The Dissertation Committee for Daniel Robert Machin Certifies that this is the approved version of the following dissertation:

The Hypotensive Effects of Conventional Non-Fat Dairy Products: The Role of Arterial Stiffness

Committee:

Hirofumi Tanaka, Supervisor

R. Matthew Brothers

Edward F. Coyle

Laura Lashinger

M. Lynne McAnelly

The Hypotensive Effects of Conventional Non-Fat Dairy Products: The Role of Arterial Stiffness

by

Daniel Robert Machin, B.S.; M.S. Kin.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

August, 2014

Acknowledgements

I first and foremost would like to thank my advisor in the Cardiovascular Aging Research Laboratory, Hirofumi Tanaka, for his guidance, motivation, and support throughout my doctoral studies. I am grateful for the freedom and independence that you provided that allowed me to pursue my own research interests and path. Your advice and honest feedback was always greatly appreciated and necessary for my growth as a scientist.

I am thankful to the members of my dissertation committee, Ed Coyle, Matt Brothers, Lauri Lashinger, and Lynne McAnelly. Your expert input and support throughout my dissertation studies has helped me immensely. In addition to my committee members, I would like to thank all faculty and staff that I have worked with in the Kinesiology and Health Education, Nutritional Sciences, and Biological Sciences departments.

During my time as a doctoral student, my Ph.D. work was funded by an external source. I would like to gratefully acknowledge the Dairy Research Institute, as without their funding my dissertation studies would not have been possible.

I have worked in two laboratories at UT. Thank you to all my lab mates: Justin Trombold, Kevin Christmas, Josh Lee, Blake McLean, Doug Van Pelt, Matt Pahnke, Ting-Heng Chou, Sarah Hill, Il-Young Kim, Sanghee Park, Ari Reinfeld, and Jim Casler from the Human Performance Laboratory, and Mohammed

Alkatan, Wonil Park, Melissa Mouton, Bennett Fallow, Takashi Tarumi, Evan Pasha, Alex Kraus, Victor Lee, Miriam Pearman, Jeff Baker, Amanda Akkari, Jitanan (Nui) Laosiripisan, Ahmed Elmenshawy, and Jisok Lim from the Cardiovascular Aging Research Laboratory. I look forward to seeing where all of your paths take you and where our paths might cross again.

Thank you to all of my friends and family for their love and support. There are too many friends to list, but more than likely if you are reading my dissertation you would have made the cut. Most importantly, to my parents, thank you for raising me the way that you did. The person that I am today is due to your encouragement to work hard and do my best.

The Hypotensive Effects of Conventional Non-Fat Dairy Products: The Role of Arterial Stiffness

Daniel Robert Machin, Ph.D.

The University of Texas at Austin, 2014

Supervisor: Hirofumi Tanaka

High consumption of dairy products, particularly non-fat dairy, is associated with reduced risk of high blood pressure and vascular dysfunction. Currently, it is not known if the solitary addition of conventional non-fat dairy products to the normal routine diet is capable of reducing blood pressure or improving vascular function. Accordingly, the primary aims of the present study were to determine if the solitary addition of conventional non-fat dairy products to the normal routine diet would reduce blood pressure and improve vascular function in middle-aged and older adults with elevated blood pressure.

Using a randomized, crossover intervention study design, forty-nine adults with elevated blood pressure underwent a High Dairy condition (+4 servings/day of conventional non-fat dairy products) and isocaloric No Dairy condition (+4 servings/day fruit products) in which all dairy products were removed. Both

dietary conditions lasted 4 weeks with a 2-week washout before crossing over into the alternate condition. In Study 1, the High Dairy condition produced reductions in brachial systolic blood pressure and pulse pressure. The hypotensive effects were observed within three weeks after the initiation of dietary intervention and in both casual seated and ambulatory (24-hour) measurements. On the contrary, pulse pressure was increased after removal of all dairy products in the No Dairy condition compared to baseline and after in the High Dairy condition. There were no changes in diastolic blood pressure after either dietary condition.

In Study 2, the High Dairy condition produced reductions in carotid systolic blood pressure, pulse pressure, and carotid-femoral pulse wave velocity with a concomitant increase in brachial flow-mediated dilation and cardiovagal baroreflex sensitivity. Brachial flow-mediated dilation decreased and carotid pulse pressure increased after removal of all dairy products in the No Dairy condition. Furthermore, Δ carotid systolic blood pressure and carotid-femoral pulse wave velocity were highly related. Taken together, we concluded that the solitary manipulation of conventional dairy products, particularly non-fat dairy, in the normal routine diet would modulate levels of blood pressure and vascular function in middle-aged and older adults with pre-hypertension and hypertension.

Table of Contents

List of Tables

List of Figures

Chapter 1: General Introduction

In most of the developed world, diseases of the cardiovascular system are responsible for more annual deaths than any other cause [1]. The progression of cardiovascular disease (CVD) is primarily an age-related phenomenon. So, for most individuals living in industrialized societies, with advancing age brings increased risk of CVD and events [2, 3]. It is well documented that elevated blood pressure (BP) (systolic BP ≥ 120 mmHg and/or diastolic BP ≥ 80 mmHg) is a major contributor to the progression of CVD; approximately 90% of those with CVD are also hypertensive (systolic BP ≥140 mmHg and/or diastolic BP ≥ 90 mmHg or currently being prescribed BP-lowering medications) [4]. Increases in systolic BP are also closely associated with aging, with the greatest increases occurring in prehypertensive and hypertensive individuals [5-7]. A major contributor to age-related increases in BP with age is change in vascular stiffness or compliance of central elastic arteries (e.g., aorta and carotid arteries) [8]. Increases in central arterial stiffness are related to aging [9] and increased BP [10] and are also predictive of cardiovascular events [11]. Thus, vascular dysfunction, namely central arterial stiffness, is an underlying risk factor associated with elevated BP and the aging process that when modified, greatly reduces the risk of CVD. Therefore, a primary focus of healthy aging is to promote a lifestyle that maintains normal BP and preserves vascular function.

The first line approach in clinical treatment of elevated BP is the adoption of healthy lifestyle modifications [12, 13]. Consuming a diet that is high in fruits, vegetables, and low- and non-fat dairy products, but low in total fat, saturated fat, and sodium is considered a healthy lifestyle modification and has been consistently shown to reduce BP [14-19]. Although the adoption of this diet reduces BP and overall risk of CVD, some dietary components may be more effective in lowering BP than others. One of the most cited and well known studies to date is the Dietary Approaches to Stop Hypertension (DASH) [20], in which over 500 subjects completed one of three, 8-week long dietary interventions: 1) typical western diet, 2) diet high in fruits and vegetables, and 3) diet high in fruits and vegetables with additional portions of low-fat dairy products (currently termed DASH eating plan). Compared to the typical western diet, both diets that increased daily servings of fruits and vegetables reduced systolic and diastolic BP, with the greatest reductions in BP occurring in the DASH group. Furthermore, reductions in BP observed in the DASH group were also significantly greater than those observed in the fruits and vegetables diet that had no added dairy products. These findings demonstrate the ability of low- and non-fat dairy products to reduce BP and have been supported by other studies with similar DASH intervention [14-19]. Most of the studies demonstrating a hypotensive effect with increased daily amounts of low- and non-fat dairy products incorporated other dietary or lifestyle changes (e.g., weight loss, increased physical activity). Less is known about whether the addition of non-fat

dairy products to the normal routine diet is able to reduce BP without other concomitant lifestyle or dietary changes. While it has been shown that individuals who consume greater daily portions of low-fat dairy products have lower central arterial stiffness [21], no study has investigated the effects on vascular function in response to these lifestyle modifications. Therefore purpose of this study was to determine whether a dietary intervention that increases daily consumption of conventional non-fat dairy products would exert hypotensive effects and/or improve vascular function.

This study employed a randomized crossover design consisting of two dietary conditions: 1) High Dairy condition (+4 servings/day non-fat dairy products) and 2) isocaloric No Dairy condition (+4 servings/day fruit products) with all dairy products removed from the diet. Using this design we determined the hypotensive effects of non-fat dairy products, as well as changes in vascular function related to these reductions in BP. A total of 53 middle-aged and older adults (35-80 years old) with elevated systolic BP (≥120-159 mmHg) were recruited for this study. All subjects were in prehypertension or stage-1 systolic hypertension classifications, as classified by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) [13]. Dietary conditions were in randomized order and four weeks in length with a washout period of at least two weeks before crossing over into the alternate dietary condition. Measurement of casual seated BP, ambulatory BP, central arterial stiffness, and endothelial vasodilatory function

were assessed prior to and at the end of each dietary condition. It was hypothesized that in the High Dairy condition, BP would be reduced and vascular function improved, compared to the No Dairy condition.

Research Purpose and Hypotheses

Study #1: The first study focused on the hypotensive effects of conventional non-fat dairy products in casual resting and ambulatory conditions. This study used a crossover design to determine the effects of a dietary condition that is high in non-fat dairy products (+4 servings non-fat dairy product; High Dairy condition), compared to an isocaloric No Dairy condition (+4 servings fruit products; No Dairy condition) with all dairy products removed from the diet. The specific hypotheses were as follows:

1. The addition of 4 servings/day of non-fat dairy products in the High Dairy condition would result in lower casual seated, supine and ambulatory BP values, compared with baseline.
2. The removal of all dairy products in the No Dairy condition would result in higher casual seated, supine, and ambulatory BP values, compared with baseline, as well as after the High Dairy condition.

Study #2: The second study focused on changes in central BP and vascular function (e.g., central arterial stiffness, endothelial vasodilatory function, etc.) that may be related to reductions in BP in the High Dairy condition, compared with No Dairy condition observed in study #1. The specific hypotheses are as follows:

1. The addition of non-fat dairy products in the High Dairy condition would result in reduced central BP and improved vascular function, compared with baseline.
2. The removal of all dairy products in the No Dairy condition would result in higher central BP and attenuated vascular function, compared with baseline, as well as after the High Dairy condition.

Chapter 2: Study 1 - Hypotensive Effects of Solitary Addition of Conventional Non-Fat Dairy Products to the Routine Diet: A Randomized Controlled Trial

Abstract

High consumption of low- and non-fat dairy products is associated with reduced risk of high blood pressure. We aimed to investigate if the solitary addition of non-fat dairy products to the normal routine diet was capable of lowering blood pressure in middle-aged and older adults with elevated blood pressure. Using a randomized, crossover intervention study design, forty-nine adults (56% women) with elevated blood pressure (53±2 yr, 135±1/80±1 mmHg) underwent a High Dairy condition (+4 servings/day of conventional non-fat dairy products) and isocaloric No Dairy condition (+4 servings/day fruit products) in which all dairy products were removed. Both dietary conditions lasted 4 weeks with a 2-week washout before crossing over into the alternate condition. The High Dairy condition produced reductions in systolic blood pressure (135±1 to 127±1 mmHg) and pulse pressure (54±1 to 48±1 mmHg) (both $P<0.05$). The hypotensive effects were observed within 3 weeks after the initiation of dietary intervention and in both casual seated and ambulatory (24-hour) measurements ($P<0.05$). Pulse pressure was increased after removal of all dairy products in the No Dairy condition (54±1 to 56±1 mmHg, $P<0.05$). There were no changes in diastolic

blood pressure after either dietary condition. We concluded that the solitary manipulation of conventional dairy products in the normal routine diet would modulate levels of blood pressure in middle-aged and older adults with pre-hypertension and hypertension. This trial was registered at clinicaltrials.gov as NCT01577030.

Introduction

Diseases of the cardiovascular system increase with advancing aging and are responsible for more annual deaths than any other causes [1]. One of the most important and predictive risk factors for CVD is elevated BP, particularly systolic BP and pulse pressure in middle-aged and older adults [22]. Although the risk of coronary heart disease is greater in hypertensive than normotensive adults at any age, the magnitude of risk elevation associated with hypertension is much greater in older adults as compared with young adults [2]. Clearly, from a societal standpoint, it is of paramount importance to reduce the incidence of systolic hypertension, particularly isolated hypertension, in middle-aged and older adults.

In most individuals with elevated BP, the initial treatment approach is the adoption of healthy lifestyle modifications, including dietary changes [13]. The identification of dietary changes that lower BP comes from the observation that dietary calcium consumption, particularly from dairy products, was lower in individuals with hypertension [23]. In a follow-up study it was shown that dairy products, fruits, and vegetables were inversely related to BP [24]. These findings were used to describe the DASH diet, which is high in fruits, vegetables, and low- and non-fat dairy products, but low in total fat and sodium, and has been shown to lower BP in individuals with elevated BP [15, 20]. The DASH diet has become a mainstay of dietary modifications to reduce BP. In one of the DASH diet

interventional studies [20], subjects completed one of 3 dietary interventions: 1) typical western diet, 2) diet high in fruits and vegetables, and 3) DASH diet. Subjects who consumed the DASH diet had the greatest reductions in BP. These reductions were even greater than in those who consumed the fruits and vegetables diet and were attributed to the addition of low- and non-fat dairy products in DASH diet. It should, however, be noted that the hypotensive effects of the DASH diet cannot be ascribed to dairy products alone because other dietary changes (e.g., a reduction in total fat and saturated fat) were also incorporated. Several other interventional studies have shown reductions in BP after consumption of a diet high in low- and non-fat dairy products [15-17, 25], although some studies have not shown this [26, 27]. However, most of these studies were controlled feeding studies or incorporated other dietary or lifestyle changes. Currently, it is not known if the solitary addition of non-fat dairy products to the normal routine diet, without any other lifestyle or dietary changes, is capable of reducing BP. We reasoned that such dietary approach would be more practical, adaptable, and generalizable for the secondary prevention of hypertension.

Accordingly, the primary aim of the present study was to determine the effects of conventional non-fat dairy products added to the normal routine diet in middle-aged older adults with elevated BP. Our working hypothesis is that the addition of non-fat dairy products would reduce BP. Blood pressure was measured both in the casual resting state as well as in the ambulatory (24-hour)

condition. In order to maximally differentiate the dietary dairy intake, all dairy products were removed from the routine diet in the control condition.

Methods

Subjects. A total of 49 adults (44% men and 56% women) with a mean age of 53±2 years were studied (**Figure 2.1**). Subjects were recruited via advertisements on The University of Texas at Austin website, fliers posted on the campus and the surrounding community, and from our database of subjects who participated in previous studies in the laboratory. Inclusion criteria were: elevated systolic BP in the seated position between 120 and 159 mmHg (pre-hypertension or stage-1 systolic hypertension) with a diastolic BP of less than 100 mmHg; no overt signs of chronic diseases on physical examination and medical history (e.g., diabetes, cancer); normal blood chemistry; ankle-brachial index ≤0.9; nonsmoker for previous 2 years; not lactose intolerant; dairy product consumption ≤3 servings/day, strenuous physical activity ≤3 days/week. Five subjects were taking antihypertensive medications, and medication dosages and routines were maintained throughout the entire study. Prior to entrance into the study, all subjects underwent a 2-week run-in period that included 3 screening visits to ensure that all subjects demonstrated stable BP within the appropriate range. The University of Texas at Austin Institutional Review Board reviewed and approved the study. All volunteers gave their written informed consent before participation.

Experimental Design. We used a controlled, randomized, crossover experimental design with two 4-week dietary conditions and a washout period of

at least 2 weeks separating dietary conditions. The rationale for 4 weeks of dietary intervention was based on: a) recommendations from health care organizations regarding the use of non-pharmacological treatment strategies to improve cardiovascular risk factors; and b) the fact that BP changes very rapidly to intervention stimuli as early as 2-4 weeks; therefore, the 4 weeks would allow a sufficient period of time for the dietary product stimulus to produce the hypothesized effects. Measurements were taken at the beginning and end of each dietary condition at the same time of day to eliminate any diurnal effects and after having abstained from food, alcohol, caffeine, and exercise for at least 12 hours. In premenopausal women, measurements were performed during the early follicular phase of the menstrual cycle.

The 2 dietary conditions were: High Dairy condition and isocaloric No Dairy condition. During the High Dairy condition, subjects added 4 daily servings of non-fat milk (HEB Grocery Company, San Antonio, TX), non-fat fruit juice sweetened yogurt (Cascade Fresh, Seattle, WA), and/or non-fat cheese Kraft Foods Group, Northfield, IL) on top of their baseline dietary dairy intake. One serving of dairy was either 245 g of milk, 170 g of yogurt, or 57 g of cheese. The research bionutritionist explained how much one serving of all dairy products was to each subject. Milk was provided to subjects by the gallon or half-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. During the No Dairy condition, all dairy products were

removed from the diet and 4 daily servings of fruit juice, applesauce, and/or fruit cups were added to the diet. Additional servings of fruit products were provided to those with regular dairy consumption at baseline to counterbalance the calories lost due to removal of dairy products during the No Dairy condition. Prior to the start of each dietary condition, subjects met with a research bionutritionist to have specific instructions about the dietary conditions explained to them. After the dietary consultation, subjects received one-week worth of food products and returned weekly for additional food product refills. Throughout the entire experimental protocol, subjects were instructed to maintain their normal lifestyle, aside from dietary changes prescribed by the laboratory research bionutritionist to reduce the overall caloric content when 4 servings of dairy or fruit products were added. Research staff enrolled participants on the study, generated the random allocation to the treatment sequence (using a coin flip simple randomization), and implemented the allocation sequence. Due to the nature of the experimental dietary conditions, it was not possible to blind subjects to what dietary condition they were currently in. Additionally, there was no true placebo condition, as a lack of treatment provision was considered unethical. The study was conducted from January 2012 to November 2013.

Seated Brachial BP Measurements. Seated brachial artery BP measurements were made with a semi-automated BP device (Omron HEM-907XL, Omron Healthcare, Inc., Lake Forest, IL) in triplicate on the right arm after 5 minutes in the upright seated position with the arm at heart level and under

quiet, comfortable, ambient (~24 ˚C) laboratory conditions [28]. In addition, in order to determine the time course of the BP changes with the dietary intervention, casual seated BP was measured during the weekly visits to the laboratory to refill food products.

Ambulatory (24-hour) BP Measurements. Ambulatory BP recordings were made over a 24-hour period of normal daily activity using a noninvasive ambulatory BP monitor (Model 90217, SpaceLabs Medical, Redmond, WA) [29]. The ambulatory system was programmed to inflate automatically every 15 min from 6 AM to 11 PM and every 20 min between 11 PM and 6 AM.

Blood Samples. A blood sample was collected by venipuncture after an overnight fast. Fasting whole blood concentrations of total cholesterol, HDL-cholesterol, triglycerides, and glucose were determined enzymatically. LDL-cholesterol was estimated using the measured concentrations of total cholesterol, HDL-cholesterol, and triglycerides. Whole blood glycated hemoglobin concentration (HbA_{1c}) was measured using a commercially available HbA_{1c} reagent kit (DCA Systems, Siemens Healthcare Diagnostics, Tarrytown, NY).

Dietary Analyses. Subjects were given detailed instructions on how to keep 3-day dietary records by the research bionutritionist. During the weekly visits to the laboratory to refill food products, dietary records were collected

before and at the end of each dietary condition and analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX). This software is based on the comprehensive food knowledge database with over 51,000 foods and ingredients. Study compliance during each dietary condition was assessed by having each subject complete daily dietary surveys that indicated consumption of the food products provided, as well as any dairy products consumed as part of their normal diet.

Statistical Analyses. Power calculations were performed using nQuery Adviser computer software. The alpha level used for power analyses was set at 0.05. Sample size calculations were based on the number of subjects needed to detect significant changes in primary dependent variables from baseline levels in response to lifestyle modifications [30-33]. With 49 subjects/group, we have greater than 80% power to detect the changes. A 2-way and 3-way mixed model analysis of variance (ANOVA) with repeated measures was used to evaluate the effect of condition x time interaction by using SPSS software version 21 (IBM, Chicago, IL). Where a significant condition x time interaction was found, paired-samples *t*-tests with a Bonferroni correction were conducted to determine difference between specific time points. Statistical significance was set at $P<0.05$ for all analyses. Data are presented as mean ± SEM.

Results

Subject compliance/adherence to the study protocol was 97 and 96% in the High Dairy and No Dairy conditions, respectively, based on the daily dietary survey. Although no subjects blatantly consumed dairy products while in the No Dairy condition, a few subjects accidentally consumed dairy products (<1 serving/wk) in the beginning of the No Dairy intervention Additionally, a few individuals did not consume all servings of study food products on a daily basis. When this occurred, we instructed subjects to consume the missing servings the following day. Because the non-compliance here is fairly minor in nature, these subjects were included in the study sample. The selected subject characteristics are presented in **Table 2.1**. Body mass and metabolic profiles did not change with either dietary condition. There were no sex (gender)-related differences in these responses to the dietary conditions or in any of the responses described, as assessed by 3-way mixed model ANOVA with repeated measures (sex x condition x time). Accordingly, sex was dropped from the analyses. Elimination/addition of subjects with antihypertensive medications (n=5) did not affect the overall results.

As shown in **Table 2.2**, there were no significant differences in total caloric intake between or within dietary conditions. Dietary protein intake increased after the High Dairy condition, but decreased after the No Dairy condition ($P<0.05$). In the High Dairy condition, total dairy intake increased significantly from 1.2±0.1 to

4.7±0.1 servings/day (P<0.05), with laboratory-provided non-fat dairy products accounting for 4.0±0.1 servings/day, with 2.0±0.1, 1.9±0.1, and 0.1±0.0 servings/day coming from milk, yogurt, and cheese, respectively. Conversely, in the No Dairy condition, total dairy intake decreased from 1.4±0.1 to 0.0±0.0 servings/day (P<0.05). The baseline dairy intake consisted of 0.4±0.1 and 0.7±0.1 servings/day of non-fat and full-fat dairy products. As expected, following the High Dairy condition, potassium, calcium, magnesium, and vitamin D intake increased (P<0.05); all of them, except for potassium, decreased after the No Dairy condition (P<0.05). Most importantly, dietary consumption of potassium, calcium, magnesium, vitamin D, and dairy products were significantly different between conditions after the 4-week interventions (P<0.05 for all).

As depicted in **Figure 2.2**, systolic BP decreased significantly after the High Dairy condition (P<0.05). No such changes were observed after the No Dairy condition. There were no changes in diastolic BP after either dietary condition. Pulse pressure was reduced by 6±1 (P<0.05) after the High Dairy condition and had an increase of 2±1 mmHg (P<0.05) after the No Dairy condition.

Analyses of weekly BP measurements revealed that both systolic BP and pulse pressure were significantly decreased as early as the week 3 of the High Dairy condition (P<0.05) (**Figure 2.3**). Additionally, these values at the weeks 3 and 4 in the High Dairy condition were significantly different from those measured

during the same weeks in the No Dairy condition (P<0.05). There were no significant changes in diastolic BP in either group.

A general trend of changes in ambulatory BP was similar to those observed during casual seated BP measurement (**Figure 2.4**). Ambulatory (24-hour) systolic BP decreased significantly after the High Dairy condition, with most of the BP reduction coming from the daytime periods (P<0.05). There were no changes in ambulatory diastolic BP after either dietary condition. Ambulatory pulse pressure decreased (P<0.05) after the High Dairy condition during the 24-hour and daytime periods.

Discussion

The primary findings from the present randomized crossover study are as follows. The addition of 4 daily servings of conventional non-fat dairy products to the normal routine diet decreased systolic BP and pulse pressure in middle-aged and older adults with elevated BP. The reduction in casual seated BP was accompanied by the similar reduction in ambulatory (24-hour) BP due mainly to the decrease in daytime BP. Conversely, when all dairy products were removed from the routine diet, pulse pressure increased significantly relative to baseline and after in the High Dairy condition. These findings indicate that the solitary manipulation of non-fat dairy products can modulate BP in middle-aged and older adults with elevated BP.

With advancing age, systolic BP increases and diastolic BP decreases resulting in a widening of pulse pressure [34]. Among middle-aged and older adults, pulse pressure is highly predictive of CVD risk, even more so than both systolic or diastolic BP [35]. In the present study, we observed significant reductions in systolic BP and pulse pressure following the High Dairy condition. The analyses of the time course indicate that the hypotensive effects of the non-fat dairy products were significant as early as 3 weeks after the initiation of the High Dairy condition. These results indicate that the solitary addition of conventional dairy products to the normal routine diet could produce significant

reductions in systolic BP and pulse pressure well within the time frame that lifestyle modifications including dietary changes are typically prescribed.

The results of the present study are generally consistent with previous studies reporting that increased low- and non-fat dairy product consumption are associated with BP-lowering effects [15-17, 20, 25]. However, a dietary intervention study with a similar research design to the present study showed no hypotensive benefit after 61 overweight or obese individuals increased consumption of low-fat dairy products (+4 servings/day) for six months [26]. The conflicting results may be due to differences in dietary calcium intake. Dietary calcium consumption has been shown to be lower in individuals with hypertension and inversely related to BP [23, 24]. There is evidence of calcium metabolism disturbances in individuals with hypertension [36], which may be due to low dietary calcium consumption [23, 24], as dietary calcium supplementation has been shown to reduce BP in hypertensive individuals [37]. When dairy product consumption is increased, dietary calcium consumption is increased accordingly. In the present study, baseline calcium consumption was lower (~750 mg/day vs. ~1,100 mg/day) and the gradient in dietary calcium after the High Dairy condition was nearly 2-fold greater (~1,350 mg/day vs. ~700 mg/day) than the previous study [26]. Thus, changes in BP may have been due to differences in dietary calcium consumption after the dietary interventions.

Not only did we observe reductions in systolic BP and pulse pressure in the casual resting state, but we also observed significant reductions in the ambulatory state. The magnitude of reductions in systolic BP and pulse pressure after the High Dairy condition was, however, less in the ambulatory measurements. While BP treatment guidelines typically refer to BP values measured in the seated resting conditions, ambulatory BP measured in 24 hours is more representative of normal life and may be a better indicator of CVD risk than casual resting BP [38]. Thus, reductions in BP we observed in the ambulatory measurements after the High Dairy condition may have a greater prognostic benefits for middle-aged and older adults with elevated BP who seek to gain benefit from dairy products.

A rather unanticipated finding of the present study is that pulse pressure increased significantly when the regular consumption of dairy products was removed from the routine diet. Compared with the High Dairy condition that 4 servings of non-fat dairy servings were added, a much lower quantity of dairy products were removed from the diet (1.4 servings/day) in the No Dairy condition. These results imply that the subjects studied in the present study were enjoying the hypotensive benefits from relatively modest consumption of dairy products in their routine diet. One may argue that the increase in pulse pressure might have been due to the addition of fruit products provided in the No Dairy condition. However, fruit consumption is inversely related to BP and CVD [24, 39]. Additionally, the national BP guidelines recommend increased dietary

consumption of fruit products, as well as low- and non-fat dairy products, to reduce BP [13]. We observed no changes in blood lipid profile, glucose, or HbA_{1c} in the No Dairy condition that are consistent with the hypertensive effects. While fruit products provided were isocaloric to non-fat dairy provided in the alternate condition, they contained minimal protein and were higher in carbohydrate content. The amount that dietary protein decreased after the No Dairy condition (10g) is the approximate amount contained in 1.4 servings of dairy products. Dietary supplementation with milk proteins has been shown to reduce BP and local arterial stiffness [30]. A recent cross-sectional analysis indicates that total dairy food consumption is inversely related to pulse pressure and arterial stiffness [21]. Thus, it is plausible that the removal of protein provided from dairy products may have increased pulse pressure, as milk proteins have been shown to act as angiotensin converting enzyme (ACE) inhibitors [40]. In addition to changes in protein intake, there were reductions in magnesium, vitamin D, and calcium consumption after the No Dairy condition. These changes were expected with the removal of dairy products, as these nutrients are abundant in dairy products. Meta-analyses have shown that dietary supplementation with magnesium, vitamin D, or calcium can result in small reductions (2-4 mmHg) in systolic BP [41, 42]. Thus, a decrease in dietary consumption of one or more of these nutrients in No Dairy condition may have increased pulse pressure.

For the prevention of hypertension and CVD, it is recommended that low- and non-fat dairy products be added to the diet [13]. This recommendation is presumably driven by a concern that high fat content in dairy products may increase the risk of obesity, diabetes, and CVD. However, recent reviews have indicated no relation or even inverse relation of total dairy intake to metabolic and CVD risks [43-45]. Future studies are warranted to determine the effect of full-fat dairy products on BP.

There are several strengths of the present study that can be emphasized. First, we kept each subject's normal routine diet, as the baseline dietary conditions and only dairy and fruit consumption were experimentally manipulated. We reason that such approach would be more generalizable to a greater number of populations who seek to conveniently adapt high dairy consumption in their normal diet. Second, BP was measured in both the casual seated and ambulatory states, which allowed us to evaluate the effects of both dietary conditions in the clinical setting as well as in normal life. There are several limitations as well. We were unable to determine which non-fat dairy product was more effective in reducing BP. Individuals in the study had the choice of non-fat yogurt, milk, and/or cheese, in addition to other dairy products consumed as part of their normal routine diet. Additionally, we cannot determine which ingredients of dairy products (milk protein, calcium, potassium, milk peptide, etc.) were responsible for hypotensive effects of dairy products.

As in many other studies, the completion of the study brings more questions than we can answer. What is the minimal and optimal dose of dietary dairy intake to reduce BP? Are there any additive effects of dairy consumption to other lifestyle modifications (e.g., regular exercise)? Could the effect of the solitary addition of dairy products to the normal routine diet be greater in ethnic minorities? What is the physiological mechanism underlying the reduction in BP? There is no question that more studies are needed to fully evaluate the hypotensive effects of dairy products.

Lifestyle modifications, including dietary changes, are the first line approach for treating elevated BP. The present findings indicate that simply adding 4 servings of conventional non-fat dairy products are effective in reducing both systolic BP and pulse pressure in middle-aged and older adults. Unlike other lifestyle modifications, including regular exercise, that are difficult to achieve high compliance and adherence, dairy products can be easily incorporated into daily routine of older adults to gain hypotensive benefits.

Table 2.1. Changes in Selected Subject Characteristics and Blood Chemistry

	No Dairy		High Dairy	
Variables	**Before**	**After**	**Before**	**After**
Height, cm	170±2	-	170±2	-
Body mass, kg	87.8±3	87.7±3	88.1±3	88.3±3
Body mass index, kg/m^2	30.4±1	30.3±1	30.5±1	30.5±1
Total cholesterol, mmol/l	5.23±0.2	5.10±0.2	5.26±0.2	5.23±0.2
HDL cholesterol, mmol/l	1.24±0.1	1.11±0.1	1.17±0.1	1.14±0.1
LDL cholesterol, mmol/l	3.39±0.2	3.39±0.1	3.47±0.1	3.42±0.1
Triglycerides, mmol/l	1.25±0.1	1.30±0.1	1.37±0.1	1.48±0.1
Blood Glucose, mmol/l	5.27±0.1	5.22±0.1	5.33±0.1	5.44±0.1
HbA_{1c}, %	5.4±0.1	5.4±0.1	5.4±0.1	5.4±0.1

Values are mean ± SEM.

All significant differences were preceded by a significant condition x time interaction.

Table 2.2. Changes in Dietary Composition

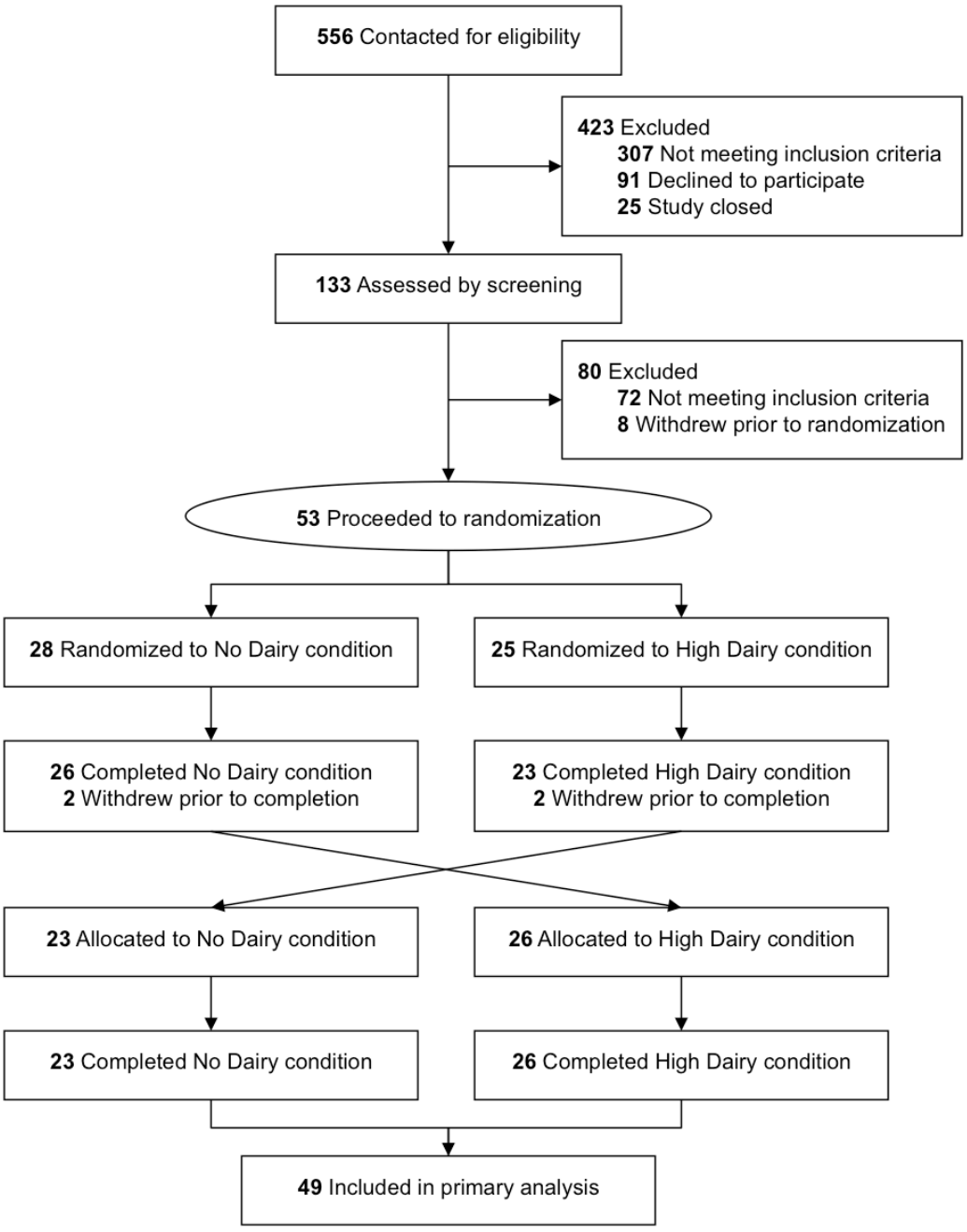
	No Dairy		High Dairy	
Variables	**Before**	**After**	**Before**	**After**
Calories, kcal/day	1,818±59	1,842±45	1,830±64	1,880±50
Fat, g/day	71±4	58±3	69±3	60±3
Carbohydrate, g/day	201±9	251±7*	203±10	227±7*†
Protein, g/day	79±4	69±3*	82±3	98±3*†
Alcohol, g/day	8±2	6±2	11±2	6±2
Sodium, mg/day	2,923±190	2,555±124	2,753±141	2,739±124
Potassium, mg/day	2,115±152	2,003±98	2,156±130	3,094±127*†
Calcium, mg/day	758±54	416±39*	777±56	1,755±64*†
Magnesium, mg/day	212±13	169±11*	213±14	271±14*†
Vitamin D, IU/day	221±66	149±66*	208±69	512±64*†
Dairy, serving/day	1.4±0.1	0.0±0.0*	1.2±0.1	4.7±0.1*†

Values are mean ± SEM.

All significant differences were preceded by a significant condition x time interaction.

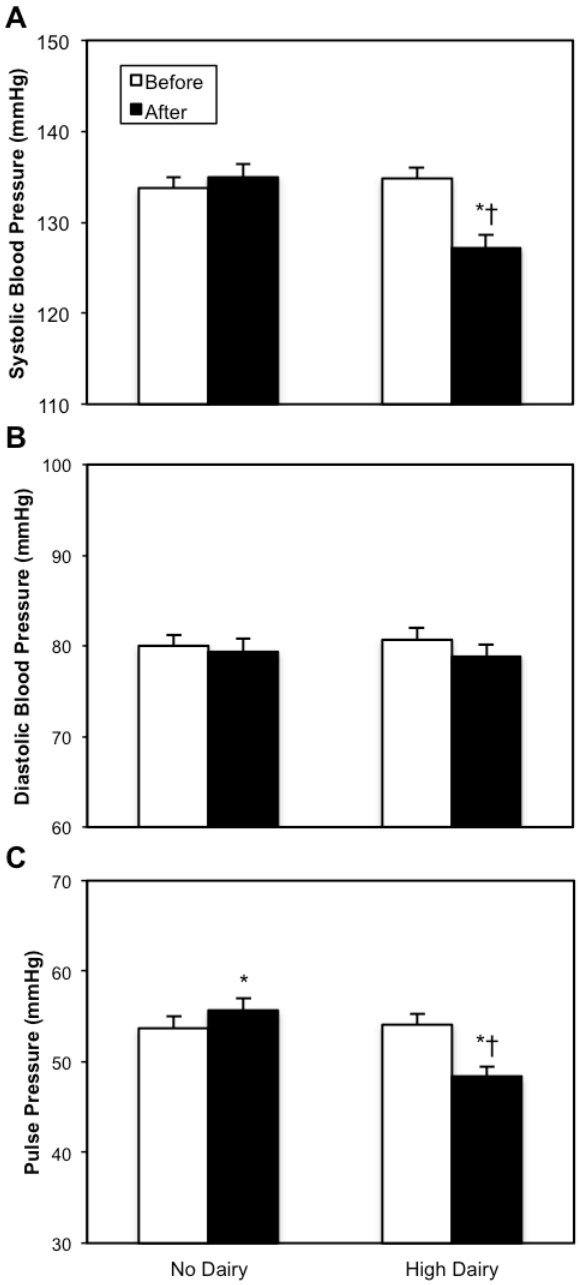
*P<0.05 vs. Before; †P<0.05 vs. After in No Dairy condition.

Figure 2.1. Participant Flow Through the Trial



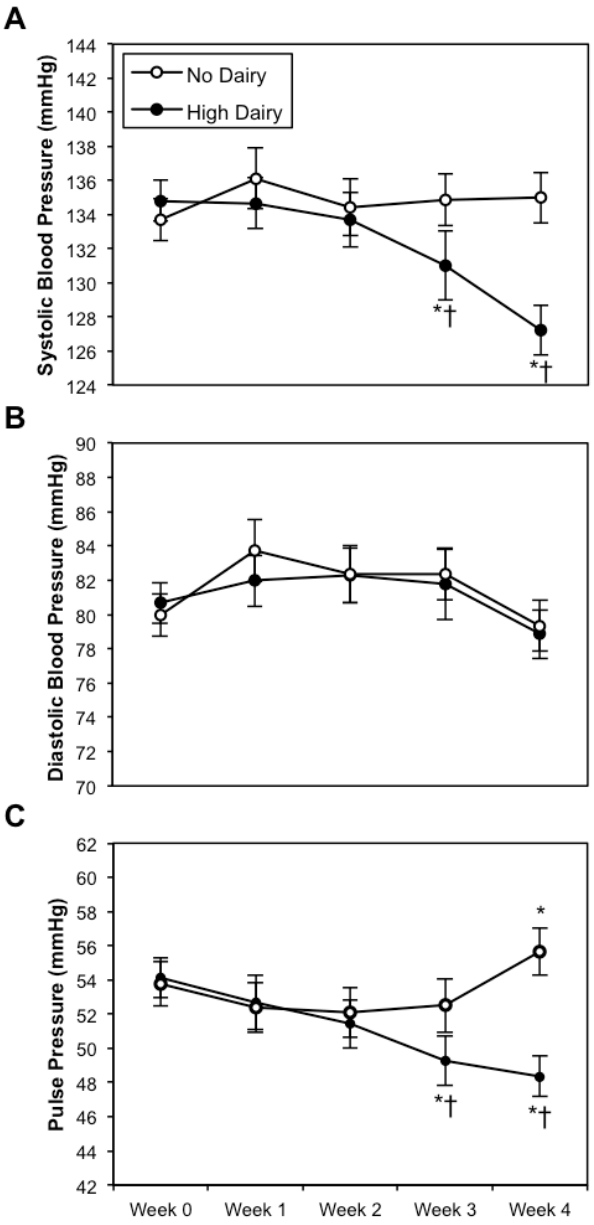
Participant flow through the trial.

Figure 2.2. Blood Pressure



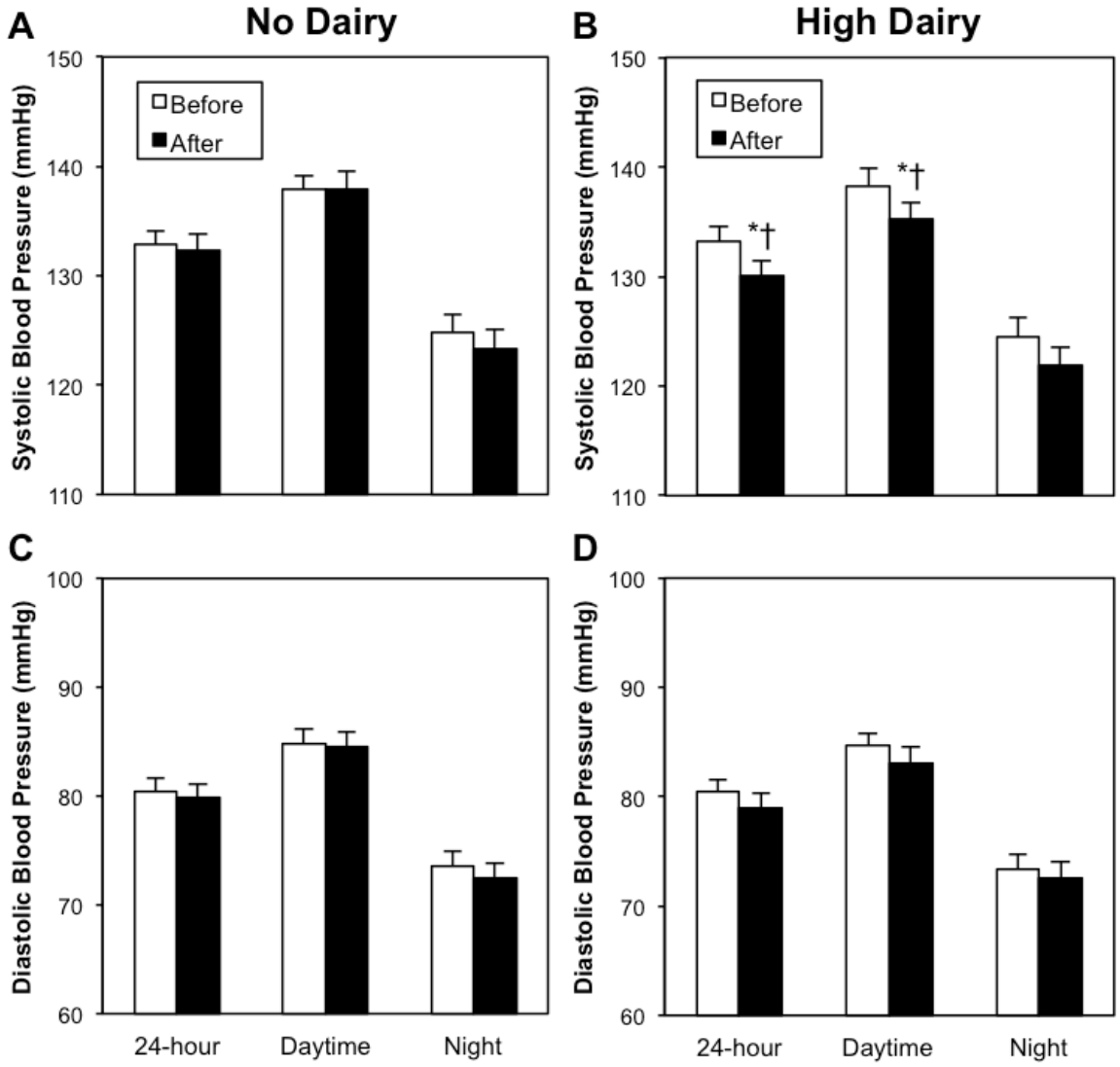
Seated resting brachial systolic blood pressure (A), diastolic blood pressure (B), and pulse pressure (C) before and after each condition (n=49). **P*<0.05 vs. Before. †*P*<0.05 vs. After in No Dairy condition. Values are mean ± SEM.

Figure 2.3. Time Course of Changes in Blood Pressure



Time course of changes in casual seated brachial systolic blood pressure (A), diastolic blood pressure (B), and pulse pressure (C) measured weekly (n=49). *P<0.05 vs. week 0 within condition. †P<0.05 vs. No Dairy. Values are mean ± SEM.

Figure 2.4. Ambulatory Blood Pressure



Ambulatory (24-hour) blood pressure before and after the High Dairy (A and C) and No Dairy (B and D) conditions (n=48). *P<0.05 vs. Before. †P<0.05 vs. After in No Dairy condition. Values are mean ± SEM.

Chapter 3: Study 2 - Effects of Non-Fat Dairy Products Added to the Routine Diet on Vascular Function

Abstract

High consumption of low- and non-fat dairy products is associated with reduced risk of high blood pressure and central arterial stiffness. Currently, it is not known if the solitary addition of non-fat dairy products to the normal routine diet is capable of reducing central blood pressure and/or improving cardiovascular function of middle-aged and older adults with elevated blood pressure. Using a randomized, crossover intervention study design, forty-nine adults (44% men, 53±2 years, 170±2 cm, 88±3 kg) with elevated BP (134±1/81±1 mmHg) underwent a High Dairy condition (+4 servings/day of conventional non-fat dairy products) and isocaloric No Dairy condition (+4 servings/day fruit products) in which all dairy products were removed. Both dietary conditions lasted 4 weeks with a 2-week washout before crossing over into the alternate condition. The High Dairy condition produced reductions in central systolic blood pressure (-3±1 mmHg) and carotid-femoral pulse wave velocity (-0.5±0.1 m/sec), with a concomitant increase in brachial flow-mediated dilation (+1.1±0.4%) and cardiovagal baroreflex sensitivity (+5±1 ms/mmHg) (P <0.05 for all). Furthermore, changes observed in central systolic blood pressure and carotid-femoral pulse wave velocity were highly related (r=0.55, P <0.05). On the contrary, brachial flow-mediated dilation was reduced after removal of all dairy

products in the No Dairy condition (-1.0±0.1%, $P<0.05$). In conclusion, the solitary manipulation of conventional dairy products in the normal routine diet modulates levels of central blood pressure and vascular function in middle-aged and older adults with elevated blood pressure. This trial was registered at clinicaltrials.gov as NCT01577030.

Introduction

Arterial BP increases with advancing age in most industrialized countries resulting in a markedly high prevalence of essential hypertension, particularly in isolated systolic hypertension, among older adults [46]. This rise in BP is the major contributor to age-associated increases in risk of a number of cardiovascular disorders, including cerebrovascular and coronary heart disease [34]. The physiological mechanisms by which arterial BP increases with increasing age are not known. However, structural and functional alterations of the large arterial wall, including stiffening of arteries, have been implicated [47]. Increases in arterial stiffness are associated with the reduction in arterial endothelial vasodilatory function and arterial baroreflex sensitivity (BRS) [48, 49] further contributing to the elevated risks of CVD in older adults.

To reduce the incidence of CVD, it is of paramount importance to identify treatments that can lower BP and restore age-related reductions in vascular function. In most individuals at risk of CVD, the initial treatment approach is the adoption of healthy lifestyle modifications [13]. Indeed, studies that have evaluated lifestyle changes such as regular exercise and salt restriction have reported a reduction in systolic BP and an improvement in vascular function in middle-aged and older adults [28, 31]. We and others have reported that the consumption of a diet high in low- and non-fat dairy products is associated with the reductions in BP [15, 16, 20, 25]. Information concerning the physiological

mechanisms by which dairy consumption lowers arterial BP is extremely limited. It is plausible to hypothesize that an improvement in vascular function in general and de-stiffening of large arteries in particular may play an important role in producing the hypotensive effects of dairy products. Indeed, previous studies have demonstrated reduced central arterial stiffness and improved endothelial function after strictly controlled dietary interventions involving supplementation with milk proteins [30, 32]. Currently, it is not known if the addition of conventional non-fat dairy products to the normal routine diet is capable of improving vascular function and whether such improvement in vascular function is associated with reductions in arterial BP.

Accordingly, the primary aims of the present study were to determine if the solitary addition of conventional non-fat dairy products to the normal routine diet would reduce central BP and improve vascular function in middle-aged and older adults with elevated BP. Central, rather than brachial, BP was used for this study since central BP seems to be a better predictor of hypertensive risks as well as the hemodynamic load imposed on the left ventricle [50]. We hypothesized that the addition of non-fat dairy products would reduce central systolic BP and that changes in BP would be accompanied by improvements in vascular function (e.g., reduced central arterial stiffness and increased endothelial function). In order to maximally differentiate the dietary dairy intake, all dairy products were removed from the routine diet in the control condition.

Methods

Subjects. A total of 49 adults (44% men, 53±2 years, 170±2 cm, 88±3 kg) with elevated BP (134±1/81±1 mmHg) were studied. Subjects were recruited via advertisements on The University of Texas at Austin website, fliers posted on the campus and the surrounding community, and from our database of subjects who participated in previous studies in the laboratory. Inclusion criteria were: elevated brachial systolic BP in the seated position between 120 and 159 mmHg (pre-hypertension or stage-1 systolic hypertension) with a diastolic BP of less than 100 mmHg; no overt signs of chronic diseases on physical examination and medical history; normal blood chemistry; ankle-brachial index ≤0.9; non-smoker for previous 2 years; not lactose intolerant; dairy product consumption ≤3 servings/day, strenuous physical activity ≤3 days/week. Five subjects were taking antihypertensive medications, and medication dosages and routines were maintained throughout the entire study. Prior to entrance into the study, all subjects underwent a 2-week "run-in period" that included 3 screening visits to ensure that all subjects demonstrated stable BP within the appropriate range. The University of Texas at Austin Institutional Review Board reviewed and approved the study. All volunteers gave their written informed consent before participation.

Experimental Design. We used a controlled, randomized, crossover experimental design with two 4-week dietary conditions and a washout period of

at least 2 weeks separating dietary conditions. The rationale for 4 weeks of dietary intervention was based on: a) recommendations from health care organizations regarding the use of non-pharmacological treatment strategies to improve cardiovascular risk factors; and b) the fact that vascular functions change very rapidly to intervention stimuli as early as 2-4 weeks [31]; therefore, the 4 weeks would allow a sufficient period of time for the dietary product stimulus to produce the hypothesized effects. Measurements were taken at the beginning and end of each dietary condition at the same time of day to eliminate any diurnal effects and after having abstained from food, alcohol, caffeine, and exercise for at least 12 hours. In premenopausal women, measurements were performed during the early follicular phase of the menstrual cycle.

The 2 dietary conditions were: High Dairy condition and isocaloric No Dairy condition (**Figure 3.1**). During the High Dairy condition, subjects added four daily servings of non-fat milk, non-fat fruit juice sweetened yogurt, and/or non-fat cheese on top of their baseline dietary dairy intake. One serving of dairy was either 245 g of milk, 170 g of yogurt, or 57 g of cheese. The research bionutritionist explained how much one serving of all dairy products was to each subject. The subjects were free to consume the required servings at any time of day as a single dose or all at once. During the No Dairy condition, all dairy products were removed from the diet and four daily servings of fruit juice, applesauce, and/or fruit cups were added to the diet. Additional servings of fruit products were provided to those with regular dairy consumption at baseline to

counterbalance the calories lost due to removal of dairy products during the No Dairy condition. Prior to the start of each dietary condition, subjects met with a research bionutritionist to have specific instructions about the dietary conditions explained to them. After the dietary consultation, subjects received 1-week worth of food products and returned weekly for additional food product refills. Throughout the entire experimental protocol, subjects were instructed to maintain their normal lifestyle, aside from dietary changes prescribed by the laboratory research bionutritionist to reduce the overall caloric content when 4 servings of dairy or fruit products were added. Research staff enrolled participants on the study, generated the random allocation to the treatment sequence (using a coin flip simple randomization procedure), and implemented the allocation sequence. Due to the nature of the experimental dietary conditions, it was not possible to blind subjects to what dietary condition they were currently in. Additionally, there was no true placebo condition, as a lack of treatment provision was considered unethical for patients at elevated risk of cardiovascular events.

Carotid Arterial Stiffness and BP. After at least 10 min of rest in the supine position, bilateral brachial and ankle BPs, left carotid and femoral pulse pressure waveforms, and heart rate were simultaneously measured by an automated vascular testing device (Colin VP-2000, Omron Healthcare, Kyoto, Japan) for 30 sec at least 3 times per testing period [51]. Arterial applanation tonometry incorporating an array of 15 micropiezoresistive transducers recorded pulse pressure waveforms from the carotid and femoral arteries. The transit time

was determined from the time delay between the tonometers placed on the left carotid and femoral arteries and was automatically measured based on the foot-to-foot method. The straight distance between the carotid and femoral arterial recording sites was measured over the surface of the body [10, 52, 53]. Measurement distance was multiplied by 0.8 in order to adjust the distance closer to the real pulse pressure waveform travel distance [54]. Subsequently, carotid-femoral pulse wave velocity (cfPWV) was calculated as pulse pressure waveform travel distance divided by the transit time. Additionally, the carotid pulse pressure waveforms, obtained using applanation tonometry [55], were recorded within an attached notebook computer and processed with dedicated software (WinDaq 2000, Dataq Instruments, Akron, OH). To correct for the hold-down pressure of applanation tonometry, carotid mean and diastolic BPs were calibrated to oscillometrically determined brachial mean and diastolic BPs (Colin VP-2000, Omron Healthcare, Kyoto, Japan), as previously described [55].

Cardiovagal BRS. After 10 min in the seated upright position, cardiovagal BRS was determined using the Valsalva maneuver, as previously described [56]. Subjects performed at least 3 Valsalva maneuvers by forcibly exhaling against a closed airway. Subjects were asked to maintain an expiratory mouth pressure 40 mmHg for 10 seconds, while R-R interval (via electrocardiography) and beat-by-beat BP (Pilot 9200, Colin Medical Instruments, San Antonio, TX) were measured continuously. Data for cardiovagal BRS were recorded and analyzed by waveform browsing software (WinDaq 2000, Dataq Instruments, Akron, OH)

during the phase IV overshoot. Systolic BP values were linearly regressed against corresponding R-R intervals from the point where R-R intervals begin to lengthen to the point of maximal systolic BP elevation [48, 57].

Brachial Flow-Mediated Dilation (FMD). After at least 10 min of rest in the supine position, FMD of the brachial artery was assessed using a noninvasive, standardized procedure [58]. Briefly, a pneumatic BP cuff was positioned 3-5 cm distal to the antecubital fossa, and connected to a rapid cuff inflation device (E20 Rapid Cuff Inflator, D.E. Hokanson, Bellevue, WA). Brachial artery diameter and blood flow velocity were measured from images derived from a Doppler ultrasound machine equipped with a high-resolution linear array transducer (Philips iE33 Ultrasound System, Bothel, WA) positioned 5-10 cm proximal to the antecubital fossa. After collecting 1 min each of baseline brachial artery diameter and blood flow velocity, the cuff was inflated to 100 mmHg above the subject's brachial systolic BP for 5 min. Blood flow velocity was recorded for 15 seconds after cuff deflation and then brachial artery diameter was recorded until 3 min past cuff deflation. All ultrasound-derived blood flow and diameter data were analyzed by the same investigator using image analysis software (Brachial Analyzer, Medical Imaging Applications, Coralville, IA). Brachial FMD was calculated using the equation: [(peak diameter – baseline diameter)/baseline diameter] x 100.

Dietary Analyses. Subjects were given detailed instructions on how to keep 3-day dietary records by the research bionutritionist. During the weekly visits to the laboratory to refill food products, dietary records were collected before and at the end of each dietary condition and analyzed using Nutritionist Pro software (Axxya Systems, Stafford, TX). This software is based on the comprehensive food knowledge database with over 51,000 foods and ingredients. Study compliance during each dietary condition was assessed by having each subject complete daily dietary surveys that indicated consumption of the food products provided, as well as any dairy products consumed as part of their normal diet.

Statistical Analyses. Power calculations were performed using nQuery Adviser computer software. Sample size calculations were based on the number of subjects needed to detect significant changes in primary dependent variables from baseline levels in response to lifestyle modifications [30-33]. With 49 subjects/group, we have greater than 80% power to detect the hypothesized changes. A 2-way (condition x time) and 3-way (sex x condition x time) mixed model ANOVA with repeated measures was used to evaluate the effect of condition x time interaction by using SPSS software version 21 (IBM, Chicago, IL). Where a significant interaction was found, paired-samples *t*-tests with a Bonferroni correction were conducted to determine difference between specific time points. Bivariate correlations were used to determine relations between

dependent variables. Statistical significance was set at $P<0.05$ for all analyses. Data are presented as mean ± SEM.

Results

Subject compliance/adherence to the study protocol was 97 and 96% in the High Dairy and No Dairy conditions based on the daily dietary survey. Although no subjects blatantly consumed dairy products while in the No Dairy condition, a few subjects accidentally consumed dairy products (<1 serving/wk) in the beginning of the No Dairy intervention Additionally, a few individuals did not consume all servings of study food products on a daily basis. When this occurred, we instructed subjects to consume the missing servings the following day. Because the non-compliance here is fairly minor in nature, these subjects were included in the study sample. There were no sex (gender)-related differences in these responses to the dietary conditions or in any of the responses described, as assessed by 3-way mixed model ANOVA with repeated measures (sex x condition x time). Accordingly, sex was dropped from the analyses. Elimination/addition of subjects with antihypertensive medications (n=5) did not affect the overall results.

In the High Dairy condition, total dairy and non-fat dairy intake increased significantly from 1.2±0.1 and 0.3±0.1 to 4.7±0.1 and 4.0±0.1 servings/day (P<0.05). Alternatively, in the No Dairy condition, in which all dairy products were removed from the diet, total dairy and non-fat dairy intake decreased significantly from 1.4±0.1 and 0.4±0.1 to 0.0±0.0 and 0.0±0.0 servings/day

(P<0.05). There were no differences in total caloric intake between the two dietary conditions.

As shown in **Table 3.1**, there were significant reductions in supine brachial systolic BP, brachial pulse pressure, and ankle pulse pressure after the High Dairy condition (P<0.05). Additionally, brachial pulse pressure increased significantly after the No Dairy condition (P<0.05). There were no changes in diastolic BP after either condition.

As depicted in **Figure 3.2**, carotid systolic BP decreased significantly after the high Dairy condition (P<0.05). No such changes were observed after the No Dairy condition. There were no changes in diastolic BP after either dietary condition. Carotid pulse pressure was reduced after the High Dairy condition, but increased after the No Dairy condition (P<0.05 for both). Although carotid augmentation index tended to be lower after the High Dairy condition, this reduction was not statistically significant. Cardiovagal BRS was significantly higher after the High Dairy condition (P<0.05), with no change after the No Dairy condition. As depicted in **Figure 3.3**, cfPWV was significantly reduced after the high dairy condition (P<0.05). Additionally, changes observed in cfPWV after either dietary condition were highly related to changes in carotid systolic BP (r=0.55), ankle systolic BP (r=0.56), and cardiovagal BRS (r=-0.27) (P<0.05 for all).

Analysis of brachial artery characteristics before and after each dietary condition revealed no differences in baseline brachial artery diameter (**Table 3.1**). However, as shown in **Figure 3.4**, relative brachial artery FMD was significantly higher after the High Dairy condition, but lower after the No Dairy condition ($P<0.05$). This trend of changes in brachial FMD was also observed with the absolute change in brachial artery diameter in both High Dairy and No Dairy conditions (0.13±0.02 to 0.16±0.02 mm and 0.13±0.01 to 0.09±0.02 mm, $P<0.05$).

Discussion

The primary findings of the present randomized crossover study are as follows. We found that the addition of four daily servings of conventional non-fat dairy products to the normal routine diet decreased central systolic BP and pulse pressure in middle-aged and older adults with elevated BP. The reductions in central BP were related to the corresponding changes in central arterial stiffness and were accompanied by increased brachial FMD and cardiovagal BRS. When all dairy products were removed the routine diet, central pulse pressure increased and brachial FMD decreased significantly. Taken together, these findings indicate that the solitary manipulation of dairy products, particularly non-fat dairy products, can modulate central BP and key vascular functions in middle-aged and older adults with elevated BP.

With advancing age, systolic BP increases, and diastolic BP decreases resulting in a widening of pulse pressure [34]. Previous studies have demonstrated reductions in BP after consumption of a diet high in low- and non-fat dairy products [15-17, 20, 25]. However, these studies only measured peripheral BP. In recent years, “central BP” has gathered a lot of attention as it is more strongly associated with vascular disease and outcome than “peripheral BP” measured on the brachial artery. To the best of our knowledge, the present study is the first to determine the effect of dairy dietary intervention on central BP. We observed that central systolic BP and pulse pressure were significantly

lower after the High Dairy condition whereas no change was observed in the No Dairy condition. These results indicate that the solitary addition of conventional non-fat dairy products to the normal routine diet produce significant reductions not only in peripheral but also in central BP.

Individuals who consume a greater number of daily servings of dairy products have lower central arterial stiffness [21]. Additionally, retinal damage, which is frequently associated with central arterial stiffening [59], is higher in those with lower dairy product consumption [60]. While there appears to be a link between dairy product consumption and vascular function, interventional studies to investigate these relations are very limited. A reduction in central artery stiffness has been reported after a DASH diet intervention that includes weight loss and other dietary changes [15]. But to the best of our knowledge, there were no intervention studies that have examined the effects of the solitary addition of non-fat dairy products on vascular function. In the present study, we observed significant reductions in central artery stiffness as measured by cfPWV after the High Dairy condition. While not statistically significant, carotid augmentation index, a measure of arterial wave reflection and arterial stiffness, was also lower after the High Dairy condition. Moreover, we found that changes in cfPWV were highly related to corresponding changes in carotid systolic BP. Our present findings indicate the solitary addition of conventional non-fat dairy products to the normal routine diet is effective in reducing arterial stiffness and

that the reductions in central BP may be mediated, at least in part, by reductions in arterial stiffness.

Arterial baroreflex is an important short-term mechanism for regulating arterial BP [61] and is associated with increased risk of ventricular tachyarrhythmias and sudden cardiac death [62]. Decreases in the compliance of carotid arteries and aorta, in which the arterial baroreceptors are located, leads to corresponding reductions in the ability of these reflexogenic regions to transduce signals, resulting in decreases in cardiovagal BRS [56]. In the present study, we found that cardiovagal BRS increased significantly after the High Dairy condition. Additionally, improvements in cardiovagal BRS were inversely related to changes in central arterial stiffness. The present findings are consistent with previous studies that utilized other lifestyle modifications, including regular exercise [28, 57]. In these studies, however, increases in cardiovagal BRS were achieved after more than 3 months of lifestyle modifications, whereas in the present study, improvements were achieved as early as 4-weeks after the initiation of the dietary intervention. Thus, dietary modifications involving non-fat dairy products, not only reduces arterial stiffness, but also improve sequela of arterial stiffening (i.e., cardiovagal BRS).

Brachial FMD has been utilized as a marker of vascular endothelial function [63] and is strongly associated with CVD and cardiovascular event risks [64]. Brachial FMD increased significantly after the High Dairy condition.

Interestingly, when the dairy products were removed from the routine diet, brachial FMD decreased significantly. Thus, the solitary manipulation of dairy products in their routine diet appears to modulate levels of endothelial function in middle-aged and older adults. The magnitude of improvements in FMD in the present dietary intervention study was comparable with that observed after a 3-month swimming exercise intervention [28]. Currently, it is not known if the effect of high dairy intervention is additive to the effects of other lifestyle modifications including exercise. However, combined DASH diet and weight loss appear to be more effective at improving vascular function than DASH diet alone [15].

There are several strengths of the present study that can be emphasized. First, we kept each subject's normal routine diet, as the baseline dietary conditions and only dairy and fruit consumption were experimentally manipulated for the High Dairy and No Dairy conditions, respectively. We reason that such approach would be more generalizable to a greater number of populations who seek to conveniently adapt high dairy consumption in their normal diet. Additionally, the noninvasive measurements of vascular function performed in this study have been shown to be highly repeatable [10, 48, 52, 55, 57, 58], as well as predictive of future CVD risk [11, 64]. There are, however, several limitations as well. We were unable to determine which non-fat dairy product was more effective in reducing BP. Individuals in the study had the choice of non-fat yogurt, milk, and/or cheese, in addition to other dairy products consumed as part of their normal routine diet. Additionally, we cannot determine which

ingredients of dairy products (milk protein, calcium, potassium, milk peptide, etc.) were responsible for hypotensive effects of dairy products.

Lifestyle modifications, including dietary changes, are the first line approach for treating elevated BP. The present findings indicate that simply adding four servings of conventional non-fat dairy products to the routine diet are effective in reducing central (carotid artery) systolic BP and pulse pressure in middle-aged and older adults. Additionally, the addition of non-fat dairy products reduced central arterial stiffness and improved endothelial function. On the contrary, removal of dairy products increased central pulse pressure and reduced endothelial function. Unlike other lifestyle modifications, including regular exercise, that are difficult to achieve high compliance and adherence, dairy products can be easily incorporated into the daily routine to gain hypotensive as well as vasoprotective benefits.

Table 3.1. Changes in Selected Subject Characteristics

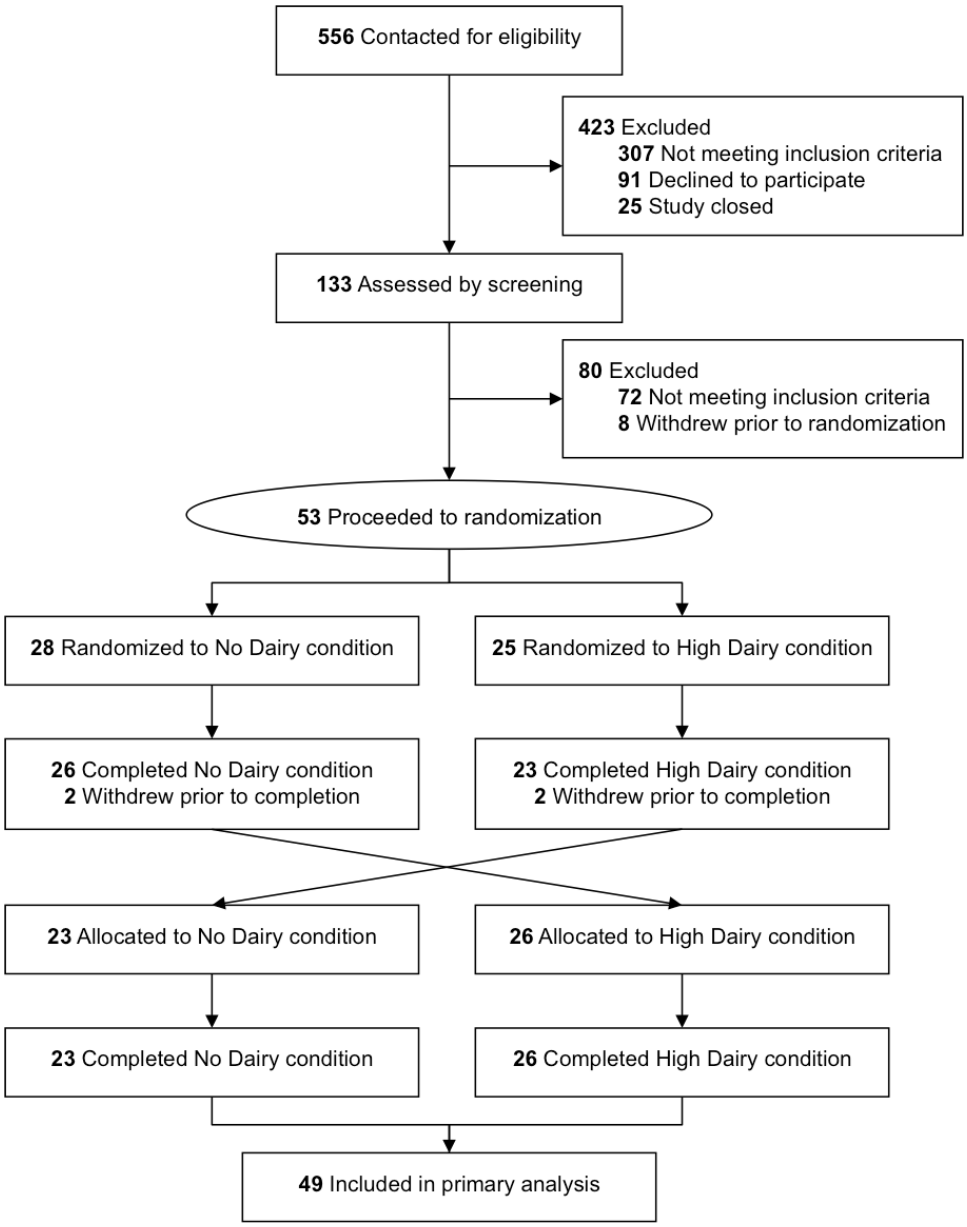
	No Dairy		High Dairy	
Variables	**Before**	**After**	**Before**	**After**
Heart rate, bpm	64±1	63±1	63±1	64±1
Supine brachial systolic BP, mmHg	134±1	135±2	135±1	132±2*†
Supine brachial diastolic BP, mmHg	80±1	80±1	80±1	78±1
Supine brachial pulse pressure, mmHg	54±1	56±1*	55±1	54±1*
Ankle systolic BP, mmHg	152±2	152±2	152±2	149±2
Ankle diastolic BP, mmHg	80±1	79±1	79±1	77±1
Ankle pulse pressure, mmHg	72±2	73±2	74±2	71±2*
Carotid augmentation index, % (n=43)	18±3	17±3	18±3	15±3
Cardiovagal BRS, ms/mmHg (n=44)	14±1	13±1	15±1	20±2*†
Baseline brachial artery diameter, mm (n=47)	3.8±0.1	3.9±0.1	3.9±0.1	3.9±0.1

Values are mean ± SEM (n=49 unless stated otherwise)

All significant differences were preceded by a significant condition x time interaction.

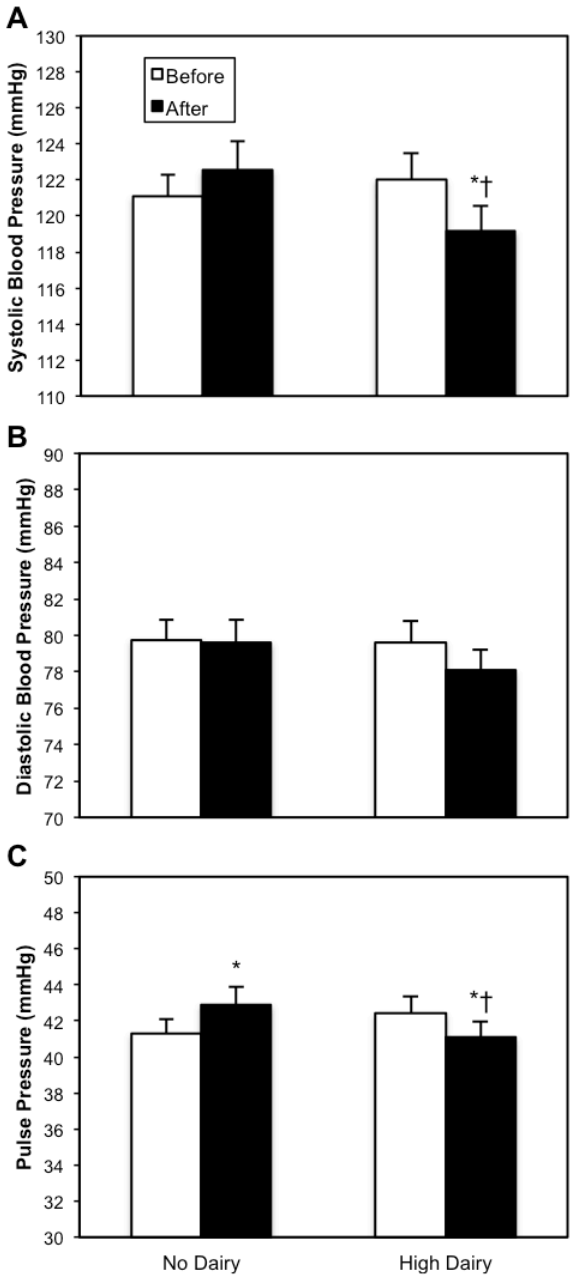
*P<0.05 vs. Before; †P<0.05 vs. After in No Dairy condition.

Figure 3.1. Participant Flow Through the Trial



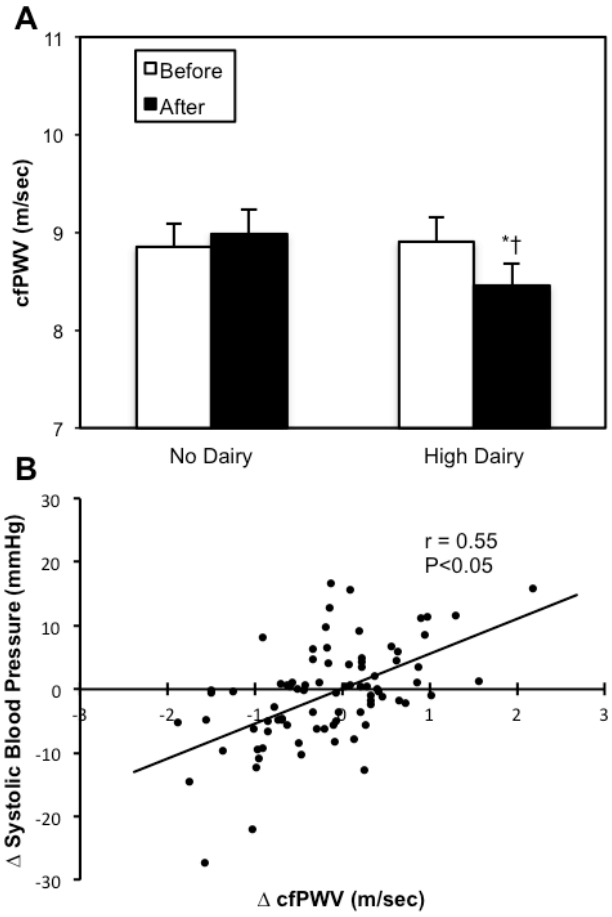
Participant flow through the trial.

Figure 3.2. Central Blood Pressure



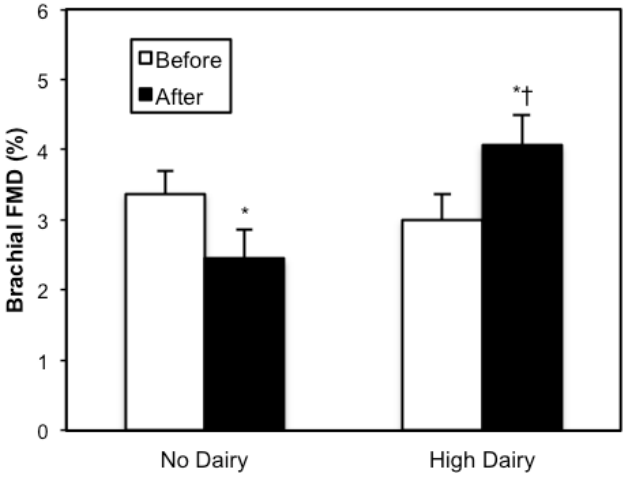
Carotid artery systolic blood pressure (A), diastolic blood pressure (B), and pulse pressure (C) before and after each dietary condition (n=49). **P* <0.05 vs. Before. †*P*<0.05 vs. After in No Dairy condition. Values are mean ± SEM.

Figure 3.3. Central Arterial Stiffness



Carotid-femoral pulse wave velocity before and after each dietary condition (n=41) (A). Relation of ΔcfPWV and Δcarotid systolic blood pressure in both conditions (r = .55) (B). *P <0.05 vs. Before. †P<0.05 vs. After in No Dairy condition. Values are mean ± SEM.

Figure 3.4. Brachial Flow-Mediated Dilation



Brachial flow-mediated dilation before and after each dietary condition (n=47). *P<0.05 vs. Before. †P<0.05 vs. After in No Dairy condition. Values are mean ± SEM.

Chapter 4: Review of Literature

Epidemiology of Cardiovascular Disease

Cardiovascular disease is the number one cause of death in the US, accounting for nearly one-third of annual deaths [4]. This is not an emerging issue, as CVD has been the leading cause of death in the US for the past 80 years [65]. Proportionally, the prevalence of CVD is higher in older adults compared to young or middle-aged adults, thus, the incidence of death from CVD is highest in older adults [34]. The reason for high prevalence of CVD in older adults is because the development of CVD takes years or decades, therefore, by nature, CVD is more prevalent in older individuals [1]. For this reason CVD is considered to be a disease of aging, with advancing age being its most predictive risk factor. Although advances in technology and medical care have increased the average lifespan [66], it is reasonable to assume, based on the nature of CVD development and progression, that with further increases in average lifespan CVD will remain a top, if not the primary, cause of death.

With aging, there are various physiological and morphological changes to throughout the cardiovascular system that alter the ability of the heart and arteries to maintain their normal function [67-71]. It is for these reasons that with increasing age there is a greater likelihood of CVD. It should be noted that despite age being the most predictive risk factor for CVD, not all individuals develop CVD as they age. Those who live a lifestyle characterized by healthy

eating habits, physical activity, and avoidance of tobacco products, as well as other risky behaviors (e.g., heavy alcohol consumption, illicit drug usage, etc.) tend to be at lower risk of CVD [1]. In addition to CVD, those that remain free from other chronic diseases associated with aging (e.g., cancer, diabetes, chronic obstructive pulmonary disorders, etc.) can attribute the absence of these diseases and CVD to their lifestyle, as well as other factors [13]. Thus, a target of many research investigations has been to determine 'what the factors are?' that contribute to the reduced incidence of CVD and other chronic diseases in these individuals. Absence of CVD in some individuals can be attributed to genetic traits and other non-modifiable factors. However, it has been suggested that a large part of CVD risk reduction can be attributed to modifiable lifestyle factors (e.g., high cholesterol, smoking status, elevated BP) [72, 73]. Identification of modifiable lifestyle factors that reduce the risk of CVD are of utmost importance, as they could provide evidence on which recommendations can be made to individuals at risk of or already diagnosed with CVD to reduce further progression or risks of death associated with CVD. Although CVD tends to be prevalent in later life, changes that occur through the aging process that lead its development begin during childhood [74]. Thus, emphasizing with even greater importance the need not only to investigate modifiable risk factors that prevent CVD, but utilize these findings to provide evidence-based recommendations that can be adopted earlier in life to reduce the risk of future disease.

Over the past several decades we have developed a greater understanding of the underlying causes of CVD. In concert with these findings is the development of preventative and treatment strategies for CVD. Thus, death from CVD has been greatly reduced worldwide, particularly in many high-income countries [75]. Unfortunately, some of these modern preventive and treatment strategies are expensive or unavailable to low- and middle-income countries. Consequently, the rates of CVD development and death are much higher in these countries [75]. While the adoption of healthy lifestyle modifications are relatively inexpensive, in some low- and middle-income countries there is less of a priority placed on early identification of risk factors associated with CVD [76, 77]. Regardless of socio-economic restrictions or a subpar emphasis on early identification of diagnosis or risk of disease, the prevention and treatment of CVD through lifestyle changes are responsible for large portion of the decline in CVD associated deaths observed over the last 40 years [65, 78, 79]. Despite the enormous reductions in death, CVD remains the number one cause of death worldwide, even in those high-income countries that have modern prevention and treatment modalities available [4].

Blood Pressure as a Risk Factor for Cardiovascular Disease

Throughout the developed world, the prevalence of CVD increases with advancing age [80]. While age is not a modifiable risk factor, there are many other risk factors for CVD that can be modified. One of the most important and predictive of these risk factors in middle-aged and older adults is elevated BP,

particularly systolic BP and pulse pressure [22, 81]. The identification and treatment of elevated BP is largely responsible for reductions in premature death over the past several decades [13, 78]. Although the risk of coronary heart disease is greater in hypertensive than normotensive adults at any age, the magnitude of risk elevation associated with hypertension is much greater in older adults as compared with young adults [2]. Additionally, as CVD risk is closely associated with aging, systolic BP also tends to increases with age [82]. Increases in BP with age are highly related to current BP. That is those with elevated BP earlier in life tend to have the greatest increases in the future [5-7]. Thus, as long-term risk of CVD in most individuals is highly related to BP, those age-related increases in BP may be both symptoms and predictors of CVD [2, 3]. It is for these reasons that maintaining normal BP values through the lifespan has become a primary focus of aging to minimize CVD risk.

Currently, there are more than 82 million Americans with CVD. Among those with CVD 92% are hypertensive (systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg or are prescribed BP lowering medications) [4]. By these numbers, hypertensive individuals represent more than one-third of the US population 20 years and older [4]. Although it appears that while high BP *per se* is not a major cause of death from CVD, accounting for only 7.5% [4], the risk of CVD is greater in hypertensive than normotensive adults at any age [2]. Not only does hypertension increase the risk of CVD, even prehypertension increases risk of future CVD [83, 84]. As elevated BP is one of the most important and

predictive risk factors for future CVD, treatment of hypertension, and even prehypertension [13], has become fairly aggressive, with current guidelines recommending the use of anti-hypertensive medications to reduce BP in most populations (except over the age of 65 years) with BP of 140/90 or greater [85]. Consequently, the economic cost of CVD has risen to upwards of 300 billion dollars per year [4]. It is estimated that these costs will continue to rise, as lifespans continue to increase and lifestyles that promote CVD risk (e.g., sedentary lifestyle) are more common. Clearly, from a socio-economical standpoint, it is of paramount importance to reduce the incidence of systolic hypertension, particularly isolated hypertension, in middle-aged and older adults.

Risk Factors for Elevated Blood Pressure

Elevated BP is highly associated with older age, but as stated previously, BP does not rise with age in all individuals. Although not all risk factors for CVD can be modified (e.g., ethnicity, sex, and age), many that are also related to elevated BP can (e.g., physical inactivity, obesity, and unhealthy diet). Therefore, those who age with a greater number of these risk factors would also be more likely to have increased BP with age. For most individuals with elevated BP the first line approach to reduce BP is the adoption of healthy lifestyle modifications [13]. However, the efficacy of lifestyle modifications can be greatly influenced by ethnicity, age, and sex. For these reasons, this section will

examine these non-modifiable risk factors and their relations to hypertension, CVD, and treatment for elevated BP.

Ethnic and Sex Differences

While there is a greater prevalence of hypertension among older individuals, some ethnicities are more affected by hypertension than others. Non-Hispanic black adults have a higher prevalence of hypertension than do Hispanic and non-Hispanic white adults [86]. Treatment and identification of hypertension has ethnic differences, as well. Although Hispanic adults have similar and sometimes lower incidence of hypertension than non-Hispanic white adults, they also have a lower awareness of their hypertension [86-88]. Thus, without being diagnosed and aware of their hypertension, Hispanic individuals remain untreated and at higher risk of CVD. It is well known that non-Hispanic black adults have a higher prevalence of hypertension, thus they represent a group at higher risk of CVD. Regardless of ethnic differences in hypertension prevalence, similar approaches are recommended to reduce BP [13], but some may be more effective than others [89]. Most notably, in non-Hispanic black adults, consuming a DASH eating plan has been shown to reduce BP to a greater extent than observed in Hispanic and non-Hispanic white adults [13, 90].

There are also differences in hypertension prevalence between sexes, most notably with age [1, 89]. Although increases in prevalence of hypertension with age occur in both men and women, the rate of increase is different between

sexes [1]. In comparison to women, men tend to be at greater risk of hypertension and CVD, but only at younger ages [1]. It is believed that the menstrual hormones exert a hypotensive and vasoprotective effect on the vasculature of premenopausal women, reducing their risk of high BP and CVD [91]. Therefore, menopause-related changes in these hormones are responsible for the increased risk of hypertension and CVD observed in women as they age. It should be noted that, while BP is elevated in women after menopause, there are other indirect changes associated with menopause that may increase BP, as well, such as weight gain and salt sensitivity [92]. Overall, there are some sex-related differences in hypertension and CVD risk, but elevations in BP occur in both men and women. While men appear to be more affected earlier in age, there is a substantial increase in prevalence of hypertension among women after menopause. Regardless of reasons for sex-related of differences in BP elevation with age, the treatments to reduce BP are similar between men and women.

Lifestyle Factors that Increase Blood Pressure

The present physiology of humans is believed to have evolved consuming a diet that was low in sodium. It is estimated that late in the Paleolithic period dietary sodium consumption was 0.8 g/day, in contrast to the current dietary sodium consumption in a typical western diet that consists of 4.0 g/day [93]. Excess dietary sodium consumption increases BP [94]. While BP tends to rise with age, this is not true in all countries. For example, in undeveloped countries that are characterized by low sodium consumption, there is a low incidence of

hypertension and BP does not rise with age [94]. Although these countries tend to have other differences in living conditions in comparison to the developed world (i.e., an active lifestyle, lower chance of excess caloric intake, etc.), hypertension prevalence is increased even in undeveloped countries that consume high sodium diets [94]. Regardless of the living conditions, in most cases, when sodium intake is reduced BP is also reduced [31, 95], even in individuals with resistant hypertension [96]

Currently, more than two-thirds of Americans are classified as overweight and more than one-third classified as obese. The present prevalence of obesity has become a major concern in regard to CVD risk [97, 98], more so, as obesity rates are predicting to continue to increase. Although the annual mortality rate due to CVD has decreased over the last half century, the growing prevalence of obesity may reverse this trend, as increased body weight is associated with increased BP [99-102] and central arterial stiffness [103]. Elevated systolic BP is by far the largest single contributor to CVD risk in adults and for most individuals living in industrialized societies, with advancing age comes increases in systolic BP [2, 3]. Thus, the impacts of an increasingly obese society will further elevate the rise in BP observed with aging, putting those affected at a greater likelihood of CVD-related risk and mortality [104]. In addition to the BP elevating effects, obesity is also associated with insulin resistance and risk of diabetes, both of which increase the risk of CVD independent of elevated BP [105]. Currently in the US, there are approximately 24 million adults diagnosed with type 2 diabetes

[106], with 79 million other Americans that are at risk or presenting preliminary symptoms of diabetes (e.g., insulin resistance, prediabetes, metabolic syndrome) [107]. This is a large number of Americans and is directly related to CVD risk as individuals with diabetes are at greater risk for CVD. Additionally, there are various other complications associated with diabetes such as blindness, amputation, nerve damage, and renal failure that may all indirectly contribute to risk of CVD by promoting a more sedentary lifestyle [108]. A link between diabetes and CVD has been shown in patients with diabetes in regards to predictive capacity for recovery from and reoccurrence of myocardial infarction (MI) or other cardiovascular events. In individuals with diabetes, the 7-year incidence rate of MI is equivalent to or greater than that of a non-diabetic MI survivor. Thus, an individual with diabetes has the same chance of having a MI than an individual who has previously had a MI. Moreover, in diabetic MI survivors the risk of MI reoccurrence is more than double that of non-diabetic MI survivors [109]. Overall, these data demonstrate that the risk of CVD and incidence of cardiovascular events (e.g., MI, stroke) is much greater in people with diabetes. This enhanced proclivity towards CVD and cardiovascular event incidence emphasizes the importance of managing risk factors for CVD in people with diabetes, as well as the nearly 80 million other Americans that are considered prediabetic [110-112].

For most adults living in industrialized societies, systolic BP, adiposity and BMI increase with advancing age. As all of these increase the risk of

hypertension, the risk for CVD is almost inevitably increased with aging [2, 3]. Aging cannot be avoided, but unhealthy aging that is accompanied by elevations in BP can be prevented with the adoption of healthy lifestyle modifications and/or pharmacological treatment [13]. The more elevated BP is the greater the increase in BP that occurs with aging [83]. Thus, a strong emphasis has been placed on the importance of reducing BP early and maintaining a normal BP (systolic BP <120 mmHg and diastolic BP <80 mmHg) to reduce the risk of future CVD. To convey the seriousness of even slightly elevated BP to the general public, the JNC) has reclassified individuals who were once considered 'normal' (systolic BP 120-129 mmHg and/or diastolic BP 80-84 mmHg) or 'high normal' (systolic BP 130-139 mmHg and/or diastolic BP 85-89 mmHg) as 'prehypertensive' (systolic BP 120-139 mmHg and/or diastolic BP 80-89 mmHg) [113]. While prehypertension is not a diseased state, it is thought of as a predecessor to diseased state as the more BP is elevated above "normal" the greater risk and rate of BP rise that occurs with aging [83]. Moreover, this reclassification places a stronger emphasis on the importance of reducing elevated BP early; without intervention it will most likely develop into the diseased state of hypertension with advancing age without intervention.

Changes in Vascular Function with Aging and Hypertension

Arterial BP, reported as systolic BP over diastolic BP, represents the pressure exerted on the walls of the arteries throughout each cardiac cycle.

Systolic BP corresponds to the maximum pressure, while diastolic BP is the minimum pressure in the arteries. Clinical measurement of BP is typically done on the right arm and assesses BP in the brachial artery. Unfortunately, measurement of BP in the clinical setting is subject to variability due to both examiner and patient error [114]. Although BP measurement in the clinical setting appears to be relatively simple, there are many factors that can result in inaccuracies including, but not limited to body position, cuff size, fasting status, anxiety, smoking, alcohol or caffeine consumption, distended bladder, and talking [114]. Therefore, guidelines have been established for the measurement of BP in the clinical setting, as accuracy of BP measurements is critical for prediction of CVD risk, as well as proper classification for BP lowering treatment [13, 115]. Guidelines set forth by the American Heart Association call for BP to be measured multiple times on the right arm with the patient in the seated position after a 5-minute period of quiet rest [115]. The right arm should be free of clothing and resting at heart level, as changes in arm position, even as little as an inch above or below heart level can affect BP leading to inaccurate measurement [116]. Cuff size is of major importance in determining accurate BP. The bladder of the BP cuff should encircle at least 80% of the circumference and cover two thirds of the length of the upper arm. If cuff size is too small, a greater pressure is required to occlude the brachial artery, artificially increasing BP and potentially diagnosing a truly normotensive individual as hypertensive [117, 118]. Fasting status is major influence on BP, as consumption of a meal prior to measurement

can result in postprandial hypotension due to increased splanchnic blood flow [119]. Postprandial hypotension is of major concern in individuals who are hypertensive, as they tend to have the greatest decreases in BP after a meal, thus the amount BP is reduced after a meal can be useful in predicting future CVD risk [120, 121]. Additionally, those who are hypertensive, but have BP measured after a meal, their true BP and risk for CVD would be unknown. On a related note, consumption of caffeine or alcohol is capable of transiently increasing BP [114]. Thus, they should be avoided prior to measurement. There are many more confounding factors that influence BP that should be controlled in order to achieve accurate measurement of BP [122]. It is the examiner's responsibility to ensure that BP measured in the clinical setting is conducted under repeatable conditions and conforms to American Heart Association guidelines. Any deviation could influence BP measurement and result in inaccurate readings or underestimation of future CVD risk or cardiovascular events.

There are many factors that contribute to error in BP measured in the clinical setting. Fortunately, by following the appropriate guidelines for BP measurement, most of these factors can be reduced, however patient anxiety is not necessarily one of them [123]. In some individuals, BP is artificially high due to anxiety, this condition is referred to as white coat hypertension and can be determined through 24-hour ambulatory BP monitoring [124]. Although white coat hypertension is not a disease state, people with white coat hypertension

may be at greater risk of future CVD, as the regular occurrence of stressful situations, as might be expected in these individuals, is related to left ventricular hypertrophy [125]. Nevertheless, in those with white coat hypertension it is sometimes impossible to get an accurate reading of BP under normal clinical setting. Therefore, 24-hour ambulatory BP monitoring is necessary to allow for measurement of BP throughout the normal daily life. 24-hour ambulatory BP monitoring may also be beneficial to individuals without white coat hypertension. Blood pressure fluctuates throughout the day, and BP measured in the clinical setting, as mentioned previously, is subject to error. For these reasons, ambulatory BP monitoring is used in those without white coat hypertension to assess BP in their normal daily life. Thus, not only can ambulatory BP monitoring be used to determine true BP and the risk of CVD in an individual with white coat hypertension, but it can also be used to determined CVD risk in those without white coat hypertension. Additionally, some individuals have 'masked' hypertension. In this condition, low BP is measured in the clinical setting, but their true hypertensive BP is observed during ambulatory measurement [126, 127]. When comparing BP measured in the clinical setting to BP measured in the ambulatory condition, BP measured in the ambulatory condition tends to be slightly lower [128, 129]. Individuals whose systolic BP is 10% lower than the average daytime BP is considered to have a nocturnal dipping of BP, these individuals are hence referred to as dippers [130]. Those who are nondippers, do not have this decrease in BP at night and are at greater risk of CVD and left

ventricular hypertrophy than those that dip [38, 127, 131]. There are many advantages to ambulatory BP monitoring, but it should be noted that it is intolerable for some patients to have patients to have BP measured throughout the day and night. Given these limitations, ambulatory BP monitoring is an accurate and predictive tool in the determination of CVD risk in normotensive and hypertensive individuals [132].

Regardless of how BP is measured at the brachial artery, whether under proper conditions and guidelines in the clinical setting or ambulatory condition, it is highly predictive of CVD risk [83]. However, brachial BP is not as predictive of future CVD risk, as BP measured in the central arteries (e.g., carotid arteries and aorta), as central BP is more representative of CVD risk [133]. Additionally, changes in brachial BP are not always related to changes in central BP [134]. Brachial BP is useful for predicting CVD risk, as it is highly related to central arterial BP [135-137]. It is the characteristics of the central arteries that are most predictive of future CVD and cardiovascular events, as it is in these arteries that many of the deleterious structural and functional changes occur with aging and hypertension [133]. In fact, a major component of increased BP with age is the changes that occur to the stiffness or compliance of central arteries [8]. Therefore, in most cases, the characteristics of central arteries are of greater importance to cardiovascular health than those of the peripheral arteries. In youth, the central arteries are highly elastic and demonstrate low stiffness and low BP [138]. With aging, increases in stiffness of the central arteries reduce

their elasticity, thereby increasing BP and the future risk of CVD and cardiovascular events [11, 103]. A primary function of the central arteries is to buffer the blood ejected from the left ventricle of the heart during each cardiac cycle. The buffering capacity of the central arteries is of essential for a normal functioning cardiovascular system, as it allows a greater stroke volume to be ejected while maintaining a lower pressure from the left ventricle [139]. In comparison to old arteries, young elastic arteries exhibit an enhanced ability to expand and recoil with each cardiac pulsation and relaxation [48, 56]. The increase in stiffness of the central arteries that occurs with aging also increases the workload of the heart, inducing left ventricular hypertrophy over time [140]. As central arteries age they become more stiff and lose compliance, resulting in an increased velocity at which pulse pressure waveform travels through the arterial system [71]. The pulse pressure waveform is the sum of the forward (incident) and the backward (reflected) waveform [141]. With advancing age or hypertension in stiff arteries with decreased compliance, the velocity in which the pulse wave travels through the arterial tree is increased [71]. The result of this is a return of the reflected wave at a higher velocity [69, 71]. A primary consequence of increased central arterial stiffness and corresponding increased pulse wave velocity is that the reflected wave collides with the incident wave at an earlier point in the cardiac cycle [133, 139]. A collision of the reflected wave and incident wave, if pulse wave velocity is high enough, is an augmenting of systolic BP and concomitant lowering of diastolic BP, which results in an

increased pulse pressure [142]. Increases in systolic BP and pulse pressure, as well as central pulse wave velocity are positively associated with CVD and cardiovascular events [5, 11, 143]. It appears that increases in BP may be preceded by increases in central arterial stiffness [144]. Thus, analysis of properties related to increases in central arterial stiffness that occur with age, may be more important in regard to future risk of future CVD. Additionally, reducing the stiffness of the large formerly elastic central arteries is a target of many therapeutic treatments to reduce the risk of CVD or cardiovascular events [28, 30-32].

The pathology of age-related increases in stiffness and reduction in elasticity of the central arteries is relatively well understood and will be explained later. This portion of the review will focus on the changes in BP that occur with aging and that are influenced with increasing central arterial stiffness. Blood pressure consists of two components: a steady-state and pulsatile component [145]. Both components of BP are useful in predicting CVD risk and cardiovascular events, but risk assessment has been shown to be different between components of BP in regard to age or sex [146-148]. The steady-state component represents the mean BP in the arteries and can be calculated from brachial BP using the equation: mean arterial pressure = 2/3 diastolic BP + 1/3 systolic BP. This formula takes into account the timing of the cardiac cycles; two-thirds of time the heart is in diastole and to one-third it is in systole. Thus, mean arterial pressure is primarily determined from diastolic BP. In younger adults

diastolic BP tends to be more indicative of CVD risk, however, with aging and increasing central arterial stiffness, diastolic BP tends to decrease [149]. While mean arterial pressure does not decrease, due to relative increases in systolic BP with increasing age, mean arterial pressure becomes less predictive of CVD risk and arterial wall thickening with age [134]. It is for these reasons that the pulsatile component of BP has been investigated, as it is more indicative to age-related abnormalities in central arterial stiffness than absolute BP [150]. The pulsatile component of BP can be calculated from brachial BP using the formula: pulse pressure = systolic BP – diastolic BP. This formula represents difference between the maximum and minimum pressure in the arteries. As an individual ages, there is an increase in the stiffness of the arteries, the velocity at which the pulse wave travels through the arterial tree is increased. This results in a collision of the reflected and incident wave at an early point of a given cardiac cycle, resulting in increased systolic BP and reduced diastolic BP. Based on this theory, increases in central arterial BP would increase pulse pressure [81]. Central arterial stiffness, as measured by aortic pulse wave velocity, has been shown to be a strong predictor of future CVD and cardiovascular events [151-155], but measurement of central arterial stiffness has yet to make its way into the clinical setting. Thus, pulse pressure can be used as a surrogate marker to estimate changes in central arterial stiffness [156]. As such, pulse pressure has been shown to be more predictive of CVD risk in older individuals than systolic or diastolic BP not only in the clinical setting [35, 157], but in the ambulatory

condition, as well [38]. Although central arterial stiffness is not measured in the clinical setting, there are techniques available that have been extensively studied. The gold-standard for measurement of central arterial stiffness is cfPWV, and is determined from the transit time of the arterial pulse from the carotid to the femoral artery, measured at the foot of the waveform [158]. While measurement of central arterial stiffness in this manner is not yet possible under ambulatory conditions, it is possible to estimate ambulatory central arterial stiffness by pulse pressure calculation or by the ambulatory arterial stiffness index. The ambulatory arterial stiffness index can be calculated as one minus the regression slope of diastolic BP plotted against systolic BP [159]. While it is not a common measurement of central arterial stiffness it has shown to be highly related to CVD risk [160, 161]. Overall, there are several ways to assess the relations of central arterial stiffness and BP to CVD risk. Although central arterial stiffness is not yet a measurement performed in the clinical setting, it is projected to make its debut in the clinical setting in the coming years.

Central arterial stiffness increases with age, resulting in a reduced compliance of the central arteries [70, 71]. The compliance or elasticity of an artery is determined by two primary elements of the arterial wall: 1) the amount/proportion of the intima-media wall elastin and collagen and 2) the vasoconstrictor tone of the smooth muscle that surrounds the intimal layer of the artery [67-69]. As arteries age, structural changes to the arterial wall result in a reduced proportion of elastin with a comparable increase in collagen content in

the arterial wall [68]. Reductions in elastin are also observed at a younger age in animals with hypertension [162], as higher BP represents a greater strain on the elastin. A common characteristic of aging is a reduced ability of the body to recover from a given stressor. Theoretically, an individual with higher BP places a higher strain on the elastin component of the arterial wall, which results in a higher rate of elastin breakdown. With aging there is a reduced ability to resynthesize elastin, resulting in a proportional increase in collagen [163]. Arteries that are higher in collagen have higher stiffness, thus the pulse pressure waveform travels at a higher velocity throughout the arterial tree in stiff arteries, as compared to normal elastic arteries [164]. Collagen is stiffer than elastin; therefore, increases in collagen in the arterial wall reduce its ability of the arterial wall to buffer blood ejected from the left ventricle. The resulting increase in BP also increases the workload of the heart. Changes to the elastic components of the intima-media wall represent a relatively chronic adaptation that occurs slowly over years and is unlikely to change over a period of days or weeks [68]. Most lifestyle interventions that reduce central arterial stiffness or improve central arterial compliance tend to do so after several weeks by reducing vasomotor tone [28, 30-32]. Changes in vasomotor tone can occur immediately and can be influenced by relatively short-term (e.g., weeks) lifestyle interventions or pharmacological treatment [165-167]. It has been shown that participation in aerobic exercise in previously sedentary middle-aged and older adults reduces central arterial stiffness and BP [28]. Similar findings have been observed after

adoption of dietary changes, such as reductions in sodium intake [31] or consumption of milk proteins [30, 32]. It is believed that changes in arterial vasoconstrictor tone may account for these reductions in central arterial stiffness. Thus, a dietary intervention that reduces arterial vasoconstrictor tone could change central arterial elasticity, thereby lowering BP and overall risk of CVD.

While increased central arterial compliance affects BP, it also likely affects the ability of arterial baroreceptors to modulate BP and heart rate through the arterial baroreflex [8]. The arterial baroreceptors are pressure-sensitive receptors in the walls of large elastic arteries, aortic arch and carotid sinus bifurcation, that stiffen and lose compliance with age [48, 56]. Changes in afferent signaling of the baroreceptors cause reflex-adjustments in the autonomic nervous system to maintain homeostatic regulation of arterial BP [168]. This reflex is impaired in hypertensive and older individuals with reduced large artery compliance [8, 169]; thus, in these individuals, there may be a reduced ability to increase heart rate or BP in response to a hypotensive stress. This condition, orthostatic hypotension, is associated with CVD [61]. However, it has been demonstrated that with reductions in BP and/or increases in large artery compliance, there are similar improvements in BRS [28]. Similar to changes in central arterial stiffness or compliance, cardiovagal BRS is improved after exercise interventions and dietary interventions that reduce BP. It is believed that changes in central arterial stiffness that occur with age, particularly in the carotid arteries, reduce cardiovagal BRS, thus improvements in elasticity or

stiffness of the central arteries would likely be accompanied by, and possibly related to, improvements in cardiovagal BRS.

An additional hemodynamic factor underlying modulation of vasoconstrictor tone is the ability of the vascular endothelium to release vasoactive substances [170]. The increased bioavailability of vasoactive substances (i.e., nitric oxide; NO) increases central arterial compliance and has been shown to improve arterial endothelial function in humans [171]. Endothelial function is reduced with aging and hypertension [172, 173], and is highly predictive of risk of CVD and future cardiovascular events [174]. Briefly, the ability of the endothelium to release vasoactive substances that increase the diameter of an artery can be measured by infusing acetylcholine in an artery [174]. Infusion of acetylcholine stimulates endothelial nitric oxide synthase (eNOS) resulting in the release of NO from the vascular endothelium and subsequent increase in diameter of the artery. Quantification of endothelial function is assessed by measuring the increase in forearm blood flow to infusion of acetylcholine in the brachial artery. Additionally, ultrasound imaging of the brachial artery can be used as a method to quantify endothelial function by recording the increase in diameter of the brachial artery to a given dose of acetylcholine infusion. Similar to measurements of central arterial stiffness, improvements in endothelial function occur after participation in an aerobic exercise program or the adoption of other healthy lifestyle modifications like dietary interventions. While this is necessary to determine the efficacy of lifestyle

modifications or pharmacological intervention, acetylcholine infusion is highly invasive and not likely to be performed in the majority of US research institutions, as a medical doctor is required in most cases. Therefore, a noninvasive measurement of endothelial function has been determined based on the principle that increases in arterial blood flow act as a shear stress that cause the endothelium to release vasoactive substances.

The common, noninvasive method of measuring markers of endothelial function is known as FMD and is performed with ultrasound analysis of the brachial artery [175] or femoral artery [176]. Although the mechanisms are similar, for this review, the focus will be on measurement of brachial artery FMD. The brachial artery diastolic diameter is recorded with an ultrasound probe placed on the upper arm. Then a BP cuff on the forearm is increased to a suprasystolic pressure distal to the ultrasound probe for 5 minutes [58]. This results in a period of ischemia and build up of metabolic byproducts below the BP cuff that cause resistance vessels below the BP cuff to dilate. Upon cuff deflation a reactive hyperemic stimulus increases blood flow to the lower arm. This transient increase in blood flow acts as a shear stress on the brachial artery wall. To normalize the shear stress vasoactive substances are released from the endothelium, which cause an increase in diameter of the brachial artery. Flow-mediated dilation is calculated as the relative increase in peak diameter after cuff deflation in comparison to baseline diastolic diameter using the equation: [(peak diameter – baseline diameter)/baseline diameter] x 100.

It should be noted that whether FMD truly represents NO release from the endothelium has recently come into question [177]. Flow-mediated dilation is highly related to the initial shear stimulus. Thus, changes in FMD may be primarily driven by changes in other vasoactive substances in response to the shear stress rather than the exclusive vasodilatory action of NO [178]. This may represent the ability of the microvasculature or resistance arteries to dilate, increasing hyperemic blood flow and causing the increase in diameter of the larger conduit artery. Other studies that have infused an eNOS inhibitor in the brachial artery have shown a minimal effect on FMD compared to a placebo saline infusion [178]. A recent meta-analysis estimates that although NO was once thought to the primary vasoactive substance released to cause FMD, likely it is only a minor vasodilator under this condition [179]. Regardless of whether NO is truly reflected from measurement of FMD, it cannot be argued that the ability of the artery to increase diameter in response to an increase in flow is highly predictive of future cardiovascular events [64].

There is no question that endothelial dysfunction is highly related to CVD risk. Many studies have shown improvements in endothelial function in response to acetylcholine infusion. It has also been shown that similar changes in lifestyle that result in improvements in endothelial function also result in improvements in FMD [180, 181].

Overall, there are multiple factors related to aging that contribute to elevated BP that occurs with advanced age. Modulation of any one of these factors (e.g., endothelial function, BRS, or arterial vasoconstrictor tone) or all can affect the others as well as the overall rise in BP observed with aging. Thus, it is difficult to determine which change in vascular function is responsible, as when one changes, they are all likely to change [165, 167]. Evidence of this has been shown through systemic infusion of an eNOS inhibitor that reduced endothelial function. In this condition BP is also increased, making it nearly impossible to determine the effect of endothelial function on central arterial stiffness, as it is highly dependent on BP. Regardless of which measurement of vascular function is responsible for reductions in BP observed after the adoption of healthy lifestyle modifications, clinical significance in terms of predicting CVD risk is high with all of the above measurements of vascular function.

Treatment of Elevated Blood Pressure

The risks of morbidity and mortality from CVD progressively rise with increasing BP [34]. Therefore, to reduce risk of adverse CVD-related health outcomes several methods of lowering BP to normal levels have been extensively studied [13, 14, 28, 31, 85, 89]. Additionally, identifying the beneficial components of a healthy lifestyle that prevent future elevations in BP have also been studied. Findings from these types of studies allow for healthy lifestyle modifications to be identified and prescribed to individuals with elevated BP.

Thus, further investigation into treatments for lowering BP in hypertensive individuals is of great public health interest.

Pharmacological Intervention

In recent years, pharmacological interventions have become more common in the treatment and prevention of hypertension [13, 85, 89, 182-186]. While this is not to say that pharmacological treatment is more effective than lifestyle modifications, risk of future cardiovascular events is reduced when pharmacological treatment and lifestyle modifications are combined as initial therapy in comparison to when lifestyle modifications are recommended as the sole treatment of hypertension [187]. Moreover, treatment of high BP through single or multiple pharmacological agents have been shown to effectively reduce the risk of stroke and MI [188-191]. Commonly, the first drug prescribed to lower BP is a thiazide-type diuretic [13, 89]. In most cases, thiazide-type diuretics are effective in reducing BP; however, there are many adverse side effects including dizziness, lightheadedness, blurred vision, loss of appetite, headache, weakness, and erectile dysfunction. Other medications commonly prescribed for the lowering of BP are ACE inhibitors, angiotensin receptor blockers, β-blockers, and calcium channel blockers. ACE inhibitors and angiotensin receptor blockers act through similar pathways. Angiotensin II is a potent vasoconstrictor produced by the action of ACE on its substrate, angiotensin I. Therefore, inhibition of action by ACE would reduce bioavailability of angiotensin II, which reduces vasoconstriction, as well as BP. Angiotensin receptor blockers also alter the

effects of angiotensin II, but by blocking the receptor for angiotensin II thereby reducing BP. Adverse side effects associated with both ACE inhibitors and angiotensin receptor blockers are similar to those observed with thiazide-type diuretics. ACE inhibitors have been shown to be effective in improving cardiovagal BRS in hypertensive individuals [192, 193]. β-blockers lower BP by acting as an antagonist of β-adrenergic receptors. β-receptors are found on cardiac, smooth muscle, kidney, and arterial tissue. Stimulation of β_1-receptors by epinephrine and norepinephrine increase heart rate, induce smooth muscle constriction, release renin from the kidneys, and promote vasoconstrictor tone in arteries. All of the above increase BP, therefore, β-blockers, which act as an antagonist of β-adrenergic receptors, are prescribed to inhibit these responses, lowering BP, as well as heart rate. Similar to β-blockers, calcium channel blockers reduce vasoconstriction of arteries, but do so by reducing contraction of the vascular smooth muscle in the artery. In this way calcium channel blockers promote vasodilation and result in lowering of BP. Although effective in lowering BP, many individuals who are prescribed thiazide BP lowering drugs are unwilling to continue due to side effects associated with pharmacological treatment. Most BP medications have similar side effects, as shown above, so there is reluctance by many patients to begin or continue pharmacological treatment. This is a major underlying issue in the successful treatment of hypertension in a population as a whole. There are not many symptoms related to hypertension, therefore, upon administration of BP lowering drugs and onset of

the adverse symptoms associated with them, many decide to discontinue treatment. Regardless of the adverse side effects associated with most pharmacological treatment of BP, it is an effective method of lowering BP in most individuals.

Currently, the first line approach to reducing BP in individuals with elevated BP at risk for, or already having developed hypertension, is the adoption of healthy lifestyle modifications [13]. Adopting a healthy lifestyle that is successful in reducing BP reduces the risk of CVD and cardiovascular events; however, in many cases individuals do not attempt, or fail, to successfully incorporate healthy lifestyle modifications into their daily routine, and remain at greater risk of CVD and events. Therefore, many individuals with elevated BP opt for pharmacological approach to lowering BP. Although BP lowering drugs are effective in most individuals [194], there are some who do not respond to typical treatment guidelines and multiple drugs are needed to control BP. Regardless of perceived benefit of BP lowering drugs, many patients refuse to medicate due to their adverse side effects.

While lower BP is related to a reduced rate of CVD and events, individuals who opt for pharmacological approach in absence of making healthy lifestyle changes may still be risk for many other diseases associated with an unhealthy lifestyle (e.g., diabetes, cancer, etc.). Moreover, when healthy lifestyle modifications are prescribed properly and incorporated with success there is

almost always an equal or greater benefit for patient outcomes [13]. While many physicians prefer the pharmacological approach to BP reductions in their patients, it should be noted that the greatest reductions in BP occur with combined pharmacological treatment and lifestyle modifications [195]. One example of this is in individuals with resistant hypertension currently taking BP-lowering drugs. When sodium intake is reduced to very low values (~0.5 g/day) BP is reduced. Therefore, regardless of the treatment plan, adopting healthy lifestyle modifications should be a primary component of any BP treatment strategy [96]. Furthermore, in most cases, pharmacological approach is expensive, contributing to the already large and continually growing cost of medical care. Currently, medical care and hospital related expenses for CVD account for upwards of 300 billion dollars per year [4]. While the pharmacological approach to lowering BP extends the life of an individual with high BP, it also may increase the individuals' medical care costs. Those taking anti-hypertensive medications will likely live longer and be taking them throughout the remainder of their lives. Thus, further investigation of lifestyle modifications that reduce BP are necessary, as they represent an inexpensive and effective means to reduce both BP and the economic burden of CVD and other chronic disease like cancer, diabetes, etc. As stated previously, most individuals who attempt to make lifestyle modifications fail to do so. Therefore, there is a need for the identification of more effective and realistically achievable

lifestyle modifications in order to improve patient outcomes while simultaneously reducing the economic cost of CVD.

Lifestyle Modifications for the Treatment of Hypertension

In most individuals with elevated BP, the initial treatment approach is the adoption of healthy lifestyle modifications, including dietary changes [13]. The identification of dietary changes that lower BP comes from the observation that dietary calcium consumption, particularly from dairy products, was lower in individuals with hypertension [23]. In a follow-up study it was shown that dairy products, fruits, and vegetables were inversely related to BP [24]. These findings were used to describe the DASH eating plan, which is high in fruits, vegetables, and low- and non-fat dairy products, but low in total fat and sodium, and has been shown to lower BP in individuals with elevated BP [14-19]. The DASH eating plan has become a mainstay of dietary modifications to reduce BP. In one of the DASH interventional studies [20], subjects completed one of 3 dietary interventions: 1) typical western diet, 2) diet high in fruits and vegetables, and 3) DASH diet. Subjects who consumed the DASH diet had the greatest reductions in BP. These reductions were even greater than in those who consumed the fruits and vegetables diet and were attributed to the addition of low- and non-fat dairy products in DASH diet. It should be noted, however, that the hypotensive effects of the DASH diet could not be ascribed to dairy products alone because other dietary changes (e.g., a reduction in total fat and saturated fat) were also incorporated.

Many interventional studies have increased daily consumption of low- and non-fat dairy products and shown reductions in BP [14-19], but not all studies found this [196-198]. Most of the studies demonstrating a hypotensive effect with increased daily amounts of low- and non-fat dairy products were controlled feeding studies or incorporated other dietary or lifestyle changes (e.g., weight loss, increased physical activity). Less is known about whether the addition of non-fat dairy products to the normal routine diet is able to reduce BP without other concomitant lifestyle or dietary changes. Additionally, the role of central arterial stiffness on reductions in BP in response to these types of lifestyle changes has not been well studied. One study did investigate the effects on vascular function (cardiovagal BRS, central arterial stiffness, and brachial FMD) in response to a 4-month intervention of one of three conditions: 1) same lifestyle (control), 2) DASH eating plan, and 3) DASH eating plan plus weight loss (DASH+WL) [15]. While reductions in systolic and diastolic BP were greatest in the DASH and DASH+WL compared to control, only endpoint data for markers of vascular function were reported. Brachial FMD tended to be higher, but not significantly different than control, central arterial stiffness, measured by cfPWV, was significantly lower in DASH groups compared to control [15]. The interpretation of these data are limited, as only endpoint data was reported and comparisons with control were made by combining both DASH groups (DASH and DASH+WL). Furthermore, risk of CVD is increased in those with a higher BMI, partially due to increases in BP that are associated with weight gain. A

modest weight loss substantially lowers the long-term risk of hypertension [199]. Thus, the differences observed in this study may have been driven by reductions in weight from the DASH+WL group. It has been shown that dietary supplementation with isolated milk proteins reduces BP while simultaneously improving carotid arterial compliance [30]. Additionally, individuals, who consume more servings of low-fat dairy products have reduced central arterial stiffness, as measured by cfPWV [21]. While it appears that consumption of low- and non-fat dairy products may in fact lower central arterial stiffness and improve other markers of vascular function, further study is warranted.

Chapter 5: Summary and Future Directions

Summary

Diseases of the cardiovascular system increase with advancing aging and are responsible for more annual deaths than any other causes [1]. One of the most important and predictive risk factors for CVD is elevated BP [22]. Arterial BP increases with advancing age in most industrialized countries resulting in a markedly high prevalence of essential hypertension, particularly in isolated systolic hypertension, among older adults [46]. This rise in BP is the major contributor to age-associated increases in risk of a number of cardiovascular disorders, including cerebrovascular and coronary heart disease [34]. Although the risk of coronary heart disease is greater in hypertensive than normotensive adults at any age, the magnitude of risk elevation associated with hypertension is much greater in older adults as compared with young adults [2]. The physiological mechanisms by which arterial BP increases with increasing age are not known. However, structural and functional alterations of the large arterial wall, including stiffening of arteries, have been implicated [47].

To reduce the incidence of CVD, it is of paramount importance to identify treatments that can lower BP and restore age-related vascular dysfunction. In most individuals at risk of CVD, the initial treatment approach is the adoption of healthy lifestyle modifications [13]. The identification of dietary changes that lower BP comes from the observation that dietary calcium consumption,

particularly from dairy products, was lower in individuals with hypertension [23]. In a follow-up study it was shown that dairy products, fruits, and vegetables were inversely related to BP [24]. These findings were used to describe the DASH diet, which is high in fruits, vegetables, and low- and non-fat dairy products, but low in total fat and sodium, and has been shown to lower BP in individuals with elevated BP [15, 20]. In one of the DASH diet interventional studies [20], reductions in BP were attributed to dairy products, but not completely as other dietary changes (e.g., a reduction in total fat and saturated fat) were also incorporated. In support of these findings, several other interventional studies have shown reductions in BP after consumption of a diet high in low- and non-fat dairy products [15-17, 25], although some studies have not shown this [26, 27]. Information concerning the physiological mechanisms by which dairy consumption lowers arterial BP is extremely limited. Indeed, a previous study has demonstrated an inverse relation between dairy intake and central arterial stiffness [21]. Furthermore, others have demonstrated reduced central arterial stiffness and improved endothelial function after strictly controlled dietary interventions involving supplementation with milk proteins [30, 32].

Although it was relatively established that higher dairy consumption was associated with lower BP, prior to this study, it was not known if the solitary addition of non-fat dairy products to the normal routine diet, without any other concomitant lifestyle or dietary changes, would be capable of reducing BP. Additionally, it was not known if adopting this type of dietary change was capable

of improving vascular function and whether potential improvements in vascular function would be associated with reductions in arterial BP. In general, de-stiffening of large arteries may play an important role in producing the hypotensive effects of dairy products. Therefore, it was plausible to hypothesize that reductions in BP would occur and be accompanied by improvements in vascular function. Accordingly, the primary purpose of this dissertation study was to determine if the solitary addition of conventional non-fat dairy products to the normal routine diet would reduce BP and improve vascular function in middle-aged and older adults with elevated BP.

The primary findings of the present study were as follows. The addition of 4 daily servings of conventional non-fat dairy products to the normal routine diet decreased systolic BP and pulse pressure in middle-aged and older adults with elevated BP. The reduction in casual seated BP was accompanied by the similar reduction in ambulatory (24-hour) BP due mainly to the decrease in daytime BP. Additionally, we found that the addition of four daily servings of conventional non-fat dairy products to the normal routine diet decreased central systolic BP and pulse pressure in middle-aged and older adults with elevated BP. The reductions in central BP were related to the corresponding changes in central arterial stiffness and were accompanied by increased brachial FMD and cardiovagal BRS. Conversely, when all dairy products were removed from the routine diet, pulse pressure increased significantly in central and peripheral arteries, while brachial FMD decreased significantly. Taken together, these findings indicate

that the solitary manipulation of dairy products, particularly non-fat dairy products, can modulate central BP and key vascular functions in middle-aged and older adults with elevated BP.

Lifestyle modifications, including dietary changes, are the first line approach for treating elevated BP. The present findings indicate that simply adding 4 servings of conventional non-fat dairy products are effective in reducing both central and peripheral systolic BP and pulse pressure in middle-aged and older adults. Additionally, the addition of non-fat dairy products reduced central arterial stiffness and improved endothelial function. On the contrary, removal of dairy products increased central pulse pressure and reduced endothelial function. Unlike other lifestyle modifications, including regular exercise, that are difficult to achieve high compliance and adherence, dairy products can be easily incorporated into daily routine of older adults to gain hypotensive and vasoprotective benefits.

Future Directions

The effects of dairy products consumption, particularly non-fat dairy, on BP and vascular function need further investigation. It is clear from these data that the hypotensive and vasoprotective effects of dairy products are present even at lower levels of dairy intake (~1 serving/day), but it unclear what the minimum daily quantity of non-fat dairy product consumption that is required for reducing

BP and improving vascular function. Therefore, it is necessary to determine the minimal and optimal dose of dietary dairy intake to reduce BP and improve vascular function. On a related note, identification of what types of dairy products (e.g., milk, yogurt, cheese, etc.) are most effective in reducing BP remains unknown. Identification of minimal/optimal dose and dairy product type that produce the greatest benefit to individuals with elevated BP is of paramount importance to provide more detailed recommendations on how dairy product consumption could reduce the impact of CVD.

In the present study, we observed that changes in BP were related to central arterial stiffness. It has been established that in addition to the reduced risk of high BP and CVD, diets high in low- and non-fat dairy products are also associated with a lower risk of diabetes [200-202]. Thus, changes observed after manipulation of dairy intake may also reduce risk factors for other chronic diseases, like diabetes. It is possible that milk products may also affect insulin release and/or sensitivity, as direct mechanistic evidence of a diabetes reducing effect of milk products consumption has been shown in studies that found attenuated rise in postprandial glycemia when milk products are added to a high carbohydrate meal [203-205]. Insulin receptors are found on endothelial and vascular smooth muscle cells, and the binding of insulin at these sites results in insulin-mediated vasodilation achieved through a NO dependent mechanism [206, 207]. Therefore, it is plausible that vascular changes observed in the present study may be related to reductions in postprandial glycemia observed

after a high carbohydrate meal when milk is included. If true, it would establish a mechanistic link between diabetes and CVD that could be modulated by increasing dairy consumption.

Appendix A: Abbreviations and Acronyms

ACE = angiotensin converting enzyme

ANOVA = analysis of variance

BP = blood pressure

BRS = baroreflex sensitivity

cfPWV = carotid-femoral pulse wave velocity

CVD = cardiovascular disease

DASH = dietary approaches to stop hypertension

DASH+WL = dietary approaches to stop hypertension plus weight loss

eNOS = endothelial nitric oxide synthase

FMD = flow-mediated dilation

HbA_{1c} = glycated hemoglobin

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

MI = myocardial infarction

NO = nitric oxide

Appendix B: Health Research 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

Subject ID_____

Dairy Study Week # _____

Date study food picked up:_____

Dairy items	# servings issued	Consume 4 provided dairy servings per day Check items off list as consumed each day Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
8 oz. fat-free milk	_____							
6 oz. fat-free yogurt	_____							
3 slices fat-free cheese (1 slice = 1/3 serving)	_____							

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
Check items off list as consumed each day

Fruit items	# servings issued	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
8 oz. juice	_____							
4 oz. fruit cup	_____							
4 oz. applesauce	_____							

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 non-fat (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.

-See reverse for sample food log-

Sample 3 Day Food Intake	**Day of Week:**		**Date:**
Time	***Amount***	***Brand***	***Food/Beverage***
8am	**8 oz**		**Non-fat 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: Analysis of Blood

After a 12-hour overnight fast, blood samples was collected by venipuncture in the antecubital veins of the arm. Approximately 1 ml of blood was obtained during each testing visit. Immediately following blood collection, whole blood concentrations of total cholesterol, HDL cholesterol, triglycerides, and glucose was measured enzymatically using commercially available diagnostic kits (Cholestech LDX Analyzer, Alere, Waltham, MA). LDL cholesterol was estimated using the measured values of total cholesterol, HDL cholesterol, and triglycerides. Whole blood HbA_{1c} was measured using a commercially available HbA_{1c} reagent kit (DCA Systems, Siemens Healthcare Diagnostics Inc., Tarrytown, NY).

Total Cholesterol and HDL Cholesterol Measurement

Total cholesterol and HDL cholesterol was measured by the Cholestech LDX Analyzer, which uses an enzymatic method. Cholesterol esters in the sample are hydrolyzed by cholesterol esterase to free cholesterol and fatty acids (FFA). Then in the presence of oxygen, free cholesterol is oxidized by cholesterol oxidase to cholest-4-ene-3-one and hydrogen peroxide (H_2O_2). Finally, in a reaction catalyzed by peroxidase (POD), the peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulfoydroxypropyl-m-toluidine, sodium salt (TOOS) to form a purple-colored quinoneimine dye proportional to the total cholesterol and HDL cholesterol concentrations in the sample.

Reaction:

1. Cholesterol esters + H_20 --*Cholesterol esterase*--> Free cholesterol + FA
2. Free cholesterol + O_2 --*Cholesterol oxidase*--> Cholest-4-ene-3-one + H_2O_2
3. 2 H_2O_2 + 4-Aminoantipyrine + TOOS --*POD*--> Quinoneimine dye + 4 H_2O

Triglyceride Measurement

Triglycerides (TG) were measured by the Cholestech LDX Analyzer, which uses an enzymatic method. Triglycerides in the sample are hydrolyzed by lipase to glycerol and FFA. Glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G-3-P) and ADP in a reaction catalyzed by glycerol kinase (GK). In a third reaction, G-3-P is oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DHAP) and H_2O_2. The color reaction utilizing POD is the same as for the total cholesterol and HDL cholesterol.

Reaction:

1. TG + H20 --*Lipase*--> Glycerol + FFA
2. Glycerol + ATP --*GK*--> G-3-P + ADP
3. G-3-P --*GPO*-->DHAP + H_2O_2
4. 2 H_2O_2 + 4-Aminoantipyrine + TOOS --*POD*--> Quinoneimine dye + 4 H_2O

Glucose Measurement

Glucose was measured by the Cholestech LDX Analyzer, which uses an enzymatic method. In the presence of oxygen, glucose in the sample is oxidized by glucose oxidase to gluconolactone and H_2O_2. The color reaction utilizing POD is the same as for total cholesterol, HDL cholesterol, and TG.

Reactions:

1. Glucose + O_2 --*Glucose oxidase*--> o-D-gluconolactone + H_2O_2
2. 2 H_2O_2 + 4-Aminoantipyrine + TOOS --*POD*--> Quinoneimine dye + 4 H_2O

LDL Cholesterol Estimation

LDL cholesterol estimation was calculated by the Cholestech LDX Analyzer software based on the rearrangement of the algebraic formula for total cholesterol (total cholesterol = HDL cholesterol + LDL cholesterol + TG × 0.20) to the following:

LDL cholesterol = total cholesterol – HDL cholesterol – TG × 0.20

HbA_{1c} Measurement

HbA_{1c} was measured by the DCA Vantage system, which also measures total hemoglobin, as % HbA_{1c} of total hemoglobin concentration is reported. For the measurement of total hemoglobin, potassium ferricyanide is used to oxidize hemoglobin in the sample to methemoglobin. The methemoglobin then complexes with thiocyanate to form thiocyan-methemoglobin, the colored species that is measured. From this reaction, the extent of color development at 531 nm is proportional to the concentration of total hemoglobin in the sample. For measurement of HbA_{1c}, an agglutinator containing multiple copies of the immunoreactive portion of HbA_{1c} causes agglutination of latex coated with HbA_{1c} specific mouse monoclonal antibody. The agglutination reaction causes increased scattering of light, which is measured as an increase in absorbance at 531 nm. HbA_{1c} in whole blood specimens

competes for the limited number of anti-body-latex binding sites causing an inhibition of agglutination and decreased scattering of light. The decreased scattering is measured as a decrease in absorbance at 531 nm. The HbA_{1c} concentration is then quantified using a calibration curve of absorbance versus HbA_{1c} concentration. The percent HbA_{1c} in the sample is then calculated using the following equation:

$$\% \text{ HbA1c} = \frac{[HbA_{1c}]}{[\text{Total hemoglobin}]} \times 100$$

Appendix F: Supplemental Data from Study 1

Table F.1. Changes in Selected Subject Characteristics and Blood Chemistry

	No Dairy Condition First (n=26)				High Dairy Condition First (n=23)			
	No Dairy		High Dairy		High Dairy		No Dairy	
Variables	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Height, cm	170±2	-	170±2	-	171±3	-	171±3	-
Body mass, kg	89.5±4	89.3±4	90.0±4	90.3±4	86.1±4	86.1±3	85.9±3	85.9±3
Body mass index, kg/m^2	31.0±1	30.9±1	31.1±1	31.2±1	29.7±1	29.7±1	29.7±1	29.7±1
Total cholesterol, mmol/l	5.40±0.2	5.17±0.2	5.37±0.3	5.36±0.2	5.14±0.2	5.09±0.3	5.04±0.3	5.03±0.3
HDL cholesterol, mmol/l	1.24±0.1	1.05±0.1	1.18±0.1	1.10±0.1	1.16±0.1	1.17±0.1	1.27±0.1	1.19±0.1
LDL cholesterol, mmol/l	3.60±0.2	3.54±0.2	3.61±0.2	3.60±0.2	3.30±0.2	3.21±0.2	3.17±0.2	3.22±0.2
Triglycerides, mmol/l	1.21±0.1	1.25±0.1	1.26±0.1	1.42±0.1	1.49±0.2	1.54±0.2	1.31±0.2	1.35±0.2
Blood glucose, mmol/l	5.36±0.1	5.26±0.2	5.47±0.2	5.56±0.2	5.20±0.1	5.27±0.1	5.18±0.1	5.21±0.1
HbA_{1c}, %	5.5±0.1	5.4±0.1	5.5±0.1	5.5±0.1	5.4±0.1	5.3±0.1	5.4±0.1	5.3±0.1
Systolic BP, mmHg	134±2	135±2	134±2	126±2	135±2	129±2	134±2	135±2
Diastolic BP, mmHg	80±2	79±2	80±2	78±2	81±2	80±2	80±2	80±2
Pulse pressure, mmHg	54±2	56±2	54±2	48±2	54±2	49±2	54±2	55±2
Heart rate, BPM	70±2	67±2	69±2	69±2	68±2	69±2	68±2	69±2

Data are presented at mean ± SEM.

Table F.2. Changes in Dietary Composition

	No Dairy Condition First (n=26)				High Dairy Condition First (n=23)			
	No Dairy		High Dairy		High Dairy		No Dairy	
Variables	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Calories, kcal/day	1872±90	1875±55	1843±96	1881±71	1814±86	1879±72	1758±73	1805±74
Fat, g/day	77±7	60±4	69±5	62±5	68±4	58±4	64±4	56±4
Carbohydrate, g/day	204±13	255±8	206±14	227±10	200±14	226±12	198±13	247±13
Protein, g/day	80±6	71±4	84±4	96±4	79±4	99±5	78±5	66±5
Alcohol, g/day	6±2	4±2	8±3	4±2	13±4	9±3	11±3	8±3
Sodium, mg/day	3,147±311	2,671±181	3,032±214	2,892±204	2,437±159	2,566±121	2,670±185	2,424±165
Potassium, mg/day	2,051±192	2,014±138	2006±153	2,918±109	2,326±213	3,291±237	2,187±243	1,992±141
Calcium, mg/day	743±72	477±63	738±88	1,686±82	822±68	1,833±99	775±83	346±38
Magnesium, mg/day	200±17	176±15	198±3	259±14	229±24	285±25	225±20	160±16
Vitamin D, IU/day	284±121	200±122	245±127	540±120	167±33	481±28	150±35	92±21

Data are presented at mean ± SEM.

Table F.3. Specific Dairy Product Consumption

	No Dairy Condition First (n=26)				High Dairy Condition First (n=23)			
	No Dairy		High Dairy		High Dairy		No Dairy	
Variables	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Dairy, serving/day	1.3±0.2	0.0±0.0	0.9±0.2	4.6±0.2	1.4±0.2	4.9±0.2	1.4±0.2	0.0±0.0
Non-fat dairy, serving/day	0.3±0.1	0.0±0.0	0.3±0.1	4.0±0.1	0.4±0.2	4.0±0.1	0.4±0.2	0.0±0.0
Full-fat dairy, serving/day	0.9±0.2	0.0±0.0	0.5±0.1	0.4±0.2	0.8±0.2	0.4±0.1	0.6±0.1	0.0±0.0
Milk, serving/day	0.4±0.1	0.0±0.0	0.3±0.1	2.2±0.2	0.6±0.2	2.4±0.2	0.5±0.1	0.0±0.0
Yogurt, serving/day	0.2±0.1	0.0±0.0	0.1±0.0	1.9±0.1	0.2±0.1	2.0±0.2	0.3±0.1	0.0±0.0
Cheese, serving/day	0.6±0.1	0.0±0.0	0.4±0.1	0.1±0.0	0.5±0.1	0.1±0.0	0.5±0.1	0.0±0.0
Other dairy, serving/day	0.1±0.0	0.0±0.0	0.1±0.0	0.4±0.1	0.1±0.0	0.4±0.1	0.1±0.0	0.0±0.0

Data are presented at mean ± SEM.

Table F.4. Changes in Blood Pressure in Antihypertensive Drug Users vs. Non-Users

Variables	No Dairy		High Dairy	
	Before	**After**	**Before**	**After**
Antihypertensive Drugs (n=5)				
Systolic BP, mmHg	142±4	141±6	144±4	136±6
Diastolic BP, mmHg	84±4	84±5	86±3	84±4
Pulse pressure, mmHg	57±5	57±3	58±3	51±5
No Drugs (n=44)				
Systolic BP, mmHg	133±1	134±2	134±1	126±1
Diastolic BP, mmHg	79±1	79±2	80±1	78±1
Pulse pressure, mmHg	53±1	56±1	54±1	48±1

Data are presented as mean ± SEM.

References

1. Go, A.S., Mozaffarian, D., Roger, V.L., Benjamin, E.J., Berry, J.D., Blaha, M.J., Dai, S., Ford, E.S., Fox, C.S., Franco, S., et al. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. Circulation 2014; 129: e28-e292.

2. Burt, V.L., Cutler, J.A., Higgins, M., Horan, M.J., Labarthe, D., Whelton, P., Brown, C., and Roccella, E.J. Trends in the prevalence, awareness, treatment, and control of hypertension in the adult US population. Data from the health examination surveys, 1960 to 1991. Hypertension 1995; 26: 60-69.

3. Kannel, W.B., D'Agostino, R.B., and Silbershatz, H. Blood pressure and cardiovascular morbidity and mortality rates in the elderly. American heart journal 1997; 134: 758-763.

4. Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Benjamin, E.J., Berry, J.D., Borden, W.B., Bravata, D.M., Dai, S., Ford, E.S., Fox, C.S., et al. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. Circulation 2012; 125: e2-e220.

5. Kannel, W.B. Blood pressure as a cardiovascular risk factor: prevention and treatment. JAMA : the journal of the American Medical Association 1996; 275: 1571-1576.

6. Kannel, W.B. Elevated systolic blood pressure as a cardiovascular risk factor. The American journal of cardiology 2000; 85: 251-255.

7. Stokes, J., 3rd, Kannel, W.B., Wolf, P.A., D'Agostino, R.B., and Cupples, L.A. Blood pressure as a risk factor for cardiovascular disease. The Framingham Study--30 years of follow-up. Hypertension 1989; 13: I13-18.

8. Lage, S.G., Polak, J.F., O'Leary, D.H., and Creager, M.A. Relationship of arterial compliance to baroreflex function in hypertensive patients. The American Journal of Physiology 1993; 265: H232-237.

9. Avolio, A.P., Chen, S.G., Wang, R.P., Zhang, C.L., Li, M.F., and O'Rourke, M.F. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. Circulation 1983; 68: 50-58.

10. Tanaka, H., DeSouza, C.A., and Seals, D.R. Absence of age-related increase in central arterial stiffness in physically active women. Arteriosclerosis, thrombosis, and vascular biology 1998; 18: 127-132.

11. Mitchell, G.F., Hwang, S.J., Vasan, R.S., Larson, M.G., Pencina, M.J., Hamburg, N.M., Vita, J.A., Levy, D., and Benjamin, E.J. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation 2010; 121: 505-511.

12. Aronow, W.S., Fleg, J.L., Pepine, C.J., Artinian, N.T., Bakris, G., Brown, A.S., Ferdinand, K.C., Ann Forciea, M., Frishman, W.H., Jaigobin, C., et al. ACCF/AHA 2011 expert consensus document on hypertension in the elderly: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus documents developed in collaboration with the American Academy of Neurology, American Geriatrics Society, American Society for Preventive Cardiology, American Society of Hypertension, American Society of Nephrology, Association of Black Cardiologists, and European Society of Hypertension. Journal of the American College of Cardiology 2011; 57: 2037-2114.

13. Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L., Jr., Jones, D.W., Materson, B.J., Oparil, S., Wright, J.T., Jr., et al. Seventh report of the Joint National Committee on Prevention,

Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 42: 1206-1252.

14. Hilpert, K.F., West, S.G., Bagshaw, D.M., Fishell, V., Barnhart, L., Lefevre, M., Most, M.M., Zemel, M.B., Chow, M., Hinderliter, A.L., et al. Effects of dairy products on intracellular calcium and blood pressure in adults with essential hypertension. Journal of the American College of Nutrition 2009; 28: 142-149.

15. Blumenthal, J.A., Babyak, M.A., Hinderliter, A., Watkins, L.L., Craighead, L., Lin, P.H., Caccia, C., Johnson, J., Waugh, R., and Sherwood, A. Effects of the DASH diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and women with high blood pressure: the ENCORE study. Archives of internal medicine 2010; 170: 126-135.

16. Buonopane, G.J., Kilara, A., Smith, J.S., and McCarthy, R.D. Effect of skim milk supplementation on blood cholesterol concentration, blood pressure, and triglycerides in a free-living human population. Journal of the American College of Nutrition 1992; 11: 56-67.

17. Svetkey, L.P., Simons-Morton, D., Vollmer, W.M., Appel, L.J., Conlin, P.R., Ryan, D.H., Ard, J., and Kennedy, B.M. Effects of dietary patterns on blood pressure: subgroup analysis of the Dietary Approaches to Stop Hypertension (DASH) randomized clinical trial. Archives of internal medicine 1999; 159: 285-293.

18. Van Beresteijn, E.C., van Schaik, M., and Schaafsma, G. Milk: does it affect blood pressure? A controlled intervention study. J Intern Med 1990; 228: 477-482.

19. Svetkey, L.P., Simons-Morton, D.G., Proschan, M.A., Sacks, F.M., Conlin, P.R., Harsha, D., and Moore, T.J. Effect of the dietary approaches to stop hypertension diet and reduced sodium intake on blood pressure control. Journal of clinical hypertension 2004; 6: 373-381.

20. Appel, L.J., Moore, T.J., Obarzanek, E., Vollmer, W.M., Svetkey, L.P., Sacks, F.M., Bray, G.A., Vogt, T.M., Cutler, J.A., Windhauser, M.M., et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. The New England journal of medicine 1997; 336: 1117-1124.

21. Crichton, G.E., Elias, M.F., Dore, G.A., Abhayaratna, W.P., and Robbins, M.A. Relations between dairy food intake and arterial stiffness: pulse wave velocity and pulse pressure. Hypertension 2012; 59: 1044-1051.

22. Sharrett, A.R., Sorlie, P.D., Chambless, L.E., Folsom, A.R., Hutchinson, R.G., Heiss, G., and Szklo, M. Relative importance of various risk factors for asymptomatic carotid atherosclerosis versus coronary heart disease incidence: the Atherosclerosis Risk in Communities Study. American journal of epidemiology 1999; 149: 843-852.

23. McCarron, D.A., Morris, C.D., and Cole, C. Dietary calcium in human hypertension. Science 1982; 217: 267-269.

24. McCarron, D.A., Morris, C.D., Henry, H.J., and Stanton, J.L. Blood pressure and nutrient intake in the United States. Science 1984; 224: 1392-1398.

25. van Meijl, L.E. and Mensink, R.P. Low-fat dairy consumption reduces systolic blood pressure, but does not improve other metabolic risk parameters in overweight and obese subjects. Nutrition, metabolism, and cardiovascular diseases : NMCD 2011; 21: 355-361.

26. Crichton, G.E., Howe, P.R., Buckley, J.D., Coates, A.M., and Murphy, K.J. Dairy consumption and cardiometabolic health: outcomes of a 12-month crossover trial. Nutrition & metabolism 2012; 9: 19.

27. Benatar, J.R., Sidhu, K., and Stewart, R.A. Effects of high and low fat dairy food on cardio-metabolic risk factors: a meta-analysis of randomized studies. PLoS One 2013; 8: e76480.

28. Nualnim, N., Parkhurst, K., Dhindsa, M., Tarumi, T., Vavrek, J., and Tanaka, H. Effects of swimming training on blood pressure and vascular function in adults >50 years of age. The American journal of cardiology 2012; 109: 1005-1010.

29. Seals, D.R., Stevenson, E.T., Jones, P.P., DeSouza, C.A., and Tanaka, H. Lack of age-associated elevations in 24-h systolic and pulse pressures in women who exercise regularly. The American Journal of Physiology 1999; 277: H947-955.

30. Yoshizawa, M., Maeda, S., Miyaki, A., Misono, M., Choi, Y., Shimojo, N., Ajisaka, R., and Tanaka, H. Additive beneficial effects of lactotripeptides and aerobic exercise on arterial compliance in postmenopausal women. American journal of physiology. Heart and circulatory physiology 2009; 297: H1899-1903.

31. Gates, P.E., Tanaka, H., Hiatt, W.R., and Seals, D.R. Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. Hypertension 2004; 44: 35-41.

32. Yoshizawa, M., Maeda, S., Miyaki, A., Misono, M., Choi, Y., Shimojo, N., Ajisaka, R., and Tanaka, H. Additive beneficial effects of lactotripeptides intake with regular exercise on endothelium-dependent dilatation in

postmenopausal women. American journal of hypertension 2010; 23: 368-372.

33. Tanaka, H., Bassett, D.R., Jr., Howley, E.T., Thompson, D.L., Ashraf, M., and Rawson, F.L. Swimming training lowers the resting blood pressure in individuals with hypertension. Journal of hypertension 1997; 15: 651-657.

34. Lewington, S., Clarke, R., Qizilbash, N., Peto, R., Collins, R., and Prospective Studies, C. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002; 360: 1903-1913.

35. Franklin, S.S., Khan, S.A., Wong, N.D., Larson, M.G., and Levy, D. Is pulse pressure useful in predicting risk for coronary heart Disease? The Framingham heart study. Circulation 1999; 100: 354-360.

36. McCarron, D.A. Calcium and magnesium nutrition in human hypertension. Annals of internal medicine 1983; 98: 800-805.

37. McCarron, D.A. and Morris, C.D. Blood Pressure Response to Oral Calcium in Persons with Mild to Moderate HypertensionA Randomized, Double-Blind, Placebo-Controlled, Crossover Trial. Annals of internal medicine 1985; 103: 825-831.

38. Verdecchia, P., Schillaci, G., Borgioni, C., Ciucci, A., Pede, S., and Porcellati, C. Ambulatory pulse pressure: a potent predictor of total cardiovascular risk in hypertension. Hypertension 1998; 32: 983-988.

39. Bazzano, L.A., He, J., Ogden, L.G., Loria, C.M., Vupputuri, S., Myers, L., and Whelton, P.K. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination

Survey Epidemiologic Follow-up Study. The American Journal of Clinical Nutrition 2002; 76: 93-99.

40. FitzGerald, R.J. and Meisel, H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. The British Journal of Nutrition 2000; 84 Suppl 1: S33-37.

41. Kass, L., Weekes, J., and Carpenter, L. Effect of magnesium supplementation on blood pressure: a meta-analysis. European journal of clinical nutrition 2012; 66: 411-418.

42. Chung, M., Balk, E.M., Brendel, M., Ip, S., Lau, J., Lee, J., Lichtenstein, A., Patel, K., Raman, G., Tatsioni, A., et al. Vitamin D and calcium: a systematic review of health outcomes. Evid Rep Technol Assess (Full Rep) 2009; 1-420.

43. Huth, P.J. and Park, K.M. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. Advances in nutrition 2012; 3: 266-285.

44. Kratz, M., Baars, T., and Guyenet, S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. European journal of nutrition 2013; 52: 1-24.

45. Soedamah-Muthu, S.S., Ding, E.L., Al-Delaimy, W.K., Hu, F.B., Engberink, M.F., Willett, W.C., and Geleijnse, J.M. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. The American Journal of Clinical Nutrition 2011; 93: 158-171.

46. Franklin, S.S., Gustin, W., Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B., and Levy, D. Hemodynamic patterns of age-related

changes in blood pressure The Framingham Heart Study. Circulation 1997; 96: 308-315.

47. O'Rourke, M.F. and Hashimoto, J. Mechanical factors in arterial aging: a clinical perspective. Journal of the American College of Cardiology 2007; 50: 1-13.

48. Monahan, K.D., Tanaka, H., Dinenno, F.A., and Seals, D.R. Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. Circulation 2001; 104: 1627-1632.

49. Seals, D.R., Jablonski, K.L., and Donato, A.J. Aging and vascular endothelial function in humans. Clin Sci (Lond) 2011; 120: 357-375.

50. Safar, M. and Jankowski, P. Central blood pressure and hypertension: role in cardiovascular risk assessment. Clinical science 2009; 116: 273-282.

51. Cortez-Cooper, M.Y., Supak, J.A., and Tanaka, H. A new device for automatic measurements of arterial stiffness and ankle-brachial index. The American journal of cardiology 2003; 91: 1519-1522, A1519.

52. Tanaka, H., DeSouza, C.A., and Seals, D.R. Arterial stiffness and hormone replacement use in healthy postmenopausal women. The journals of gerontology. Series A, Biological sciences and medical sciences 1998; 53: M344-346.

53. Tanaka, H., Dinenno, F.A., Hunt, B.E., Jones, P.P., DeSouza, C.A., and Seals, D.R. Hemodynamic sequelae of age-related increases in arterial stiffness in healthy women. The American journal of cardiology 1998; 82: 1152-1155, A1110.

54. Sugawara, J., Hayashi, K., Yokoi, T., and Tanaka, H. Age-associated elongation of the ascending aorta in adults. JACC Cardiovasc Imaging 2008; 1: 739-748.

55. Tanaka, H., Dinenno, F.A., Monahan, K.D., Clevenger, C.M., DeSouza, C.A., and Seals, D.R. Aging, habitual exercise, and dynamic arterial compliance. Circulation 2000; 102: 1270-1275.

56. Monahan, K.D., Dinenno, F.A., Seals, D.R., Clevenger, C.M., Desouza, C.A., and Tanaka, H. Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. American journal of physiology. Heart and circulatory physiology 2001; 281: H284-289.

57. Monahan, K.D., Dinenno, F.A., Tanaka, H., Clevenger, C.M., DeSouza, C.A., and Seals, D.R. Regular aerobic exercise modulates age-associated declines in cardiovagal baroreflex sensitivity in healthy men. The Journal of Physiology 2000; 529 Pt 1: 263-271.

58. Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. Journal of the American College of Cardiology 2002; 39: 257-265.

59. Liao, D., Wong, T.Y., Klein, R., Jones, D., Hubbard, L., and Sharrett, A.R. Relationship between carotid artery stiffness and retinal arteriolar narrowing in healthy middle-aged persons. Stroke; a journal of cerebral circulation 2004; 35: 837-842.

60. Gopinath, B., Flood, V.M., Wang, J.J., Burlutsky, G., and Mitchell, P. Lower dairy products and calcium intake is associated with adverse retinal

vascular changes in older adults. Nutrition, metabolism, and cardiovascular diseases : NMCD 2014; 24: 155-161.

61. La Rovere, M.T., Bigger, J.T., Jr., Marcus, F.I., Mortara, A., and Schwartz, P.J. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998; 351: 478-484.

62. Billman, G.E., Schwartz, P.J., and Stone, H.L. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. Circulation 1982; 66: 874-880.

63. Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Sullivan, I.D., Lloyd, J.K., Deanfield, J.E., and Spiegelhalter, D.J. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. The Lancet 1992; 340: 1111-1115.

64. Green, D.J., Jones, H., Thijssen, D., Cable, N.T., and Atkinson, G. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension 2011; 57: 363-369.

65. Hoyert, D.L. 75 years of mortality in the United States, 1935-2010. NCHS Data Brief 2012; 1-8.

66. Rice, D.P. and Fineman, N. Economic implications of increased longevity in the United States. Annu. Rev. Public Health 2004; 25: 457-473.

67. Rucker, R.B. and Tinker, D. Structure and metabolism of arterial elastin. Int Rev Exp Pathol 1977; 17: 1-47.

68. O'Rourke, M. Mechanical principles in arterial disease. Hypertension 1995; 26: 2-9.

69. O'Rourke, M.F. Mechanical principles. Arterial stiffness and wave reflection. Pathol Biol (Paris) 1999; 47: 623-633.

70. van der Heijden-Spek, J.J., Staessen, J.A., Fagard, R.H., Hoeks, A.P., Boudier, H.A., and van Bortel, L.M. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. Hypertension 2000; 35: 637-642.

71. Kelly, R., Hayward, C., Avolio, A., and O'Rourke, M. Noninvasive determination of age-related changes in the human arterial pulse. Circulation 1989; 80: 1652-1659.

72. Greenland, P., Knoll, M.D., Stamler, J., Neaton, J.D., Dyer, A.R., Garside, D.B., and Wilson, P.W. Major risk factors as antecedents of fatal and non-fatal coronary heart disease events. JAMA : the journal of the American Medical Association 2003; 290: 891-897.

73. Wilson, P.W.F., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., and Kannel, W.B. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97: 1837-1847.

74. Raitakari, O.T., Juonala, M., Kähönen, M., Taittonen, L., Laitinen, T., Mäki-Torkko, N., Järvisalo, M.J., Uhari, M., Jokinen, E., and Rönnemaa, T. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA : the journal of the American Medical Association 2003; 290: 2277-2283.

75. Lawes, C.M., Vander Hoorn, S., Rodgers, A., and International Society of, H. Global burden of blood-pressure-related disease, 2001. Lancet 2008; 371: 1513-1518.

76. Beaglehole, R., Epping-Jordan, J., Patel, V., Chopra, M., Ebrahim, S., Kidd, M., and Haines, A. Improving the prevention and management of chronic disease in low-income and middle-income countries: a priority for primary health care. The Lancet 2008; 372: 940-949.

77. Lim, S.S., Gaziano, T.A., Gakidou, E., Reddy, K.S., Farzadfar, F., Lozano, R., and Rodgers, A. Prevention of cardiovascular disease in high-risk individuals in low-income and middle-income countries: health effects and costs. The Lancet 2007; 370: 2054-2062.

78. Levy, R.I. Causes of the decrease in cardiovascular mortality. The American journal of cardiology 1984; 54: 7C-13C.

79. Levy, R.I. and Moskowitz, J. Cardiovascular research: decades of progress, a decade of promise. Science 1982; 217: 121-129.

80. Uemura, K. and Pisa, Z. Trends in cardiovascular disease mortality in industrialized countries since 1950. World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales 1987; 41: 155-178.

81. Millar, J.A., Lever, A.F., and Burke, V. Pulse pressure as a risk factor for cardiovascular events in the MRC Mild Hypertension Trial. Journal of hypertension 1999; 17: 1065-1072.

82. Sagie, A., Larson, M.G., and Levy, D. The natural history of borderline isolated systolic hypertension. The New England journal of medicine 1993; 329: 1912-1917.

83. Vasan, R.S., Larson, M.G., Leip, E.P., Evans, J.C., O'Donnell, C.J., Kannel, W.B., and Levy, D. Impact of high-normal blood pressure on the risk of cardiovascular disease. The New England journal of medicine 2001; 345: 1291-1297.

84. Liszka, H.A., Mainous, A.G., 3rd, King, D.E., Everett, C.J., and Egan, B.M. Prehypertension and cardiovascular morbidity. Ann Fam Med 2005; 3: 294-299.

85. Lackland, D.T. Hypertension: Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure guidelines. Curr Opin Neurol 2013; 26: 8-12.

86. Hertz, R.P., Unger, A.N., Cornell, J.A., and Saunders, E. Racial disparities in hypertension prevalence, awareness, and management. Archives of internal medicine 2005; 165: 2098-2104.

87. Hertz, R.P., McDonald, M., Unger, A.N., and Lustik, M.B. Racial and ethnic disparities in the prevalence and management of cardiovascular risk factors in the United States workforce. J Occup Environ Med 2007; 49: 1165-1175.

88. Cutler, J.A., Sorlie, P.D., Wolz, M., Thom, T., Fields, L.E., and Roccella, E.J. Trends in hypertension prevalence, awareness, treatment, and control rates in United States adults between 1988-1994 and 1999-2004. Hypertension 2008; 52: 818-827.

89. Dennison-Himmelfarb, C., Handler, J., Lackland, D.T., LeFevre, M.L., MacKenzie, T.D., Ogedegbe, O., Smith Jr, S.C., Svetkey, L.P., Taler, S.J., and Townsend, R.R. 2014 Evidence-Based Guideline for the Management of High Blood Pressure in Adults Report From the Panel Members Appointed to the Eighth Joint National Committee (JNC 8). 2013.

90. Eckel, R.H., Jakicic, J.M., Ard, J.D., Miller, N.H., Hubbard, V.S., Nonas, C.A., de Jesus, J.M., Sacks, F.M., Lee, I.M., Smith, S.C., Jr., et al. 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk: A Report of the American College of Cardiology/American Heart

Association Task Force on Practice Guidelines. Journal of the American College of Cardiology 2013.

91. Rosano, G.M.C., Vitale, C., Marazzi, G., and Volterrani, M. Menopause and cardiovascular disease: the evidence. Climacteric 2007; 10: 19-24.

92. Schulman, I.H., Aranda, P., Raij, L., Veronesi, M., Aranda, F.J., and Martin, R. Surgical menopause increases salt sensitivity of blood pressure. Hypertension 2006; 47: 1168-1174.

93. Eaton, S.B. and Eaton, S.B., 3rd. Paleolithic vs. modern diets--selected pathophysiological implications. European journal of nutrition 2000; 39: 67-70.

94. Prior, I.A.M., Evans, J.G., Harvey, H.P.B., Davidson, F., and Lindsey, M. Sodium intake and blood pressure in two Polynesian populations. New England journal of medicine 1968; 279: 515-520.

95. Whelton, P.K., Appel, L.J., Espeland, M.A., Applegate, W.B., Ettinger, W.H., Jr., Kostis, J.B., Kumanyika, S., Lacy, C.R., Johnson, K.C., Folmar, S., et al. Sodium reduction and weight loss in the treatment of hypertension in older persons: a randomized controlled trial of nonpharmacologic interventions in the elderly (TONE). TONE Collaborative Research Group. JAMA : the journal of the American Medical Association 1998; 279: 839-846.

96. Pimenta, E., Gaddam, K.K., Oparil, S., Aban, I., Husain, S., Dell'Italia, L.J., and Calhoun, D.A. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension results from a randomized trial. Hypertension 2009; 54: 475-481.

97. Wang, Y.C., McPherson, K., Marsh, T., Gortmaker, S.L., and Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet 2011; 378: 815-825.

98. Malik, V.S., Willett, W.C., and Hu, F.B. Global obesity: trends, risk factors and policy implications. Nat Rev Endocrinol 2013; 9: 13-27.

99. Kannel, W.B., Brand, N., Skinner, J.J., Jr., Dawber, T.R., and McNamara, P.M. The relation of adiposity to blood pressure and development of hypertension. The Framingham study. Annals of internal medicine 1967; 67: 48-59.

100. Kannel, W.B., LeBauer, E.J., Dawber, T.R., and McNamara, P.M. Relation of body weight to development of coronary heart disease. The Framingham study. Circulation 1967; 35: 734-744.

101. Fields, L.E., Burt, V.L., Cutler, J.A., Hughes, J., Roccella, E.J., and Sorlie, P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. Hypertension 2004; 44: 398-404.

102. Shihab, H.M., Meoni, L.A., Chu, A.Y., Wang, N.Y., Ford, D.E., Liang, K.Y., Gallo, J.J., and Klag, M.J. Body mass index and risk of incident hypertension over the life course: the Johns Hopkins Precursors Study. Circulation 2012; 126: 2983-2989.

103. Sutton-Tyrrell, K., Newman, A., Simonsick, E.M., Havlik, R., Pahor, M., Lakatta, E., Spurgeon, H., and Vaitkevicius, P. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension 2001; 38: 429-433.

104. Hubert, H.B., Feinleib, M., McNamara, P.M., and Castelli, W.P. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up

of participants in the Framingham Heart Study. Circulation 1983; 67: 968-977.

105. Mokdad, A.H., Ford, E.S., Bowman, B.A., Dietz, W.H., Vinicor, F., Bales, V.S., and Marks, J.S. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA : the journal of the American Medical Association 2003; 289: 76-79.

106. Huang, E.S., Basu, A., O'Grady, M., and Capretta, J.C. Projecting the future diabetes population size and related costs for the U.S. Diabetes Care 2009; 32: 2225-2229.

107. Shaw, J.E., Sicree, R.A., and Zimmet, P.Z. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87: 4-14.

108. Nathan, D.M. Long-term complications of diabetes mellitus. The New England journal of medicine 1993; 328: 1676-1685.

109. Haffner, S.M., Lehto, S., Ronnemaa, T., Pyorala, K., and Laakso, M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. The New England journal of medicine 1998; 339: 229-234.

110. Stamler, J., Vaccaro, O., Neaton, J.D., and Wentworth, D. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; 16: 434-444.

111. Kannel, W.B. and McGee, D.L. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care 1979; 2: 120-126.

112. Kannel, W.B. and McGee, D.L. Diabetes and cardiovascular risk factors: the Framingham study. Circulation 1979; 59: 8-13.

113. Lenfant, C., Chobanian, A.V., Jones, D.W., Roccella, E.J., Joint National Committee on the Prevention, D.E., and Treatment of High Blood, P. Seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): resetting the hypertension sails. Hypertension 2003; 41: 1178-1179.

114. Reeves, R.A. Does this patient have hypertension?: How to Measure Blood Pressure. JAMA : the journal of the American Medical Association 1995; 273: 1211-1218.

115. Pickering, T.G., Hall, J.E., Appel, L.J., Falkner, B.E., Graves, J., Hill, M.N., Jones, D.W., Kurtz, T., Sheps, S.G., and Roccella, E.J. Recommendations for blood pressure measurement in humans and experimental animals part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005; 111: 697-716.

116. Netea, R.T., Lenders, J.W.M., Smits, P., and Thien, T. Both body and arm position significantly influence blood pressure measurement. J Hum Hypertens 2003; 17: 459-462.

117. Aylett, M., Marples, G., Jones, K., and Rhodes, D. Evaluation of normal and large sphygmomanometer cuffs using the Omron 705CP. J Hum Hypertens 2001; 15.

118. Bakx, C., Oerlemans, G., Van den Hoogen, H., Van Weel, C., and Thien, T. The influence of cuff size on blood pressure measurement. J Hum Hypertens 1997; 11.

119. Jansen, R.W.M.M. and Lipsitz, L.A. Postprandial hypotension: epidemiology, pathophysiology, and clinical management. Annals of internal medicine 1995; 122: 286-295.

120. Aronow, W.S. and Ahn, C. Association of postprandial hypotension with incidence of falls, syncope, coronary events, stroke, and total mortality at 29-month follow-up in 499 older nursing home residents. J Am Geriatr Soc 1997; 45: 1051-1053.

121. Kohara, K., Jiang, Y., Igase, M., Takata, Y., Fukuoka, T., Okura, T., Kitami, Y., and Hiwada, K. Postprandial hypotension is associated with asymptomatic cerebrovascular damage in essential hypertensive patients. Hypertension 1999; 33: 565-568.

122. Kaplan, N.M., *Kaplan's clinical hypertension*. 2010: Lippincott Williams & Wilkins.

123. Palatini, P., Palomba, D., Bertolo, O., Minghetti, R., Longo, D., Sarlo, M., and Pessina, A.C. The white-coat effect is unrelated to the difference between clinic and daytime blood pressure and is associated with greater reactivity to public speaking. Journal of hypertension 2003; 21: 545-553.

124. Owens, P., Atkins, N., and O'Brien, E. Diagnosis of white coat hypertension by ambulatory blood pressure monitoring. Hypertension 1999; 34: 267-272.

125. Devereux, R.B., Pickering, T.G., Harshfield, G.A., Kleinert, H.D., Denby, L., Clark, L., Pregibon, D., Jason, M., Kleiner, B., Borer, J.S., et al. Left ventricular hypertrophy in patients with hypertension: importance of blood pressure response to regularly recurring stress. Circulation 1983; 68: 470-476.

126. Ohkubo, T., Kikuya, M., Metoki, H., Asayama, K., Obara, T., Hashimoto, J., Totsune, K., Hoshi, H., Satoh, H., and Imai, Y. Prognosis of "masked" hypertension and "white-coat" hypertension detected by 24-h ambulatory blood pressure monitoring 10-year follow-up from the Ohasama study. Journal of the American College of Cardiology 2005; 46: 508-515.

127. Redon, J. and Lurbe, E. Nocturnal blood pressure versus nondipping pattern what do they mean? Hypertension 2008; 51: 41-42.

128. Li, Y., Staessen, J.A., Lu, L., Li, L.H., Wang, G.L., and Wang, J.G. Is isolated nocturnal hypertension a novel clinical entity? Findings from a Chinese population study. Hypertension 2007; 50: 333-339.

129. O'Brien, E., Asmar, R., Beilin, L., Imai, Y., Mallion, J.-M., Mancia, G., Mengden, T., Myers, M., Padfield, P., and Palatini, P. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. Journal of hypertension 2003; 21: 821-848.

130. Henskens, L.H., Kroon, A.A., van Oostenbrugge, R.J., Haest, R.J., Lodder, J., and de Leeuw, P.W. Different classifications of nocturnal blood pressure dipping affect the prevalence of dippers and nondippers and the relation with target-organ damage. Journal of hypertension 2008; 26: 691-698.

131. Li, L.H., Li, Y., Huang, Q.F., Sheng, C.S., Staessen, J.A., and Wang, J.G. Isolated nocturnal hypertension and arterial stiffness in a Chinese population. Blood Press Monit 2008; 13: 157-159.

132. Clement, D.L., De Buyzere, M.L., De Bacquer, D.A., de Leeuw, P.W., Duprez, D.A., Fagard, R.H., Gheeraert, P.J., Missault, L.H., Braun, J.J., Six, R.O., et al. Prognostic value of ambulatory blood-pressure recordings

in patients with treated hypertension. The New England journal of medicine 2003; 348: 2407-2415.

133. Laurent, S. Arterial wall hypertrophy and stiffness in essential hypertensive patients. Hypertension 1995; 26: 355-362.

134. Boutouyrie, P., Bussy, C., Lacolley, P., Girerd, X., Laloux, B., and Laurent, S. Association between local pulse pressure, mean blood pressure, and large-artery remodeling. Circulation 1999; 100: 1387-1393.

135. Franklin, S.S. Beyond blood pressure: arterial stiffness as a new biomarker of cardiovascular disease. Journal of the American Society of Hypertension 2008; 2: 140-151.

136. Roman, M.J., Okin, P.M., Kizer, J.R., Lee, E.T., Howard, B.V., and Devereux, R.B. Relations of central and brachial blood pressure to left ventricular hypertrophy and geometry: the Strong Heart Study. Journal of hypertension 2010; 28: 384-388.

137. Pini, R., Cavallini, M.C., Palmieri, V., Marchionni, N., Di Bari, M., Devereux, R.B., Masotti, G., and Roman, M.J. Central But Not Brachial Blood Pressure Predicts Cardiovascular Events in an Unselected Geriatric PopulationThe ICARe Dicomano Study. Journal of the American College of Cardiology 2008; 51: 2432-2439.

138. Van Merode, T., Hick, P.J., Hoeks, A.P., Rahn, K.H., and Reneman, R.S. Carotid artery wall properties in normotensive and borderline hypertensive subjects of various ages. Ultrasound Med Biol 1988; 14: 563-569.

139. Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I., and Struijker-Boudier, H. Expert consensus document on arterial stiffness:

methodological issues and clinical applications. European heart journal 2006; 27: 2588-2605.

140. Nitta, K., Akiba, T., Uchida, K., Otsubo, S., Otsubo, Y., Takei, T., Ogawa, T., Yumura, W., Kabaya, T., and Nihei, H. Left ventricular hypertrophy is associated with arterial stiffness and vascular calcification in hemodialysis patients. Hypertension research: official journal of the Japanese Society of Hypertension 2004; 27: 47-52.

141. Safar, M.E. Pulse pressure, arterial stiffness and wave reflections (augmentation index) as cardiovascular risk factors in hypertension. Therapeutic advances in cardiovascular disease 2008; 2: 13-24.

142. London, G., Guerin, A., Pannier, B., Marchais, S., Benetos, A., and Safar, M. Increased systolic pressure in chronic uremia. Role of arterial wave reflections. Hypertension 1992; 20: 10-19.

143. Vlachopoulos, C., Aznaouridis, K., and Stefanadis, C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. Journal of the American College of Cardiology 2010; 55: 1318-1327.

144. Weisbrod, R.M., Shiang, T., Al Sayah, L., Fry, J.L., Bajpai, S., Reinhart-King, C.A., Lob, H.E., Santhanam, L., Mitchell, G., Cohen, R.A., et al. Arterial stiffening precedes systolic hypertension in diet-induced obesity. Hypertension 2013; 62: 1105-1110.

145. Franklin, S.S. Ageing and hypertension: the assessment of blood pressure indices in predicting coronary heart disease. Journal of hypertension. Supplement: official journal of the International Society of Hypertension 1999; 17: S29.

146. Darne, B., Girerd, X., Safar, M., Cambien, F., and Guize, L. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. Hypertension 1989; 13: 392-400.

147. Scuteri, A., Cacciafesta, A.M., Di Bernardo, M.G., De Propris, A.M., Recchi, D., Celli, V., Idone, G., Nocco, M.L., and Marigliano, V. Sex differences in correlates of steady state and pulsatile component of blood pressure. Clin Sci (Lond) 1996; 91: 385-389.

148. Scuteri, A., Cacciafesta, M., Di Bernardo, M.G., De Propris, A.M., Recchi, D., Celli, V., Cicconetti, P., and Marigliano, V. Pulsatile versus steady-state component of blood pressure in elderly females: an independent risk factor for cardiovascular disease? Journal of hypertension 1995; 13: 185-191.

149. Franklin, S.S., Larson, M.G., Khan, S.A., Wong, N.D., Leip, E.P., Kannel, W.B., and Levy, D. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. Circulation 2001; 103: 1245-1249.

150. McVeigh, G.E., Bratteli, C.W., Morgan, D.J., Alinder, C.M., Glasser, S.P., Finkelstein, S.M., and Cohn, J.N. Age-related abnormalities in arterial compliance identified by pressure pulse contour analysis: aging and arterial compliance. Hypertension 1999; 33: 1392-1398.

151. Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P., and Benetos, A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001; 37: 1236-1241.

152. Blacher, J., Safar, M.E., Guerin, A.P., Pannier, B., Marchais, S.J., and London, G.M. Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int 2003; 63: 1852-1860.

153. Blacher, J., Asmar, R., Djane, S., London, G.M., and Safar, M.E. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. Hypertension 1999; 33: 1111-1117.

154. Safar, M.E., Henry, O., and Meaume, S. Aortic pulse wave velocity: an independent marker of cardiovascular risk. Am J Geriatr Cardiol 2002; 11: 295-298.

155. Mattace-Raso, F.U., van der Cammen, T.J., Hofman, A., van Popele, N.M., Bos, M.L., Schalekamp, M.A., Asmar, R., Reneman, R.S., Hoeks, A.P., Breteler, M.M., et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006; 113: 657-663.

156. Mackenzie, I.S., Wilkinson, I.B., and Cockcroft, J.R. Assessment of arterial stiffness in clinical practice. QJM 2002; 95: 67-74.

157. Franklin, S.S., Sutton-Tyrrell, K., Belle, S.H., Weber, M.A., and Kuller, L.H. The importance of pulsatile components of hypertension in predicting carotid stenosis in older adults. Journal of hypertension 1997; 15: 1143-1150.

158. Van Bortel, L.M., Laurent, S., Boutouyrie, P., Chowienczyk, P., Cruickshank, J.K., De Backer, T., Filipovsky, J., Huybrechts, S., Mattace-Raso, F.U.S., and Protogerou, A.D. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. Journal of hypertension 2012; 30: 445-448.

159. Li, Y., Wang, J.-G., Dolan, E., Gao, P.-J., Guo, H.-F., Nawrot, T., Stanton, A.V., Zhu, D.-L., O'Brien, E., and Staessen, J.A. Ambulatory arterial stiffness index derived from 24-hour ambulatory blood pressure monitoring. Hypertension 2006; 47: 359-364.

160. Dolan, E., Thijs, L., Li, Y., Atkins, N., McCormack, P., McClory, S., O'Brien, E., Staessen, J.A., and Stanton, A.V. Ambulatory arterial stiffness index as a predictor of cardiovascular mortality in the Dublin Outcome Study. Hypertension 2006; 47: 365-370.

161. Leoncini, G., Ratto, E., Viazzi, F., Vaccaro, V., Parodi, A., Falqui, V., Conti, N., Tomolillo, C., Deferrari, G., and Pontremoli, R. Increased ambulatory arterial stiffness index is associated with target organ damage in primary hypertension. Hypertension 2006; 48: 397-403.

162. Briones, A.M., González, J.M., Somoza, B., Giraldo, J., Daly, C.J., Vila, E., Carmen González, M., McGrath, J.C., and Arribas, S.M. Role of elastin in spontaneously hypertensive rat small mesenteric artery remodelling. The Journal of Physiology 2003; 552: 185-195.

163. Martyn, C.N. and Greenwald, S.E. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. The Lancet 1997; 350: 953-955.

164. Farrar, D.J., Green, H.D., Bond, M.G., Wagner, W.D., and Gobbeé, R.A. Aortic pulse wave velocity, elasticity, and composition in a nonhuman primate model of atherosclerosis. Circulation research 1978; 43: 52-62.

165. Bank, A.J., Wilson, R.F., Kubo, S.H., Holte, J.E., Dresing, T.J., and Wang, H. Direct effects of smooth muscle relaxation and contraction on in vivo

human brachial artery elastic properties. Circulation research 1995; 77: 1008-1016.

166. O'Rourke, M. Arterial stiffness, systolic blood pressure, and logical treatment of arterial hypertension. Hypertension 1990; 15: 339-347.

167. Boutouyrie, P., Lacolley, P., Girerd, X., Beck, L., Safar, M., and Laurent, S. Sympathetic activation decreases medium-sized arterial compliance in humans. The American Journal of Physiology 1994; 267: H1368-1376.

168. La Rovere, M.T., Pinna, G.D., and Raczak, G. Baroreflex sensitivity: measurement and clinical implications. Ann Noninvasive Electrocardiol 2008; 13: 191-207.

169. Ebert, T.J., Morgan, B.J., Barney, J.A., Denahan, T., and Smith, J.J. Effects of aging on baroreflex regulation of sympathetic activity in humans. The American Journal of Physiology 1992; 263: H798-803.

170. Luscher, T.F. and Barton, M. Biology of the endothelium. Clin Cardiol 1997; 20: II-3-10.

171. DeSouza, C.A., Shapiro, L.F., Clevenger, C.M., Dinenno, F.A., Monahan, K.D., Tanaka, H., and Seals, D.R. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. Circulation 2000; 102: 1351-1357.

172. Taddei, S., Virdis, A., Mattei, P., Ghiadoni, L., Fasolo, C.B., Sudano, I., and Salvetti, A. Hypertension causes premature aging of endothelial function in humans. Hypertension 1997; 29: 736-743.

173. Taddei, S. and Salvetti, A. Endothelial dysfunction in hypertension. Adv Exp Med Biol 1997; 432: 235-246.

174. Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., and Münzel, T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001; 104: 2673-2678.

175. Gokce, N., Keaney, J.F., Hunter, L.M., Watkins, M.T., Menzoian, J.O., and Vita, J.A. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function A prospective study. Circulation 2002; 105: 1567-1572.

176. Kooijman, M., Thijssen, D.H.J., De Groot, P.C.E., Bleeker, M.W.P., Van Kuppevelt, H.J.M., Green, D.J., Rongen, G.A., Smits, P., and Hopman, M.T.E. Flow-mediated dilatation in the superficial femoral artery is nitric oxide mediated in humans. The Journal of Physiology 2008; 586: 1137-1145.

177. Green, D. Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. Journal of Applied Physiology 2005; 99: 1233-1234; discussion 1237-1238.

178. Wray, D.W., Witman, M.A., Ives, S.J., McDaniel, J., Trinity, J.D., Conklin, J.D., Supiano, M.A., and Richardson, R.S. Does brachial artery flow-mediated vasodilation provide a bioassay for NO? Hypertension 2013; 62: 345-351.

179. Green, D.J., Dawson, E.A., Groenewoud, H.M., Jones, H., and Thijssen, D.H. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. Hypertension 2014; 63: 376-382.

180. Eskurza, I., Monahan, K.D., Robinson, J.A., and Seals, D.R. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. The Journal of Physiology 2004; 556: 315-324.

181. Eskurza, I., Myerburgh, L.A., Kahn, Z.D., and Seals, D.R. Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. The Journal of Physiology 2005; 568: 1057-1065.

182. Moser, M., Guyther, J.R., Finnerty, F., Richardson, D.W., and Langford, H. Report of the joint national committee on detection, evaluation, and treatment of high blood pressure: A cooperative study. JAMA : the journal of the American Medical Association 1977; 237: 255-261.

183. Hill, M.N., Levine, D.M., and Whelton, P.K. Awareness, use, and impact of the 1984 Joint National Committee consensus report on high blood pressure. American journal of public health 1988; 78: 1190-1194.

184. Nickey, A. The 1988 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Archives of internal medicine 1988; 148: 1023-1038.

185. Chobanian, A.V., Alderman, M.H., DeQuattro, V., Frohlich, E.D., Gifford Jr, R.W., Hill, M.N., Kaplan, N.M., Langford, H.G., Moore, M.A., and Nickey, W.A. The 1988 report of the Joint National Committee on detection, evaluation, and treatment of high blood pressure. Archives of internal medicine 1988; 148: 1023.

186. Joint National, C. The sixth report of the Joint National Committee on Detection, Eveluation and Treatment of High Blood Pressure (JNCIV). Archives of internal medicine 1997; 157: 2413-2446.

187. Gradman, A.H., Parise, H., Lefebvre, P., Falvey, H., Lafeuille, M.H., and Duh, M.S. Initial combination therapy reduces the risk of cardiovascular events in hypertensive patients: a matched cohort study. Hypertension 2013; 61: 309-318.

188. Collins, R., Peto, R., Godwin, J., and MacMahon, S. Blood pressure and coronary heart disease. Lancet 1990; 336: 370-371.

189. Collins, R., Peto, R., MacMahon, S., Hebert, P., Fiebach, N.H., Eberlein, K.A., Godwin, J., Qizilbash, N., Taylor, J.O., and Hennekens, C.H. Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. Lancet 1990; 335: 827-838.

190. Hebert, P.R., Moser, M., Mayer, J., Glynn, R.J., and Hennekens, C.H. Recent evidence on drug therapy of mild to moderate hypertension and decreased risk of coronary heart disease. Archives of internal medicine 1993; 153: 578-581.

191. Salerno, C.M., Demopoulos, L., Mukherjee, R., and Gradman, A.H. Combination angiotensin receptor blocker/hydrochlorothiazide as initial therapy in the treatment of patients with severe hypertension. Journal of clinical hypertension 2004; 6: 614-620.

192. Egan, B.M., Fleissner, M.J., Stepniakowski, K., Neahring, J.M., Sagar, K.B., and Ebert, T.J. Improved baroreflex sensitivity in elderly hypertensives on lisinopril is not explained by blood pressure reduction alone. Journal of hypertension 1993; 11: 1113-1120.

193. Ebert, T.J. Captopril potentiates chronotropic baroreflex responses to carotid stimuli in humans. Hypertension 1985; 7: 602-606.

194. Law, M.R., Morris, J.K., and Wald, N.J. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. BMJ 2009; 338: b1665.

195. Group, T.T.o.M.H.R. The treatment of mild hypertension study. A randomized, placebo-controlled trial of a nutritional-hygienic regimen along with various drug monotherapies. . Archives of internal medicine 1991; 151: 1413-1423.

196. Huggins, C.E., Margerison, C., Worsley, A., and Nowson, C.A. Influence of dietary modifications on the blood pressure response to antihypertensive medication. The British Journal of Nutrition 2011; 105: 248-255.

197. Barr, S.I., McCarron, D.A., Heaney, R.P., Dawson-Hughes, B., Berga, S.L., Stern, J.S., and Oparil, S. Effects of increased consumption of fluid milk on energy and nutrient intake, body weight, and cardiovascular risk factors in healthy older adults. J Am Diet Assoc 2000; 100: 810-817.

198. Wennersberg, M.H., Smedman, A., Turpeinen, A.M., Retterstol, K., Tengblad, S., Lipre, E., Aro, A., Mutanen, P., Seljeflot, I., Basu, S., et al. Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. The American Journal of Clinical Nutrition 2009; 90: 960-968.

199. Moore, L.L., Visioni, A.J., Qureshi, M.M., Bradlee, M.L., Ellison, R.C., and D'Agostino, R. Weight loss in overweight adults and the long-term risk of hypertension: the Framingham study. Archives of internal medicine 2005; 165: 1298-1303.

200. Choi, H.K., Willett, W.C., Stampfer, M.J., Rimm, E., and Hu, F.B. Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. Arch Intern Med 2005; 165: 997-1003.

201. van Dam, R.M., Rimm, E.B., Willett, W.C., Stampfer, M.J., and Hu, F.B. Dietary patterns and risk for type 2 diabetes mellitus in U.S. men. Ann Intern Med 2002; 136: 201-209.

202. Liu, S., Choi, H.K., Ford, E., Song, Y., Klevak, A., Buring, J.E., and Manson, J.E. A prospective study of dairy intake and the risk of type 2 diabetes in women. Diabetes Care 2006; 29: 1579-1584.

203. Nilsson, M., Stenberg, M., Frid, A.H., Holst, J.J., and Bjorck, I.M. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. Am J Clin Nutr 2004; 80: 1246-1253.

204. von Post-Skagegard, M., Vessby, B., and Karlstrom, B. Glucose and insulin responses in healthy women after intake of composite meals containing cod-, milk-, and soy protein. Eur J Clin Nutr 2006; 60: 949-954.

205. Ostman, E.M., Liljeberg Elmstahl, H.G., and Bjorck, I.M. Inconsistency between glycemic and insulinemic responses to regular and fermented milk products. Am J Clin Nutr 2001; 74: 96-100.

206. Scherrer, U., Randin, D., Vollenweider, P., Vollenweider, L., and Nicod, P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 1994; 94: 2511-2515.

207. Steinberg, H.O., Brechtel, G., Johnson, A., Fineberg, N., and Baron, A.D. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 1994; 94: 1172-1179.