BP [39, 40]. Aside from LTPs, other nutraceutical features of dairy proteins include relaxation of vascular smooth muscle cells [190, 191], and suppression of vasoconstricting peptide hormones [192], vasoconstriction-responding receptors [193], and proinflammatory markers [194, 195] linked to early atherosclerotic plaque development [196]. The capabilities of dairy protein supplementation to lower elevated BP while concomitantly promoting antioxidative status, should be regarded as an appropriate dieting strategy to modify CVD risk.

Nevertheless, general saturated fat intake has been established as a cause of elevating systolic BP when compared to consuming unsaturated fatty acids [32, 33]. The mechanisms involving saturated fats contributing to elevated BP have been linked to reduced insulin sensitivity [60, 197] and increased oxidative stress promoting vascular dysfunction [198], increasing proinflammatory cytokines, and the RAS response that is associated with elevated BP [47, 48]. Prolonging exposure of saturated fats within the circulation through reduced insulin sensitivity, especially among overweight and obese individuals, aggravates the renin angiotensin response through oxidative mechanisms [48]. Dietary proteins have been demonstrated to offset the oxidative effects to exert vascular protective effects [91, 199]. However, no study has yet demonstrated the degree to which dairy proteins may offset the hypertensive effects contributable to saturated fats found in dairy.

Saturated fatty acids emanating from dairy sources are emerging as potential candidates for cardiovascular health. In an abbreviated 3-week high-fat DASH trial [16], whole-fat dairy products were substituted in place of low- and non-fat dairy products and found to comparably lower BP as the original DASH eating plan. However, data surrounding saturated fat in dairy has been mixed and inconclusive as observational studies have demonstrated inverse trends of BP and levels of whole-fat dairy consumption [15, 27, 175] or no relationship [17, 30]. The lack of sensitivity to detect hypotensive trends with whole-fat dairy intake may be limited from the blending of low- and whole-fat dairy consumers, often ingesting higher levels of low-fat dairy compared to whole-fat dairy [17, 30], or not accounting for distinctive dietary patterns linking low-fat than whole-fat dairy consumers with healthier eating habits [17].

However, saturated fats indicative of heart healthy benefits has challenged conventional thought in context of heart-healthy benefits. Two saturated fats from dairy, pentadecanoate (C15:0) and transpalmitoleate (C16:1n-7) [200-202], endogenously produced in bovine milk, have demonstrated favorable BP effects despite a miniscule presence. Self-reported data based on dairy fat intake revealed significant reductions in systolic and diastolic BP when compared from the lowest to highest quintiles of C15:0 and C16:1n-7 [203]. Other observational studies establishing associations in BP between low and high dairy fat intake have shown either no significant reductions in BP for C16:1n-7 [204], C15:0 [202], or positive findings for reduced BP with C16:1n-7 [205]. The previously mentioned studies finding no hypotensive trends [202, 204], also did not demonstrate hypertensive effects as other common saturated dairy fats such as myristic acid [203], stearic acid [32], or coconut oil rich in lauric acid [33]. Therefore, the effectiveness of

two fatty acids to lower BP may be limited in their ability to attenuate the hypertensive properties of alternative saturated fats identified in whole milk products.

SATURATED FAT CONSUMPTION AND CVD RISK

Conventional understanding of dietary saturated fat intake linked with CVD risk has been controversial. Ancel Keys is identified for establishing the relationship between heart disease and overnutrition by total dietary fat and consequential elevation of total cholesterol [206, 207]. Early in Keys' career, he discovered a strong association between dietary fat and CVD mortality, postulating evidence in support of a low-fat dietary regimen [208]. Some viewed Keys' conclusions as valid [209] while others proved the study insufficient when held up to scrutiny [210]. Keys' findings were simply dismissed based on several revelations: 1) weaker association when investigator bias was eliminated by including all countries opposed to a select few, 2) limited significance as the measure for total dietary fat intake was based on amounts available for consumption rather than what was actually consumed, and 3) lack of specificity as total protein available revealed similar relationships to CVD mortality in comparison to total fat [210]. Later, Keys' differentiated between the hypo- and hypercholesterolemic effects of polyunsaturated and saturated fats, respectively [211, 212], establishing saturated fats from dairy and meat sources as the major contributors of total cholesterol and subsequent CVD risk [212]. Yet, extensive study of the cholesterol-lowering properties of polyunsaturated fat, particularly vegetable oils rich in linoleic acid [211, 213], has established no clear benefits to corresponding CVD risk [214, 215]. Vascular consequences of excessive dietary linoleic acid intake may originate from a natural proclivity towards oxidized metabolites. Oxidized linoleic acid derivatives have been established to be a major factor concerning oxidized LDL particles [216, 217] mechanistically releasing

proatherogenic compounds [218]. Given the little relevant scientific knowledge of dietary fats at the time, Keys was a significant figure to put the arguments forward despite surmising findings in a "blunt and cocksure manner [206]." Ensuing debates that argued for greater scientific relevance prior to making presumptuous claims concerning saturated fats and CVD risk [219] were dismissed by Keys [220]. Despite debates among the scientific community, declining whole- and inclining low- and non-fat milk sales have spoken to the consensus among Americans since 1975 [221]. Since the mid-2000's, butter sales have also increased [222] as well as whole-fat milk in 2015-2016 [221], suggesting a potential rise in dairy fat consumption.

In 1977, the release of the first U.S. dietary guidelines endorsing a low-fat diet and replacement of fats for carbohydrates [223] has seemingly prompted additional health issues over time. Since that time, diabetic and obesity prevalence accelerated [224, 225] along with ingestion of refined carbohydrates in place of complex carbohydrates [225]. Validation for such trends may be due to greater satiety levels following a meal with high saturated fat content than a carbohydrate-enriched meal [226] as endorsed by the 1977 guidelines. Tradeoffs in the macronutrient profile of saturated fat for carbohydrates yields unfavorable health outcomes as refined sugars promote a greater CVD risk than saturated fats or carbohydrates from whole-grain products [35]. Furthermore, it has been well established that progression from the lowest to highest quintiles of saturated fat consumption, also among high-fat dairy consumption [227], is not associated with elevated CVD risk [20, 34, 35]. While the dining habits of Americans cannot be firmly ascribed to the 1977 guidelines, saturated fat may not be the exclusive culprit responsible for elevated CVD risk.

The integration of certain dietary habits through incorporation of heart-healthy foods may be the key to further offset potential CVD risk when consuming saturated fat. Two European countries, Finland and France, are infamous consumers of saturated fat being among the highest worldwide [228]. Yet, despite similarly elevated cholesterol [229] and BP levels [229, 230], France has the lowest CVD mortality risk than any other country [228], formerly identified as the "French paradox". Several dietary trends may explain the existence of the French paradox including daily moderate wine consumption to prevent platelet aggregation [229, 231], high consumption of vegetable and whole food items [228], and small portion sizes [232]. In contrast, the Finnish had a dietary regimen rich in saturated fat, protein, carbohydrates [228, 233], and sodium [104] with little vegetable and fruit sources. Since the late 1970s, CVD risk has been reduced among the Finnish [234] when it was first identified by Keys as a high risk country [235]. Although high dairy fat consumption is associated with greater CVD risk among the Finnish [228], the French have lower risk undeterred by similar levels of dairy fat intake as other developed countries [229]. Therefore, all contributable dietary CVD risk factors should be assessed with their complementary or uncomplimentary roles in conjunction with saturated fat.

Results from studies revealing that saturated fats have minimal cardiovascular consequence [20, 34, 35], and reduced prevalence of hypertension in populations consuming high amounts of whole-fat dairy products [15, 27, 175], has many advocates jumping onto the "butter is back!" campaign. However, current understanding is still very limited, and navigation of potential health implications is ongoing. Due to LDL cholesterol being a major CVD risk factor, <7% of total energy provided by saturated fats are recommended by the AHA based on previous literature linking saturated fats to increased cholesterol levels [118]. The negative hypercholesterolemic

effects of saturated fat consumption are offset by increasing HDL cholesterol levels [36]. Partitioning saturated fatty acids according to chain length also has varying effects on cholesterol. Specifically, shorter fatty acids markedly raise LDL and HDL cholesterol levels to a greater degree than longer saturated fatty acids [36, 236]. Palmitic acid (16:0), which constitutes the highest concentration of saturated fats in dairy [51], has demonstrated hypocholesterolemic properties in men and women with normal cholesterol levels [237, 238]. Moreover, the thromboxane/prostacyclin ratio, respectively platelet aggregate promoter/platelet aggregate inhibitor ratio [239, 240], was demonstrated to be quite favorable in participants consuming palmitic-rich oils in comparison to coconut or olive oils [237]. The singular effects of other major saturated fatty acids found in dairy, differentially affect cholesterol levels by respectively exerting no effects or decreases in LDL and HDL cholesterol with stearic acid supplementation (18:0) [241] and increases in both LDL and HDL cholesterol with myristic supplementation (14:0) [242] when compared to baseline in healthy participants. Additional nutrients in particular foods may also play a role in reducing cholesterol levels such as dairy calcium reducing absorption of fats in the gut associated with lowered LDL serum cholesterol levels when analyzed against a controlled isocaloric diet [243]. Thus, the contrasting hyper- and hypocholesterolemic effects of solitary saturated fatty acids, among other nutrients, could potentially counterbalance the heart health paradigm.

Overall, the cardiovascular benefits associated with whole-fat dairy product consumption seem optimistic. However, a lack of causal evidence remains to determine the efficacy of whole-fat dairy on adults with elevated BP. The high-fat DASH trial, which substituted non- and low-fat for whole-fat dairy products, effectively demonstrated a similar hypotensive response to the

normal DASH eating plan [16]. From these results, it could be argued that a reduction in BP was the result of a holistic nutritive approach that incorporated whole foods to increase ingestion of complex carbs, vegetables, and fruits apart from the normalized eating patterns of western civilization. Therefore, to assess the potential hypotensive properties of whole-fat dairy, studies that provide additional whole-fat dairy products to the normal routine diet are necessary.

VASCULAR FUNCTIONS ASSOCIATED WITH AGING, HYPERTENSION, AND DAIRY CONSUMPTION

BP is the measure of circumferential force exerted on the arterial wall perpendicular to blood flow. Under pulsatile conditions, the corresponding distension and recoil properties of the arteries, from the respective aftereffects of contraction (systole) and relaxation (diastole) of the myocardium [244], responsible for controlling the conductance of blood, diminish with advancing age. The distension of the arterial wall acts as a buffer to the systolic pressure by storing blood to be later released during recoil, or diastole, also known as the Windkessel model [245]. Sustained forces from chronic hypertension cause eventual fatigue the elastic components of the arterial wall [246], a property of the central arterial elastic arteries [77], leading to enhanced collagen synthesis and overall arterial stiffness [247-249]. Aside from fatiguing elastic lamellae of the arterial wall, elevated cholesterol bolsters arterial stiffness through plaque calcification [250]. Yet, arterial stiffness and elevated BP can be lowered through cholesterol reduction [251]. Arterial stiffness is an independent predictor of CVD mortality [12], particularly among the hypertensive population [252]. Inherent arterial stiffening is also subsequently associated with incidental systolic hypertensive risk [66], demonstrating the potential elastic properties of the arterial wall to affect BP. Advancing age is also directly associated with rising BP and arterial stiffness [92, 253, 254].

Therefore, therapeutic intervention trials aimed at reducing hypertension may inadvertently reduce arterial stiffness.

The endothelium is the innermost lining of the arterial wall and serves as the first-line defense against atherogenesis [110]. Responsible for the balance between vasoconstriction and vasodilation, the simple squamous layer of cells produce nitric oxide (NO) [255], a potent vasodilator, and endothelin-1 (ET-1) [256, 257], a powerful vasoconstrictor. Due to their proximity of the lumen, endothelial cells are primary targets for cellular destruction from reactive oxygen species and inflammatory markers [258-260]. Studies have validated endothelial dysfunction progressing with arterial stiffness [67, 68] and arterial stiffness promoting endothelial dysfunction [261]. Therefore, maintaining arterial elasticity is important for endothelial function despite origins of pathology. Additional initiating factors contributing to endothelial dysfunction has been linked to hypertension, atherosclerosis, thrombus development, and myocardial infarctions from plaque buildup in the coronary arteries [110, 258, 259, 262]. Patients with apparent CVD risk factors exhibit reduced blood flow in comparison with healthy individuals [263]. Blood flow is important for arterial vasomotor activity [264], particularly in the production of endothelium-dependent NO [265, 266] to inhibit ET-1 production and subsequent platelet aggregation [267, 268]. Therefore, reductions in blood flow are significantly associated with CVD-related events compared to those with adequate blood flow [260]. Clinical research has demonstrated reversal of hypertensive-associated endothelial dysfunction through antihypertensive treatments [269], suggesting the importance of regulating elevated BP to modulate endothelial function. Age-associated endothelial dysfunction naturally decreases to a

similar magnitude for normo- and hypertensive individuals [270, 271], but a greater degree of impairment is visible with the aging hypertensive population [270].

Together, endothelial dysfunction, arterial stiffness, and hypertension are factors that blunt baroreceptor sensitivity (BRS). With nerve endings located within the medial layer of the carotid arteries and aorta [272], baroreceptors are sensitive to excessive distension of the arterial wall from short-term elevated systolic BP [132]. The baroreceptors mediate sympathetic output regulating vessel diameter [273]. Sustained hypertension results in a heightened operational threshold of the baroreceptors [132] resulting in reduced function of the baroreceptors [85]. Moreover, arterial stiffening is an independent risk factor of impaired BRS [274], limiting the functional capacity of the baroreceptors by reducing afferent inputs [275]. In animal models, prostaglandins released from endothelial cells stimulate baroreceptors during episodic hypertension to promote vasodilation in a paracrine manner [276, 277]. Thus, endothelial dysfunction results in less prostaglandins released contributing to an impaired BRS, especially in animal models with chronic hypertension [277, 278]. Based on these functional components of BRS, impairment has been classified as a strong prognostic indicator of CVD-related mortality [279].

Dietary intervention strategies have been used to improve vascular function and overall CVD risk. Aimed at reducing elevated BP, clinical trials have also demonstrated favorable effects toward vascular functional parameters. The original DASH eating plan validated the effectiveness of the diet to improve central arterial stiffness [71] and endothelial function [71, 280] in contrast to those consuming a typical western diet. Moreover, addition of non-fat dairy products to the normal routine diet improved central arterial stiffness, endothelial function, and BRS while concomitantly reducing elevated BP when compared to an isocaloric control [11]. Despite positive

findings surrounding non-fat dairy consumption, the controversial data surrounding whole-fat dairy product consumption has not been thoroughly examined. To date, observational studies have provided mixed results concerning whole-fat dairy consumption by demonstrating increased central arterial stiffness compared to no consumption [74], or no effect when examined across the lowest and highest quartiles [83]. Aside from observational studies, no clinical investigations have yet assessed the efficacy of whole-fat dairy intake on the vascular function profile.

NERVOUS SYSTEM INVOLVEMENT IN HYPERTENSION

Excessive sympathetic nervous system activity has been demonstrated to be directly related to advancing age [281-283] and hypertension [284], contributing to the increased essential hypertension with aging. Furthermore, blunted sensitivity to inhibitory sympatholytic drugs within the aged [285], represents a predicament that the geriatric population faces in overpowering such sympathetic outflow [286]. Systemically, elevated whole blood norepinephrine concentrations, from sympathetic neuronal spillover or low reuptake [286, 287], activates downstream α-adrenergic receptors to intensify vasoconstrictor tone and subsequent BP. The ability to modify arterial stiffness is believed to be partly due to the contractile state of vasoconstrictor tone exerted by the smooth muscle cells in the arterial wall [288-290]. Therefore, suppression of vascular tone, via augmented NO bioavailability, is likely to reduce arterial stiffening in hypertensive individuals by promoting elasticity [291, 292]. Other factors including weight gain [293] and saturated fat intake [48] are also associated with increased sympathetic activity further contributing to an elevated BP response.

Systemic sympathetic nervous system activity is modulated through the central nervous system. Baroreceptors are the primary mediators relaying elevated BP to central command,

dampening sudden rises through a feedback loop mechanism within the central nervous system [132, 294]. Episodic elevations in BP exceeding the set point of the baroreceptors generates afferent sympathetic signals to the nucleus tractus solitaries (NTS). From the NTS, the neuronal tracts bifurcate into the parasympathetic branch of the heart to reduce cardiac output [273] and inhibitory interneurons within the caudal ventrolateral medulla [295]. In turn, the caudal ventrolateral medulla exerts inhibitory effects on tonically-active neurons within the rostral ventrolateral medulla (RVLM) inducing reduced arterial vasoconstrictor tone [296]. Majority of neurons within the RVLM are glutamatergic and synthesize adrenaline thereby continuously evoking sympathetic efferent signals when uninhibited [297, 298]. The RVLM also selectively facilitates the production of norepinephrine from neuroendocrine cells located within the adrenal medulla. Unlike epinephrine adrenal preganglionic neurons that are insensitive to the baroreflex, the norepinephrine neurons are inhibited upon the BRS reflex [299]. At rest, sympathetic signals arising from the RVLM are unlikely to raise BP unless other sympathoexcitatory stressors are present.

The central nervous system is also responsible for regulation of blood volume regulation through the kidneys. The paraventricular nucleus of the hypothalamus, responsible for detecting increases in blood osmolality from water deprivation, contains sympathetic afferent projections to the RVLM [300, 301]. The RVLM then sends efferent inputs to the kidney glands promoting renin production to preserve BP to end-target organs from blood volume loss [302, 303], initiating RAS. Renin activates angiotensinogen to advance AngII production [304, 305] stimulating the release of aldosterone from the adrenal glands to reuptake sodium and induce arterial vasoconstriction to maintain blood perfusion to end-target organs [306]. In turn, circulating AngII elevates

sympathetic efferents arising from the RVLM [307] and is appropriately regarded as a sympathetic modulator of BP. Moreover, AngII has exhibited pressure-independent resetting of carotid baroceptor regulation of mean arterial BP and heart rate [308]. Therefore, AngII blunts baroreceptor sensitivity to detect changes in BP thereby exerting central pressor effects. AngII release can propagate other issues such as suppressing production of NO by obstructing the uptake of its precursor into the endothelial cells [89, 309]. Additionally, AngII fosters collagen synthesis [89] and inflammation associated with atherosclerotic lesion development [310, 311] and successive arterial stiffness. Angiotensin converting enzyme (ACE), responsible for converting AngI to AngII, has been viewed as an essential component of AngII-induced hypertension and targeted for inhibition as a strategy to lower hypertensive and CVD risk [89, 312].

Antihypertensive casein-derived proteins found in dairy products contain ACE inhibiting properties, known as LTPs. Despite a multitude of ACE inhibitory-like proteins found in dairy [313-315], the most common and potent lactotripeptides are isoleucine-proline-proline and valine-proline-proline [88]. Long-term pharmaceutical ACE inhibitors have demonstrated similar properties in comparison to vasodilators [316] reducing BP, vascular resistance, intima media thickness, and left ventricular hypertrophy [317]. LTPs were among the first biopeptides to enlighten the scientific community regarding health implications from diminished BP [187]. *In vitro* analyses have validated that LTPs are advantageous toward increasing NO levels to reduce vasoconstrictor tone [89, 318]. With respect to sympathetic neuronal activity, a 12-week hypocaloric DASH trial demonstrated significantly reduced sympathetic nerve activity among hypertensive individuals and reduced norepinephrine levels compared to baseline [319]. Yet, the participants also demonstrated 12-week weight loss, which has also been associated to reduced

sympathetic nerve activity, reduced norepinephrine, and restored baroreflex function [320]. Full-fat dairy products have not been thoroughly examined with respect to sympathetic nerve activity. It would be reasonable to assume that increased whole-fat dairy would elicit similar effects to LTP-isolated nutraceuticals or low-fat dairy intake as they contain similar hypotensive constituents. However, previous studies have established a compromised norepinephrine uptake [321] and release [322] impairing storage and release in rats. A diet high in saturated fat has been shown to increase oxidation to diminish endothelial function and subsequent NO production [323]. As oxidation increases AngII production, saturated fat most likely contributes to arterial smooth muscle tone to elevate BP.

BLOOD PRESSURE MEASURES

Accurate methodology surrounding BP measurements is of imperative importance when assessing CVD risk to avoid false readings. Despite mercury sphygmomanometers being identified as the gold standard for BP determination, automated devices have increased in popularity within the clinical setting [41]. Automated devices have been shown to provide similar accuracy to mercury sphygmomanometers [324, 325] while limiting inter-individual variability. Due to the ease of use, home-based measurements by the patient are highly encouraged to avoid false readings associated with white coat hypertension [124]. Seated BP measurements are notorious for misdiagnosing hypertension by providing false readings due to patient anxiety, known as “white coat hypertension”, which occurs among 20-40% of patients [326-328]. Physicians are more likely to trigger alarm in patients than nurses during office visits, especially within the geriatric population [329]. Home and 24-hour ambulatory BP monitoring are better prognostic indicators of CVD risk than office-based measurements [41, 330-332].

Precision and accuracy are not the only benefits to wearing a 24-hour ambulatory BP monitor, as other CVD risk factors may also be detected. Generally, the circadian rhythms cause BP to steadily rise from early morning hours and remain elevated until late afternoon when BP starts to fall and dips while sleeping [333]. Dipping patterns in BP are important when considering CVD risk as higher CVD-related events and mortality have been linked to non-dipping individuals [334], which is more typical among non-Hispanic blacks than whites [335]. Patients with dipping BP are more likely to experience a sudden increase upon awakening, known as the "morning surge" [336], which is associated with higher CVD risk than those whose rise is slower and more steady [333, 337]. Comparatively, it is unknown whether morning surgers or non-dipping individuals are at a greater CVD risk, but therapeutic interventions can reverse non-dippers into dippers to help eliminate morning surge, especially when hypertensive medication is taken upon awakening [333, 334]. Hormonal regulators follow circadian BP patterns such as plasma norepinephrine has been linked to postural changes that occur upon awakening [338, 339]. The same effect is also seen with plasma renin levels [340].

Chapter 5: Summary and Future Directions

SUMMARY

Lifestyle modifications in the form of diet are the first line approach to manage elevated BP [25]. A diet high in dairy intake, especially non- and low-fat dairy products, have been demonstrated to lessen hypertension risk [8-10]. However, the effects of whole milk and full-fat dairy intake on blood pressure were unknown from an interventional perspective.

The DASH eating plan is a widely recommended dietary approach to reduce elevated blood pressure [8]. The DASH study demonstrated that a combined diet of fruits and vegetables with low-fat dairy products resulted in a greater magnitude of BP reduction than a diet rich in fruits and vegetables. Based on such results, we conducted a randomized controlled dietary intervention trial adding skim milk and non-fat dairy products to the normal routine diet of adults with elevated blood pressure [10]. Subjects reduced blood pressure by solitarily adding 4 daily servings of dairy for 4 weeks. Further, the effects of skim milk and non-fat dairy products also improved vascular function by reducing central arterial stiffening, improving endothelial function, and increasing baroreceptor sensitivity [11]. The results from that previous investigation are consistent with the findings from observational studies [17, 175] demonstrating the effectiveness of non-fat dairy to reduce BP in adults with elevated BP.

A modified higher-fat DASH diet incorporating full-fat dairy products in place of non- and low-fat dairy products from the original DASH diet [16], reduced blood pressure to a similar degree as the original DASH. Among observational cohort studies, there are mixed and inconclusive results demonstrating no reductions in BP [29, 30] to some showing significant reductions in blood pressure with higher intake of full-fat dairy [15, 27]. To determine the causal

implications of full-fat dairy, we performed a randomized controlled dietary intervention study to assess the effects of whole milk and full-fat dairy products on adults with elevated BP. The results from the current dissertational study did not demonstrate a hypotensive trend through the solitary addition of 4 daily servings of full-fat dairy for 4 weeks. In addition, there were no improvements in central arterial stiffness, endothelial function, or cardiovagal baroreceptor sensitivity.

Based on these findings, the solitary addition of whole milk and full-fat dairy consumption are not consistent with the previous investigations showing the hypotensive impacts of skim milk and non-fat dairy in adults with elevated blood pressure. Therefore, adding full-dairy products to the normal routine diet is not a recommended approach to reduce BP in adults with elevated BP.

FUTURE DIRECTIONS

The research design of the current dissertational project was chosen given the success of our previous investigation of non-fat dairy products eliciting hypotensive effects [10, 11]. However, our current understanding of the isolated interaction between dairy fat and protein are still not understood. Previous investigations have noted acute postprandial effects of saturated fats to increase RAS, proinflammatory markers, and oxidative species to promote endothelial dysfunction [48, 60, 198]. When proteins are added to a meal rich in saturated fat, the effects of endothelial dysfunction from elevated saturated intake are ameliorated [91, 199]. Based on the limitation of the current investigations to isolate the effects of the various dairy constituents, mechanistic factors attributable to various dairy constituents could not be examined. Future investigations should study the mechanistic effects of acute postprandial effects of isolated dairy fat and protein in adults with elevated blood pressure. Such investigations would aid further understanding concerning how saturated fat interferes with the hypotensive properties of dairy.

Given the negative attributes of saturated fat on the blood pressure and the vasculature, it is also of interest to understand if lowering the saturated fat content of dairy through incorporation of both low- and full-fat dairy products to the normal routine diet exert hypotensive effects and improvements in vascular function.

Appendices

Appendix A: Abbreviations and Acronyms

ACE = angiotensin converting enzyme

Ang = angiotensin

ANOVA = analysis of variance

BMI = body mass index

BP = blood pressure

BRS = baroreflex sensitivity

cfPWV = carotid-femoral pulse wave velocity

CVD = cardiovascular disease

DASH = dietary approaches to stop hypertension

ET-1 = endothelin-1

FMD = flow-mediated dilation

HbA_{1c} = glycated hemoglobin

HDL = high density lipoprotein

JNC = Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

LDL = low density lipoprotein

LTP = lactotripeptide

NO = nitric oxide

RAS = renin angiotensin system

RVLM = rostral ventrolateral medulla

Appendix B: Research Health Questionnaire

Cardiovascular Aging Research Laboratory
University of Texas at Austin

Personal Information

Today's Date _____ Please print your name _____
Phone Number _____ Email _____
Date of Birth _____ Age _____ Sex ☐ Male ☐ Female
Who is your physician? _____ Phone _____
In case of emergency, contact _____ Phone _____

Please circle the highest grade in school you have completed:

Elementary school	1	2	3	4	5	6	7	8
High school	9	10	11	12				
College/Post Grad	13	14	15	16	17	18	19	20+

What is your marital status? ☐ Single ☐ Married; ☐ Widowed ☐ Divorced; Separated
Ethnic Background: ☐ Hispanic or Latino ☐ Not Hispanic or Latino
Race:
☐ White ☐ American Indian/Alaskan Native ☐ Pacific Islander
☐ Black or African American ☐ Asian

Symptoms or Signs Suggestive of Disease
Check appropriate box:

Yes	No		
☐	☐	1.	Have you experienced unusual pain or discomfort in your cheek, neck, jaw, arms or other areas that may be due to heart problems?
☐	☐	2.	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling)?
☐	☐	3.	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you suffer from swelling of the ankles (ankle edema)?
☐	☐	6.	Have you experienced an unusual and rapid throbbing or fluttering of the heart?
☐	☐	7.	Have you experienced severe pain in your leg muscles during walking?
☐	☐	8.	Has a doctor told you that you have a heart murmur?

Chronic Disease Risk Factors
Check appropriate box:

Yes	No		
☐	☐	9a.	Are you a male over age 45 years or a female over age 55 years?
☐	☐		b. Are you a female who has experienced premature menopause?
☐	☐		c. If you answered "yes" to 9b, are you on estrogen replacement therapy?
☐	☐	10.	Has your father or brother had a heart attack or died suddenly of heart disease before the age of 55; has your mother or sister experienced these heart problems before the age of 65?

Yes	No		
☐	☐	11.	Are you a current cigarette smoker?
☐	☐	12.	Has a doctor told you that you have high blood pressure (more than 140/90 mm Hg) or a heart condition?
☐	☐	13.	Is your total serum cholesterol greater than 200 mg/dl, or has a doctor told you that your cholesterol is at a high risk-level?
☐	☐	14.	Do you have diabetes mellitus?
☐	☐	15.	Are you physically inactive and sedentary (little physical activity on the job or during leisure time)?
☐	☐	16.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	17.	During the past year, would you say that you have experienced enough stress, strain, and pressure to have a significant effect on your health?
☐	☐	18.	Do you eat foods nearly every day that are high in fat and cholesterol such as fatty meats, cheese, fried foods, butter, whole milk, or eggs?
☐	☐	19.	Do you weigh 30 or more pounds than you should?
☐	☐	20.	Do you know of any other reason you should not do physical activity?

Medical History

21. *Please check which of the following conditions you have had or now have. Also check medical conditions in your family (father, mother, brother(s), or sister(s)). Check as many as apply.*

Self	Family	Medical Condition	Self	Family	Medical Condition
☐	☐	Coronary heart disease, heart attack; by-pass surgery	☐	☐	Major injury/fracture to foot, leg, knee
☐	☐	Arrhythmias	☐	☐	Major injury to back or neck
☐	☐	Angina	☐	☐	Major injury/fracture to hip or shoulder
☐	☐	High blood pressure	☐	☐	Rheumatoid Arthritis
☐	☐	Peripheral vascular disease	☐	☐	Osteoarthritis
☐	☐	Phlebitis or emboli	☐	☐	Gout
☐	☐	Other heart problems	☐	☐	Osteoporosis
☐	☐	Stroke	☐	☐	Fibromyalgia
☐	☐	Asthma	☐	☐	Diabetes mellitus
☐	☐	Bronchitis	☐	☐	Kidney disease
☐	☐	COPD (emphysema)	☐	☐	Cataracts
☐	☐	Lung cancer	☐	☐	Glaucoma
☐	☐	Breast cancer	☐	☐	Hearing loss
☐	☐	Prostate cancer	☐	☐	Depression
☐	☐	Skin cancer	☐	☐	Anxiety, phobias
☐	☐	Colorectal cancer	☐	☐	Eating disorders
☐	☐	Other cancer. Specify:	☐	☐	Sleeping problems
☐	☐	Gallstones/gallbladder disease	☐	☐	Substance abuse problems (alcohol, other drugs, etc.)
☐	☐	Liver disease (cirrhosis)	☐	☐	Chronic Fatigue Syndrome
☐	☐	Hepatitis	☐	☐	Thyroid problems

Self	Family	Medical Condition	Self	Family	Medical Condition
☐	☐	Anemia (low iron)	☐	☐	Hysterectomy
☐	☐	Stomach/duodenal ulcer	☐	☐	Problems with menstruation
☐	☐	Rectal growth or bleeding	☐	☐	Post-menopausal (date:
☐	☐	Crohne's disease	☐	☐	Raynaud's disease
☐	☐	Irritable bowel syndrome	☐	☐	Allergies
☐	☐	Marfan's syndrome			

Any other health problems. Please specify and include information on any recent illnesses, hospitalizations, or surgical procedures.

22. *Please check any of the following medications you take regularly and give the name of the medication.*

Medication	Name of Medication
☐ Heart medicine	_____
☐ Blood pressure medicine	_____
☐ Blood cholesterol medicine	_____
☐ Hormones	_____
☐ Birth control medicine	_____
☐ Medicine for breathing/lungs	_____
☐ Insulin	_____
☐ Other medicine for diabetes	_____
☐ Arthritis medicine	_____
☐ Medicine for depression	_____
☐ Medicine for anxiety	_____
☐ Thyroid medicine	_____
☐ Medicine for ulcers	_____
☐ Painkiller medicine	_____
☐ Allergy medicine	_____
☐ Other (please specify)	_____
☐ Do you have any drug allergies?	_____
☐ Dietary supplements (please specify)	_____

Body Weight

23. What is the most you have ever weighed? _____ pounds`
24. Are you now trying to:

☐ Lose weight ☐ Gain weight ☐ Stay about the same ☐ Not trying to do anything

Stress

25. During the past month, how would you rate your overall level of stress?

☐ Very high ☐ High ☐ Moderate ☐ Low

26. In the past year, how much effect has stress had on your health?

☐ A lot ☐ Some ☐ Hardly any or none

27. On average, how many hours of sleep do you get in a 24-hour period?

☐ Less than 5 ☐ 5-6.9 ☐ 7-9 ☐ More than 9

28. How would you describe your cigarette smoking habits?
□ Never smoked
□ Used to smoke. How many years has it been since you smoked? _____ years
□ Still smoke. How many cigarettes a day do you smoke on average? ___cigarettes/day

29. How many alcoholic drinks do you consume? (A "drink" is a glass of wine, a wine cooler, a 16oz bottle/12oz can of beer, a shot glass of liquor, or a mixed drink).
□ Never use alcohol □ Less than 1 per week □ 1-6 per week □ 1 per day
□ 2-3 per day □ More than 3 per day

30. In one sitting, how many drinks do you typically consume? _____
31. How many cups (8 ounces) of coffee do you drink per day? _____
32. How many ounces of sodas containing caffeine do you drink per day? _____

Physical Fitness, Physical Activity/Exercise

33. Considering a **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free time** (write on each line the appropriate number).

a) **STRENUOUS EXERCISE (HEART BEATS RAPIDLY)** **Times Per Week**
(i.e.,running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling) _____

b) **MODERATE EXERCISE (NOT EXHAUSTING)**
(i.e.,fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing) _____

c) **MILD EXERCISE (MINIMAL EFFORT)**
(i.e.,yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking) _____

34. Considering a 7-Day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)

□ OFTEN □ SOMETIMES □ NEVER/RARELY

35. How long have you exercised or played sports regularly?
□ I do not exercise regularly □ Less than 1 year □ 1-2 years
□ 2-5 years □ 5-10 years □ More than 10 years

Occupational Health

36. Please describe your main job title and duties.

_____

37. How much hard physical work is required on your job?
□ A great deal □ A moderate amount □ A little □ None

Reproductive Health

38. What is the date of your last menstrual cycle?

_____

X-ray testing

39. Have you recently had or are you planning to have barium tests or a nuclear medicine scan or injection with an x-ray dye?

□ No □ Yes If yes, when? _____

Appendix C: Daily Dietary Surveys

Dairy Study Week # _____

Subject ID_____

Date study food picked up:_____		**Consume 4 provided dairy servings per day items off list as consumed each day**						**Check**
Dairy items	**# servings issued**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
8 oz. whole milk	_____							
8 oz. full-fat yogurt	_____							
1.5 slices Swiss cheese	_____							

List below any additional dairy products consumed that were not provided by laboratory

Non-Laboratory Provided Dairy items	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**

Date & Time for next study food pick up:

Dairy Study Week # _____

Subject ID_____

Date study food picked up:_____		**Consume 4 provided fruit servings per day items off list as consumed each day**						**Check**
Fruit items	**# servings issued**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
8 oz. Coconut Milk	_____							
8 oz. Orange Juice	_____							
Peanuts or Sunflower Seeds	_____							
Fruit Cup								

List below any fruit/fruit juice consumed that was not provided by laboratory

Non-Laboratory Provided Fruit items	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**

Date & Time for next study food pick up:

Appendix D: Three-Day Dietary Record

3-Day Food Intake Record Instructions

1. Record day of the week and date for everything you eat and drink for three days (two week days & one weekend day) prior to arriving at your appointment.
2. Include the time, amount and type of food/beverage consumed. Provide as much detail as possible, including brand names when available. For example, instead of recording "cereal with milk", record "1.5 cups Kashi GoLean cereal with 6 oz low-fat milk". Instead of "1 slice wheat toast with jam", record "1 slice Orowheat 100% whole-wheat toast with 1tsp Smucker's low-sugar strawberry preserves". See sample food log for more examples.
3. For combination foods such as chili, soup, casseroles, sandwiches, list all items in the food and amounts of each item.
4. For dairy products (milk, cheese, yogurt, etc) record whether, regular (whole), lowfat (1%), reduced fat (2%), or nonfat (skim).
5. Include sweeteners (sugar, honey, syrup, etc) and fats (cream, half&half, milk, etc) added to coffee, tea, etc; as well as spreads on breads and dressings on salads.
6. For meats, indicate type (ground, sirloin, etc) and % lean, if known.

Sample 3 Day Food Intake	**Day of Week:**		**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***
8am	**8 oz**		**Nonfat milk (in cereal)**
	12 oz		**Black coffee**
	1 Tsp		**Sugar in coffee**
	1.5 Cups	**Nature's Path**	**Heritage Heirloom Whole Grains Cereal**
	1 T	**Sun-Maid**	**Fruit bits**
	1 medium		**Cara Cara navel orange**
12pm	**1.3 Cups**	**Homemade**	**Chili: ½ Cup 70% lean ground beef, 1 T onion, 2 T garbanzo beans, 2T black beans, 2 T red sweet pepper**
	3 T		**Grated cheddar/jack cheese, regular**
	½ Cup		**Fresh strawberries**
	½ Cup	**Stoneyfield**	**Lowfat vanilla yogurt**
	2 T		**Raw almonds**
3pm	**1**	**Cliff**	**Chocolate Builder's Bar**
6pm	**5 oz**		**Grilled chicken breast, skinless**
	¾ Cup		**Slaw: ¼ cup cabbage, ¼ grated carrots, ¼ broccoli, 1 tsp olive oil, 1 tsp cider vinegar**
	1 piece	**Kirkland Signature**	**Multigrain bread**
	2 tsp		**honey**
	½ tsp		**butter**
	¾ Cup		**Grilled vegetables: ¼ cup yellow squash, ¼ red pepper, ¼ cup eggplant**

Day ___

3 Day Food Intake		**Day of Week:**	**Date:**
Time	***Amount***	***Brand***	***Food / Beverage***

Appendix E: Supplemental Data

Table E.1 Changes in select subject characteristics and blood chemistry.

	No Dairy Condition First (n=30)				High Dairy Condition First (n=30)			
	No Dairy		High Dairy		High Dairy		No Dairy	
Variables	Before	After	Before	After	Before	After	Before	After
Height (cm)	168±2	-	168±2	-	170±2	-	170±2	-
Body mass (kg)	85.7±3.6	86.3±3.5	86.4±3.6	86.9±3.5	82.9±3.5	83.3±3.4	83.5±3.5	83.7±3.4
BMI (kg/m^2)	30.3±1.1	30.5±1.1	30.5±1.1	30.6±1.1	28.8±1.1	29.1±1.1	29.2±1.1	29.3±1.1
Total cholesterol (mmol/L)	5.2±0.2	5.1±0.2	5.1±0.2	5.4±0.2	5.0±0.2	5.0±0.2	5.0±0.2	4.9±0.2
HDL cholesterol (mmol/L)	1.4±0.1	1.3±0.1	1.3±0.1	1.4±0.1	1.3±0.1	1.3±0.1	1.3±0.1	1.2±0.1
LDL cholesterol (mmol/L)	3.1±0.2	3.0±0.2	3.1±0.2	3.4±0.2	3.0±0.2	3.2±0.2	3.0±0.2	3.1±0.2
Triglycerides (mmol/L)	1.5±0.2	1.5±0.2	1.3±0.3	1.5±0.2	1.5±0.2	1.5±0.2	1.6±0.3	1.3±0.3
Blood glucose (mmol/L)	5.3±0.1	5.3±0.1	5.2±0.1	5.5±0.1	5.3±0.1	5.5±0.1	5.3±0.1	5.2±0.1
Glycated hemoglobin (%)	5.6±0.1	5.6±0.1	5.6±0.1	5.6±0.1	5.5±0.1	5.6±0.1	5.6±0.1	5.6±0.1
Insulin (pmol/L)	66.5±9.5	63.3±6.4	68.6±7.8	65.8±6.1	64.9±6.1	66.6±6.4	64.7±7.6	63.4±6.0
Systolic BP (mmHg)	139±2	130±2	135±2	133±2	136±2	132±2	134±2	135±2
Diastolic BP (mmHg)	81±2	79±2	81±2	80±2	85±2	84±2	84±2	85±2

Values are means±SEM.

Table E.2 Changes in dietary composition.

	No Dairy Condition First (n=30)				High Dairy Condition First (n=30)			
	No Dairy		High Dairy		High Dairy		No Dairy	
Variables	Before	After	Before	After	Before	After	Before	After
Calories (kcal/d)	2086±97	1986±93	1968±97	2103±104	1960±95	2081±89	1899±96	2023±103
Total_Fat (g/d)	88±5	91±4	89±5	98±5	81±5	94±4	79±5	88±5
Sat_Fat (g/d)	28±2	30±2	27±2	40±2	27±2	38±2	27±2	27±2
Monounsat (g/d)	21±2	25±1	21±2	20±1	20±2	20±1	19±2	24±1
Polyunsat (g/d)	12±1	10±1	12±1	11±1	10±1	10±1	10±1	9±1
Total_CHO (g/d)	239±15	234±11	229±14	205±13	216±14	216±10	210±14	237±13
Total Fiber (g/d)	20±2	21±1	19±1	17±1	19±2	17±1	18±1	20±1
Total_Sugar (g/d)	99±8	114±8	89±8	83±7	82±8	86±7	81±8	110±6
Total_Pro (g/d)	82±5	72±7	78±4	92±5	77±5	96±5	73±4	74±5
Alcohol (g/d)	7±3	5±2	4±2	4±2	4±3	3±2	5±2	2±2
Sodium (mg/d)	3052±205	2326±205	3010±226	2692±272	3188±201	2929±196	3009±223	2968±269
Potassium (mg/d)	2147±166	2602±161	2072±144	2284±142	1965±163	2232±154	1769±142	2733±140
Calcium (mg/d)	818±75	2083±179	786±86	1530±79	866±74	1412±170	994±85	1894±79
Magnesium (mg/d)	218±20	200±21	219±19	209±19	201±19	219±20	202±19	217±18
Vit_D (IU/d)	470±202	796±207	424±193	496±164	537±196	608±200	514±188	591±161

Values are means±SEM.

Table E.3 Changes in blood pressure for blood pressure medication users and non-users.

Variables	No Dairy		High Dairy	
	Before	After	Before	After
Antihypertensive Drugs (n=23)				
Systolic BP (mmHg)	137±2	132±2	138±2	135±2
Diastolic BP (mmHg)	82±3	80±2	83±2	83±2
No Drugs (n=37)				
Systolic BP (mmHg)	136±2	134±2	134±2	131±2
Diastolic BP (mmHg)	83±2	83±2	83±2	81±2

Values are means±SEM.

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