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Dietary protein, dairy, yogurt, and risk of high blood pressure and subsequent cardiovascular disease in middle-aged adults

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Dissertation

**DIETARY PROTEIN, DAIRY, YOGURT, AND RISK OF HIGH BLOOD
PRESSURE AND SUBSEQUENT CARDIOVASCULAR DISEASE IN
MIDDLE-AGED ADULTS**

by

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Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

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DEDICATION

To my parents, Rene and Elizabeth Buendia, words cannot describe how crucial your support and unconditional love has been throughout my life. You have supported my educational career and constantly believed and pushed me to strive for the best. Both of you have sacrificed so much to see me achieve my dreams and for that, I can never repay you. So I dedicate my work to you.

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ABSTRACT

High blood pressure (HBP) is a primary risk factor for cardiovascular disease (CVD). Identification of modifiable dietary risk factors for HBP is crucial. The objective of this dissertation is to estimate the effects of long-term protein, dairy, and yogurt intakes on risk of HBP and subsequent CVD risk among those with HBP.

Data from four prospective studies of middle-aged adults were used: the Framingham Offspring Study (FOS) (n=1,361), Nurses' Health Study (NHS) (n=66,987), NHS II (n=84,368), and Health Professionals Follow-Up Study (HPFS) (n=30,512). Diet was assessed via 3-day diet records in FOS and semi-quantitative food frequency questionnaires in NHS, NHS II, and HPFS. HBP was assessed by mercury sphygmomanometer or anti-hypertensive medication use in FOS and

self-report in NHS, NHS II, and HPFS. Self-reported incident CVD was validated by physician review in the NHS and HPFS. Cox proportional hazard models were used to calculate hazard ratios, 95% confidence intervals (CI) associated with cumulative average dairy intakes while controlling for potential confounding by age, sex, height, activity, smoking, energy, diet quality scores, fruits and vegetables (FV), fiber, and fat intake.

Higher total protein intake led to a 40% (95% CI: 0.45-0.78) HBP risk reduction in FOS. When combined with higher FV or fiber consumption, higher protein intake resulted in a HBP risk reduction of 39% and 51%, respectively. Higher total dairy (3-<6 s/day) led to 13% (95% CI: 0.83-0.92; *p*-trend<0.0001), 26% (95% CI: 0.69-0.78; *p*-trend<0.0001), and 9% (95% CI: 0.84-0.98; *p*-trend<0.0001) reduced HBP risks across NHS, NHS II, and HPFS, respectively. In pooled analyses across these cohorts, higher total dairy (3-<6 s/day) or yogurt (≥ 5 s/week) intakes in combination with higher diet quality scores resulted in 23% (95% CI: 0.73-0.80) and 31% (95% CI: 0.65-0.74) HBP risk reductions, respectively. Among hypertensives, regular yogurt consumers (≥ 2 s/week) had statistically significant 17% and 18% lower risks of CVD in NHS and HPFS, respectively; HPFS men consuming 2-<6 s/d of total dairy also had an 11% lower CVD risk.

These results suggest that higher usual intakes of total protein, dairy and yogurt have beneficial effects on HBP risk and subsequent CVD in middle-aged adults.

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LIST OF ABBREVIATIONS

ACE.....	Angiotensin-Converting Enzyme
AHA.....	American Heart Association
ANCOVA.....	Analysis of Covariance
ARIC.....	Atherosclerosis Risk in Communities
BCAA.....	Branched-Chain Amino Acids
BMI.....	Body Mass Index
BP.....	Blood Pressure
C.....	Cup
CABG.....	Coronary Artery Bypass Grafting
CAD.....	Coronary Artery Disease
CARDIA.....	Coronary Artery Risk Development in Young Adults
CCK.....	Cholecystokinin
CHD.....	Coronary Heart Disease
CHF.....	Congestive Heart Failure
CI.....	Confidence Interval
CVD.....	Cardiovascular Disease
D.....	Day
DASH.....	Dietary Approaches to Stop Hypertension

DBP.....	Diastolic Blood Pressure
DGA.....	Dietary Guidelines for Americans
DGAC.....	Dietary Guidelines Advisory Committee
DHHS.....	Department of Health and Human Services
EQ.....	Equivalents
FFQ.....	Food Frequency Questionnaire
FHS.....	Framingham Heart Study
FOS.....	Framingham Offspring Study
FV.....	Fruits and Vegetables
G.....	Grams
GLP-1.....	Glucagon-Like Peptide-1
GLUT-4.....	Glucose Transporter Type 4
HBP.....	High Blood Pressure
HDL-C.....	High Density Lipoprotein-Cholesterol
HEI.....	Healthy Eating Index
HPFS.....	Health Professionals Follow-Up Study
HR.....	Hazard Ratio
HTN.....	Hypertension
IPP.....	Isoleucine-Proline-Proline

IRB.....	Institutional Review Board
JNC.....	Joint National Committee
KCALs.....	Kilocalories
KG.....	Kilograms
LDL-C.....	Low Density Lipoprotein-Cholesterol
M.....	Meters
MET.....	Metabolic Equivalent of Task
MG.....	Milligrams
MI.....	Myocardial Infarction
MM HG.....	Millimeters of Mercury
NC.....	North Carolina
NDS.....	Nutrition Data System
NHANES.....	National Health and Nutrition Examination Survey
NHS.....	Nurses' Health Study
NHS II.....	Nurses' Health Study II
NO.....	Nitric Oxide
OZ.....	Ounce
OZ EQ.....	Ounce Equivalents
PREDIMED.....	Prevention with Mediterranean Diet

P-YRS.....	Person-Years
RAS.....	Renin-Angiotensin System
REF.....	Referent Group
S.....	Serving
SAS.....	Statistical Analysis System
SBP.....	Systolic Blood Pressure
SD.....	Standard Deviation
SE.....	Standard Error
SES.....	Socioeconomic Status
SFA.....	Saturated Fatty Acids
SHR.....	Spontaneously Hypertensive Rats
SUN.....	Seguimiento Universidad de Navarra
T.....	Tertile
T2DM.....	Type 2 Diabetes Mellitus
TBS.....	Tablespoon
TOHP.....	Trials of Hypertension Prevention
USDA.....	United States Department of Agriculture
VPP.....	Valine-Proline-Proline
VSMC.....	Vascular Smooth Muscle Cells

WES.....Western Electric Study

WK.....Week

CHAPTER 1: INTRODUCTION

1.1 HIGH BLOOD PRESSURE PREVALENCE AND IMPLICATIONS

High blood pressure (HBP) is a major risk factor for cardiovascular and renal diseases and continues to be a growing public health concern worldwide. Data from the National Health and Nutrition Examination Survey (NHANES) 2005-2008 showed that the overall prevalence of HBP among American adults 18 years of age and older was 30.9%.¹ The 2015 Heart Disease and Stroke Statistics from the American Heart Association (AHA) estimates that about a third of Americans (80 million) have HBP, with 77% of them on antihypertensive medication but only 54% of those on antihypertensive medication having their HBP controlled.² HBP was estimated to contribute to nearly half of all cardiovascular disease-associated deaths in the United States (US) in 2007-08.³ The prevalence of HBP is concerning because of its direct, independent relationship with chronic disease risk. In 2007, HBP was cited as the primary or contributing cause of death for over 330,000 Americans³ and is responsible for more than 7 million annual deaths worldwide.¹ Moreover, HBP-related cost of healthcare and loss of productivity in 2010 was estimated to be \$76.6 billion in the US.¹

The 2014 AHA update estimated that 41% of adult Americans will have a diagnosis of HBP by the year 2030.⁴ The 2010 AHA update reported that HBP prevalence is similar among men and women over 20 years old (34.4% and 32.6%, respectively) with an additional 37.4% of American adults have prehypertension.⁵ HBP risk increases with age, being male, smoking, having high cholesterol, diabetes, a sedentary lifestyle, and being African American (43% having HBP).⁵ Being one of the major risk factors for cardiovascular disease (CVD),⁴ HBP prevention is imperative.

According to the AHA, high blood pressure, or hypertension (HTN), is defined as a medical condition with which the systolic blood pressure (SBP), or the force that occurs when blood pumps out of the heart and into the arteries, is greater than or equal to 140 mm Hg; or when the diastolic blood pressure (DBP), or the force that is created as the heart rests between heart beats, is greater than or equal to 90 mm Hg.⁴ Prehypertension is having SBP levels between 120-139 mm Hg and DBP levels between 80-89 mm Hg based on two properly measured seated BP readings and is considered as an indicator for HTN.⁶ Maintaining healthy blood pressure (BP) levels is important in order to allow the oxygen-rich blood to reach aerobic tissues of the body. Over time, the higher the BP (or the

more forcefully the heart pumps out blood), the more the tissues that comprise the arterial walls are stretched beyond its healthy limits and becomes damaged. These damaged blood vessels are more prone to rupture, which can cause hemorrhagic strokes and aneurysms. Vascular scars can catch debris such as cholesterol and plaques, which makes it more difficult for the blood to reach the other parts of the body. This would then lead to an increased workload in the circulatory system, which puts a heavier strain on the heart. Plaque buildup, or atherosclerosis, could ultimately block the blood flow, which causes heart attacks and strokes (two major types of CVD). HBP is the most likely cause of a hemorrhagic stroke (when a blood vessel within the brain bursts).² Other types of CVD include: heart failure, heart valve conditions, and arrhythmia. Heart failure, or congestive heart failure (CHF), occurs when the heart works too hard in order to pump blood to other areas of the body. Arrhythmias refer to the abnormal heart rhythms. This affects how well the heart pumps blood throughout the body. Heart valve problems include: stenosis – when the heart valves do not open sufficiently to allow normal blood flow; regurgitation – when the heart valves do not properly close and blood is allowed to leak through; and mitral valve prolapse - when the valve leaflets bulge or return back into the upper chamber, which causes them not to close properly⁷.

1.2 PREVALENCE AND IMPLICATIONS OF CARDIOVASCULAR DISEASE

CVD is the leading global cause of death with 17.3 million attributable deaths annually.² It is projected to grow to more than 23.6 million by 2030. In the US, 787,000 Americans (1 out of 3) died from heart disease, stroke, or other CVD outcomes in 2011.² Furthermore, total costs attributed to CVD totaled more than \$320 billion.² The correlation between HBP and CVD is striking: 69% of people worldwide who have their first heart attack, 77% who have their first stroke, and 74% who have their first CHF incident are hypertensive.² CVD risk increases as BP levels rise. Results from the Multiple Risk Factor Intervention Trial (MRFIT) of over 360,000 middle aged men across the country, showed that both systolic blood pressure (SBP) and diastolic blood pressure (DBP), taken independently from each other and other major risk factors, were significantly related to CVD risk.⁸ Similar observations were observed for middle-aged Caucasian and African American women in the Charleston Heart Study.⁹ Although these studies were done in the 1980s and 1990s, the trend continues well into the 2000s.¹⁰⁻¹² According to the World Health organization, two-thirds of the cerebrovascular disease burden as well as half of the ischemic heart disease burden are attributable to pre-HBP or HBP levels.¹³ CVD and HBP incidence statistics are

projected to grow in the near future, warranting identification of modifiable risk factors to mitigate this public health burden.

1.3 DIETARY PROTEIN AND BLOOD PRESSURE

BP is affected by many complex factors. Diet plays a key modifiable role thus making it important to identify specific foods and nutrients that can beneficially affect BP and also be easily incorporated into population-based strategies for HBP prevention. Evidence from recent reviews^{14,15} and meta-analyses^{16,17} suggest that dietary protein consumption may benefit BP. These aforementioned meta-analyses of short-term clinical trials concluded that compared with carbohydrates, higher dietary protein intake led to modest reductions in BP. In the Rebholtz meta-analysis, both animal and plant proteins were found to have similar short-term BP-lowering effects.¹⁷ Observational studies, which have been largely cross-sectional, have shown weak beneficial effects of plant proteins in particular on BP.¹⁵ There are few observational studies (and no clinical trials) that have addressed the long-term effects of animal and plant proteins on BP, and results are conflicting.¹⁸⁻²⁰ The Western Electric Study (WES), an 8 year longitudinal study that followed 1,714 middle-aged working men in Chicago, and the Seguimiento *Universidad* de Navarra (SUN), an 11 month prospective

study that followed 5,880 Spanish men and women 20 years of age and older, found only vegetable protein consumption to be inversely correlated with BP.^{18,20} When total protein was assessed among 4,100 young adults 18-30 years of age in the Coronary Artery Risk Development in Young Adults (CARDIA) study, total protein was inversely associated with both SBP and DBP, with stronger associations for DBP.¹⁹ The inconsistencies of the above prospective cohort studies warrant the need for more longitudinal studies examining the independent effects of dietary protein on BP.

Only one trial has attempted to elucidate the independent effect of a high protein diet on BP. In a randomized crossover design with three dietary interventions: high protein, high carbohydrate, and high monounsaturated fats, the investigators of the OmniHeart trial concluded that both the monounsaturated fat diet and the higher protein diet led to lower BP as well as improvement in the overall lipid profile. More importantly, it was the higher-protein diet rather than a lower-carbohydrate diet that had the greatest BP-lowering effect in adults.²¹

1.4 DAIRY AND BLOOD PRESSURE

Various studies suggest that dietary protein may have a beneficial effect on blood pressure although a recent review emphasizes the need for additional studies to delineate the role of specific protein sources (e.g., dairy).¹⁴ Dietary patterns such as the Dietary Approaches to Stop Hypertension (DASH), is one of the five preventive strategies that The Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of HBP recommends.⁶ Weight loss, reduced sodium intake, moderate physical activity, and moderation of alcohol intake being the other four preventive strategies.⁶ The DASH diet is characterized by higher intakes of fruits and vegetables (FV) and low-fat dairy and has been shown to have BP-lowering effects in clinical trial settings.²² In fact, a later study examining the effect of a high FV diet compared to a DASH diet (high FV and low-fat dairy), showed that across all the subgroups in the trial (Caucasians, African Americans, hypertensive, and non-hypertensives), the DASH diet led to a greater BP decrease than just the FV diet alone.²³ This landmark trial led to the possible independent, antihypertensive effect of dairy, a rich source of complete protein.

The 2010 Dietary Guidelines Advisory Committee (DGAC) reviewed the evidence for the association between dairy intake and BP. They concluded that overall, there is a moderate body of evidence pointing to an inverse relationship between the intake of milk and milk products and BP, particularly SBP; the evidence for a reduction in DBP is weaker.²⁴ However, the DGAC summary suggests that the evidence is not completely consistent and that differences in results across studies may result from actual differences in effects of different types of dairy products or from residual confounding associated with other dietary factors, weight loss and demographic factors.²⁴

Dairy intake has been inversely associated with BP and risk of HTN in several prospective studies. Data from the CARDIA study²⁵, the Women's Health Study²⁶, and the Rotterdam study²⁷ indicated that a high intake of total dairy was associated with a lower HTN risk. Although some studies have observed a positive association between dairy and blood pressure, there are a few such as one in elderly women^{28,29}, the Hoorn Study¹⁹, and the general Dutch population³⁰ that show no association between dairy intake and BP.

BP-effects of dairy has also been studied within the context of an eating pattern. Most notably, the DASH eating pattern, which was derived from a multicenter randomized controlled feeding trial that enrolled 459 prehypertensive and stage 1 hypertensive (SBP: 140-159 mm Hg or DBP: 90-99 mm Hg) adults with a mean age of 44 years (youngest being 22 years old) for eight weeks.³¹ The DASH pattern (vs. the control diet with low intakes of fruits, vegetables and dairy) lowered SBP and DBP by 5.5 and 3.0 mm Hg, respectively. The high FV diet reduced BP by approximately half as much as the DASH pattern. Not only was the DASH diet effective in lowering BP in all of the subjects in the study, reductions were greatest among those with established HTN and African Americans.²² There were very limited number of studies that looked at differential effects of diet on African Americans at the time and one of the strengths of the DASH trial was that many subgroups were represented: younger individuals, older subjects, blacks, whites, Asians, Native Americans, prehypertensives, and stage 1 hypertensives. The DASH diet contained greater amounts of potassium, calcium, fiber, and magnesium, which were all hypothesized to have BP-lowering effects.^{31,32} At the same time, the it also contained lower amounts of red and processed meats, sodium, and sugar-sweetened beverages, which were all believed to have BP-raising effects.³²

Although it was only a short-term trial, it has been replicated a number of times including in the PREMIER study.³³ The PREMIER Clinical Trial enrolled 810 individuals with prehypertension or stage 1 HTN. After the 6-month follow up, the intervention group that included DASH had a mean SBP level that was 4.3 mm Hg lower than that in the control group (given advice only) while the intervention arm without DASH was 3.7 mm Hg lower than in the control group.³³ The follow-up at 18 months found that subjects in the group that included DASH had a 23% lower risk of HTN compared with the “advice only” group while those in the established lifestyle intervention had a 17% lower HTN risk.³⁴

In summary, while some observational and clinical trial evidence to date suggests a beneficial effect of dairy in BP control, considerable inconsistency is present across study designs and results. Further long-term epidemiological data are needed to address these issues.

1.5 YOGURT AND BLOOD PRESSURE

Most studies of dairy intake and BP have combined all types of dairy into one exposure. The nutrient profiles of the different dairy subtypes vary. For example,

vitamin D, which in low levels can activate the renin-angiotensin-aldosterone system and lead to increases in BP directly by vasoconstriction³⁵, is only fortified in milk but not in cheese or yogurt products. Cheese products have a higher sodium content than yogurt and milk products.³⁶ Albeit controversial, sodium intake has been hypothesized to have harmful effects on BP but no such association has been observed on cheese-derived sodium.³⁷ Recent evidence suggesting a protective role of branched-chain amino acids (BCAA) on CVD and its ability to form hypotensive lactotripeptides³⁸ also vary with different dairy source. Table 1.1 below compares BP-related nutrients between the three major sources of dairy: milk, cheese, and yogurt. Yogurt contains the highest amounts of BCAA, potassium, magnesium, and calcium while having the lowest amount of sodium.³⁶

Table 1.1: Nutrient profiles of one serving of yogurt, milk, and hard cheese

Nutrients	1 c. plain yogurt	1 c. whole milk	1.5 oz. hard cheese
Calories (kcal)	149	146	173
Total fat (g)	7.96	7.98	14.38
Total protein (g)	8.5	7.9	10.2
Leucine (mg)	858	647	842
Isoleucine (mg)	463	403	523
Valine (mg)	703	468	609
Vitamin D (IU)	5.0	5.0	10
Total carbohydrates (g)	11.4	12.8	0.57
Calcium (mg)	296	276	115

Magnesium (mg)	29.4	24.4	11
Potassium (mg)	380	349	32
Sodium (mg)	113	97.6	274

Fermented dairy products have been around for centuries. Yogurt is one of the major fermented dairy products worldwide and according to the United States Department of Agriculture (USDA), a food product can only be called “yogurt” if it was produced via fermentation with a bacterial strain containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.³⁹ For the past 20 years, fermented dairy products such as yogurt have gained much attention for their health benefits.^{40,41}

Preliminary evidence indicates that fermented dairy products may play a hypotensive role in BP control.⁴⁰ It was reported that the consumption of fermented milk, which contained 2.5-3.5 mg/kg/day of two casein-derived tripeptides: valine-proline-proline (VPP) and isoleucine-proline-proline (IPP), resulted in a reduction of SBP by 177 mm Hg in Spontaneously Hypertensive Rats (SHR).⁴² One of the largest human double-blind, placebo-controlled randomized trials that involved 94 hypertensive subjects, in which the experimental group received 150 mL of milk that was fermented by *Lactobacillus helveticus* twice a day for ten weeks, resulted in a SBP decrease of 4.1 mm Hg and their DBP was lowered by 1.8 mm Hg.⁴³ Although only for a short duration,

fermented dairy products have shown beneficial effects on BP. This warrants long-term, well-controlled human studies to evaluate fermented dairy benefits on CVD risk and its attributed risk factors including HTN.

Few observational studies and no clinical trials have examined the benefits of yogurt consumption on BP.⁴⁴ The Women's Health Study of 28,886 middle-aged US women found that decreased HBP risk was independently associated with higher intakes of low-fat dairy products, calcium, and vitamin D after a 10-year follow-up.²⁶ There was no association between high-fat dairy and risk of HBP. Of the four categories of low-fat dairy products including skim milk, yogurt, cottage cheese, and sherbet, only skim milk was inversely associated with HBP risk. Another study in a French cohort observed a slight decrease in DBP with a higher intake of any dairy product except for cheese but there was no association with SBP.⁴⁵ With this inconsistency in findings, it is imperative to question the extent in which different dairy products affect BP.

Yogurt intake was shown to be inversely associated with weight gain in combined analysis of data from the Nurses' Health Study (NHS), NHS II, and Health Professionals Follow-Up Studies (HPFS).⁴⁶ Cross-sectional studies

indicate that more than 85% of hypertensive individuals are overweight or obese.⁴⁷ These effects on body weight maybe responsible for other yogurt-related metabolic effects. Extensive clinical trial data show a substantial and BP-lowering effect of weight loss and an overall strong relationship between obesity and HTN. These reductions in BP occur in overweight and obese individuals regardless of reaching a normal BMI (body mass index of <25 kg/m²).⁴⁸ Neter et al performed a meta-analysis of 25 randomized, controlled trials, and found that a weight loss of 1 kg was associated with 1 mm Hg reduction in SBP and DBP in prehypertensive subjects.⁴⁹ The largest of the trials included in the meta-analysis, Trials of Hypertension Prevention (TOHP), demonstrated a larger weight loss effect combining a behavioral weight loss intervention along with the dietary intervention in prehypertensive adults. This combination led to mean weight loss of 2 kg in a 6-month follow-up. The weight loss was accompanied by a mean decrease of SBP and DBP of 3.7 and 2.7 mm Hg respectively⁵⁰ along with a 42% decreased risk of incident HBP⁵¹. The TOHP trial results show strong clinical evidence that reductions in body weight and body fat is strongly correlated on BP reductions. Yogurt intake has also been associated with a better metabolic profile. In the Framingham Offspring Study, consumption of yogurt has been linked with lower levels of triglycerides, glucose, and lower presence of insulin

resistance, even after controlling for diet quality.⁵² BMI adjusted led to some but not a complete attenuation of most of these effects.

While the specific mechanisms by which dairy products may benefit blood pressure are not known, several have been hypothesized. The original DASH trial cited the mineral content of dairy (i.e., its calcium, potassium and magnesium content) as having potentially beneficial effects on blood pressure. While calcium is involved in blood pressure regulation, it appears that its effects are relatively modest and may only aid those with low baseline calcium levels.^{53,54} Out of the three BP-related minerals in dairy, the effect of potassium seems to be the strongest, with higher levels of intake being shown to relate to lower BP levels in a number of individual studies^{55,56} and several meta-analyses.^{57,58}

Studies of magnesium and blood pressure have been inconclusive,⁵⁹ although several studies have linked magnesium to being a natural calcium channel blocker via steric inhibition with sodium on vascular smooth muscle cell binding sites. This induces endothelial-dependent vasodilation and lower BP levels.⁶⁰⁻⁶²

Yogurt is a concentrated source of calcium, potassium, and magnesium,⁶³ lending to its appeal for further investigation of yogurt-specific mechanisms on BP.

1.6 DAIRY AND CARDIOVASCULAR DISEASE

There is an ample amount of evidence to recommend that a generally healthier lifestyle can prevent a substantial proportion of CVD.⁶⁴ The public have long been advised by the medical and scientific community to reduce saturated animal fats, which includes dairy fat, in the diet to improve health and reduce the risk of CVD. But the evidence supporting this notion is lacking. A 2010 meta-analysis of 21 prospective observational studies with over 300,000 participants of whom around 11,000 developed coronary artery disease (CAD) or stroke during 5-23 years of follow-up showed that there is no evidence that dietary saturated fat is linked with an increased risk of CAD or CVD.⁶⁵ Dairy products, especially whole milk, cheese, and butter can be high in saturated fat. It is estimated that dairy products (excluding butter) contribute to 24% of the saturated fat intake of the typical American diet.⁶⁶ Similar percentages were observed in European countries (25-30%).⁶⁷

In several studies, particularly in Europe, dairy consumption has been inversely associated with risk of CVD⁶⁸. In the Honolulu Heart Program⁶⁹, investigators observed that middle-aged men who did not drink milk, had a two-fold increase in rates of stroke than men who consumed 2 cups of milk per day. A recent meta-analysis concluded that every 200 mL increase in milk consumption per day led to a 6% reduction in risk of CVD⁷⁰. In that study, the protective effects were stronger for risk of stroke than for coronary heart disease (CHD). These findings may suggest that the inverse association between dairy consumption and cardiovascular risk is linked with its effects on blood pressure.

Even fewer studies examined the yogurt-specific effect on CVD risk. Some have found that yogurt intake reduced low density lipoprotein cholesterol (LDL-C):total cholesterol levels⁷¹⁻⁷³ and benefitted arterial intima-media thickness²⁸, suggesting that yogurt consumption may protect against development of CVD. A meta-analysis of short term intention studies of the effect of a probiotic milk product on plasma cholesterol showed a 4% decrease in total cholesterol and a 5% decrease in LDL-cholesterol in European cohorts.⁷⁴ The effects of yogurt on HBP risk is less clear but the intermediate effects of yogurt intake may reduce overall cardiovascular risk by benefitting cholesterol levels and vascular integrity.

1.7 OVERVIEW OF STUDIES

1.7.1 Framingham Offspring Study

The Framingham Offspring Study (FOS) is a second-generation cohort consisting of the offspring from the Framingham Heart Study (FHS). The FHS started in 1949 with the examination of 5,209 subjects (1,644 spouse pairs), aged 30-62, to evaluate risk factors and determine certain characteristics of CHD in the town of Framingham, Massachusetts.⁷⁵ At that time, very little was known about the etiology of CVD but CVD-related deaths continue to increase steadily and has become a burden in American public health. It is a longitudinal study, which is still ongoing today, and in 1971, gave rise to the FOS.

The FOS had two major aims: to determine whether there have been secular changes in the levels of CVD risk factors between the FHS and FOS and to examine the presence of genetic and familial effects in determining levels of these risk factors.⁷⁶ 5,135 subjects were enrolled in the FOS, with 5,124 attending the first exam. Diet was assessed via 3-day diet records starting on exam 3 (1983-1987) and also on exam 5 (1991-1995)⁷⁷. For these analyses, the baseline exam is the first exam when diet was collected. Although most the subject had exam 3 diet data, some only had exam 5 and thus for those who only had exam 5 diet

data, exam 5 serves as their baseline exam. For those who had both exam 3 and exam 5 dietary data, the mean was calculated and exam 3 remains their baseline exam. Along with BP triplicate measurements via the standard mercury sphygmomanometer, anthropometric, blood chemistries, urinalysis, medical histories, and lifestyle habits at each exam occurring every four years. Follow-up was through the end of the exam 7 cycle (up to 2001). The timeline for FOS in these analyses is illustrated in figure 1.1.

1.7.2 Nurses' Health Study

The Nurses' Health Study (NHS) was established in 1976 with the primary purpose of investigating the potential long-term effects of oral contraceptive use. At that time, it was a potent drug that was being prescribed to many women and little was known of its health effects. The study has been broadened over time to include the effects of many health related and lifestyle practices including diet and physical activity.⁷⁸

Registered nurses were selected with the assumption that with their nursing education, they would be able to respond accurately to brief, biennial questionnaires. Along with their education, they would most likely be motivated

and interested in the overall goal of this prospective study. The original nurses were between the age of 30-55 years, married, and lived in 11 populous states: California, Texas, Florida, Ohio, Michigan, Maryland, New Jersey, Pennsylvania, New York, Connecticut, and Massachusetts.

From the initial 170,000 mailed responses, around 122,000 responded (71.7% response rate). A follow-up questionnaire was sent every two years thereafter. These biennial questionnaires asked the nurses about diseases and other health-related behaviors such as: alcohol use, smoking, menopausal status, and hormone use. A dietary component was added to the biennial questionnaires in 1980 to recognize the importance of nutrition in the development of chronic disease. For each food, a commonly used household unit or portion was specified. The nurses were asked how often, on average over the previous year, they had consumed each food item. Subsequent dietary questionnaires were collected in 1984, 1986, 1990, and four years thereafter. As expected, a high response rate was observed at each biennial questionnaire cycle (~90%) starting in 1980. Figure 1.1 below shows the study timeline for NHS that was used in these analysis. Eight sets of food frequency questionnaires (FFQ) were used, with follow-up continuing through 2008.

Quality of life questions were added in 1992 and every four years thereafter. In order to have a more accurate and objective measure of certain dietary biomarkers, genetic markers, and hormone levels, 33,000 blood samples were collected in 1989-90, with 18,700 of these participants having a follow-up collection in 2000-01.

1.7.3 Nurses' Health Study II

The Nurses' Health Study II (NHS II) was established in 1989 to study the effects of oral contraceptives along with diet and lifestyle risk factors in a younger population (ages 25-42). This younger generation of women nurses included those who began use of oral contraceptives during adolescence and were thus maximally exposed during their early reproductive life. Such early use of oral contraceptives were hypothesized to have an adverse effect on cancer risk. The upper age group in NHS II represented the younger age group in the original NHS in order to examine health effects over time. Nursing boards in the following states were able to provide information on birth date and age on the target population of nurses: California, Texas, Missouri, Kentucky, Iowa, Indiana, Michigan, Ohio, Pennsylvania, New York, Connecticut, Massachusetts, North Carolina, and South Carolina. After a 24% baseline overall response rate

(123,000 of 517,000), exclusions of incomplete forms and ineligible women led to a total of 116,686 remaining in the NHS II.

Every two years starting in 1989, members of the NHS II cohort received a follow-up questionnaire with questions regarding disease diagnoses and health/lifestyle behaviors including alcohol use, smoking, menopausal status, and hormone use. The first FFQ was collected in 1991 and subsequent diet questionnaires were administered every four years. A two-page quality-of-life supplement was included in the first mailing of the 1993 and 1997 questionnaires.

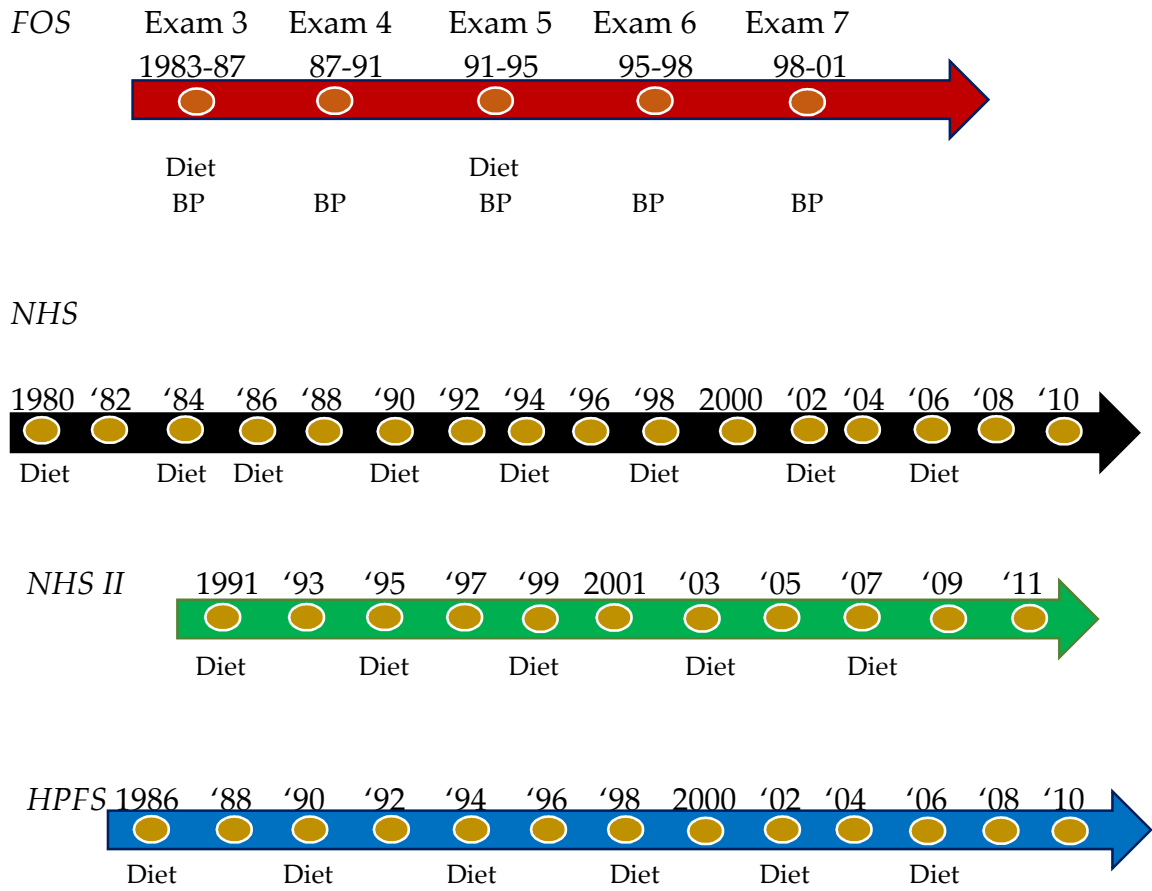
Blood and urine samples for dietary and genetic markers from approximately 30,000 nurses were collected in the late 1990's. Response rates were similar to NHS (approximately 90%) for each two year questionnaire cycle. Figure 2 below shows the study timeline for NHS II. Follow-up began in 1991 and continued through 2011 for these analyses

1.7.4 Health Professionals Follow-Up Study

The Health Professionals Follow-Up Study (HPFS) began in 1986 when 51,529 American male health professionals, ages 40-75 years, provided information on their medical history, lifestyle, and dietary intake. The HPFS was designed to evaluate effects of nutrition factors on men's health and incidence of chronic diseases such as CVD and cancer. It was designed to complement the all-female NHS cohort, which examines similar factors on chronic disease outcomes. Of the participants recruited, 29,683 were dentists, 4,185 were pharmacists, 3,745 were optometrists, 2,220 were osteopath physicians, 1,600 were podiatrists, and 10,098 were veterinarians. Similar to the NHS, male health professionals were targeted under the assumption that being a medical provider, they would be motivated and committed to participate in a long-term study and would be highly interested in the findings from such study on various health outcomes.

Every two years, HPFS cohort members received questionnaires similar to NHS participants inquiring disease diagnoses and various health-related topics which included: smoking behaviors, physical activity, and medications taken. FFQ's were first administered in four-year intervals starting in 1986. Figure 2 below illustrates the timeline of HPFS that was used for these analyses. Six sets of FFQs were used, with follow-up continuing through 2010.

Figure 1.1: Study Timelines for the FOS, NHS, NHS II, and HPFS



1.8 GOALS OF THE DISSERTATION

HBP is a growing public health burden that is strongly associated with CVD, the number one cause of death in America and worldwide. As BP levels increase, the risk of CVD increases significantly. With an aging population and with BP strongly correlated with older age, the need to identify modifiable risk factors and behaviors to alleviate BP increases is crucial. There is a moderate amount of mostly cross-sectional and short-term experimental evidence that implicates dietary protein to have BP-lowering effects alone or in a context of healthy eating pattern such as the DASH diet but long-term studies are less clear. Specific protein sources such as dairy, has been shown to have a beneficial effect on BP as well. With inconsistent observational studies and only several long-term prospective studies, the antihypertensive effects of protein and dairy need to be examined further. With only several in-vitro and in-vivo studies and a few cross-sectional evidence that suggest a beneficial effect of fermented dairy, especially yogurt, on BP levels, a longitudinal study is warranted to elucidate yogurt's long-term BP effects.

1.9 OBJECTIVES OF THE DISSERTATION

The objectives of this dissertation are to use dietary and BP data from over 11 years of follow-up in the Framingham Offspring Study to quantify the long-term effects of total protein intake on mean BP levels and incident HBP risk. Next, three prospective cohorts: Nurses' Health Study I, II, and the Health Professionals Follow-Up Study of over 180,000 participants, will be used to estimate the longitudinal effects of total dairy and yogurt, on incident HBP risk and subsequent risk of CVD among those with HBP.

CHAPTER 2: HIGHER PROTEIN DIETS PREDICT LOWER HIGH BLOOD PRESSURE RISK IN FRAMINGHAM OFFSPRING STUDY ADULTS

2.1 ABSTRACT

Background: Short-term clinical trials suggest that dietary protein has blood pressure-lowering effects but long-term effects of total, animal and plant proteins are less clear.

Objective: To evaluate effects of total, animal, and plant proteins on mean systolic (SBP) and diastolic blood pressure (DBP) and incident high blood pressure (HBP) risk among middle-aged adults in the Framingham Offspring Study.

Methods: Men and women (ages 30-54) without prevalent HBP, cardiovascular disease or diabetes with three-day dietary records from exams 3 or 5 (n=1,361) were included and followed for a mean of 11.3 years for development of HBP. Protein intakes adjusted for body size were derived using the residual method. Analysis of covariance and Cox proportional hazard's models were used to adjust for age, sex, education, height, activity, smoking, fat calories, diet quality, and body mass index.

Results: Higher adjusted protein intakes were associated with lower mean SBP and DBP. Both animal and plant proteins lowered BPs and led to statistically significant reductions in HBP risk (hazard ratios (HR) of 0.68 and 0.51, respectively). Subjects in the highest tertile of total protein intake had a 40% lower long-term risk (95% confidence interval (CI): 0.45-0.78) of developing HBP. Beneficial effects of protein were apparent for both men and women and for normal weight and overweight individuals. Higher protein diets also characterized by higher fiber intakes led to a 51% reduction (95% CI: 0.37-0.66) in HBP risk.

Conclusion: Adults consuming more dietary protein from both plant and animal sources had lower long-term risks of HBP.

2.2 BACKGROUND

High blood pressure (HBP) is a major cause of cardiovascular disease and renal failure.⁷⁹ Evidence from recent reviews^{14,15} and meta-analyses^{16,17} suggest that dietary protein consumption may benefit BP. Both of these meta-analyses of short-term clinical trials concluded that compared with carbohydrates higher dietary protein led to modest BP reductions. In the Rebholtz meta-analysis, both animal and plant proteins were found to have similar short-term effects on BP.^{16,17} Observational studies, which have been largely cross-sectional, have shown weak beneficial effects of plant proteins in particular on BP.¹⁴ There are few observational studies (and no clinical trials) that have addressed the long-term effects of animal and plant proteins on BP, and results are conflicting.¹⁸⁻²⁰

The Dietary Approaches to Stop Hypertension (DASH) studies have documented the importance of diet patterns to BP.⁵⁷ The greatest reductions in BP in the DASH trials have been seen with higher intakes of FV (fruits and vegetables) plus higher intakes of low-fat dairy products (an important source of dietary protein). In general, the effects of FV alone are modest.⁸⁰ Higher dietary fiber intakes have also been associated with a BP-lowering effect, particularly among hypertensive individuals.⁸¹ Combined effects of dietary protein and fiber intakes

on BP are less clear although at least one review suggests that they may be additive.⁸²

The goal of the current study was to evaluate the longitudinal effects of the amount and type of dietary protein on mean BP and the risk of incident HBP among middle-aged adults in the Framingham Offspring Study (FOS). The interactive effects of dietary protein with FV and dietary fiber were also examined.

2.3 METHODS

2.3.1 Study Population

The Framingham Offspring Study (FOS) began in 1971 with enrollment of 5,124 offspring (and spouses) from the original Framingham Heart Study cohort. Subjects were evaluated at roughly four-year intervals following the baseline visit and BP was measured at each exam. Diet was assessed using three-day diet records at the third (starting in 1984) and fifth examination visits.⁸³

Subjects (n=3,284) were included who had complete dietary data at examination 3, 5, or both. For those with complete data at both visits, mean dietary protein

intake was estimated. For those with dietary data only at exam 3 or 5, protein intake from that exam was used. Exam 3 served as the baseline visit for eligible subjects with dietary data at that visit; exam 5 served as baseline for those with missing dietary data at exam 3.

Additional exclusions included 1,284 who were outside of the age range (30-54 years) at the time of dietary assessment; 294 with extreme values for total energy (<1200 or >4000 kilocalories/day for men; <1000 or >3500 kilocalories/day for women), alcohol (>20% of calories) or foods (e.g., >35 eggs/wk), a BMI<18.5, missing data on potential confounders, or lacking all follow-up data; and 345 subjects with prevalent type 2 diabetes, CVD, or hypertension (HTN) at baseline, leaving 1,361 subjects available for proportional hazard's modeling. For calculation of mean BP at the first follow-up exam after baseline, an additional 26 subjects were excluded who were missing that data.

2.3.2 Dietary Measurement

Approximately 16,000 days of diet records were collected during exams 3 and 5. A trained nutritionist instructed families in the completion of diet records (on two weekdays and one weekend day) and the use of two-dimensional food

models for estimating portion sizes. Diet records were entered into the Nutrition Data System (NDS), developed at the University of Minnesota.⁸⁴ The NDS program calculated each subject's daily intake of protein (grams) in addition to other macro- and micro-nutrients, including dietary fiber. The NDS provided estimated daily intakes for total, animal, and plant protein. FV intake per day (quantified in cup equivalents) was calculated by linking food codes output from the NDS system with USDA Pyramid food codes derived from the Continuing Survey of Food Intake of Individuals (CSFII).⁸⁵ FV and dietary fiber were combined with dietary protein in selected analyses.

2.3.3 Blood Pressure Outcomes

BP was measured by a Framingham physician using a standard mercury sphygmomanometer. Two measurements were taken after subjects sat quietly for 5 minutes. Mean baseline SBP and DBP values were estimated during the same examination cycle in which baseline dietary assessment was completed. Follow-up SBP and DBP were measured at four year intervals at the routine examination visits. Incident HBP was defined as any of the following: (1) mean SBP \geq 140 mm Hg or DBP \geq 90 mmHg at two consecutive exams, or (2) mean SBP \geq 160 mm Hg or DBP \geq 95 mm Hg on a single exam, or (3) use of anti-hypertensive medication

for BP-lowering purposes. Follow-up for incident HBP started at the time of the last dietary protein assessment and continued until the first of the following: incident HBP, loss to follow-up, death, or end of follow-up (through the end of exam 7).

2.3.4 Potential Confounders

The following were considered as potential confounding factors: age, sex, education level (high school or less vs. beyond high school), physical activity, cigarette smoking, Healthy Eating Index (HEI) score, baseline BP, height, baseline BMI, and dietary fat intake (% of calories from fat and saturated fat). A physical activity index, a modification of the original method by Kannel⁸⁶, was calculated as the number of self-reported hours per day spent doing moderate or vigorous activities multiplied by a numeric weight derived from the oxygen consumption required (liters/min) for that activity. Cigarette smoking was assessed at every exam visit. Subjects were considered current smokers if they smoked at any time during the baseline exam period. Mean cigarettes smoked per day during the same period were estimated. Body weight was measured at baseline using a calibrated spring balance scale and height were measured at each exam with a standard stadiometer. To reduce the effect of measurement error, average adult height from all available measures between ages 30 and 54

years of age was used to calculate baseline BMI [weight (in kilograms) divided by average height (in meters squared)]. To measure overall diet quality, Healthy Eating Index (HEI) scores were derived from the 2005 MyPyramid Food Guidance System which incorporates key recommendations in the 2005 Dietary Guidelines for Americans.⁸⁷ Other specific dietary factors such as the, total energy, dietary sodium, monounsaturated fatty acids, alcohol intake, FV, and fiber were assessed as potential confounders and dropped from the final models as they led to less than a 5% change in the effect estimates.

2.4 STATISTICAL ANALYSIS

To derive estimates of protein intake (total, animal, plant) that were adjusted for body size, mean intakes were computed using the residual method, regressing each subject's protein intake on body weight. This method was modeled from Willett's energy-adjustment method⁸⁸ with resulting protein residuals being uncorrelated with (and therefore not confounded by) body weight. For the initial analyses, each protein variable was classified into tertiles using the weight-adjusted residual variables. To assess the effects of protein combined with intakes of FV and fiber, weight-adjusted protein residuals were dichotomized (<median vs. ≥median), as were intakes of total FV and fiber, using sex-specific

medians (for FV: 2.92 cup-equivalents/day for men and 2.65 cup-equivalents/day for women; for fiber: 16.83 grams/day for men and 13.38 grams/day for women). Diet combinations were created by classifying each subject into one of four dietary intake categories (for example: (a) low intakes of both protein and FV, (b) low protein and high FV intakes, (c) high protein and low FV intakes, and (d) high intakes of both).

Analysis of covariance (ANCOVA) modeling was used to compare adjusted mean SBP and DBP levels after four years of follow-up across tertiles of protein consumption as well as protein combined with FV and fiber intakes. In this prospective analysis, it was necessary to consider subjects who developed HBP during the follow-up period (since HTN treatment would impact follow-up BP levels). For those who developed HBP but were not on drug treatment, no adjustment to the follow-up SBP or DBP measures was needed. However, for new cases of treated HBP, mean baseline BP levels were substituted for follow-up BP.

Cox proportional hazard's models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for long-term risk of developing HBP

associated with dietary protein intake independently and combined with FV and fiber intakes. Final multivariable models included the following potential confounders: age, sex, education level, height, physical activity, smoking status, HEI score, and percent of energy from fat. Since BMI could be a causal intermediate in these analyses, the final models were run with and without follow-up BMI. In addition, stratified analyses were completed by protein type (animal vs. plant), baseline BMI (<25 vs. ≥ 25 kg/m²), and sex. All analyses were performed using Statistical Analysis Systems software, version 9.1 (SAS Institute).

2.5 RESULTS

Baseline characteristics of FOS subjects in each tertile of total protein intake are shown in table 2.1. Those with higher protein intakes were taller, more educated, leaner, and were more likely to be male ($p < 0.001$, for all). Those with lower protein intakes consumed fewer calories, FV, and fiber ($p < 0.001$, for all). Baseline blood pressure was slightly lower among individuals with the highest tertile baseline protein intakes ($p = 0.02$ for SBP and $p = 0.06$ for DBP).

Table 2.1. Baseline characteristics by tertile of total protein intake in the Framingham Offspring Study

Baseline Characteristics	Tertiles of estimated protein intake ¹			<i>p</i> value
	1 (low)	2	3 (high)	
<i>n</i>	444	445	446	
Age, (y)	44.4 (6.2)	43.2 (6.4)	43.4 (6.5)	0.01
Height, cm	167.3 (8.9)	168.1 (8.6)	171.3 (9.3)	<0.001
BMI, kg/m ²	26.3 (4.7)	24.8 (3.6)	25.0 (3.5)	<0.001
Male, %	27.7	37.8	60.0	<0.001
Current smoker, %	28.4	24.5	24.7	0.21
Attained high school education, %	58.2	70.1	70.8	<0.001
Physical activity index	12.2 (7.3)	12.3 (8.2)	12.8 (8.1)	0.42
SBP, mm Hg	117.7 (13.0)	115.7 (11.6)	114.6 (11.9)	0.02
DBP, mm Hg	76.3 (8.4)	75.2 (8.5)	75.1 (8.0)	0.06
Energy, kcals/d	1555 (364)	1876 (414)	2393 (535)	<0.001
Total protein ¹ , g/d	58.0 (8.5)	77.7 (4.8)	102.6 (13.8)	<0.001
Animal protein ¹ , g/d	39.3 (8.5)	54.8 (7.6)	75.1 (13.5)	<0.001
Plant protein ¹ , g/d	17.7 (5.0)	21.7 (6.7)	26.2 (8.9)	<0.001
Calories from carbohydrates, %	47.6 (8.3)	45.1 (7.6)	43.4 (7.7)	<0.001
Calories from fat, %	35.4 (6.3)	36.2 (6.4)	36.4 (6.5)	0.049
Calories from saturated fat, %	12.2 (2.9)	12.3 (2.9)	12.6 (3.0)	0.06

Fruits/vegetables, cup eq/d	2.6 (1.2)	3.0 (1.3)	3.5 (1.7)	<0.001
Fiber, g/d	13.1 (4.5)	15.5 (5.4)	18.9 (7.3)	<0.001
Whole grains, oz eq/d	0.5 (0.6)	0.6 (0.8)	0.7 (0.8)	<0.001

Values are mean (SD) for continuous variables and % for categorical variables.

p denotes the significance of the linear trend across tertiles of protein intake.

¹Protein intakes expressed as weight-adjusted residuals (g/d)

Table 2.2 shows that protein consumption (total, animal, plant) was inversely associated with both SBP and DBP after four years of follow-up. Overweight subjects (BMI \geq 25 kg/m²) had generally higher BP levels than leaner individuals (BMI < 25 kg/m²) but protein intake was linked with lower BPs for both groups. Among the overweight, both animal and plant proteins led to lower DBP levels while leaner subjects consuming more animal and plant proteins had lower SBP levels.

Table 2.2. Multivariable-adjusted blood pressure levels by tertiles of total, animal, and plant protein intake among adults

Baseline intake	All ¹			Baseline BMI < 25 ²			Baseline BMI \geq 25 ²		
	n	SBP	DBP	n	SBP	DBP	n	SBP	DBP
Total protein ³									
T1	444	120.5	77.1	195	116.5	73.7	249	124.4	80.4
		(0.6)	(0.4)		(0.9)	(0.6)		(0.9)	(0.5)

T2	445	119.1	76.5	251	115.8	74.4	194	122.7	79.0
		(0.6)	(0.4)		(0.8)	(0.5)		(0.9)	(0.6)
T3	446	117.7	75.6	237	113.1	72.9	209	122.6	78.3
		(0.6)	(0.4)		(0.8)	(0.6)		(0.9)	(0.6)
<i>p</i> for trend		0.001	0.02		0.006	0.29		0.17	0.02
Animal protein ³									
T1	445	120.0	77.0	207	116.2	73.8	238	124.1	80.2
		(0.6)	(0.4)		(0.9)	(0.6)		(0.9)	(0.6)
T2	442	119.8	76.7	238	115.7	74.2	204	124.2	79.5
		(0.6)	(0.4)		(0.8)	(0.6)		(0.9)	(0.6)
T3	448	117.6	75.6	238	113.5	73.1	210	121.7	78.2
		(0.6)	(0.4)		(0.8)	(0.6)		(0.9)	(0.6)
<i>p</i> for trend		0.02	0.049		0.03	0.41		0.08	0.02
Plant protein ³									
T1	445	120.7	77.5	192	116.4	74.4	253	125.1	80.7
		(0.6)	(0.4)		(1.0)	(0.6)		(0.8)	(0.5)
T2	446	118.4	76.5	247	115.4	73.7	199	121.3	79.3
		(0.6)	(0.4)		(0.8)	(0.5)		(0.9)	(0.6)
T3	444	118.3	75.3	244	113.8	73.0	200	123.1	77.6
		(0.6)	(0.4)		(0.9)	(0.6)		(1.0)	(0.6)
<i>p</i> for trend		0.03	0.001		0.04	0.11		0.06	0.0002

Values are mean (SE).

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; T, tertile.

¹Adjusted for age, sex, education, height, activity, smoking status, % energy from fat, BMI status (normal, overweight or obese).

²Adjusted for age, sex, education, height, activity, smoking status, % energy from fat.

³Protein intakes expressed as weight-adjusted residuals in g/d.

The effects of dietary protein combined with total FV or fiber consumption on adjusted mean BP levels are shown in Table 2.3. We explored first independent effects of the sex-specific tertiles of FV and fiber intake on mean BP levels (data not shown) and found that those in the highest tertile of FV intake had a mean SBP that was 2.1 mm Hg lower ($p=0.02$) and a DBP that was 0.8 mm Hg lower ($p=0.16$) than those with the lowest intakes. The highest dietary fiber intakes were associated with stronger BP-lowering effects ($p=0.001$ for SBP; $p=0.002$ for DBP). In Table 2.3, subjects consuming more protein combined with either higher FV or fiber intakes had lowest mean SBP and DBP levels. For example, adults with higher protein and fiber intakes had SBP levels that were 4.0 mm Hg lower (and DBP levels that were 2.3 mm Hg lower) than those with lower intakes of both ($p<0.001$ for both SBP and DBP). In general, the effects of dietary fiber on BP seemed were stronger than those for FV. Finally, more protein consumption led to beneficial effects on BP for both men and women while the benefits of dietary fiber seemed stronger for women than for men.

Table 2.3. Multivariable-adjusted blood pressure levels according to combined intakes of total protein with FV or fiber

Baseline diet pattern ¹	Mean blood pressures after 4 years of follow-up				
	N	SBP ²	P value	DBP ²	P value
<i>All</i>					
Protein + FV					
Low protein, low FV (reference)	387	120.3 (0.7)	-	76.8 (0.4)	-
Low protein, high FV	279	120.4 (0.8)	0.88	77.4 (0.5)	0.35
High protein, low FV	280	118.6 (0.8)	0.10	76.2 (0.5)	0.31
High protein, high FV	389	117.4 (0.7)	0.002	75.5 (0.4)	0.02
Protein + fiber					
Low protein, low fiber (reference)	413	121.1 (0.6)	-	77.3 (0.4)	-
Low protein, high fiber	253	119.0 (0.8)	0.04	76.7 (0.5)	0.32
High protein, low fiber	256	119.2 (0.8)	0.07	77.0 (0.5)	0.63
High protein, high fiber	413	117.1 (0.6)	<0.001	75.0 (0.4)	<0.001
<i>Men</i>					
Protein + FV					
Low protein, low FV (reference)	113	124.2 (1.1)	-	80.5 (0.8)	-
Low protein, high FV	80	122.7 (1.4)	0.40	80.2 (0.9)	0.75
High protein, low FV	165	120.0 (1.0)	0.13	79.5 (0.6)	0.31
High protein, high FV	200	120.2 (0.9)	0.006	78.3 (0.6)	0.02
Protein + fiber					
Low protein, low fiber (reference)	128	123.2 (1.1)	-	80.4 (0.7)	-
Low protein, high fiber	65	124.5 (1.5)	0.49	80.2 (1.0)	0.88

High protein, low fiber	150	121.7 (1.0)	0.30	79.7 (0.7)	0.46
High protein, high fiber	215	120.5 (0.8)	0.055	78.2 (0.6)	0.02
<i>Women</i>					
Protein + FV					
Low protein, low FV (reference)	274	117.8 (0.8)	-	74.4 (0.5)	-
Low protein, high FV	199	118.5 (1.0)	0.55	75.4 (0.6)	0.21
High protein, low FV	115	116.1 (1.2)	0.24	73.6 (0.8)	0.39
High protein, high FV	189	115.2 (1.0)	0.04	73.4 (0.6)	0.22
Protein + fiber					
Low protein, low fiber (reference)	285	119.5 (0.8)	-	75.2 (0.5)	-
Low protein, high fiber	188	115.9 (1.0)	0.004	74.2 (0.6)	0.24
High protein, low fiber	106	117.8 (1.3)	0.25	75.3 (0.8)	0.94
High protein, high fiber	198	114.4 (0.9)	<0.001	72.6 (0.6)	0.001

Values are mean (SE).

BMI, body mass index; DBP, diastolic blood pressure; FV, fruits and vegetables; SBP, systolic blood pressure.

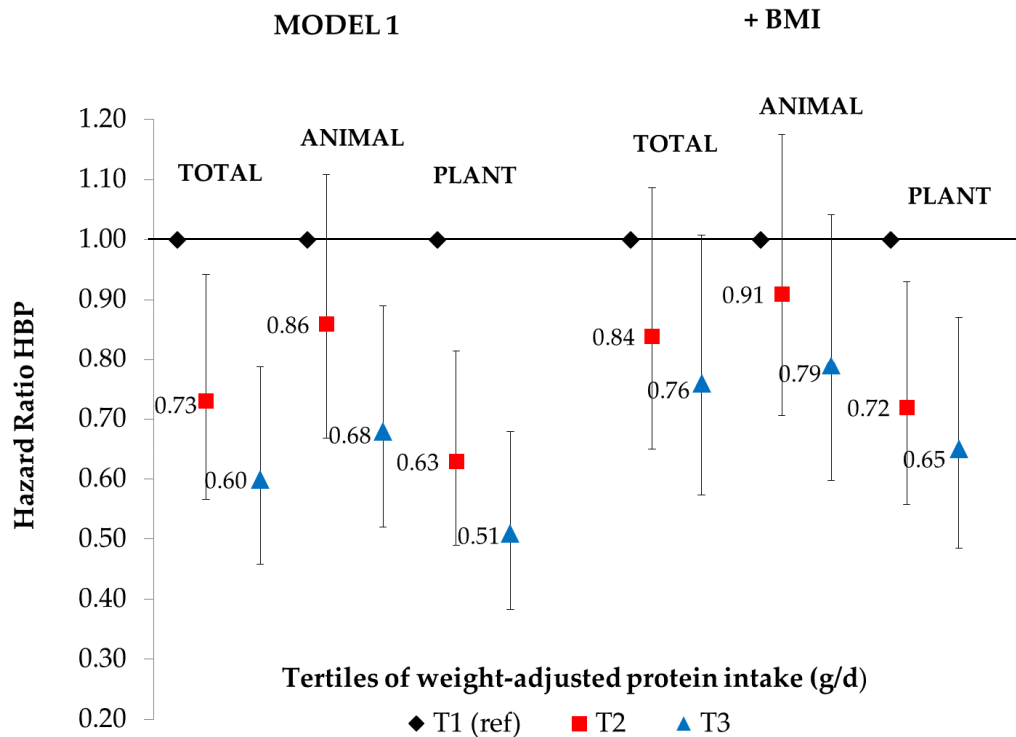
¹Cutpoint for dichotomous baseline intakes was set at the median (< median vs. ≥median) for protein intake (as weight-adjusted residuals, g/d), FV (cup equivalents/d), and fiber (g/d).

²Adjusted for age, sex (only for all subjects model), education, height, activity, smoking status, % energy from fat, baseline BMI.

There were 346 cases of incident HBP that occurred during the follow-up period (mean time = 11.3 years). Figure 2.1 illustrates the hazard ratios for incident HBP associated with total, animal, and plant protein intakes. After adjusting for age, sex, education, height, physical activity, smoking, HEI score, and percent of calories from fat, subjects in the highest tertile of total protein intake had a 40% lower risk of incident HBP (HR: 0.60, 95% CI: 0.45-0.78) compared with those in

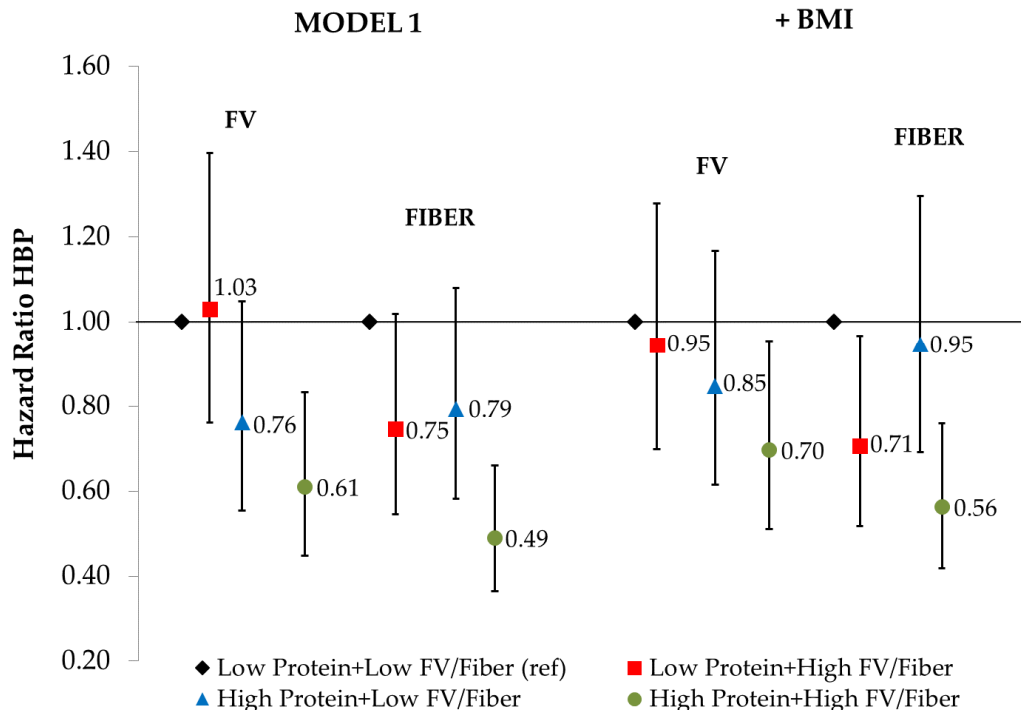
the lowest tertile. Both animal (HR: 0.68, 95% CI: 0.52-0.89) and plant (HR: 0.51, 95% CI: 0.38-0.68) protein consumption led to statistically significant reductions in HBP risk. To determine whether BMI might explain any of the effects of protein on HBP risk, baseline BMI was added to Model 2; statistically significant reductions in HBP risk remained for high plant protein intakes but were somewhat attenuated for total and animal proteins.

Figure 2.1 Adjusted hazard ratios for incident HBP by tertile of total, animal, and plant protein intake



In Figure 2.2, adjusted HRs for incident HBP associated with protein combined with either FV or fiber are shown. Overall, subjects who consumed more protein combined and more FVs intakes had a statistically significant 39% reduction in risk of HBP compared with those in the referent group (Model 1). Those who consumed more protein with a higher fiber diet, had a 51% lower risk (95% CI: 0.37-0.66) of incident HBP than those with lower intakes of both. These effects were slightly weakened by the addition of BMI to Model 2.

Figure 2.2 Adjusted hazard ratios for incident HBP by category of total protein combined with FV or fiber



High vs. low protein: <median vs. ≥median). High vs. low FV (and fiber): <sex-specific vs. ≥sex-specific median. Model 1 is adjusted for age, sex, education level, height, physical activity, smoking status, HEI score, and percent of calories from fat. Model 2 is additionally adjusted for BMI.

2.6 DISCUSSION

In this study, adults who consumed more protein, whether from animal or plant sources, had statistically significantly lower SBP and DBP levels after four years of follow-up. In general, these beneficial effects were evident for both overweight ($\geq 25 \text{ kg/m}^2$) and normal weight ($< 25 \text{ kg/m}^2$) individuals. Consuming more dietary protein was also associated with lower long-term risks of incident HBP and, when the diet was also characterized by higher intakes of fiber, higher protein intakes led to 40-60% reductions in risk of HBP.

The current analyses add to a very limited number of longer-term prospective studies of protein intake and BP in adults.¹⁴ Our results contrast with those of both the Western Electric Study (WES) and the Seguimiento Universidad de Navarra (SUN) study that found only vegetable protein consumption to be inversely correlated with BP.^{18,20} In the Coronary Artery Risk Development in Young Adults (CARDIA) study, total protein intake among 4,100 young adults, ages 18-30 years, was inversely associated with both SBP and DBP, but the effects

were strongest for DBP.¹⁹ In Framingham with a somewhat older study population, the protein effects were generally similar for SBP and DBP. Short-term clinical trials of protein biomarkers also suggest beneficial effects on BP.^{14,89,90} These clinical trials have typically compared higher-protein diets with higher-carbohydrate diets, making it difficult to separate beneficial effects of higher protein intakes from those of a lower-carbohydrate diet. The OmniHeart Trial compared three “healthy dietary” interventions among adults with prehypertension or stage 1 HTN: a diet similar to DASH with 58% of calories from carbohydrates (vs. 48% in the two other arms), a diet higher in unsaturated fats, particularly monounsaturated fats (21% vs. 13% from monounsaturated fats), and a diet higher in protein (25% of calories from protein vs. 15%).²¹ Partial substitution of carbohydrates with either protein or unsaturated fats led to greater BP reductions than the higher carbohydrate diet alone. The current long-term data from the FOS offer important evidence to suggest that both animal and plant proteins have BP-lowering effects in non-hypertensive adults.

Dietary proteins may affect BP through a number of pathways and those pathways may differ according to the amino acid composition of the food source.¹⁵ Arginine, an amino acid found in many plant and animal sources

including eggs, acts as a vasodilator through nitric oxide pathways, contributing to lower BP.⁹¹ Also, the increased plasma amino acid levels from a higher protein diet may affect proximal sodium reabsorption or lead to alterations in cell permeability, thereby enhancing BP-related renal dynamics.⁹² Dairy products are common sources of animal protein in the American diet. Some studies including at least one meta-analysis⁹³ suggest that biologically-active peptides from milk protein, including two casein-derived tripeptides (isoleucine-proline-proline and valine-proline-proline) may directly impact BP by inhibiting the angiotensin-converting enzyme (ACE) pathway.⁹⁴

It has been suggested in a number of studies, including those of the landmark DASH diet trials, that FV intakes are linked with a lower risk of HTN.^{57,95} Mechanisms could involve antioxidant defense capacity and the ability to combat oxidative stress.⁹⁶ FV contain phytochemicals including flavonols, phytosterols and polyphenols that are thought to have BP-lowering effects.⁹⁷ They are also important sources of other nutrients such as magnesium and potassium with known BP-lowering effects.⁹⁵ Despite these purported mechanisms, the overall effects of FV on BP are generally modest.⁸⁰

This study found a strong beneficial effect of dietary fiber on BP when combined with a higher protein diet. Several meta-analyses of randomized controlled trials provide strong support for BP-lowering effects of fiber, particularly among hypertensive subjects.^{81,98} While exact underlying mechanisms are unclear, it has been hypothesized that dietary fiber enhances insulin sensitivity and improves vascular endothelial function which may, in turn, benefit BP.^{99,100}

It is also possible that the observed benefits of FV or dietary fiber on BP could be a consequence of intermediate effects on BMI, as was seen in a recent study.¹⁰¹

Our analyses suggest that while there is modest attenuation of the results by the inclusion of BMI in the models, independent beneficial effects of these diet patterns characterized by higher intakes of protein and higher intakes of FV or fiber remain.

Epidemiologic studies of diet-disease relations share a number of limitations. Of necessity, dietary data for adults are obtained by self-report and are thus subject to both random error and potentially biased reporting. In addition, of the 5,124 subjects enrolled in the FOS, only 3,284 (64%) provided dietary data. Further, we had no protein biomarker information in this study to validate reported intakes.

The FOS has a number of important strengths as well. The available diet record data were collected in a standardized fashion and, of those included in these analyses, most subjects (62%) contributed six days of diet records. Our study is one of very few long-term studies that have separated the effects of animal and plant proteins in their effects on BP. Further, the study has extensive and systematically-collected information on potential confounders, enhancing both the precision and validity of the results.

2.7 CONCLUSION

The longitudinal data from this study suggest that higher intakes of dietary protein from both animal and plant sources may be linked with significantly lower risks for developing HBP during the middle adult years. The observed beneficial effects on BP in these data were partially attenuated by controlling for intermediate effects of protein on body fat. These results provide no evidence to suggest that individuals concerned about the development of HBP should avoid dietary protein. Rather, protein intake may play a role in the long-term prevention of HBP.

**CHAPTER 3: HIGHER LONG-TERM INTAKES OF TOTAL DAIRY AND
YOGURT ARE LINKED WITH A LOWER RISK OF INCIDENT HIGH
BLOOD PRESSURE IN MIDDLE-AGED ADULTS**

3.1 ABSTRACT

Background: Observational studies suggest a beneficial effect of increased dairy intake on risk of high blood pressure (HBP) but the independent effects from specific types of dairy, such as yogurt, cheese, and milk are lacking.

Objective: To estimate the independent effects of total dairy, yogurt, cheese, and milk, on risk of incident HBP among middle-aged nurses in the Nurses' Health Study (NHS), NHS II, and male health professionals in the Health Professionals Follow-Up Study (HPFS). A secondary objective was to evaluate the modifying effects of a healthy diet or BMI on the independent effects of the dairy exposures.

Methods: Data from three prospective studies of middle-aged adults were used: the NHS (n=66,987), NHS II (n=84,368), and HPFS (n=30,512) without prevalent HBP, cardiovascular disease, cancer, or diabetes with reported dietary intakes from validated, semi-quantitated food frequency questionnaires were included. NHS, NHS II, and HPFS participants were followed for a maximum of 30, 20, and 26 years respectively for development of HBP, which was ascertained via

self-report in updated biennial questionnaires. Time dependent Cox proportional hazard's models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) and to control for potential confounding by age, race, HBP family history, calories, activity, intakes of fruits and vegetables (FV), and total protein. The addition of updated BMI was added in a separate model to evaluate the potential intermediate effects of body composition on risk of HBP. Fixed effects meta-analysis was used to combine the results from each cohort and to evaluate between-study heterogeneity.

Results: Participants who had a usual yogurt intake of 5 or more servings/week (s/wk) in the NHS, NHS II, and HPFS cohorts respectively saw a 20% (95% CI: 0.74-0.87), 17% (95% CI: 0.77-0.90), and a 6% (95% CI: 0.83-1.07) HBP risk reduction compared to the referent group of <1 serving/month. The addition of BMI attenuated the effects to 14% and 11% in the NHS and NHS II cohorts respectively, but the effects remained statistically significant. The effect of yogurt was modified by an overall healthy diet. Those with a high usual yogurt intake of ≥ 5 s/wk combined with a high DASH diet score (tertile 3), had a statistically significant 31% lower risk of among the three cohorts. Pooled analyses among the three cohorts showed that participants with a high milk (2-<6 s/d) or high cheese (1-4 s/d) intake experienced a 13% (95% CI: 0.85-0.89) and an 8% (95% CI:

0.88-0.96) reduced risk of HBP. Total dairy intake was inversely associated with risk of HBP in each of the three cohorts (p for linear trend <0.0001 for all). Those with the highest usual total dairy intake (3- <6 s/d) saw a 13% (95% CI: 0.83-0.92), 26% (95% CI: 0.69-0.78), and a 9% (95% CI: 0.84-0.98) lower risk of HBP in the NHS, NHS II, and HPFS respectively. BMI slightly attenuated the effects but each remained statistically significant. When combined with a high DASH diet score, those with high dairy intakes (3- <6 s/d) among the three cohorts saw a 23% reduced HBP risk compared to those with low dairy intakes (<1.5 s/d) and a low DASH score.

Conclusion: Higher usual intakes of total dairy and yogurt are associated with a lower risk of HBP across the NHS, NHS II, and HPFS cohorts, with yogurt having a greater effect than total dairy. Our study adds solid evidence that high long-term dairy intakes, especially yogurt, is linked with lower incidence of HBP.

3.2 BACKGROUND

In NHANES 2009-2012, it was estimated that 32.6% (or 80 million) American adults 20 years or older had high blood pressure (HBP).¹⁰² Annual HBP-attributable deaths were estimated to be 65,123 in 2013,² a 39.3% increase from 2001.¹⁰³ The direct and indirect cost of HBP is \$46.4 billion with a projected cost of \$274 billion by 2030.¹⁰⁴ With the increasing cost of HBP-associated healthcare, and HBP being regarded as the most important attributable risk factor for mortality¹⁰⁵, modifiable behaviors factors that may affect blood pressure (BP) are needed to be determined in order to alleviate the public health burden of HBP.

Understanding the effects of dietary patterns and implementing them remains the primary prevention tool for HBP. The Dietary Approaches to Stop Hypertension (DASH) trial was the first feeding trial that provided innovative, solid evidence of how certain food groups consumed together in an eating pattern can influence BP.²² The randomized feeding trial tested the DASH diet, which had lower amounts of total and saturated fat, and cholesterol while providing high intakes of fiber and protein along with antihypertensive minerals such as calcium, potassium, and magnesium, at intakes around the 75th percentile of the usual American consumption, along with the typical American

diet as the control group, and a diet with increased intakes of 8-9 servings/d of fruits and vegetables (FV) only (without enrichment of dairy). The benefits were greatest for both SBP and DBP in the DASH diet group, which additionally contained 2 to 3 servings of low-fat dairy per day, compared to the diet enriched with 8-9 servings of fruits and vegetables (FV) only.²² The most recent Dietary Guidelines for Americans (DGA) released by the United States Department of Agriculture (USDA) and Department of Health and Human Services (DHHS) in 2015 echoed a similar sentiment that eating an overall healthy diet of whole grains, FV, lean meats, poultry, seafood, legumes, nuts, and low-fat or fat-free dairy products can help combat preventable diet-related diseases¹⁰⁶. All of the components promoted by the 2015 DGA are already constituents of the DASH diet. Several longitudinal studies in middle-aged Americans in the NHS¹⁰⁷ and the Insulin Resistance Atherosclerosis Study¹⁰⁸ have linked adherence to the DASH diet to lower risks of chronic diseases such as HBP, CVD, and type 2 diabetes (T2DM). Although the DGA are not specifically targeting BP, its evidence-based guidelines mirroring the DASH diet sends a powerful and influential message to the American public that having an overall balanced diet is consistently associated with positive health outcomes and lower risk of several chronic diseases.

Several longitudinal studies including those in middle-aged Americans in the National Heart, Lung, and Blood Institute Family Heart Study¹⁰⁹ and the Women's Health Study²⁶ observed that increased overall dairy intake is linked with lower mean BP and HBP risk. In 2010, the Dietary Guidelines for Americans committee concluded that there was moderate evidence from mostly observational studies (eight cross-sectional and three prospective cohort studies) and several randomized experimental trials of a beneficial effect of dairy consumption to lower BP, and dairy intake was associated with lower risk of CVD and T2DM¹¹⁰. A recent meta-analysis and systematic review of five observational studies from US and European cohorts by Ralston et al. concluded that total dairy and low-fat dairy intakes were inversely associated with risk of HBP.³⁷ Although the evidence for the beneficial effects of dairy intake on HBP seems consistent based on cross-sectional and longitudinal studies, some have shown mixed findings. Alonso et al showed a beneficial effect of low-fat dairy in the Atherosclerosis Risk in Communities (ARIC) study on BP but no effect of total dairy on BP in African Americans over an 8 year follow up.¹¹¹ This finding is in contrast to findings from the original DASH trial where a greater BP-lowering effect was observed among African Americans than Caucasian participants. One reason for this disparity is likely attributed to the randomized feeding study

design of the DASH trial, where all foods were provided and dietary intake was followed more closely, measured with greater accuracy than with simple dietary recall methods used in observational cohort studies like ARIC.

Some clinical trials have also shown inconsistent results of dairy's effect on BP.

Van Meijl et al conducted a 16 week, randomized crossover feeding trial, in which weight was kept constant, in overweight and obese subjects in which they saw a decrease in SBP with the addition of low-fat dairy (500 mL of low-fat milk and 150 g. of low-fat yogurt at each day of the intervention with both milk and yogurt containing 1.5% fat) but no effect on DBP.¹¹² In contrast, a 6-month clinical trial evaluating the effects of increased total dairy intake on body composition and metabolic health while keeping weight stable saw no effects of 3-5 s/d of dairy supplementation on BP.¹¹³ Although it has been shown that dairy has BP-lowering effects independent of body weight²², it is difficult to tease apart a BP effect from weight change, since most clinical interventions have unintended effects on weight¹¹⁴. Since most of the dairy interventions related to BP have incorporated small changes to eating patterns, these small changes can have sizeable impact on macronutrient intakes and on body weight. The primary focus is how specific changes in dairy consumption impact blood pressure and even

though body composition is an important determinant of BP, the intervention has to be applied in a broader scale in both weight-stable and weight-loss settings. More carefully conducted longitudinal and experimental studies that carefully account for the amount and types of dairy being consumed is the best way to specify what the effect of each dairy product is on BP. This is key as different populations respond to dairy interventions in different capacities.¹¹⁵

In terms of antihypertensive effects of dairy, especially fermented dairy such as yogurt, the casein protein has been studied most extensively¹¹⁶. It has been observed in animal models^{41,117} and in vitro^{118,119} that the caseins in milk facilitate calcium and phosphate absorption in the small intestine.¹¹⁶ Calcium and phosphate are the main substrates needed for the production of small biopeptides which are made from either milk protein digestion in the enterocyte or by fermentation of milk by *lactobacilli* bacteria typically added to yogurt.¹¹⁶ These biopeptides have the potential to be a non-pharmacologic means to lower BP. Observational studies that specifically evaluate yogurt consumption in relation to BP are scarce but beginning to emerge. Beydoun et al showed that yogurt intake was associated with lower SBP using the NHANES 1999-2004 data.⁴⁴ Yogurt was recently shown to be associated with a lower incidence of

metabolic syndrome in an elderly Mediterranean population in the PREDIMED study¹²⁰ and a lower risk of hypertension (HTN) in the Framingham Offspring Study (FOS) Cohort.¹²¹ These observational studies do not have ample statistical power in order to capture effects of usual daily intakes of yogurt.

Long-term observational studies on the effect of dairy, and especially yogurt, on BP are lacking. Only one long-term longitudinal study specifically quantified yogurt's BP-lowering effect. Wang et al conducted their analysis using data from the FOS, a cohort of 2,636 participants followed for over 10 years.¹²¹ This study was limited in its statistical power to only estimate the potential beneficial effect of 1 serving of yogurt per week¹²¹ – an intake level that falls below the USDA recommended 2-3 servings/day of total dairy from various subtypes considered together (milk, cheese, yogurt).¹²² Yogurt is usually combined with other dairy foods as a “total dairy” exposure in epidemiologic analyses, and its specific effects may therefore be underestimated. Yet, there has been a substantial increase in yogurt consumption in the past decade. Only 4.3% of American adults consumed yogurt on a given day in 1999, compared to 9.3% in 2011.¹²³ Thus, investigations into yogurt-specific health effects are warranted. Yogurt is highly concentrated with casein and whey proteins, as well as calcium,

magnesium, and potassium⁶³, all of which have been observed to have BP-lowering effects in animal studies as well as observational and experimental studies in humans¹¹⁵. Well-conducted long-term studies are needed in order to assess the potential benefits of daily yogurt consumption on cardiometabolic risk factors such as HBP in order to refine dietary guidance for the population. The aim of this study is to estimate the long-term effects of total dairy and yogurt intake on incident HBP among middle-aged women and men in the Nurses' Health I, II, and Health Professionals Follow-Up cohorts. These three cohorts are comprised of over 300,000 participants and were followed up for 20-30 years, which enables us to observe enough variation in yogurt intake to be able to estimate yogurt-specific effects on risk of HBP.

3.3 METHODS

3.3.1 Study Population

Data from three prospective cohort studies will be used for these analyses: the Nurses' Health Study (NHS), Nurses' Health Study II (NHS II), and the Health Professionals Follow-Up (HPFS) Study. The NHS was composed of 121,741 female registered nurses, ages 30-55 years at enrollment in 1976 when the first medical history and lifestyle questionnaires were mailed. Follow-up

questionnaires were sent every two years thereafter up through 2010. The NHS II was initiated in 1989 with enrollment of 116,430 female registered nurses, ages 25-42 years, who similarly provided medical history and lifestyle information and completed a follow-up health-related questionnaire every two years up through 2011. The HPFS began in 1986 with 51,529 American men ages 40-75 years, who worked in a variety of medical fields and completed biennial medical and lifestyle questionnaires through 2010. Baseline dietary assessments using a standardized food frequency questionnaire (FFQ) were completed in 1980, 1991, and 1986 for subjects in the NHS, NHS II, and HPFS, respectively.¹²⁴ The goal of these three large cohorts was to examine diet and lifestyle factors that affected overall health and risk of chronic diseases.

For the current analyses, we excluded men and women at the time of the baseline dietary assessments who had diagnoses of HBP, angina, stroke, myocardial infarction (MI), coronary artery bypass grafting (CABG), diabetes (including type 1, type 2, or gestational diabetes), or cancer. Additional exclusion criteria included the following: participants who left >70 of the 131 food items blank on the baseline food frequency questionnaire (FFQ) or who reported unusual total energy intakes (<500 or >3,500 kcals/d for women and <800 or >4,200 kcals/d for

men), had missing follow-up HBP information, missing dairy intake, and high total dairy (≥ 6 s/d), cheese (> 4 s/d), and milk (≥ 6 s/d) intakes. After exclusions, data from 69,351 NHS, 84,368 NHS II, and 30,512 HPFS participants were left for analysis. The study protocol was approved by the institutional review boards of the Boston University School of Medicine and Brigham and Women's Hospital.

3.3.2 Assessment of Dairy and Yogurt Consumption

In the 1980 NHS questionnaire, a 61-item FFQ was administered to NHS participants to collect dietary information on usual intake of foods and beverages in the previous year. To assess updated dietary intake during the extended follow-up, an expanded 131-item FFQ was mailed in 1984, 1986, 1990, 1994, 1998, 2002 and 2006 in the NHS. The expanded FFQ was used for NHS II in 1991, 1995, 1999, 2003, and 2007; similarly for HPFS in 1986, 1990, 1994, 1998, 2002, and 2006.

All of the FFQs asked participants how often, on average, they consumed each food or beverage item in a standard portion size within the past year. There were nine possible responses that ranged from "never, or less than once per month" to "6+ per day." Each FFQ response was converted to a continuous value representing average daily servings consumed. For example, if someone chose

“2-3 per day,” the midpoint value (2.5 servings/day) was used to represent their intake. Table 3.1 below shows the conversion of each possible FFQ response to its corresponding intake value.

Table 3.1 Conversion of FFQ intake categories into continuous frequency intakes (servings/day)

FFQ Category	Daily Intake Value assigned ¹
Never	0.00
1-3/month	0.07
1/week	0.14
2-4/week	0.43
5-6/week	0.79
1/day	1.00
2-3/day	2.50
4-5/day	4.50
6+/day	6.00

¹These values reflect the actual intake as servings/day (s/d). For categories with a range of intake, the midpoint of the range was used and converted to servings/day.

Nutrient intake was calculated by multiplying the frequency of consumption of each food item by the nutrient composition in the standard portion size of that food from the USDA’s food composition database.¹²⁵ The reproducibility and validity of these FFQs have been shown elsewhere.^{124,126-128} The correlation coefficients for individual dairy foods in the NHS are: 0.69 for skim milk, 0.56 for whole milk, 0.97 for yogurt, 0.73 for ice cream, 0.70 for cottage cheese, and 0.57 for hard cheese.¹²⁶

For these analyses, serving sizes are reported in terms of USDA's MyPyramid serving sizes⁸⁵. For a few of the dairy foods on the FFQ, serving sizes did not match the standard MyPyramid serving sizes. These were converted in order to reflect standard dairy food servings as defined by USDA's MyPyramid⁸⁵. For example, the MyPyramid serving size for ice cream, sherbet, and frozen yogurt is 1 ½ cups⁸⁵. Therefore, ice cream, sherbet, and frozen yogurt intakes reported on the FFQ were divided by 3 in order to represent a fraction of the MyPyramid serving of dairy. Using the MyPyramid definition of a dairy product (foods that are made from milk and retain their calcium content,)⁸⁵ cream cheese, butter, and cream were excluded as part of the total dairy variable⁸⁵. Total dairy intake in these analyses includes: all milk (skim, low-fat, reduced-fat, and whole), ice cream, sherbet/frozen yogurt, cheese (cottage/ricotta, hard, sliced), and yogurt. Total yogurt intake will be used as the exposure in these analyses. Therefore, all varieties of yogurt were summed together to create the total yogurt exposure for these analyses.

3.3.3 High Blood Pressure Outcome Ascertainment

At every biennial questionnaire, participants in each cohort were asked if they were diagnosed with HBP by a physician. This method of self-report has been

previously validated in the NHS cohort: a subsample of 161 women who did not report HBP had their BP checked by a physician; all measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels were below 140 and 90 mm Hg, respectively. Furthermore, self-reported HBP diagnoses were validated for 51 out of 62 NHS participants who agreed to have their medical records checked.¹²⁹ Validity was similarly observed in the NHS II: relevant medical records were obtained from a subset of randomly selected NHS II participants who self-reported a new diagnosis of HBP on the 2005 questionnaire, along with randomly selected nurses who denied this diagnosis in 2005 and in every previous year. The sensitivity of self-reported HBP was 94% in NHS II and the specificity of a nurse reporting no diagnosis of HBP was 85%¹³⁰. In HPFS, a random sample of 100 participants reporting a diagnosis of HBP on the 1988 questionnaire was followed-up, where 39 agreed to have their medical records accessed. The records confirmed that all of them had HBP or were receiving antihypertensive treatment¹³¹.

Subjects with prevalent HBP at the time of the baseline dietary assessments were excluded from these analyses. A participant was considered to have prevalent HBP if he or she reported this diagnosis at the baseline questionnaire: 1980

(NHS), 1991 (NHS II), or 1986 (HPFS). Participants were considered to be incident HBP cases if they reported a new physician-diagnosed HBP diagnosis on the routine follow-up questionnaires.

3.3.4 Covariate assessment

Updated information on chronic disease risk factors such as body weight (in kg), age (years), physical activity, and family history of HTN were asked in the biennial follow-up questionnaires and race was asked in the baseline questionnaire. Height was converted to meters and used to calculate BMI (weight/height²). Participants reported their average weekly time spent in several recreational activities including: walking or hiking, jogging, running, bicycling, and heavy outdoor work. Participants also reported their usual walking pace and the number of flights of stairs climbed daily. Total metabolic equivalent (MET) hours of activity per week (MET-hr/week)^{132,133} were calculated in order to incorporate activity duration, intensity, and frequency. The reproducibility and validity of these questionnaire have been described previously.¹³⁴ In a subgroup of NHS II participants (N=151), the correlation over a 1-year period between activity reported via questionnaire vs past-week recalls was 0.79. For vigorous activities, the correlation was 0.62.¹³⁴

Since yogurt has been associated with an overall healthy diet⁵², A DASH diet score was constructed in order to observe potential effect modification of an overall healthy diet on the yogurt-specific effect on HBP. The calculation of the DASH diet score has been previously described¹⁰⁷. In brief, 8 foods and nutrients that characterize the DASH diet comprised the DASH diet score: high intakes of low-fat dairy products, fruits, vegetables, nuts and legumes, and whole grains and low intakes of red and processed meats, sugar-sweetened beverages and sodium¹³⁵. A DASH diet score was calculated using data from each FFQ by classifying the participants into quintiles of intake for each of the 8 aforementioned components. For whole grains, low-fat dairy, fruits, vegetables, and nuts and legumes, those in the highest quintile received a score of 5 and those in the lowest quintile of intake received a score of 1. For sodium, sugar-sweetened beverages, and red and processed meats, those in the lowest quintile of intake received a score of 5 and those in the highest quintile of intake received a score of 1. The DASH score was calculated by summing the quintile rankings of the 8 components, yielding a range from 8-40, with 40 being the score that most closely resembled a DASH diet.

3.4 STATISTICAL ANALYSES

Cumulative averages of dietary intakes were used in these analyses and their calculation has been described previously elsewhere.¹³⁶ In these analyses, dietary intakes from each diet questionnaire were given equal weight in the calculation of the cumulative average. For example, the cumulative average intake of yogurt in 1999 is the simple average of yogurt intake from 1991, 1995, and 1999. Figure 1 below illustrates the calculation of cumulative average yogurt intake in the NHS II at each dietary questionnaire year.

Figure 3.1 Study diagram for NHS II



Cumulative average yogurt at 2003= $\text{mean}(\text{yogurt}_{91}+\text{yogurt}_{95}+\text{yogurt}_{99}+\text{yogurt}_{03})$

Cumulative averages were calculated from baseline up to the censoring events of death, end of study, or lost to follow-up. When a participant reported HBP, diet at the previous exam was carried forward in order to best represent long-term dietary intake and to minimize within-person variation¹³⁷. In addition, the updating of cumulative average dietary intake was stopped when participants developed MI, CABG, stroke, T2DM, or cancer as these diagnoses may prompt a

change in diet, which may confound the relationship between dairy and HBP.^{137,138} Total dairy and yogurt intakes at previous questionnaires were carried forward for those with missing follow-up dairy and yogurt intakes. While those with missing dietary data at all exam years were excluded from these analyses.

Total dairy was separated into high-fat and low-fat to assess whether dairy fat had differential effects on risk of HBP. High-fat dairy included whole milk, high-fat cheese, and ice cream. Low-fat dairy was comprised of reduced fat and skim milk, yogurt, low-fat cheese, sherbet, and cottage cheese. Total, high-fat, and low-fat dairy, as well as yogurt, milk, and cheese were categorized in order to estimate the independent effect of each dairy exposure. Cutpoints were chosen to optimize analytic power and to represent intakes that are easily interpretable and applicable to both daily recommendations and the FFQ categories. For example, yogurt intake was categorized as: <1 serving/month (s/mo) (ref), 1 s/mo-<1 serving/week (s/wk), 1-<2 s/wk, 2-<5 s/wk, and ≥ 5 s/wk. Total dairy was categorized into the following categories: <0.5 s/d (ref), 0.5-<1.5 s/d, 1.5-<3 s/d, 3-<6 s/d.

To determine whether the effects of yogurt were modified by a healthy diet pattern, we removed dairy and yogurt in the DASH diet score and used two new DASH scores (one without dairy and one without yogurt) to make combined categories with dairy and yogurt intake. This enabled us to see the independent and combined effect of a high dairy/yogurt intake and an overall healthy diet as measured by the DASH score. Both derived DASH scores included: fruits, vegetables, nuts and legumes, and whole grains and low intakes of red and processed meats, sugar-sweetened beverages and sodium. The only difference is that the DASH score without yogurt has all the other low-fat dairy components in the score while the DASH score without dairy does not have dairy included in the score.

Sensitivity analyses were used in order to further collapse yogurt intake into three categories: <1/month (low), 1/month-<5/week (moderate), ≥5/week (high). These three yogurt categories were then combined with tertiles of the DASH diet score (without yogurt) in order to see possible additive effects of a high yogurt intake and varying levels of the DASH diet score. Similar combined categories were created via sensitivity analyses for total dairy with tertiles of the DASH diet score (without dairy).

BMI can also act as a potential effect modifier or a causal intermediate in the association between total dairy or yogurt on HBP. Therefore, yogurt and dairy intakes were combined with three BMI categories (<25 , $25-<30$, ≥ 30 kg/m²) in order to see the differential effects of dairy and yogurt among the normal weight, overweight and obese.

Each participant's person years of follow-up began from the date of return of the baseline FFQ to the first occurrence of any of the following events: HBP diagnosis, death, loss to follow-up, or end of follow-up (30 June 2010 for NHS, 30 June 2009 for NHS II, and 31 January 2010 for HPFS). Time-dependent Cox proportional hazard regression models were used to estimate the hazard ratio (HR) and the 95% confidence interval (CI) for all dairy exposure variables in relation to incident HBP risk. Multivariable models to estimate the independent effect of yogurt on HBP included: age, race, family history of HBP, physical activity, total energy, total protein intake, intakes of fruit and vegetable, cheese, and milk. Updated BMI was added in a separate model to determine potential mediating effects of body composition on HBP risk.

To test for linear trend, the median value was assigned to each exposure category and this value was modeled as a continuous variable. All of the analyses were conducted separately in each cohort. A fixed-effects model was used to combine all three cohorts by a meta-analysis approach, having ruled out substantial enough heterogeneity of effects that would have required the use of a random effects model. All statistical tests were two-sided using alpha levels of 0.05 for all statistical tests and the construction of confidence intervals. SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) was used to perform all statistical analyses.

3.5 RESULTS

There were 81,908 total cases of incident HBP, including 41,584 cases during a maximum of 30 years of follow-up in the NHS, 26,134 cases during a maximum of 20 years in the NHS II, and 14,190 cases during a maximum of 24 years in the HPFS.

Table 3.2 Age-standardized demographic characteristics of the NHS, NHS II, and HPFS study cohort at baseline by yogurt intake categories

A. NHS (N=69,351)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 22,456)	1/mo-<1/wk (N = 14,700)	1-<2/wk (N = 20,134)	2-<5/wk (N = 10,721)	≥5/wk (N = 1,340)
Age (years) ²	46.7 (7.2)	45.2 (7.2)	44.6 (7.0)	44.5 (6.9)	45.2 (7.1)

Activity (MET-hrs/wk)	11.5 (15.5)	12.8 (19.2)	14.4 (18.9)	16.3 (21.3)	19.0 (27.1)
BMI (kg/m ²)	23.9 (4.1)	24.0 (4.0)	23.9 (3.9)	23.7 (3.9)	23.5 (3.9)
<25 kg/m ² , %	69.2	69.7	71.5	73.5	74.8
25-<30 kg/m ² , %	22.3	21.7	21.0	19.7	19.6
≥30kg/m ² , %	8.5	8.5	7.6	6.8	5.7
Race (white, %)	97.8	97.4	98.0	97.9	97.7
Current smoker, %	37.9	27.3	23.9	22.5	22.7
Family history HBP (%)	39.2	44.0	46.0	45.8	37.5
BP-lowering medication, %	5.7	5.5	5.8	5.8	5.6
Current post-menopausal hormone use, %	5.9	6.0	5.7	6.2	5.6

B. NHS II (N=84,368)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 23,429)	1/mo-<1/wk (N = 18,196)	1-<2/wk (N = 23,540)	2-<5/wk (N = 16,598)	≥5/wk (N = 2,605)
Age (years) ²	36.0 (4.7)	35.9 (4.7)	35.9 (4.7)	36.0 (4.7)	36.1 (4.6)
Activity (MET-hrs/wk)	18.0 (24.8)	19.3 (25.4)	21.5 (26.9)	25.1 (31.3)	30.4 (36.1)
BMI (kg/m ²)	24.7 (5.4)	24.4 (5.0)	23.9 (4.5)	23.9 (4.6)	24.1 (5.0)
<25 kg/m ² , %	64.2	66.9	70.9	71.3	69.3
25-<30 kg/m ² , %	21.2	20.8	19.8	19.3	18.9
≥30kg/m ² , %	14.6	12.3	9.4	9.4	11.9
Race (white, %)	95.3	96.2	97.3	97.8	97.5
Current smoker, %	16.6	11.7	10.3	9.3	9.5
Family history HBP, %	52.9	51.6	50.8	51.5	53.3
BP-lowering medication, %	2.5	2.2	1.59	1.8	2.1
Current post-menopausal hormone use, %	2.6	2.24	2.3	2.2	3.0
Oral contraceptive use, %	11.1	11.1	11.3	11.0	10.4

C. HPFS (N=30,512)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 15,856)	1/mo-<1/wk (N = 6,201)	1-<2/wk (N = 4,901)	2-<5/wk (N = 2,910)	≥5/wk (N = 644)
Age (years) ²	52.6 (9.3)	50.7 (8.9)	50.0 (8.7)	51.2 (9.2)	51.5 (9.6)
Activity (MET-hrs/wk)	19.8 (27.1)	22.9 (33.0)	25.7 (33.7)	26.0 (29.2)	32.5 (41.6)
BMI (kg/m ²) [*]	25.3 (3.0)	25.2 (3.0)	25.0 (3.0)	24.9 (3.1)	24.4 (2.8)
<25 kg/m ² , %	50.0	50.7	54.7	55.5	63.5
25-<30 kg/m ² , %	43.6	43.3	40.2	38.5	33.2
≥30kg/m ² , %	6.5	6.0	5.1	6.0	3.3
Race (white, %)	95.3	95.4	96.2	95.2	95.6
Current smoker, %	11.6	6.4	4.6	3.6	2.6
Family history HBP, %	34.0	34.2	35.9	36.2	37.4
BP-lowering medication, %	2.00	2.33	1.88	2.21	2.29

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population.

²Value is not age adjusted

The age-adjusted baseline demographic characteristics by 1 cup servings/d (1 C s/d) of yogurt in all three cohorts are shown in Table 3.2. Mean age at baseline for NHS, NHS II, and HPFS were: 44.7, 35.8, and 50.8 years respectively. Those in the highest yogurt intake category were more active, least likely to be overweight or obese and smokers. In both NHS II and HPFS, those with the highest yogurt intake (≥ 5 /week) had the highest percentage of those having a family history of HBP while the opposite was observed in the NHS. We also observed that the percentage of participants on BP-lowering medication was similar in all yogurt categories among all cohorts

Table 3.3. Age-standardized diet and food intakes of the NHS, NHS II, and HPFS study cohorts at baseline by yogurt intake categories

A. NHS (N=69,351)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 22,456)	1/mo-<1/wk (N = 14,700)	1-<2/wk (N = 20,134)	2-<5/wk (N = 10,721)	≥ 5 /wk (N = 1,340)
Alcohol (g/d)	6.7 (11.5)	6.1 (10.0)	6.1 (9.5)	5.9 (9.0)	5.8 (9.3)
DASH diet score	22.0 (4.5)	23.5 (4.5)	24.5 (4.4)	25.7 (4.4)	26.5 (4.4)
FOODS					
Whole grains (g/d)	11.5 (12.1)	14.0 (13.1)	15.1 (13.1)	16.4 (13.8)	17.1 (13.9)
Total fiber (g/d)	15.6 (3.9)	16.3 (4.0)	16.8 (4.2)	17.4 (4.4)	17.6 (4.4)
Fruits & vegetables (s/d)	3.5 (1.9)	3.9 (2.0)	4.1 (2.0)	4.6 (2.2)	5.0 (2.4)
SSB (12 oz. or 1 can s/d)	0.53 (0.93)	0.41 (0.75)	0.37 (0.71)	0.34 (0.72)	0.34 (0.77)
Red & processed meats (s/d)	1.2 (0.99)	1.2 (0.96)	1.1 (0.94)	1.0 (0.91)	1.0 (0.95)
B. NHS II (N=84,368)	Yogurt Intake Categories (1 C s/d)¹				

	<1/mo (N = 23,429)	1/mo-<1/wk (N = 18,196)	1-<2/wk (N = 23,540)	2-<5/wk (N = 16,598)	≥5/wk (N = 2,605)
Alcohol (g/d)	2.8 (6.3)	3.1 (6.0)	3.3 (6.0)	3.4 (5.7)	3.3 (5.9)
DASH score	21.9 (4.7)	23.8 (4.7)	25.0 (4.8)	26.5 (4.8)	27.8 (4.9)
FOODS					
Whole grains (g/d)	17.1 (15.1)	20.4 (15.3)	21.7 (15.7)	23.3 (15.9)	23.6 (17.1)
Total fiber (g/d)	17.0 (5.3)	18.1 (5.3)	18.7 (5.3)	19.5 (5.6)	20.1 (7.0)
Fruits & Vegetables (s/d)	4.3 (2.59)	4.8 (2.6)	5.3 (2.8)	6.0 (3.1)	6.7 (3.6)
SSB (12 oz. s/d)	0.63 (1.0)	0.47 (0.83)	0.41 (0.74)	0.35 (0.67)	0.31 (0.69)
Red & Processed meats (s/d)	1.2 (0.73)	1.2 (0.68)	1.1 (0.66)	1.1 (0.67)	1.0 (0.69)
C. HPFS (N=30,512)					
	Yogurt Intake Categories (1 C s/d)¹				
	<1/mo (N = 15,856)	1/mo-<1/wk (N = 6,201)	1-<2/wk (N = 4,901)	2-<5/wk (N = 2,910)	≥5/wk (N = 644)
Alcohol (g/d)	11.8 (15.8)	10.2 (13.7)	9.7 (13.0)	9.7 (13.2)	9.4 (12.5)
DASH score	22.3 (5.0)	24.4 (5.0)	25.6 (4.9)	26.8 (4.9)	28.1 (4.9)
FOODS					
Whole grains (g/d)	18.9 (18.4)	23.6 (19.3)	25.3 (20.0)	27.4 (21.2)	28.5 (22.5)
Total fiber (g/d)	19.6 (6.7)	21.5 (6.7)	22.3 (7.2)	23.2 (7.1)	23.2 (7.8)
Fruits & vegetables (s/d)	4.8 (2.5)	5.5 (2.7)	6.0 (2.9)	6.5 (3.0)	7.1 (3.5)
SSB (12 oz. s/d)	0.43 (0.69)	0.35 (0.58)	0.30 (0.49)	0.29 (0.52)	0.27 (0.49)
Red & processed meats (s/d)	1.28 (0.87)	1.07 (0.77)	1.01 (0.75)	0.92 (0.75)	0.88 (0.80)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population. SSB=sugar-sweetened beverages

Table 3.3 illustrates that those in the highest yogurt intake (≥5/week) had the highest caloric intake as well as diet quality as measured by the DASH score among all three cohorts. This is consistent with higher intakes of foods that are associated with a healthy diet such as fruits and vegetables, beans and legumes, whole grains, and fiber. Conversely, those in the highest yogurt intake had the lowest intakes of foods associated with a lower overall diet quality such as sugar-sweetened beverages (SSB) and red and processed meats.

Table 3.4 Age-standardized nutrient intakes of the NHS, NHS II, and HPFS study cohorts at baseline by yogurt intake categories

A. NHS (N=69,351)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 22,456)	1/mo-<1/wk (N = 14,700)	1-<2/wk (N = 20,134)	2-<5/wk (N = 10,721)	≥5/wk (N = 1,340)
Calories (kcal/d)	1539 (504)	1538 (490)	1566 (490)	1639 (500)	1739 (542)
Total protein (g/d)	72.8 (14.7)	75.3 (14.7)	76.9 (14.8)	78.5 (15.2)	79.4 (15.7)
Total carbohydrates (g/d)	152 (38.2)	154 (36.2)	156 (35.2)	163 (35.3)	172 (37.1)
Total fat (g/d)	72.5 (14.0)	70.8 (13.5)	69.1 (13.4)	65.7 (13.4)	61.6 (14.4)
MINERALS					
Sodium (mg/d)	1287 (290)	1306 (277)	1316 (275)	1331 (274)	1348 (297)
Potassium (mg/d)	2600 (602)	2719 (596)	2805 (589)	2934 (592)	3066 (647)
Calcium (mg/d)	628 (348)	680 (350)	729 (354)	830 (393)	997 (525)
Magnesium(mg/d)	266 (88.8)	280 (89.8)	292 (92.5)	314 (98.4)	338 (109)
B. NHS II (N=84,368)	Yogurt Intake Categories (1C s/d) ¹				
	<1/mo (N = 23,429)	1/mo-<1/wk (N = 18,196)	1-<2/wk (N=23,540)	2-<5/wk (N=16,598)	≥5/wk (N = 2,605)
Calories (kcal/d)	1704 (547)	1752 (538)	1810 (534)	1893 (540)	1992 (571)
Total protein (g/d)	84.6 (16.0)	86.1 (15.0)	86.8 (14.7)	87.9 (14.7)	89.3 (15.9)
Total carbohydrates (g/d)	220 (36.1)	223 (32.8)	226 (32.1)	230 (31.7)	237 (33.8)
Total fat (g/d)	65.8 (11.7)	63.8 (10.8)	62.4 (10.6)	60.0 (10.6)	57.1 (11.4)
MINERALS					
Sodium (mg/d)	2149 (411)	2162 (363)	2159 (343)	2146 (337)	2119 (356)
Potassium (mg/d)	2763 (546)	2894 (511)	2975 (503)	3098 (506)	3254 (574)
Calcium (mg/d)	927 (436)	997 (429)	1045 (424)	1111 (421)	1225 (461)
Magnesium (mg/d)	292 (73.3)	312 (72.8)	322 (71.8)	336 (72.3)	348 (75.0)
C. HPFS (N=30,512)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 15,856)	1/mo-<1/wk (N = 6,201)	1-<2/wk (N = 4,901)	2-<5/wk (N = 2,910)	≥5/wk (N = 644)
Calories (kcal/d)	1969 (617)	1985 (615)	2056 (615)	2123 (627)	2296 (673)
Total protein (g/d)	90.5 (16.4)	92.8 (16.0)	93.0 (15.6)	94.0 (16.0)	94.2 (16.7)
Total carbohydrates (g/d)	229 (42.0)	238 (40.7)	244 (39.3)	251 (39.9)	261 (41.6)
Total fat (g/d)	74.1 (13.7)	70.9 (13.5)	69.1 (13.1)	66.0 (13.3)	62.2 (14.2)
MINERALS					
Sodium (mg/d)	3348 (1160)	3307 (1124)	3216 (1014)	3104 (997)	2876 (961)
Potassium (mg/d)	3256 (669)	3431 (671)	3533 (666)	3635 (675)	3770 (729)
Calcium (mg/d)	741 (379)	795 (383)	867 (390)	938 (399)	1156 (533)
Magnesium (mg/d)	321 (108)	343 (112)	364 (119)	385 (123)	423 (139)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population.

Table 3.4 lists the baseline macro- and micronutrient intake by yogurt intake categories among all three cohorts. Those with the highest yogurt intake (≥ 5 /week) had the highest total protein and carbohydrate intake as well as all four minerals: sodium, potassium, magnesium, and calcium. Conversely, high yogurt eaters had the lowest total fat intakes with health professionals (mean fat = 71.1 g/d) and NHS (mean fat = 68.4 g/d) participants having a slightly higher mean intake than the NHS II (mean total fat = 64.5 g/d).

Table 3.5 Age-standardized dairy of the NHS, NHS II, and HPFS study cohorts at baseline by yogurt intake categories

A. NHS (N=69,351)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 22,456)	1/mo-<1/wk (N = 14,700)	1-<2/wk (N = 20,134)	2-<5/wk (N = 10,721)	≥ 5 /wk (N = 1,340)
Total dairy (s/d)	1.2 (0.93)	1.3 (0.92)	1.4 (0.92)	1.8 (0.98)	2.1 (1.2)
Low-fat dairy (s/d)	0.54 (0.77)	0.69 (0.79)	0.84 (0.82)	1.1 (0.89)	1.52 (1.2)
High-fat dairy (s/d)	0.51 (0.63)	0.49 (0.58)	0.49 (0.54)	0.50 (0.55)	0.50 (0.60)
Cheese (1 oz. s/d)	0.35 (0.43)	0.38 (0.42)	0.43 (0.44)	0.45 (0.44)	0.46 (0.52)
Yogurt (1 C s/d)	0.01 (0.02)	0.05 (0.08)	0.11 (0.19)	0.27 (0.37)	0.61 (0.83)
Total milk (8 oz. s/d)	0.80 (0.98)	0.87 (0.98)	0.90 (0.96)	0.96 (1.0)	1.1 (1.1)
% dairy from yogurt	1.6 (8.5)	8.0 (16.5)	12.9 (20.3)	21.3 (24.6)	31.8 (29.1)
B. NHS II (N=84,368)	Yogurt Intake Categories (1 C s/d) ¹				
	<1/mo (N = 23,429)	1/mo-<1/wk (N = 18,196)	1-<2/wk (N = 23,540)	2-<5/wk (N=16,598)	≥ 5 /wk (N = 2,605)
Total dairy (s/d)	1.5 (1.2)	1.7 (1.2)	1.8 (1.2)	2.1 (1.2)	2.5 (1.3)
Low-fat dairy (s/d)	0.94 (1.0)	1.1 (1.0)	1.3 (1.1)	1.6 (1.1)	1.9 (1.2)
High-fat dairy (s/d)	0.53 (0.58)	0.53 (0.56)	0.55 (0.53)	0.55 (0.54)	0.55 (0.59)
Cheese (1 oz. s/d)	0.41 (0.42)	0.43 (0.43)	0.46 (0.42)	0.48 (0.44)	0.49 (0.49)
Yogurt (1 C s/d)	0.01 (0.03)	0.06 (0.07)	0.14 (0.18)	0.29 (0.30)	0.63 (0.63)
Total milk (8 oz. s/d)	0.96 (1.0)	1.1 (1.0)	1.1 (1.0)	1.2 (1.0)	1.2 (1.1)

% dairy from yogurt	1.2 (4.5)	5.4 (8.6)	9.1 (12.4)	15.5 (16.5)	25.9 (21.5)
C. HPFS (N=30,512)	Yogurt Intake Categories (1 C s/d)¹				
	<1/mo	1/mo-<1/wk	1-<2/wk	2-<5/wk	≥5/wk
	(N = 15,856)	(N = 6,201)	(N = 4,901)	(N = 2,910)	(N = 644)
Total dairy (s/d)	1.3 (1.1)	1.4 (1.1)	1.6 (1.1)	1.8 (1.2)	2.4 (1.4)
Low-fat dairy (s/d)	0.72 (0.98)	0.88 (0.97)	1.0 (1.0)	1.2 (1.1)	1.8 (1.5)
High-fat dairy (s/d)	0.63 (0.75)	0.57 (0.61)	0.59 (0.63)	0.58 (0.64)	0.63 (0.72)
Cheese (1 oz. s/d)	0.42 (0.47)	0.41 (0.44)	0.44 (0.44)	0.44 (0.44)	0.52 (0.58)
Yogurt (1 C s/d)	0.01 (0.02)	0.06 (0.08)	0.16 (0.20)	0.30 (0.29)	0.78 (0.76)
Total milk (8 oz. s/d)	0.85 (1.0)	0.89 (0.98)	0.94 (1.0)	0.97 (1.0)	1.1 (1.1)
% dairy from yogurt	0.96 (4.63)	6.6 (10.5)	12.7 (16.2)	19.8 (19.9)	34.0 (23.2)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population.

From Table 3.5, we observed that those in the highest yogurt intake had higher intakes of dairy protein as well as higher intakes of total, low-fat, and high-fat dairy. Most of the total dairy intake in all three cohorts were from low-fat dairy, with milk comprising most of the low-fat dairy intake.

Table 3.6 The beneficial effects of higher yogurt intake on incident HBP

Yogurt Intake	P-yrs	Cases	I /100K p-yrs	NHS Multivariable		+BMI	
				HR ¹	95% CI	HR ²	95% CI
<1/mo	613915	18014	2934	1.00	-	1.00	-
1/mo-<1/wk	291261	8998	3089	1.00	(0.98-1.03)	0.99	(0.97-1.02)
1-<2/wk	271482	9397	3461	0.98	(0.95-1.01)	0.98	(0.96-1.01)
2-<5/week	141841	4495	3169	0.92	(0.89-0.96)	0.95	(0.92-0.98)
≥5/week	28333	680	2400	0.80	(0.74-0.87)	0.86	(0.80-0.93)
<i>P for linear trend³</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.81	(0.76-0.86)	0.87	(0.82-0.92)
Yogurt Intake	P-yrs	Cases	I /100K p-yrs	NHS II Multivariable		+BMI	
				HR ¹	95% CI	HR ²	95% CI

<1/mo	491776	9422	1916	1.00	-	1.00	-
1/mo-<1/wk	307914	5668	1841	0.95	(0.92-0.98)	0.96	(0.93-0.99)
1-<2/wk	300582	5981	1990	0.90	(0.87-0.93)	0.94	(0.91-0.97)
2-<5/week	228960	4330	1891	0.89	(0.85-0.92)	0.92	(0.89-0.96)
≥5/week	47026	733	1559	0.83	(0.77-0.90)	0.89	(0.82-0.96)
<i>P for linear trend</i> ³				<0.0001		<0.0001	
Per 1 s/d increase				0.79	(0.74-0.85)	0.86	(0.81-0.92)

HPFS

Multivariable

+BMI

Yogurt Intake	P-yrs	Cases	I /100K		HR ¹	95% CI	HR ²	95% CI
			P-yrs	p-yrs				
<1/mo	265024	7844	2960	1.00	-	1.00	-	
1/mo-<1/wk	107086	3026	2826	0.97	(0.93-1.02)	0.97	(0.93-1.01)	
1-<2/wk	67656	1943	2872	0.93	(0.88-0.98)	0.93	(0.88-0.98)	
2-<5/week	40237	1119	2781	0.92	(0.87-0.98)	0.94	(0.88-1.00)	
≥5/week	10123	258	2549	0.94	(0.83-1.07)	1.01	(0.89-1.14)	
<i>P for linear trend</i> ³				0.0172		0.1738		
Per 1 s/d increase				0.89	(0.81-0.98)	0.94	(0.85-1.03)	

META-ANALYSIS

Multivariable

+BMI

Yogurt Intake	P-yrs	Cases	I /100K		HR ¹	95% CI	HR ²	95% CI
			P-yrs	p-yrs				
<1/mo	864199	35280	2605	1.00	-	1.00	-	
1/mo-<1/wk	679150	17692	2710	0.98	(0.96-1.00)	0.98	(0.96-1.00)	
1-<2/wk	438457	17321	2456	0.95	(0.93-0.96)	0.96	(0.94-0.98)	
2-<5/week	115596	9944	2190	0.91	(0.89-0.93)	0.94	(0.92-0.96)	
≥5/week	10123	1671	2549	0.84	(0.79-0.88)	0.89	(0.85-0.94)	
<i>P for linear trend</i> ³				<0.0001		<0.0001		
<i>P for heterogeneity</i> ⁴				0.3194		0.5835		
<i>I</i> ²				13.4		0.0		
Per 1 s/d increase				0.81	(0.78-0.85)	0.87	(0.84-0.91)	

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, milk, and cheese

²Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, milk, and cheese, BMI

³Linear trend across yogurt intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable.

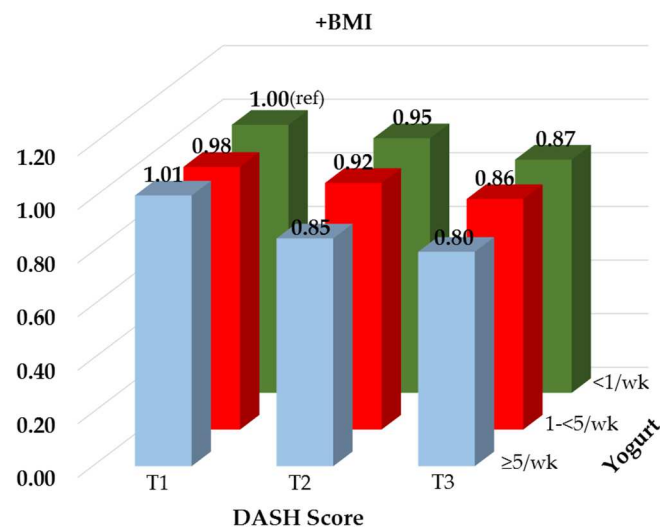
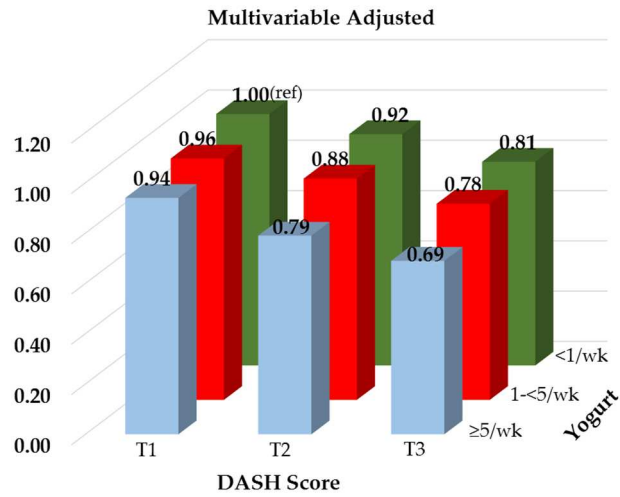
⁴Test for between-study heterogeneity by using a fixed effects meta-analysis model

The beneficial effects of increased long-term yogurt intakes are shown in Table 3.6. After adjusting for race, physical activity, energy intake, family history of HTN, and intakes of FV, milk, and cheese, a 20% (95% CI: 0.74-0.87), 17% (95% CI: 0.77-0.90), and a 6% (95% CI: 0.83-1.07) HBP risk reduction was observed for those who had a usual yogurt intake of 5 or more servings/wk compared to those who ate yogurt less than once a month in the NHS, NHS II, and HPFS cohorts respectively.

Since BMI may be in the causal pathway between yogurt consumption and HBP risk, we included BMI in a separate multivariable model. This led to some attenuation of the beneficial effect of yogurt intake in the models for all three cohorts. Finally, we carried out a meta-analysis, pooling the results from the three cohorts. Here, we observed that those with a usual yogurt intake of 5 or more servings/week had an overall 16% decreased risk of long-term incident HBP (95% CI: 0.79-0.88) compared to those with a yogurt intake of less than once a month. A significant linear decreasing trend of HBP risk with increasing yogurt intake was also observed (p for trend <0.0001). We modeled yogurt as a continuous variable in order to conceptualize the effect for each one serving increase per day. In the multivariable model, we observed a 19% decreased risk

(95% CI: 0.78-0.85) of incident HBP for each one-serving increase in yogurt consumption. The p value for heterogeneity from the fixed effects meta-analysis for both the multivariable model and the BMI-added model were both non-significant ($p>0.05$) and suggests that there is no significant heterogeneity among the three cohorts. This was further shown by quantification of the heterogeneity by the I^2 statistic: 13.4% for the multivariable model and 0% when BMI was added.

Figure 3.2 Higher yogurt intake combined with higher DASH diet score is linked with a lower risk of incident HBP



We performed sensitivity analyses on the effects of yogurt intake collapsed into three categories (low, moderate, and high) from the original five categories in Table 3.6 in order to combine the yogurt intake categories into three groups with the DASH diet score and simplify the illustration of the combined effect of

yogurt intake and diet quality. Then collapsed yogurt categories are shown in the Appendix tables A.1-A.4.

Figure 3.2 shows the combined effect of the DASH diet score and yogurt intake on HBP risk across all three cohorts. Compared to those with low yogurt intake and a low DASH score (the referent group), those who had a higher DASH score alone had a 19% (95%CI: 0.79-0.83) reduction in HBP risk. Higher yogurt intakes with a low DASH score led to very modest reductions in HBP risk. In contrast, consuming five or more servings of yogurt per week combined with a high DASH score (T3) was associated with a 31% reduced risk of HBP (95%CI: 0.65-0.74). This finding suggests that the effects of yogurt were modified by the overall diet pattern, since the joint effect (31% lower risk) was greater than the sum of the independent effects of a high yogurt intake (6% reduced risk) and a high DASH dietary score (19% reduced risk in tertile 3). The addition of BMI to the multivariable model attenuated HBP risk reduction associated with the combined yogurt and DASH score to 20% (95%CI: 0.74-0.85). We observed a range of heterogeneity estimates in the yogurt and DASH groups when the three cohorts were pooled. The cohort-specific results are shown in the Appendix Tables A.5-A.7.

Figure 3.3 BMI has a greater effect on HBP risk than yogurt

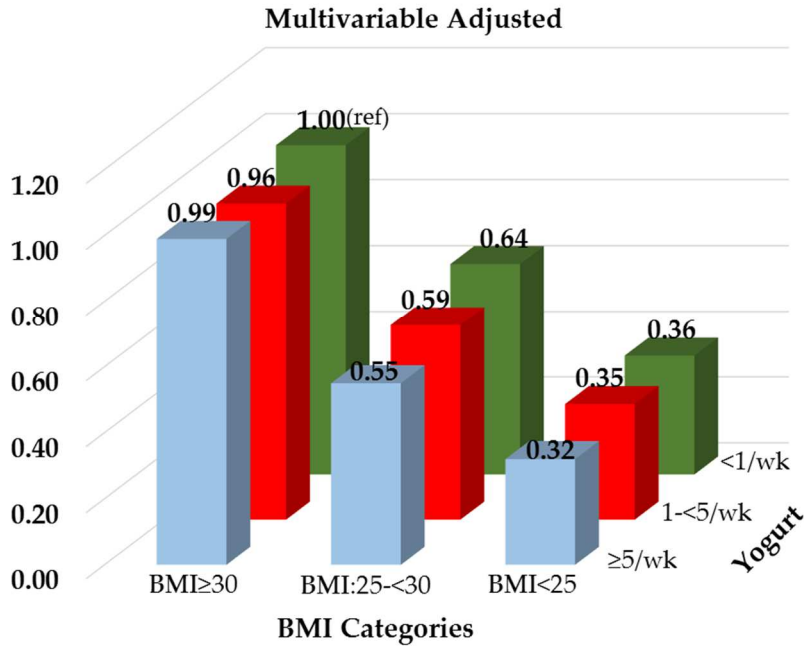


Figure 3.3 illustrates the differential effects of yogurt intake among three different BMI groups: lean (BMI < 25 kg/m²), overweight (BMI: 25-30 kg/m²) and obese (BMI ≥ 30 kg/m²). In all three cohorts, the effects of BMI alone are substantially greater than the effects of yogurt alone on HBP risk. For example, those with the highest yogurt intake (≥5 s/week) who were obese had a non-statistically significant 1% decreased risk of long-term incident HBP (95% CI: 0.90-1.10) compared to the referent group of low yogurt (<1/week) and obese. However, those with low yogurt intake but were overweight saw a 36 % decreased BMI risk (95% CI: 0.62-0.65) and those who were lean and also had a low

yogurt intake saw a 64% decreased risk of HBP (95% CI: 0.30-0.35) compared to the referent group of low yogurt (<1/week) and obese. Overall, the pooled results suggest that the combined effects of yogurt consumption and BMI are approximately additive. There was a wide range of heterogeneity observed as quantified by the *p* for heterogeneity and *I*² values for each yogurt and BMI category. Cohort specific results are illustrated in the Appendix Tables A.8-A.10.

Table 3.7 Higher milk intake is associated with a lower risk of HBP

Milk Intake	P-yrs	Cases	META-ANALYSIS				
			I /100K	Multivariable		+BMI	
			p-yrs	HR ¹	95% CI	HR ²	95% CI
<4/wk	1364819	35607	2609	1.00	-	1.00	-
4/wk-<1/d	586561	16424	2800	0.98	(0.96-0.99)	0.98	(0.96-1.00)
1-<2/d	780951	19469	2493	0.95	(0.93-0.96)	0.96	(0.94-0.98)
2-<6/d	480886	10408	2164	0.87	(0.85-0.89)	0.92	(0.90-0.94)
				<i>P for linear trend</i> ³		<0.0001	
				<i>P for heterogeneity</i> ⁴		0.0024	
			<i>I</i> ²	93.9		84.3	
Per 1 s/d increase				0.94	(0.93-0.95)	0.96	(0.95-0.97)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and cheese

²Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and cheese, BMI

³Linear trend across milk intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable.

⁴Test for between-study heterogeneity by using a fixed effects model

Table 3.7 shows that increased milk intake has slight, but linear beneficial effects on incident HBP across all three cohorts (*p* for trend<0.001). After adjustment for

age, race, physical activity, energy intake, family history of HBP, and intakes of total protein, FV, yogurt, and cheese, a high milk intake of 2-<6 s/d was associated with a 13% (95% CI: 0.85-0.89) decreased risk of incident HBP with a slight attenuation when BMI was added to the model. An increase of 1 s/d of milk was associated with a modest 6% risk reduction in HBP. There was a high level of heterogeneity among the three studies, with an I^2 of 93.9% and 84.3% in the multivariable and the BMI-adjusted model, respectively. The cohort specific effects are shown in the Appendix Tables A.11-A.13.

Table 3.8 Increased cheese consumption is inversely associated with risk of HBP in women

Cheese Intake ¹	P-yrs	Cases	I /100K p-yrs	META-ANALYSIS		+BMI	
				HR ¹	95% CI	HR ²	95% CI
<1/week	920076	22529	2449	1.00	-	1.00	-
1-4/week	1746029	46656	2672	0.99	(0.97-1.01)	0.97	(0.95-0.99)
5/week-<1/d	396193	9672	2441	0.96	(0.94-0.99)	0.94	(0.91-0.96)
1-4/d	150918	3051	2022	0.92	(0.88-0.96)	0.90	(0.86-0.93)
				<i>P for linear trend</i> ³		<0.0001	
				<i>P for heterogeneity</i> ⁴		0.0003	
				I^2	88.1		66.3
Per 1 s/d increase				0.93	(0.90-0.96)	0.90	(0.88-0.93)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and milk

²Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and milk, BMI

³Linear trend across cheese intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

Table 3.8 shows that after adjusting for the covariates in the multivariable model, those in the highest cheese intake category (1-4 s/d) experienced a 10% (95%: 0.84-0.97) and 14% (95%: 0.81-0.91) decreased risk in long-term incident HBP in the NHS and NHS II cohorts respectively. The addition of BMI did not change these effects in either cohort. Higher cheese intake had no effect on incident HBP in the HPFS. After pooling the three cohorts, significant heterogeneity was observed ($I^2=88.1\%$ for the multivariable model and $I^2=66.3$ for the BMI-adjusted model). Those with the highest cheese intake had a modest but statistically significant 8% (95%: 0.88-0.96) decreased HBP risk compared to the referent group consuming <1 s/week, and a significant inverse linear trend was observed (p for trend <0.0001). A 1 s/d increase was associated with a 7% HBP risk reduction. Cohort specific effects are shown in the Appendix Tables A.14-A.16.

Table 3.9 Increased total dairy intake has beneficial effects on HBP risk

Dairy Intake ¹	P-yrs	Cases	I /100K p-yrs	NHS		+BMI	
				HR ¹	95% CI	HR ²	95% CI
<0.5	232919	6218	2670	1.00	-	1.00	-
0.5-<1.5	638802	19994	3130	0.97	(0.94-0.99)	0.96	(0.93-0.99)
1.5-<3	393942	12813	3253	0.91	(0.88-0.94)	0.91	(0.88-0.94)

3-<6	81170	2559	3153	0.87	(0.83-0.92)	0.91	(0.86-0.96)
<i>P for linear trend</i> ³				<0.0001		<0.0001	
Per 1 s/d increase				0.95	(0.94-0.97)	0.96	(0.95-0.98)
NHS II							
				Multivariable		+BMI	
I /100K							
Dairy Intake	P-yrs	Cases	p-yrs	HR¹	95% CI	HR²	95% CI
<0.5	115675	2369	2048	1.00	-	1.00	-
0.5-<1.5	579951	11728	2022	0.95	(0.91-0.99)	0.95	(0.91-0.99)
1.5-<3	493490	9380	1901	0.87	(0.83-0.92)	0.90	(0.86-0.95)
3-<6	187142	2657	1420	0.74	(0.69-0.78)	0.80	(0.75-0.85)
<i>P for linear trend</i> ⁴				<0.0001		<0.0001	
Per 1 s/d increase				0.91	(0.89-0.92)	0.94	(0.92-0.95)
HPFS							
				Multivariable		+BMI	
I /100K							
Dairy Intake	P-yrs	Cases	p-yrs	HR¹	95% CI	HR²	95% CI
<0.5	66701	1948	2921	1.00	-	1.00	-
0.5-<1.5	240324	7118	2962	0.98	(0.93-1.03)	0.96	(0.91-1.01)
1.5-<3	133590	3763	2817	0.89	(0.84-0.95)	0.89	(0.84-0.94)
3-<6	49511	1361	2749	0.91	(0.84-0.98)	0.90	(0.83-0.97)
<i>P for linear trend</i> ³				<0.0001		<0.0001	
Per 1 s/d increase				0.96	(0.94-0.98)	0.96	(0.94-0.98)
META-ANALYSIS							
				Multivariable		+BMI	
I /100K							
Dairy Intake	P-yrs	Cases	p-yrs	HR¹	95% CI	HR²	95% CI
<0.5	415295	10535	2537	1.00	-	1.00	-
0.5-<1.5	1459077	38840	2662	0.96	(0.94-0.99)	0.96	(0.94-0.98)
1.5-<3	1021022	25956	2542	0.90	(0.87-0.92)	0.90	(0.88-0.93)
3-<6	317823	6577	2069	0.83	(0.80-0.86)	0.87	(0.84-0.90)
<i>P for linear trend</i> ³				<0.0001		<0.0001	
<i>P for heterogeneity</i> ⁴				<0.0001		0.0203	
				<i>I</i> ²	92.3	75.3	
Per 1 s/d increase				0.94	(0.93-0.95)	0.95	(0.94-0.96)

¹Adjusted for age, race, physical activity, FV, total protein, energy intake, HBP family history

²Adjusted for age, race, physical activity, FV, total protein, energy intake, HBP family history, BMI

³Linear trend across total dairy intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

The effects of total dairy intake among the NHS, NHS II, and HPFS cohorts are demonstrated in Table 3.9. Compared to the referent group of <0.5 s/d, those in the highest intake group (3-<6 s/d) had a statistically significant decreased HBP risk of 13% (95% CI: 0.83-0.92), 26% (95% CI: 0.69-0.78), and 9% (95% CI: 0.84-0.98) in the NHS, NHS II, and HPFS cohorts. The addition of BMI to the multivariable model attenuated these effects slightly but they remained statistically significant. Using a fixed effects model in the meta-analysis, we observed significant heterogeneity ($I^2=92.3\%$ for the multivariable model) and a 17% (95% CI: 0.80-0.86) decreased risk of long-term HBP incident to those with a total dairy intake of 3-<6 s/d compared to the referent group of <0.5 s/d. The addition of BMI decreased the heterogeneity ($I^2=72.3\%$) and slightly attenuated the beneficial effect to 13% risk reduction (95% CI: 0.84-0.90). A 1 s/d increase was linked with a 6% reduced HBP risk. Only the 3-<6 s/d intake group had a high I^2 (> 80%) whereas the other dairy intake categories had low heterogeneity indicators ($I^2 < 10\%$).

Figure 3.4 Higher DASH diet scores modifies the beneficial effect of total dairy intake on incident risk of HBP

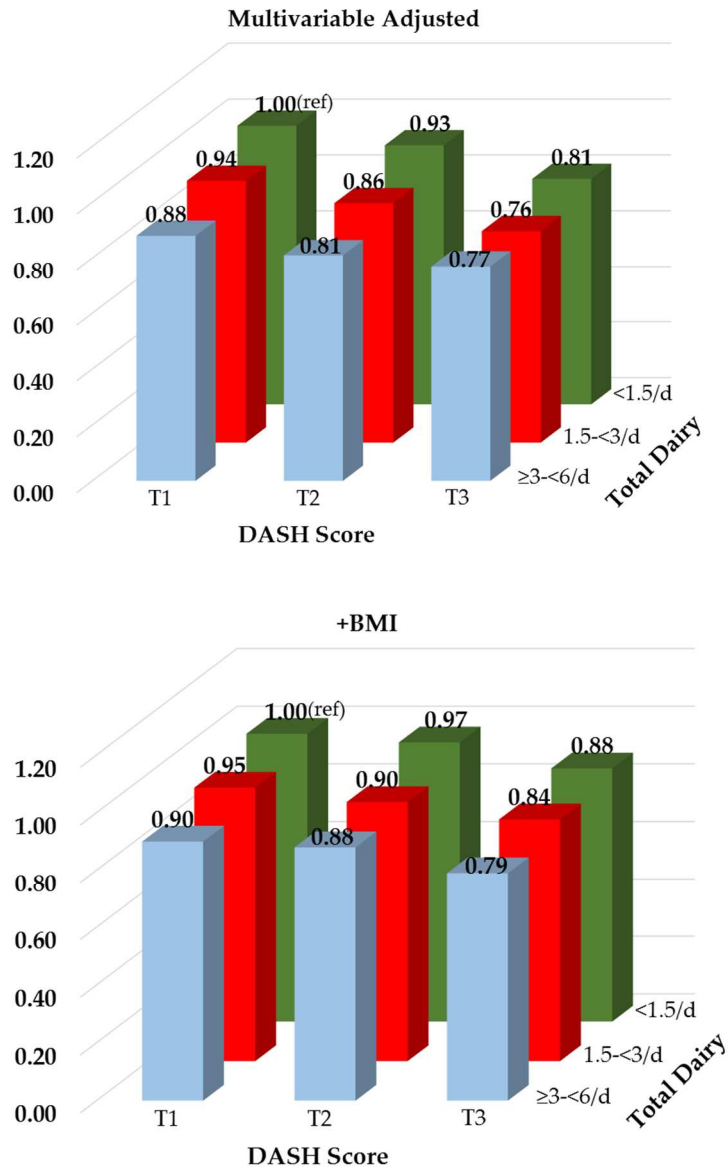


Figure 3.4 illustrates the combined effects of total dairy intake and an overall healthy dietary pattern as measured by the DASH diet score. When the three cohorts were pooled, compared to those with the lowest total dairy intake (<1.5 s/d) and the lowest DASH score (referent group), those with the highest total dairy intake (3-<6 s/d) but the lowest DASH score experienced a 12% decreased

HBP risk (95% CI: 0.83-0.92). Those with the lowest dairy intake but the highest DASH score had a 19% decreased risk in incident HBP (95% CI: 0.79-0.83). The joint effects of both a high total dairy intake and a high DASH score had contributed to a 23% decreased risk of HBP compared to the referent group (95% CI: 0.77-0.83). The addition of BMI to the model attenuated the effect to a 21% decreased HBP risk (95% CI: 0.76-0.83). After performing a meta-analysis to combine all three cohorts, we saw a range of within-group heterogeneity estimates. Cohort-specific results are shown in the Appendix Tables A.17-A.19.

3.6 DISCUSSION

We observed a 20%, 17%, and a 6% decreased long-term incident HBP risk among those who consumed a 1C serving of yogurt ≥ 5 servings/week in the NHS, NHS II, and the HPFS cohorts, respectively. The beneficial effects of yogurt consumption were generally weaker in the HPFS cohort, which may be due to the low number of men in the highest yogurt intake category (≥ 5 /week). The men in the HPFS were also older compared to the women in both NHS cohorts. Therefore, many men were excluded at baseline due to prevalent HBP, limiting statistical power in the HPFS. Since age is a strong determinant of HBP risk, it is

possible that the remaining non-hypertensive men at baseline reflect those who are relatively resistant to the development of HBP.

Yogurt has been shown to have inverse associations with several cardiometabolic risk factors such as weight gain⁴⁶, metabolic syndrome¹³⁹, common carotid artery intima-media thickness²⁸, as well as incidence of CVD⁷⁰ and diabetes¹⁴⁰. A cross-sectional study using NHANES data reported a lower SBP and prevalence of metabolic syndrome among those with higher yogurt intake⁴⁴. We observed that those who have the highest usual yogurt intake had an overall healthier diet as measured by the DASH diet score. Those in the highest yogurt intake category combined with a high DASH diet score, experienced the greatest reduction in HBP risk. A previous analysis of the Framingham Offspring and Third Generation cohorts found that higher yogurt consumers tended to have lower risks of developing cardiometabolic abnormalities such as HBP, lower circulating triglycerides, glucose, and insulin resistance even after adjusting for diet quality.⁵² The Framingham study only dichotomized yogurt intake as consumers and non-consumers whereas our study had enough statistical power to observe a linear trend of HBP risk reduction across categories of yogurt consumption for both NHS cohorts.

For total dairy, those with a high intake (3-6 s/d) had an overall 17% risk reduction in incident HBP risk among the three cohorts. This observation is in alignment with other longitudinal studies across several populations such as the Framingham Offspring Study¹²¹, middle-aged Caucasian men and women in the ARIC cohort¹¹¹, and a middle-aged (30-65 years) free-living French cohort,⁴⁵ all of which demonstrated modest decreases in BP associated with an increased dairy consumption. Recent clinical trials have also shown reductions in BP among hypertensives individuals with a dairy intervention^{141,142}. Stancliffe and colleagues designed a 12-week randomized, parallel clinical trial in 40 overweight and obese middle-aged American adults, who were randomized to either a low-dairy (<0.5 dairy serving/d and <600 mg calcium/d) or adequate-dairy (>3.5 dairy servings/d from milk and/or yogurt, ≥1200 mg calcium/d) weight-maintenance diet. The investigators found a dairy intervention to lower SBP and DBP throughout the study, as well as lower levels of inflammatory markers such as tumor necrosis factor- α and interleukin-6¹⁴¹. Although there is a moderate amount of evidence from both observational and intervention studies that supports a BP-lowering effect of dairy intake, several observational studies found no association with dairy intake on BP^{28,30}. These null association may be

explained by having different types of dairy in varying proportions across studies. In our analyses, milk was the primary source of dairy (61.7% of dairy coming from milk) and yogurt constituting a small portion (8.4%). With the combination of all dairy subtypes serving as the exposure of interest in these analyses, the independent effects of each type of dairy may be underestimated. For example, our pooled analysis estimated the beneficial effect of a one- serving- per- day increase in milk intake was not as robust as the magnitude of effect associated with a one-serving-per-day increase in yogurt intake (6% risk reduction with milk vs. 19% risk reduction with yogurt).

In accordance with the 2010 DGAC report, we observed that high intakes of total dairy alone and combined with an overall healthy diet (high DASH score) were linked with lower risk of HBP in all three cohorts¹¹⁰. Several observational studies and clinical trials^{26,112,143}, including the original DASH trial²², observed a BP- lowering effect of increased low-fat dairy intake combined with a DASH dietary pattern. This was similarly observed in our analyses where those with high dairy intake and a high DASH diet score had a 41% reduced risk of HBP. Our results suggest that all dairy products have modest beneficial effects on HBP incidence. Individuals who consume more yogurt (and more dairy) were also consuming

less red and processed meats, SSB, refined carbohydrates, and added sugars in our cohorts. Therefore, the observed beneficial effects of dairy could be interpreted as a replacement effect: high dairy consumers ate less refined carbohydrates and SSB, which may affect their risk of HBP. We controlled for many potential dietary confounders, including SSB, red meats, processed meats, and refined grains, but none of them seem to confound the associations, which is why they were not included in the final multivariable model. The observational nature of our analyses limits the interpretation of whether the observed beneficial effects of dairy on BP was because of the higher dairy intake or because of the lower SSB, refined grains, red and processed meats, or other foods that are associated with a poor overall diet.

BP is affected by a variety of mechanisms. Potassium is concentrated in certain foods, including many fruits, vegetables, and dairy products and has been shown to lower BP in a dose-response manner among both normotensive and hypertensive individuals¹⁴⁴. Hyperpolarization of endothelial cells relies on adequate potassium levels and results in a lower concentration of calcium in the cytosol, which results in smooth muscle cell relaxation and vasodilation, which lowers BP¹⁴⁵. During fermentation, biologically active peptides are formed when

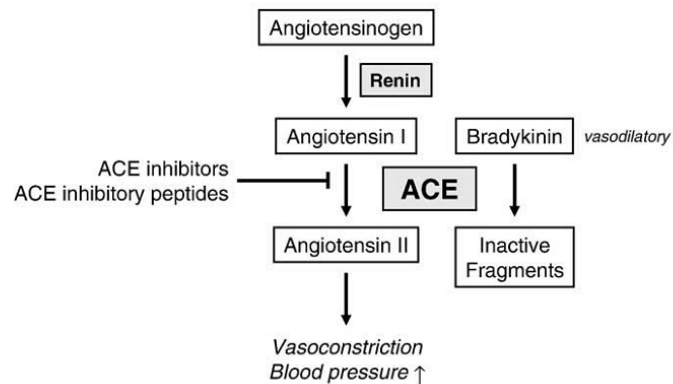
milk proteins are catalyzed by proteolytic lactic acid bacteria, such as *Lactobacillus helveticus*¹⁴⁶. Two of these peptides that have been extensively studied for their BP-lowering effects are isoleucine-proline-proline (IPP) and valine-proline-proline (VPP). Both have been shown to acutely reduce BP in spontaneously hypertensive rats (SHR) after a single administration⁴⁰. A long-term study in SHR showed that fermented milk with added IPP and VPP and antihypertensive minerals magnesium, calcium, and potassium attenuated HBP development more effectively than just fermented alone, fermented milk with magnesium, calcium, potassium only, or fermented milk with IPP and VPP but without minerals. This suggests that the overall nutrient profile of fermented dairy may have the most potent antihypertensive effects¹⁴⁷. A meta-analysis on 14 randomized feeding trials with duration from 4-24 weeks in both normotensive and hypertensive adults aged 35-75 years estimated a 2 mm Hg decrease in SBP with a fermented milk intervention compared to the control placebo¹⁴⁸. Although these human trials are of short duration, they suggest a potential, non-pharmacologic route to lower BP.

The main mechanism that has been studied extensively with regards to the BP-lowering effects of milk-derived peptides is the inhibition of the angiotensin-

converting enzyme (ACE). ACE is the rate-determining enzyme in the function of the renin-angiotensin system (RAS), a key regulator of BP, fluid, and electrolyte balance¹⁴⁹. In the RAS, ACE converts angiotensin I to angiotensin II, which is a potent vasoconstrictor that induces aldosterone release, increases sodium levels, and leads to higher BP levels. IPP and VPP from casein-derived peptides have exhibited ACE inhibiting properties¹⁵⁰ by reducing the rate of production of angiotensin II¹⁵¹ and promoting the vasodilator release¹⁵¹. IPP and VPP-specific ACE inhibition has been shown in-vitro⁹⁴ and in SHR¹⁵² via increasing renin concentrations during long-term HTN treatment.

Antihypertensive milk-derived peptides have also been observed to have no ACE-inhibitor activity in those without HTN¹⁵³, which suggests that these peptides could affect BP via ACE-independent mechanisms. Figure 8 below shows a diagram adapted from Erdmann et al,¹⁵⁴ depicting how these dairy-derived biopeptides affect BP mechanistically.

Figure 8. An overview of the renin-angiotensin system and the potential hypotensive effects of dairy biopeptides (adapted from Erdmann et al., 2008).



ACE not only converts angiotensin I to angiotensin II but it also hydrolyzes bradykinin, which has vasodilatory effects.

Weight loss has been observed to have a major beneficial impact on HBP risk.

Our results correlate with that hypothesis as those who were normal weight at baseline had significantly lower incident HBP risk regardless of yogurt intake compared with overweight and obese subjects. In fact, previous analyses in these same three cohorts showed an inverse association between yogurt consumption and weight gain⁴⁶. The addition of BMI as a potential confounder in our multivariable model partially attenuated the beneficial effects of yogurt intake alone and combined with the DASH diet (as a marker of an overall healthy diet) on HBP risk, suggesting a potential intermediate effect of yogurt on body weight. A recent systematic review of 6 prospective cohort and 7 cross-sectional studies linked higher dairy, especially yogurt on lower long-term weight gain¹⁵⁵.

Although some intervention trials showed a beneficial effect of dairy intake on weight loss³³, via increased fat loss¹⁵⁶ and prevention of lean body mass,¹⁵⁷ others reported no evidence that a diet high in dairy enhanced weight loss during energy restriction in obese individuals^{158,159}. It should be noted that incorporation of dairy into a weight loss regimen had no adverse effect on weight loss across the majority of the trials^{33,156,157}. Although there are inconsistencies between epidemiologic and intervention studies on the effects of dairy on weight loss, evidence suggests a modest short-term weight loss of increased dairy intake. A recent meta-analysis of 29 randomized controlled trials of over 2400 participants (median age=41.4 years) found no long-term benefits of increased dairy consumption on body weight and body fat¹⁶⁰. The meta-analysis did suggest that increased dairy intake may have facilitate weight loss in short-term or energy-restricted RCTs¹⁶⁰. The observational nature of our study limits our interpretation of the role of body composition as either a confounder or a potential causal intermediate in the association of dairy on HBP, although it seems likely that the true effect may lie somewhere between the effects seen with and without BMI in the model.

Aside from their BP-lowering mechanism, milk proteins have also been shown to stimulate insulin secretion¹⁶¹. Insulin secretion may directly affect food intake regulation by suppressing appetite, which consequently, indirectly affects body weight¹⁶¹. Dairy protein also contains a high amount of the three branched-chain amino acids (BCAAs) leucine, valine, and isoleucine, which have a unique role in protein synthesis and lean body mass preservation during weight loss¹⁶². Milk proteins have also been shown to increase plasma concentrations of satiety hormones such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1)¹⁶³. Whey and casein have differing effects on appetite regulation: whey has been classified to suppress food intake in the short-term¹⁶⁴ while casein, a bigger protein than whey, has a delayed transit time through the GI tract, delaying digestion time¹⁶⁵. The literature supports the obesity-lowering effects of dairy but the exact mechanism of actions by different types of dairy (milk, cheese, or yogurt) remains unclear.

Our study has several methodologic strengths including a large sample size that enabled us to study a wide range of yogurt intakes. The high follow-up rates and availability of repeated measures of dietary intake as well as demographic and lifestyle variables are also important strengths. To our knowledge, this is the first

longitudinal study with sufficient power to estimate the long-term dose-response relation of usual yogurt intake and incident HBP risk. A recent study by Wang et al using FOS data found that for every additional serving of yogurt per *week*, there was a 4% reduction in HBP risk¹²¹. The wider range of intakes and greater statistical power across our three cohorts enabled us to estimate with notable precision a 19% reduction in HBP risk for each additional one serving of yogurt consumed per *day*.

Our study is also subject to several limitations. First, the NHS, NHS II, and HPFS cohorts consisted predominantly of Caucasian nurses and health professionals of European ancestry. While the homogeneity of racial, educational and socioeconomic status may help to reduce confounding, our results cannot be generalized to other populations. There is strong evidence of racial differences in HBP risk in the literature¹⁶⁶ but our results are limited to middle-aged Caucasians. Although our study suffers from a lack of generalizability, the relatively high educational status of our participants is also a strength because of the increased likelihood that the data collected were reliable. Dietary assessment by FFQs have been shown to suffer from measurement and random error. However, the FFQs used in our cohorts were validated against multiple diet

records, and reasonable correlation coefficients were observed for dairy foods. Moreover, cumulative averages of all dietary variables were calculated in order to minimize the random measurement error caused by within-person variation and to accommodate diet and secular changes over time. Although we had ample statistical power (particularly in women) to observe associations of usual yogurt intake up to what is recommended by the USDA (1 s/d), the type and brand name of yogurt consumed was not available from the FFQs. Therefore, it is difficult to attribute the observed benefits to various components of yogurt such as protein or probiotic content, that have been observed in other lines of research to have antihypertensive effects. Although we adjusted for known and potential risk factors for HBP and even created a DASH diet score to consider possible confounding and effect modification by a healthy dietary pattern associated with yogurt intake, unmeasured and residual confounding is still a possibility.

We carried out a meta-analysis of the three cohorts in this study to estimate the overall effect of total dairy and the individual dairy components on incident HBP risk. We observed a wide range of heterogeneity estimates for most of the meta-analyses except for yogurt. This could be due to the inherent differences among the cohorts in terms of various BP-related risk factors such as sex and age (mean

baseline ages were 45, 36, and 51 years in the NHS, NHS II, and HPFS cohorts respectively). Additionally, men have a higher risk of HBP than women until age 45 and women having a higher risk of HBP at age 65 years and above¹⁶⁷, which highlights how age is a strong predictor of HBP risk especially between men and women. Yogurt is usually eaten as an individual food and may be susceptible to less bias and misreporting compared to milk and cheese, which has been shown to be susceptible to under-reporting and misclassification when eaten as part of mixed dishes¹⁶⁸. These reasons may have contributed to the high heterogeneity estimates for milk ($I^2=93.9\%$) and cheese ($I^2=88.1\%$). All of these factors suggest that the effect estimates from the meta-analyses may not necessarily be from the same underlying population, pooling all three cohorts may suffer from excess heterogeneity, and less heterogeneity may be observed if the cohorts were presented separately.

The only dairy exposure in which there was little to no heterogeneity when the results were pooled for all three cohorts was for yogurt intake. This is consistent with the finding that yogurt was associated with a healthy diet pattern. Yogurt eaters tend to eat higher intakes of FV, fiber, lean meats, and dairy⁵². As we observed, yogurt eaters also tend to consume fewer SSB and eat less red and

processed meats. Having a similar underlying dietary pattern would reduce the heterogeneity among the yogurt eaters, which may explain the low heterogeneity in our meta-analysis of yogurt intake categories.

3.7 CONCLUSION

We found that higher usual intakes of total dairy and all subtypes (low-fat, high-fat, milk, cheese, and yogurt) was associated with a lower risk of HBP across the NHS, NHS II, and HPFS cohorts, with yogurt having the greatest effect of all dairy foods. The effects of each dairy exposure seemed to be strongest in the younger women in NHS II. The consistent findings for yogurt suggest that this specific dairy food can be recommended with confidence for inclusion into a healthy dietary pattern, which may lessen the necessity of pharmacologic HBP treatment. However, more long-term studies are needed to further elucidate what bioactive component(s) in yogurt are driving its antihypertensive effects. Randomized clinical trials are also warranted to further examine the causal effects of yogurt consumption, its bioactive nutrient profile, as well as its probiotic content on body weight and HBP. Finally, these results suggest that regular intake of yogurt may have important public health benefits in terms of

reducing the burden of HBP and its consequences, as least within Caucasian adults.

**CHAPTER 4: THE BENEFICIAL EFFECTS OF INCREASED YOGURT
CONSUMPTION ON RISK OF CARDIOVASCULAR DISEASE AMONG
MIDDLE-AGED ADULTS WITH HIGH BLOOD PRESSURE**

4.1 ABSTRACT

Background: High blood pressure (HBP) is a major risk factor for cardiovascular disease (CVD), the leading cause of worldwide mortality. Short-term clinical trials have indicated beneficial effects of higher dairy intakes, especially yogurt, among those with HBP on CVD, but long-term observational data among hypertensives have not been published.

Objective: To estimate the independent effects of total dairy and yogurt on risk of incident CVD among middle-aged female nurses in the Nurses' Health Study (NHS) and male health professionals in the Health Professionals Follow-Up Study (HPFS) with HBP.

Methods: Analyses include subjects from NHS and HPFS participants (age 30-55 years, n=57,768 and age 40-75 years, n=18,593, respectively) with reported HBP and without prevalent CVD, diabetes, type 2 diabetes (T2DM), or cancer were used. Cumulative average dairy intakes were derived from validated, semi-quantitative food frequency questionnaires. CVD, defined as myocardial

infarction (MI) and stroke, was ascertained via self-report and validated by medical record review. NHS and HPFS subjects were followed for development of CVD over a maximum period of 30 and 26 years, respectively. Time dependent Cox proportional hazard's models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) while controlling for potential confounding by age, race, family history of HBP or MI, BP medication use, smoking, alcohol, energy intake, physical activity, BMI, and intakes of fiber and *trans* fats.

Results: Yogurt intake was inversely associated with risk of CVD among these hypertensive participants (p for linear trend <0.01 for both NHS and HPFS). Participants who had a usual yogurt intake of ≥ 2 s/wk in the NHS and HPFS had 17% (95% CI: 0.75-0.92) and 18% (95% CI: 0.68-0.97) CVD risk reductions, respectively. The effect of yogurt was further modified by a healthy diet pattern as measured by a DASH score. Regular yogurt consumers (≥ 2 s/wk in the NHS; ≥ 1 s/wk in the HPFS) who also had higher DASH diet scores, had 19% (95% CI: 0.71-0.92) and 31% (95% CI: 0.57-0.83) lower risks of CVD, respectively. Men consuming 2- <5 s/d of milk had a 16% (95% CI: 0.72-0.98) lower risk of CVD while in women, higher cheese intake (1-4 s/d) was linked with an 8% (95% CI: 0.83-1.01) lower risk of CVD. Total dairy intake was not associated with risk of

CVD in women but HPFS men with intakes of 2-<6 s/d of total dairy had a 15% (95% CI: 0.75-1.05) CVD risk reduction. Those with a high dairy intake (1-<6 s/d) combined with a high DASH score (≥ 22) experienced a 12% (95% CI: 0.79-0.97) and a 16% (95% CI: 0.73-0.97) lower risk of CVD in NHS and HPFS subjects, respectively.

Conclusion: In both women and men with prevalent HBP, higher usual yogurt intake was associated with a lower risk of developing CVD. Milk and total dairy were associated with a modest CVD risk reduction in hypertensive men while cheese consumption was associated with a small CVD risk reduction in hypertensive women. When combined with a higher DASH score, higher total dairy and yogurt intakes led to statistically significant reductions in risk of CVD in both men and women. These results suggest that the incorporation of yogurt into a healthy diet pattern in hypertensive adults may provide a non-pharmacological approach to CVD prevention.

4.2 BACKGROUND

While the prevalence of high blood pressure (HBP) in the American adult population of 18 years and older is currently estimated to be approximately 33%, this figure is projected to increase to around 41% by 2030². In 2010, HBP-related health care costs and productivity losses in the US were estimated at \$76.6 billion¹⁶⁹. HBP remains one of the major critical risk factors for cardiovascular disease (CVD), which is the leading cause of death amongst Americans,⁵ and makes up 17% of overall national health spending¹⁷⁰. The total medical costs for CVD exceed \$273 billion and are projected to triple to \$818 billion by 2030 with an additional \$276 billion in indirect costs.¹⁰⁴ Recent health metrics estimate that 40.6% of CVD mortality in the US population is attributable to HBP¹⁷¹. A meta-analysis of 29 prospective cohort studies with over 1 million participants, found that HBP and pre-HBP were associated with stroke, myocardial infarction (MI), and CVD incidence.¹⁷² HBP not only affects about one billion people worldwide but there is compelling evidence that it is a major cause of cardiovascular morbidity and overall mortality², making it a widespread and significant health concern. As the key risk factor for CVD, HBP is a particularly valuable target for the control of future total costs of CVD.¹⁰⁴

According to the American Heart Association (AHA), 69%, 77%, and 74% of those who experienced their first heart attack, stroke, or congestive heart failure, respectively, had HBP⁴. Starting with a systolic blood pressure (SBP) level of 155 mm Hg or a diastolic blood pressure (DBP) level of 75 mm Hg, Lewington et al observed that across 61 longitudinal studies, an increase of 20/10 mm Hg in SBP/DBP levels was associated with more than a twofold increase in death rates from stroke, ischemic heart disease and other vascular causes.¹⁷³ Previous studies found that those who were pre-hypertensive (SBP of 130–139; DBP 85–89 mm Hg) had an elevated risk of CVD¹⁷⁴, especially stroke, cardiovascular death, and MI¹⁷⁵ compared with those with BP levels <120/80 mmHg.^{174,175}

HBP is linked to an increased risk of CVD through a variety of mechanisms. Arterial stiffness, which is associated with atherogenesis¹⁷⁶, is also an independent predictor of cardiovascular morbidity and mortality in hypertensives¹⁷⁷ and may lead to higher BP levels¹⁷⁸. A higher SBP reflects the stiffening of arterial walls in the areas that are exposed to the increased BP¹⁷⁸.

Adequate blood flow to the myocardium depends on the myocardial perfusion pressure during diastole¹⁷⁹. Higher overall BP levels can be attributed in part to arterial stiffening, which could lead to increased systemic circulatory load and subsequent risk of CVD death.¹⁸⁰ Another pathway by which HBP can affect

CVD progression is through its deteriorating effect on vascular endothelial function¹⁸¹. Vascular endothelial cells protect blood vessels and prevent arteriosclerosis by releasing various BP-regulating substances that have the ability to inhibit monocyte adhesion and/or thrombus formation¹⁸².

While the *Dietary Guidelines for Americans* (DGA) called for limiting saturated fat (SFA) intake in order to lower CVD risk¹⁶⁷, increased dairy consumption, particularly from yogurt, has paradoxically been shown to have beneficial effects on CVD-related comorbidities. These include: antihypertensive effects^{183,184}, lower abdominal body fat^{184,185}, lower risk of type 2 diabetes and insulin resistance^{185,186}, and higher high density lipoprotein cholesterol (HDL-C) levels¹⁸⁴. Yogurt intake in the US has increased significantly within the past decade¹²³. According to NHANES, 4.7% of American adults consumed yogurt on a given day in 1999-2000, which doubled to 9.3% by 2011-2012¹²³. With a recent significant rise in yogurt intake, it's important to recognize yogurt's unique health benefits. Earlier studies suggested that regular consumption of fermented dairy products such as yogurt was associated with a lower risk of atherosclerotic vascular disease (ASVD)¹⁸⁷ and a reduction in arterial stiffness¹⁸⁸ in hypertensive subjects. In one study, investigators recruited 89 hypertensive Danish adults (mean age=49 years) to participate in a 24-week randomized, placebo-controlled, double-blind parallel

trial where they observed a reduction in augmentation index (a surrogate marker of arterial stiffness) with *Lactobacillus helveticus*-fermented milk containing bioactive peptides (valine-proline-proline (VPP) and isoleucine-proline-proline (IPP)) compared to fermented milk without the bioactive peptides¹⁸⁹.

A meta-analysis performed by Dong et al on 13 randomized controlled trials in both normotensive and hypertensive adults 35 years or older found that an intervention containing fermented milk was associated with an overall 2 mm Hg decrease in SBP.¹⁴⁸ This decrease in SBP was found to be associated with 7% and 10% lower CHD and stroke mortality risks, respectively¹⁷³. The trials among hypertensives in the meta-analysis by Dong and colleagues had an overall 4 mm Hg decrease in SBP with fermented milk supplementaion¹⁴⁸, suggesting a greater CVD benefit among hypertensives compared to non-hypertensives¹⁷³. Hirota et al showed that fermented milk tripeptides VPP and IPP improved vascular endothelial function independent of their BP-lowering effects in a randomized trial of hypertensive males,¹⁹⁰ suggesting that fermented dairy products have CVD-lowering capabilities that are independent of their BP-lowering effects.

While yogurt is known to be a nutrient-rich food as well as a potential source of probiotic bacteria, there are few, large, longitudinal studies that substantiate the health effects of yogurt. Studies have often included yogurt within overall dairy

consumption and therefore, its potential unique effects may be underestimated due to the varying nutrient profiles of other dairy foods. To our knowledge, there is no published evidence on the effects of long-term yogurt intake on CVD risk among those with HBP.

Several longitudinal studies have examined the effects of total dairy intake on incident CVD among those without HBP. Their findings have been conflicting: several reported that total milk was inversely associated with stroke^{69,191-193} and CHD¹⁹¹, while other studies suggest that milk intake is associated with an increased risk of both CHD¹⁹⁴ and stroke¹⁹⁵. Additionally, a few reported no appreciable association between total dairy intake and CHD^{196,197}. The use of differing categories of dairy intake in observational studies as well as differences in the population groups across studies make the results difficult to compare. Most studies used quintiles or quartiles of intake to estimate the distribution of milk consumption, thus making it difficult to compare the dose-response effects across studies. Also, the likely misclassification of reported dairy intakes inherent in dietary assessment is another potential source of inconsistency in findings due to measurement error. Finally, there is the potential for biased reports of dairy exposure given the long-term public health messaging associated with dairy products and heart disease in the US¹⁹⁸.

The Dietary Approaches to Stop Hypertension (DASH) Trial was a landmark trial that showed that a diet rich in fruits and vegetables (FV), whole grains, and low-fat dairy products (particularly low-fat milk and yogurt) had a strong BP-lowering effect²². It should be noted that along with 2-3 servings of low-fat dairy, the DASH diet had higher servings of full-fat dairy (0.7 s/d) compared to the control and FV only groups (0.4 and 0.3 s/d respectively).²² This suggests that moderate consumption of full-fat dairy, in conjunction with a DASH diet that already contains 2-3 s/d of low-fat dairy, may have beneficial effects on BP. The original DASH intervention trial lowered SBP by 11 mm Hg, on average, compared to the typical American diet among hypertensive participants²². The DASH diet has been incorporated into the recommended lifestyle changes for those with HBP by the Eighth Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC)¹⁹⁹. In observational research, a prospective cohort study of 5,532 hypertensive adults in the Third National Health and Nutrition Examination Survey (NHANES III) were followed up for an average of 8.2 years for incidence of all-cause mortality, along with mortality associated with overall CVD, stroke, and ischemic heart disease²⁰⁰. The investigators found that individuals who consumed an eating pattern that was similar to the DASH diet experienced 31%

and 89% lower risk of all-cause and stroke-related mortality, respectively²⁰⁰.

Taken together, the evidence supports an association between a DASH diet, which includes low-fat dairy and a modest amount of full-fat dairy, and lower CVD risk among those with or without HBP.

The literature supports the strong correlation between HBP and CVD but there are limited long-term data on the specific effects of dairy subtypes such as cheese, milk, and especially yogurt among non-hypertensives and no studies among hypertensives. Therefore, using two large prospective cohorts, our objective is to estimate the effects of total dairy, milk, cheese, and yogurt intakes on risk of CVD among those with HBP and evaluate if the dairy-specific effects are modified by an overall healthy diet.

4.3 METHODS

4.3.1 Study Population

The participants included in these analyses were subjects from the Nurses' Health Study (NHS, N=121,741, aged 30-55 at the time of cohort inception) and the Health Professionals Follow-Up Study (HPFS, N =51,529, aged 40-75 at cohort inception) who had reported prevalent HBP. Participants in both cohorts were mailed questionnaires every two years inquiring about any physician-diagnosed

conditions such as stroke, MI, and HBP as well as other lifestyle habits.

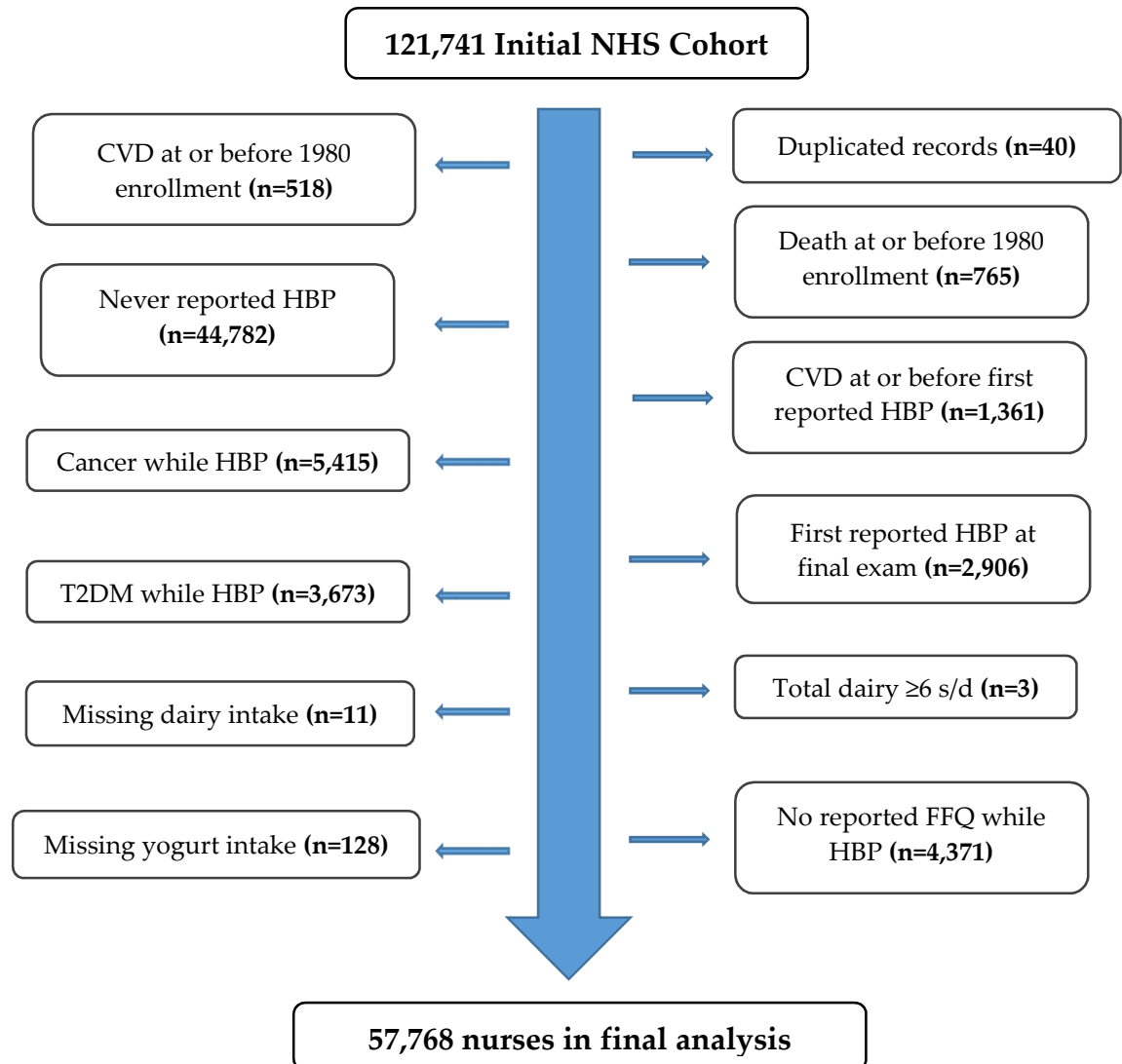
Participants also answered semi-quantitative food frequency questionnaires (FFQs) every four years, reporting their usual daily intake of more than 130 foods and beverages¹²⁴.

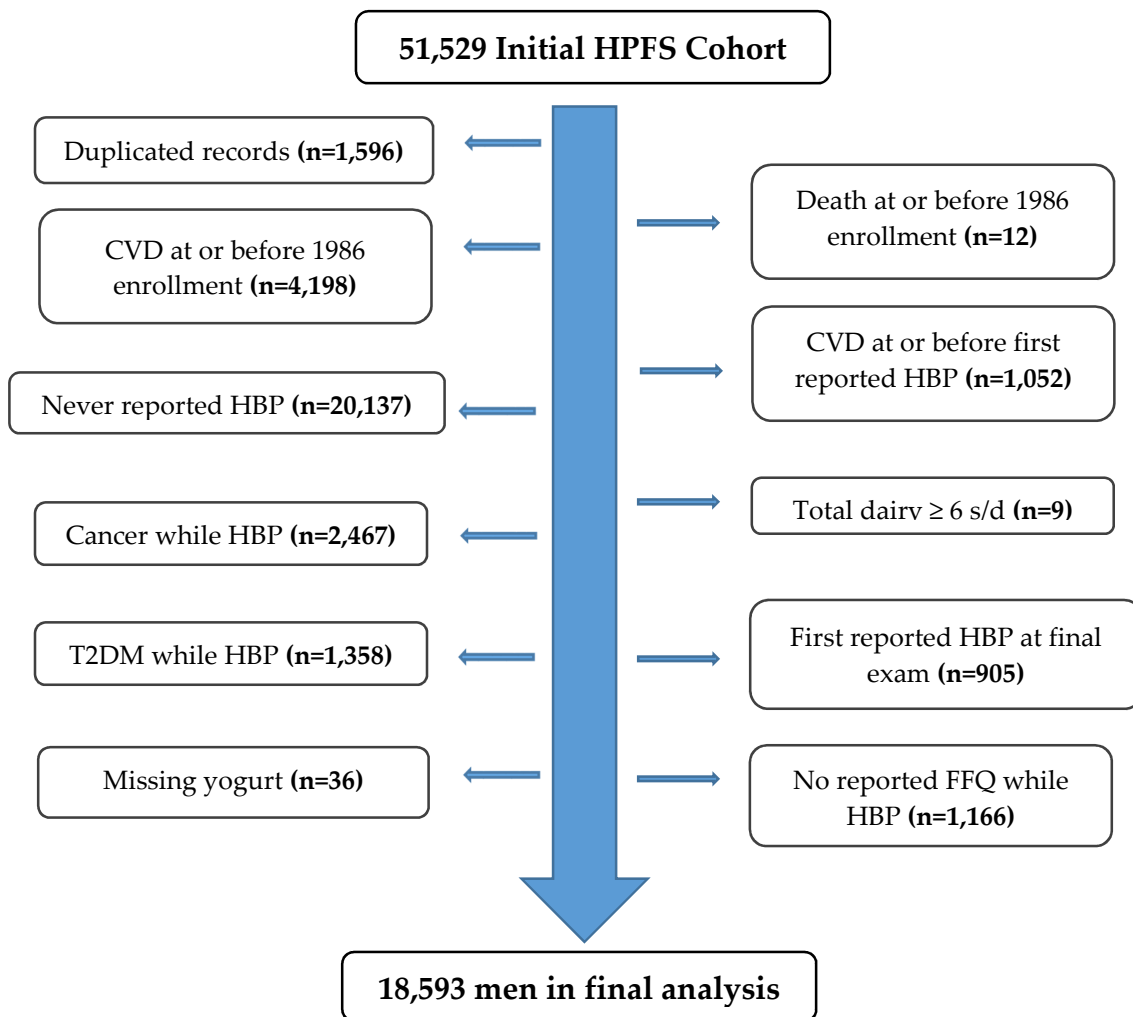
The date of the first-reported HBP served as the baseline for these analyses.

Hence, participants entered the study at different time points depending on when they first reported HBP. The enrollment exam was 1980 for NHS and 1986 for the HPFS subjects in these analyses. Since our goal was to study CVD risk among those with prevalent HBP, subjects who did not report a diagnosis of HBP at enrollment or during follow-up were excluded from these analyses. Other exclusions included: those who were missing dairy or yogurt intake, those who died or had prevalent cancer, diabetes, or cardiovascular disease (including coronary artery bypass grafting (CABG), angina, myocardial infarction (MI), or stroke) at or before the time of first report of HBP, and those with missing or implausible daily caloric intake (<500 or ≥3500 kcal/d for NHS and <800 or ≥4200 kcal/d in the HPFS). Figure 4.1 below illustrates the sample selection for the NHS and HPFS cohorts. The resulting study population consisted of 57,768 women with HBP from the NHS and 18,593 men with HBP from the HPFS. The

Institutional Review Board of Boston University School of Medicine approved the study. By virtue of voluntarily returning their questionnaires, participants provided implied consent.

Figure 4.1 Study Diagram: Sample Selection for NHS and HPFS Cohorts for Analyses of Subsequent CVD Risk among Hypertensives





4.3.2 Assessment of Dairy and Yogurt Intake

In 1980, a 61-item FFQ was mailed to NHS participants inquiring about their habitual dietary intake in the preceding year. An expanded, 131-item questionnaire was then sent in 1984, 1986, and every four years thereafter until 2006. Similar expanded FFQs were mailed every four years beginning in 1986 for HPFS¹²⁴ through 2006. Participants answered how often, on average, they

consumed each listed food item from the questionnaire using nine possible response categories. These ranged from: “never or less than once a month” to “6 or more a day”.

The frequency of usual consumption was multiplied by the nutrient composition from the USDA food composition database in order to calculate nutrient intake from each food item²⁰¹. The following food items were included as part of the dairy variable in our analyses: skim milk, low-fat and reduced-fat milk, whole milk, yogurt, ice cream, sherbet, frozen yogurt, cottage/ricotta cheese, and other cheese. MyPyramid servings as defined by the USDA were derived for each of these food items and summed to estimate total dairy servings per day⁸⁵. For example, ice cream, sherbet, and frozen yogurt FFQ servings (of ½ cup) were divided by 3 to derive a standard USDA serving of dairy. Cream cheese and cream were excluded from the dairy variable creation as they did not meet the MyPyramid definition of dairy: foods that are made from milk and retain their calcium content⁸⁵.

The reproducibility and validity of the 131-item FFQ were evaluated in both the NHS^{124,126} and HPFS¹²⁸ cohorts by comparing reported intake with multiple one-

week diet records. For example, the deattenuated correlation coefficients between FFQs and a seven-day dietary record in 173 women from NHS were 0.97 for yogurt, 0.81 for skim milk, 0.62 for whole milk, 0.57 for cheese¹²⁶. In the HPFS, a similar validation study was conducted in which 127 participants answered the 131-item FFQ and completed two, one-week diet records. Specific deattenuated Pearson correlation coefficients were 0.88 for skim milk, 0.86 for yogurt, and 0.56 for cheese.¹²⁸

4.3.3 Assessment of High Blood Pressure

A participant was considered to have prevalent HBP if she or he reported this diagnosis on the enrollment questionnaire in 1980 (NHS) or 1986 (HPFS). Those without HBP at the first exam were asked again on each biennial questionnaire to report new diagnoses of HBP. Once a participant reported HBP, they were considered to have prevalent HBP throughout the rest of the study.

The validity of self-reported diagnoses of HBP was examined in both the NHS and HPFS cohorts^{129,202}. In NHS, 77% of 51 cases of self-reported HBP in the validation study had a SBP greater than 160 mm Hg or DBP above 95 mm Hg via standard measured BP measurement¹²⁹. In the HPFS, medical records from all of

the randomly selected sample of 100 participants reporting a diagnosis of HBP on the 1988 questionnaire confirmed that all of them had HBP or were receiving antihypertensive treatment¹³¹. In addition, self-reported HBP by the validation study participants was shown to be highly predictive of subsequent cardiovascular events²⁰².

4.3.4 CVD Outcome Ascertainment

Assessment of CVD in these cohorts was previously described²⁰³. In brief, CVD outcomes in these analyses include CHD (comprised of nonfatal MI and fatal MI), and stroke (nonfatal and fatal cases). Participants were asked to report if they had been diagnosed by a physician with any chronic illnesses, including MI and stroke, within the past two years (since the last exam). Permission was then requested to access medical records to confirm reported new diagnoses of MI or stroke. The World Health Organization's criteria (presence of typical symptoms along with either diagnostic/electrocardiographic findings or elevated cardiac enzyme concentrations) was used to confirm nonfatal MI²⁰⁴. Study physicians reviewed computed tomography or magnetic resonance imaging to confirm nonfatal stroke cases. In accordance with the National Survey of Stroke, a stroke diagnosis was made if medical records showed a neurological deficit with

sudden or rapid onset that persisted more than 24 hours or until death²⁰⁵. Fatal MI and stroke were validated via a physician review of death or medical records. CVD events in which there were no medical records available to check self-reported diagnosis were deemed probable and were included in these analyses. Previous CVD analyses by Sun et al. in NHS and HPFS, excluded probable CVD cases and the exclusion showed no effect on CVD risk.²⁰³ Thus, to increase statistical power, probable CVD cases were retained in these analyses.

4.3.5 Assessment of Covariates

A wide range of potential confounding variables were explored including socio-demographic factors, family history, and other diet and lifestyle factors. While most of the participants were Caucasian (97.0% and 94.5% for NHS and HPFS respectively), analyses were adjusted for self-reported race. Family history of HBP was assessed in 1992 for the NHS and 1990 and 1992 for HPFS. Family history of MI was asked on the 1976 and 1996 questionnaires for NHS and the 1986 and 1996 questionnaires for HPFS. The FFQs were used to ascertain participants' intakes of foods and nutrients such as fruits and vegetables, whole grains, dietary fat, and total energy intake. On biennial questionnaires, NHS and HPFS participants were asked to report updated information on smoking status, physical activity (estimated as metabolic equivalent tasks [METs] per week) and

other lifestyle factors. Body mass index (BMI) was calculated as the self-reported weight (in kg) divided by the height squared (in meters). Self-reported weight was validated in 123 men in the HPFS and 140 women in the NHS with standardized weight measurements during at-home visits by study technicians²⁰⁶ and physical activity was validated against 7-day activity records¹³⁴. The correlation coefficient between self-reported and measured estimates was 0.97 for weight²⁰⁶ and 0.79 for physical activity¹³⁴.

Since yogurt intake is likely to be associated with a healthy diet pattern⁵², we calculated a DASH eating pattern score for each subject and combined this with yogurt intake categories as a potential effect modifier of the relationship between yogurt (and other dairy-related exposure variables) and CVD outcomes. Creation of the DASH diet score has been previously described¹⁰⁷. In brief, the following eight foods are reflected in the DASH diet score: high intakes of low-fat dairy products, fruits, vegetables, nuts and legumes, whole grains, and low intakes of red and processed meats, sugar-sweetened beverages (SSB) and sodium¹³⁵. A DASH diet score was calculated from each FFQ by classifying participants into quintiles of intake for each of the 8 aforementioned DASH food components. For whole grains, low-fat dairy, fruits, vegetables, and nuts and legumes, those in the

highest quintile received a score of 5 and those in the lowest quintile of intake received a score of 1. For sodium, SSB, and red and processed meats, those in the lowest quintile of intake received a score of 5 and those in the highest quintile of intake received a score of 1. The DASH score was calculated by summing the quintile rankings of the 8 components, yielding a score that ranged from 8-40, with 40 being the score that most closely resembled a DASH diet.

4.4 STATISTICAL ANALYSIS

Cumulative average intake levels of total dairy, yogurt, and other dietary variables were calculated starting at the time of the first-reported HBP occurrence to the time of first occurrence of one of the following censoring events: date of CVD diagnosis, date of death, loss to follow-up, or end of follow-up (30 June 2010 for NHS and 31 January 2010 for HPFS). The calculation of cumulative averages has been previously explained¹³⁸ but in brief, the nutrient intakes at each dietary questionnaire year when a subject reported HBP had equal weight in the calculation of cumulative average of dietary intakes. Figure 4.2 below illustrates how participants were followed up for these analyses and how their cumulative averages were calculated. In the first example, a nurse first reported HBP on the 1984 questionnaire. Thus, the calculation of her cumulative

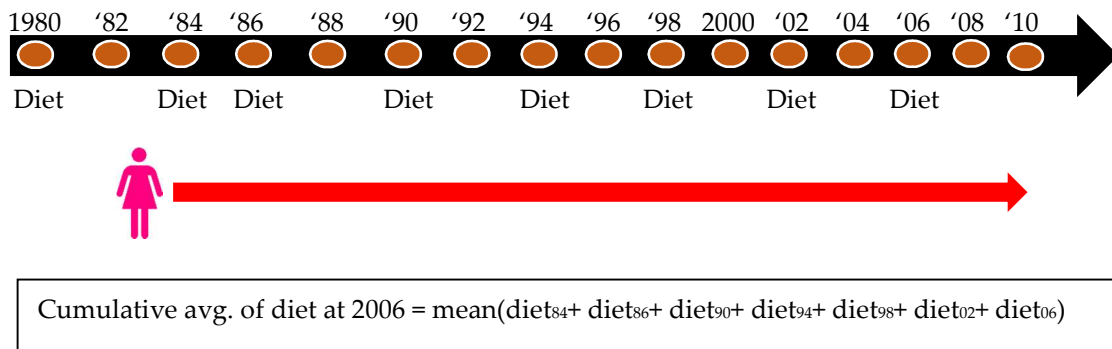
average of dietary intake began with her reported intake on the 1984 questionnaire. She did not report any of the final end points of the study (CVD, death, or lost to follow-up) thus, subsequent reported dietary intakes were also used, given equal weight, in calculating her cumulative average intake. In the second example, a health professional first reported HBP in 1994, which marks the commencement of his follow-up time and the first reported dietary intake used for calculating his cumulative average intake. He reports an MI on the 2002 questionnaire, which is when his follow-up time is stopped and his reported dietary intakes from the previous questionnaire (1998) are carried forward to 2002.

Nutrient and food intakes were not updated when participants first reported angina, coronary artery bypass grafting (CABG), or high cholesterol since these diagnoses may influence subsequent dietary behaviors^{138,207}. For these participants, cumulative averages of dietary intake were carried forward before the occurrence of the aforementioned disease diagnoses (see Figure 4.2 for illustration). We conducted a further secondary analysis by allowing the update of dietary information after participants reported angina, CABG, or high cholesterol (No Diet Update). This was done to see if there was potential bias or

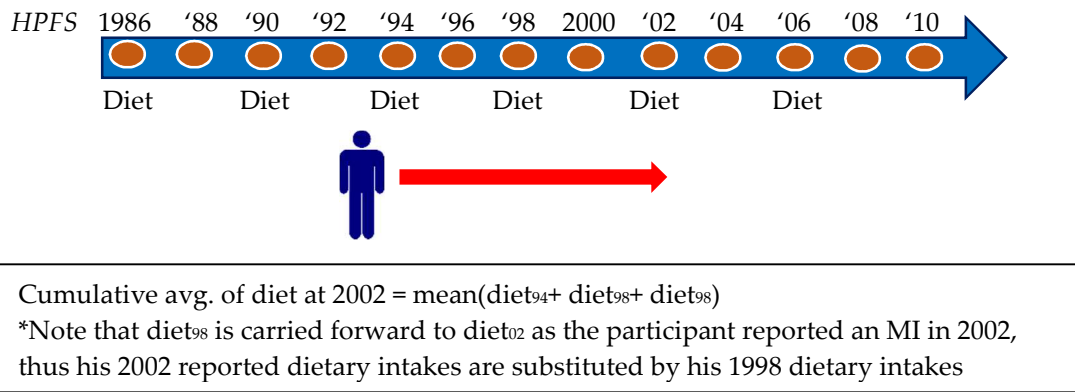
misreporting of dietary intakes once subjects developed CABG, angina, or high cholesterol.

Figure 4.2 Illustration of Two Hypothetical Follow-Up and Cumulative Average Calculation Scenarios for the CVD Analyses

#1: Nurse first reports HBP at 1984 questionnaire and does not develop CVD, nor dies throughout the rest of the study



#1: Male health professional first reports HBP at 1994 questionnaire and suffers an MI and reports it in the 2002 questionnaire



After evaluating the direct effects of yogurt and other dairy products on CVD risk among hypertensives, potential effect modification by the DASH diet score

was examined using stratified multivariate analysis. To do this, dichotomous categories (low vs. high) for each dairy exposure variable were determined using sensitivity analyses and then combined with dichotomous (low vs. high) categories of the DASH diet scores. Since yogurt and dairy foods are components of the DASH score, the score used in these analyses was modified to exclude yogurt and other dairy products for the respective analyses. Thus, we combined each dairy-related exposure with the DASH score excluding that dairy variable from its calculation. In this way, four exposure categories were created. For example, the yogurt analyses yielded these four independent categories: (1) low yogurt + low DASH score (ref); (2) low yogurt + high DASH score; (3) high yogurt + low DASH score; (4) high yogurt + high DASH score. The cutpoints for NHS were as follows: yogurt (<2 servings/week vs. ≥ 2 servings/week) and DASH score without yogurt (<25 vs. ≥ 25). These cutpoints were selected by considering the sensitivity analysis results for both yogurt and the DASH score, while optimizing analytical power.

Time-dependent Cox proportional hazards regression models were used to calculate the hazard ratios (HR) and 95% confidence intervals (CI) for subsequent total CVD, CHD, and stroke risks. The following potential confounders were

analyzed individually and together: age, race, family history of HBP, family history of MI, anti-hypertensive medication use, hypercholesterolemia, physical activity, smoking status, alcohol intake, post-menopausal hormone use (NHS), aspirin and multivitamin use, BMI (baseline and updated every two years), family history of diabetes, and cumulative updates of the following dietary components: energy intake, carbohydrates, total fat and fat subtypes (saturated, monounsaturated, polyunsaturated, omega-3, *trans*), protein (total, animal, and plant), whole grains, fiber (total, cereal), nuts, FV, sugar-sweetened beverages, potatoes, beans, red meat, processed meat, red and processed meats, sodium, potassium, calcium, and magnesium. Only covariates that changed the HRs by >10% were retained in the final models. These included: age, race, physical activity, energy intake, family history of hypertension and MI, anti-hypertensive medication use, and intakes of total fiber and *trans* fats. We also adjusted for the other dairy subtypes. In the yogurt analyses, we adjusted for milk and cheese, while in the milk analyses, we adjusted for yogurt and cheese. Next, we included the dairy variables on a continuous scale in the multivariable Cox regression analyses to estimate the risk of CVD associated with each additional serving per day. All analyses were performed with SAS software (version 9.4; SAS Institute

Inc, Cary, NC). All *P* values are two-sided and a *P* value < 0.05 is considered statistically significant.

4.5 RESULTS

With over 30 years of follow-up in the NHS and 26 years in the HPFS, there were 5,421 total CVD cases in both cohorts, with 3,631 and 1,790 incident CVD cases in the NHS and HPFS, respectively. Of the 3,631 cases of CVD in NHS, there were 1,946 CHD events and 1,685 strokes. In HPFS, there were 1,267 CHD events and 523 incident strokes.

Table 4.1 Age-standardized characteristics of the NHS and HPFS study subjects with prevalent HBP by yogurt intake categories

A. NHS (N=57,768)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 22,126)	1/mo-<1/wk (N = 10,920)	1-<2/wk (N = 12,262)	≥2/wk (N = 12,460)
Age (years) ²	48.4 (6.9)	47.2 (7.0)	46.5 (7.0)	45.3 (7.0)
Activity (MET-hrs/wk)	13.0 (17.8)	14.5 (19.0)	15.9 (21.8)	18.3 (22.3)
BMI (kg/m ²)	26.8 (5.3)	27.1 (5.4)	27.0 (5.1)	27.0 (5.3)
Current smoker, %	22.8	14.9	12.3	11.0
Anti-hypertensive medication use, %	45.3	47.3	48.7	50.4
Family history HBP, %	48.0	52.8	55.2	53.1
Family history MI, %	21.2	20.7	20.1	20.4
T2DM, %	15.5	16.0	14.8	12.0
Cancer, %	16.3	16.3	16.4	13.8
Postmenopausal hormone use, %	21.2	24.6	26.2	27.6
B. HPFS (N=18,593)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 9,992)	1/mo-<1/wk (N = 3,452)	1-<2/wk (N = 2,877)	≥2/wk (N = 2,272)
Age (years) [*]	54.6 (9.3)	52.8 (9.2)	52.4 (9.0)	52.4 (9.3)
Activity (MET-hrs/wk)	25.9 (35.5)	28.2 (33.3)	31.1 (34.8)	34.5 (42.2)
BMI (kg/m ²)	24.6 (7.8)	24.7 (7.9)	24.7 (7.7)	24.2 (8.0)

Current smoker, %	8.4	3.9	3.5	2.7
Anti-hypertensive medication use, %	55.0	52.4	51.6	49.5
Family history HBP, %	47.8	48.8	53.3	49.1
Family history MI, %	33.8	32.8	33.0	35.8
T2DM, %	12.1	12.0	12.7	9.9
Cancer, %	19.1	19.0	19.2	18.7

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population; C=cup; s/d=serving/day; mo=month; wk=week.

²Value is not age adjusted

Table 4.1 illustrates the baseline characteristics of the NHS and HPFS participants at the time of their first report of HBP. Those with the highest yogurt intakes (≥ 2 s/wk) tended to be non-smokers, more physically active, and had lower cancer and diabetes prevalences.

Table 4.2 Age-standardized diet and food intakes of the NHS and HPFS subjects at baseline by yogurt intake categories

A. NHS (N=57,768)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 22,126)	1/mo-<1/wk (N = 10,920)	1-<2/wk (N = 12,262)	≥ 2 /wk (N = 12,460)
Alcohol (g/d)	6.8 (12.3)	6.2 (11.2)	6.0 (10.4)	5.8 (9.6)
DASH diet score	22.4 (4.6)	23.7 (4.5)	24.5 (4.4)	25.7 (4.4)
Total fiber (g/d)	17.3 (5.1)	18.3 (5.3)	18.7 (5.3)	19.4 (5.4)
Fruits & vegetables (s/d)	4.3 (2.0)	4.7 (2.0)	5.1 (2.0)	5.6 (2.2)
Red & processed meats (s/d)	1.2 (0.94)	1.1 (0.86)	1.1 (0.84)	0.93 (0.76)
B. HPFS (N=18,593)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 9,992)	1/mo-<1/wk (N = 3,452)	1-<2/wk (N = 2,877)	≥ 2 /wk (N = 2,272)
Alcohol (g/d)	13.9 (17.7)	11.6 (15.0)	11.2 (13.9)	10.9 (14.0)
DASH diet score	22.6 (4.9)	24.4 (4.8)	25.2 (4.7)	26.7 (4.6)
Total fiber (g/d)	20.8 (7.4)	22.5 (6.8)	23.3 (7.1)	24.3 (7.3)
Fruits & vegetables (s/d)	5.1 (2.4)	5.8 (2.6)	6.1 (2.6)	6.7 (3.0)
Red & processed meats (s/d)	1.2 (0.83)	1.0 (0.80)	0.99 (0.75)	0.87 (0.72)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population; C=cup; s/d=serving/day; mo=month; wk=week.

Table 4.2 shows the intakes of selected dietary factors according to yogurt categories in the NHS and HPFS. Higher yogurt intake was associated with a healthier diet as indicated by a higher DASH score, higher fiber and FV intakes, as well as lower intakes of red and processed meats and alcohol.

Table 4.3 Age-standardized nutrient intakes of the NHS and HPFS subjects at baseline by yogurt intake categories

A. NHS (N=57,768)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 22,126)	1/mo-<1/wk (N = 10,920)	1-<2/wk (N = 12,262)	≥2/wk (N = 12,460)
Energy intake (kcal/d)	1597 (529)	1628 (520)	1702 (524)	1806 (543)
Total protein (g/d)	71.6 (14.9)	73.5 (14.4)	74.4 (13.9)	75.7 (13.9)
Total fat (g/d)	61.6 (14.6)	59.3 (14.2)	57.6 (13.4)	53.6 (12.8)
<i>Trans</i> fats (g/d)	1.91 (0.75)	1.79 (0.71)	1.71 (0.68)	1.54 (0.62)
Total carbohydrates (g/d)	183 (44.2)	188 (43.1)	192 (40.5)	202 (38.0)
Sodium (mg/d)	1722 (597)	1745 (553)	1767 (539)	1788 (500)
Potassium (mg/d)	2801 (619)	2929 (604)	3011 (588)	3167 (595)
Calcium (mg/d)	673 (340)	740 (340)	810 (360)	964 (399)
Magnesium(mg/d)	276 (95.6)	296 (96.6)	315 (102)	346 (108)
B. HPFS (N=18,593)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 9,992)	1/mo-<1/wk (N = 3,452)	1-<2/wk (N = 2,877)	≥2/wk (N = 2,272)
Energy intake (kcal/d)	1917 (598)	1965 (602)	2017 (601)	2131 (631)
Total protein (g/d)	88.9 (16.6)	91.7 (16.4)	91.6 (15.9)	91.9 (15.8)
Total fat	71.7 (14.5)	68.8 (13.8)	67.6 (13.6)	63.6 (14.4)
<i>Trans</i> fats (g/d)	3.3 (1.4)	3.1 (1.3)	3.0 (1.4)	2.8 (1.3)
Total carbohydrates (g/d)	233 (45.3)	242 (42.2)	247 (40.8)	258 (43.0)
Sodium (mg/d)	2774 (1076)	2772 (1059)	2604 (901)	2494 (820)
Potassium (mg/d)	3347 (649)	3504 (641)	3573 (658)	3742 (675)
Calcium (mg/d)	719 (343)	789 (345)	850 (378)	1008 (414)
Magnesium(mg/d)	322 (110)	349 (112)	363 (120)	398 (130)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population; C=cup; s/d=serving/day; mo=month; wk=week; kcal=kilocalories.

Table 4.3 highlights several macro and micronutrients in the NHS and HPFS by categories of yogurt intake. In both cohorts, those with the highest yogurt intakes

(≥ 2 s/wk) consumed the most protein, carbohydrates, and potassium but had the lowest intakes of total fat and *trans* fats.

Table 4.4 Age-standardized dairy intakes of the NHS and HPFS study cohorts at baseline by yogurt intake categories

A. NHS (N=57,768)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 22,126)	1/mo-<1/wk (N = 10,920)	1-<2/wk (N = 12,262)	≥ 2 /wk (N = 12,460)
Dairy fat (g/d)	11.5 (7.2)	11.2 (6.6)	11.2 (6.4)	11.0 (5.9)
Total dairy (s/d)	1.1 (0.99)	1.3 (0.99)	1.5 (1.0)	1.9 (1.2)
Low-fat dairy (s/d)	0.71 (0.89)	0.92 (0.91)	1.1 (0.98)	1.6 (1.1)
High-fat dairy (s/d)	0.42 (0.57)	0.37 (0.51)	0.38 (0.51)	0.36 (0.49)
Cheese (1 oz s/d)	0.35 (0.36)	0.36 (0.34)	0.38 (0.36)	0.40 (0.37)
Total milk (8 oz s/d)	0.80 (0.89)	0.89 (0.90)	0.96 (0.92)	1.04 (0.95)
Yogurt (1 C s/d)	0.00 (0.02)	0.06 (0.06)	0.14 (0.16)	0.46 (0.45)
B. HPFS (N=18,593)	Yogurt Intake Categories (1 C s/d) ¹			
	<1/mo (N = 9,992)	1/mo-<1/wk (N = 3,452)	1-<2/wk (N = 2,877)	≥ 2 /wk (N = 2,272)
Dairy fat (g/d)	12.7 (7.3)	12.2 (6.5)	12.1 (6.2)	12.3 (6.4)
Total dairy (s/d)	1.3 (1.0)	1.4 (1.0)	1.5 (1.0)	2.0 (1.2)
Low-fat dairy (s/d)	0.74 (0.89)	0.91 (0.91)	1.1 (0.91)	1.5 (1.1)
High-fat dairy (s/d)	0.51 (0.60)	0.46 (0.53)	0.44 (0.50)	0.45 (0.55)
Cheese (1 oz s/d)	0.39 (0.43)	0.39 (0.39)	0.40 (0.38)	0.42 (0.43)
Total milk (8 oz s/d)	0.81 (0.92)	0.88 (0.92)	0.91 (0.91)	1.02 (1.00)
Yogurt (1 C s/d)	0.00 (0.01)	0.06 (0.06)	0.15 (0.16)	0.47 (0.40)

¹Values are means (SD) or percentages and are standardized to the age distribution of the study population; C=cup; s/d=serving/day; mo=month; wk=week.

Table 4.4 shows the dairy intakes of hypertensive NHS and HPFS participants by yogurt intake categories. Those with the highest usual yogurt intake (≥ 2 s/wk) also consumed the highest amounts of total dairy and all dairy subtypes (milk and cheese) but had a lower high-fat dairy intake. The average yogurt consumption in each yogurt intake category was similar in the two cohorts

although women had a higher overall mean yogurt (0.139 s/d) than the men (0.094 s/d) since many more women were in the highest yogurt consumption category. Overall average milk intake was slightly higher for women (0.91 s/d) than men (0.88 s/d) whereas men and women had similar mean cheese intake (HPFS: 0.39 s/d; NHS: 0.38 s/d).

Table 4.5 The beneficial effects of yogurt intake on CVD risk in NHS and HPFS

Yogurt Intake	P-yrs	CVD Cases	NHS I /100K py	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<1/mo	338120	1875	555	1.00	-	1.00	-
1/mo-<1/wk	156546	698	446	0.91	(0.83-0.99)	0.90	(0.82-0.98)
1-<2/wk	135973	561	413	0.84	(0.76-0.93)	0.87	(0.79-0.95)
≥2/wk	131054	497	379	0.83	(0.75-0.92)	0.83	(0.75-0.92)
<i>P for linear trend³</i>				0.0004		0.0005	
Per one serving/day				0.64	(0.50-0.82)	0.65	(0.51-0.83)

Yogurt Intake	P-yrs	CVD Cases	HPFS I /100K py	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<1/mo	118814	1143	962	1.00	-	1.00	-
1/mo-<1/wk	40780	309	758	0.92	(0.81-1.04)	0.90	(0.79-1.02)
1-<2/wk	27580	186	674	0.80	(0.68-0.94)	0.84	(0.72-0.98)
≥2/wk	21512	152	707	0.82	(0.68-0.97)	0.83	(0.70-0.99)
<i>P for linear trend³</i>				0.0083		0.0200	
Per one serving/day				0.57	(0.38-0.87)	0.62	(0.41-0.93)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, milk and cheese.

²Diet not updated upon report of CABG, angina, or high cholesterol.

³Linear trend across yogurt intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable. mo=month; wk=week; p-yrs=person years.

Table 4.5 shows an inverse linear association between increasing yogurt intakes and CVD risk among the NHS (p for trend=0.0004) and HPFS (p for trend=0.0083) participants. Hypertensive nurses and health professionals who had the highest yogurt intakes (≥ 2 s/wk) had a 17% (95% CI: 0.75-0.92) and an 18% (95% CI: 0.68-0.97) reduced risk of CVD compared to with those who consumed the lowest yogurt intakes (<1 s/mo). In the second multivariable model, dietary intakes were not updated following a reported diagnosis of CABG, angina, or hypercholesterolemia; the effects were similar across yogurt categories in both cohorts. Results from the linear regression analysis show that for every additional serving per day of yogurt, the risk of CVD declined by 36% (95% CI: 0.50-0.82) in women and 50% (95% CI: 0.33-0.74) in men. Similar associations were observed for CHD (see table A.9 in the appendix) and stroke (see table A.10 in the appendix) for both cohorts.

Table 4.6 Higher yogurt intake combined with a higher DASH score lowered risk of CVD

Yogurt, DASH score ¹	P-yrs	NHS		Multivariable		No Diet Update	
		CVD Cases	I /100K p-yrs	HR ²	95% CI	HR ³	95% CI

<2/wk, <25	391385	1953	499	1.00	-	1.00	-
<2/wk, ≥25	239254	1181	494	0.91	(0.84-0.98)	0.88	(0.81-0.95)
≥2/wk, <25	54791	206	376	0.87	(0.75-1.01)	0.88	(0.76-1.01)
≥2/wk, ≥25	76263	291	382	0.81	(0.71-0.92)	0.78	(0.68-0.88)

Yogurt, DASH score ¹	P-yrs	HPFS					
		CVD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ²	95% CI	HR ³	95% CI
<1/wk, <25	105289	908	862	1.00	-	1.00	-
<1/wk, ≥25	64103	554	864	0.87	(0.77-0.97)	0.88	(0.78-0.99)
≥1/wk, <25	16465	155	941	1.07	(0.90-1.27)	1.07	(0.90-1.27)
≥1/wk, ≥25	25736	210	816	0.79	(0.67-0.92)	0.80	(0.68-0.93)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, and *trans* fat.

²Diet not updated upon report of CABG, angina, or high cholesterol.

wk=week; p-yrs=person years.

Table 4.6 shows the independent and combined effects of a high yogurt intake with overall diet quality as measured by the DASH diet score. Women who consumed 2 or more yogurt servings per week, despite a low DASH score (<25) had a 13% (95% CI: 0.75-1.01) lower CVD risk compared with the referent group (low yogurt and low DASH score). Women who had a higher DASH score (≥25) but a lower yogurt intake (<2 s/wk) experienced a 9% (95% CI: 0.84-0.98) reduction in CVD risk compared to the referent group. The effect modification of yogurt intake by the DASH score was approximately additive since those with both a high yogurt intake and a high DASH score had a 19% (95% CI: 0.71-0.92) CVD risk reduction compared to the referent group. Among men, higher DASH

score alone led to a 13% lower risk compared to the referent group (yog<1/wk + DASH<25). We had insufficient power to use the previous cutoff value of 2 or more servings per week of yogurt for these analyses in men. Those consuming one or more servings of yogurt alone had no beneficial effect. However, the combined effects among men consuming one or more servings of yogurt with a higher DASH score were stronger (a 31% lower risk of CVD) than those seen in women and reflect effect modification that is more than additive.

Table 4.7 Higher milk intake is associated with a lower risk of CVD in men

NHS							
Milk Intake ¹	P-yrs	CVD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.5/d	295869	1337	452	1.00	-	1.00	-
0.5-<2/d	362604	1763	486	1.05	(0.97-1.13)	1.04	(0.97-1.12)
2-<5/d	103219	531	514	1.10	(0.99-1.22)	1.07	(0.96-1.20)
<i>P for linear trend³</i>				0.0830		0.1844	
Per one serving/day				1.04	(0.99-1.09)	1.03	(0.99-1.08)
HPFS							
Milk Intake ¹	P-yrs	CVD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.5/d	91811	783	853	1.00	-	1.00	-
0.5-<2/d	87511	753	860	0.94	(0.85-1.04)	0.93	(0.84-1.04)
2-<5/d	29363	254	865	0.84	(0.72-0.98)	0.86	(0.74-1.00)
<i>P for linear trend³</i>				0.0235		0.0426	
Per one serving/day				0.93	(0.87-0.99)	0.94	(0.88-1.00)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fats, fiber, yogurt and cheese.

²Diet not updated upon report of CABG, angina, or high cholesterol.

³Linear trend across milk intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable. d=day; p-yrs=person years.

Table 4.7 shows that increasing milk consumption was associated with a lower CVD risk in men (p for trend=0.0235) but not women. Men who consumed 2-<5 s/d of milk had a 16% (95% CI: 0.72-0.98) lower CVD risk that those who consumed <0.5 s/d. Each 1 s/d increase in intake was associated with a 7% reduced risk of CVD in men. The effects of milk intake were slightly attenuated in women when diet was not updated for the occurrence of CABG, angina, or diagnoses of high cholesterol.

Table 4.8 Higher milk intake combined with a higher DASH diet score is associated with a lower risk of CVD in men

				NHS			
		CVD	I/100K	Multivariable		No Diet Update	
Milk, DASH score	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/d, <22	294335	1351	459	1.00	-	1.00	-
<1/d, ≥22	171783	790	460	0.97	(0.88-1.06)	0.94	(0.86-1.04)
1-<5/d, <22	161691	834	516	1.10	(1.01-1.20)	1.10	(1.01-1.21)
1-<5/d, ≥22	133884	656	490	1.01	(0.91-1.12)	0.97	(0.87-1.07)
				HPFS			
		CVD	I/100K	Multivariable		No Diet Update	
Milk, DASH score	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/d, <22	79673	696	874	1.00	-	1.00	-
<1/d, ≥22	55250	471	852	0.87	(0.76-0.98)	0.83	(0.73-0.95)
1-<5/d, <22	41409	347	838	0.88	(0.77-1.00)	0.87	(0.76-0.99)
1-<5/d, ≥22	32353	276	853	0.80	(0.67-0.93)	0.79	(0.68-0.92)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, yogurt, and cheese.

²Diet not updated upon report of CABG, angina, or high cholesterol.

d=day; p-yrs=person years.

Table 4.8 shows the independent and combined effects of milk intake and adherence to the DASH diet on CVD risk. In men, those with a higher milk intake but a lower DASH score (<22) had a 12% reduced risk of CVD whereas those with lower milk intakes (<1 s/d) and a higher DASH score (≥22) had a 13% lower risk compared to the referent group. The combined effect of a high milk and a high DASH score (HR: 0.80, 95% CI: 0.67-0.93) indicates a slight modification of the effect of milk consumption on CVD risk by the DASH diet score. In the NHS, a high milk intake (1-<5 s/d) combined with a low DASH score (<22) was associated with a 10% increased risk of CVD. There was also no beneficial effect of a higher DASH score, either alone or in combination with milk consumption.

Table 4.9 Higher cheese intake is associated with a lower CVD Risk in women

Cheese Intake	P-yrs	CVD Cases	NHS		Multivariable		No Diet Update	
			I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI	
<0.25/d	369440	1823	493	1.00	-	1.00	-	
0.25-<0.5/d	251516	1180	469	0.93	(0.87-1.01)	0.93	(0.86-1.01)	
0.5-4/d	140737	628	446	0.92	(0.83-1.01)	0.91	(0.82-1.00)	
<i>P for linear trend³</i>					0.0476		0.0301	
Per one serving/day					0.85	(0.72-1.00)	0.84	(0.71-0.98)

Cheese Intake	P-yrs	HPFS		Multivariable		No Diet Update	
		CVD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.25/d	87370	725	830	1.00	-	1.00	-
0.25-<0.5/d	71476	616	862	1.06	(0.94-1.18)	1.08	(0.96-1.20)
0.5-4/d	49840	449	901	1.10	(0.97-1.25)	1.10	(0.97-1.25)
<i>P for linear trend³</i>				0.1210		0.1355	
Per one serving/day				1.16	(0.96-1.39)	1.16	(0.95-1.41)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, yogurt and milk.

²Diet not updated upon report of CABG, angina, or high cholesterol.

³Linear trend across cheese intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable. d=day; p-yrs=person years.

Results in Table 4.9 are in some ways opposite to those of Table 4.8. Here, there is a suggestion that higher cheese intakes (0.5-4 s/d) among hypertensive women may be associated with a slightly lower risk of CVD (HR: 0.92; 95% CI: 0.83-1.01).

There was also an inverse linear trend across categories of cheese intake in women (*p* for trend=0.0476). In contrast, higher cheese intake among men was not associated with CVD risk. In the multiple linear regression modeling, each additional serving of cheese in women was linked with a 15% lower CVD risk (95% CI: 0.72-1.00) and a non-statistically significant 16% increased risk in men (95% CI: 0.96-1.39).

Table 4.10 Higher cheese consumption with a higher DASH diet score is associated with a lower risk of CVD in women

Cheese, DASH score	P-yrs	CVD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	219639	1084	494	1.00	-	1.00	-
<0.25/d, ≥22	149801	739	493	0.96	(0.87-1.06)	0.93	(0.84-1.03)
0.25-4/d, <22	236387	1101	466	0.94	(0.86-1.02)	0.94	(0.86-1.03)
0.25-4/d, ≥22	155866	707	454	0.87	(0.79-0.97)	0.84	(0.76-0.93)

Cheese, DASH score	P-yrs	CVD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	47525	391	823	1.00	-	1.00	-
<0.25/d, ≥22	39845	334	838	0.91	(0.78-1.07)	0.88	(0.75-1.02)
0.25-4/d, <22	73557	652	886	1.11	(0.98-1.27)	1.11	(0.97-1.26)
0.25-4/d, ≥22	47758	413	865	0.96	(0.83-1.11)	0.94	(0.81-1.09)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, yogurt, and milk.

²Diet not updated upon report of CABG, angina, or high cholesterol.

d=day; p-yrs=person years.

Table 4.10 shows the independent and combined effects of cheese intake and the DASH score on risk of CVD. Hypertensive women with a high cheese intake but a low DASH score (<22) had a 6% lower CVD risk compared to those with low intakes of both (ref). A high DASH score alone led to only a 4% lower CVD risk. The combined effect of higher cheese intakes and a higher DASH score was slightly more than additive, that is a statistically significant 13% reduced risk of CVD (95% CI: 0.79-0.97). In men, there was no effect of higher cheese intake alone, a higher DASH score alone, or the combination of the two.

Table 4.11 Total dairy intake is inversely associated with CVD Risk in men

				NHS			
				Multivariable		No Diet Update	
Dairy Intake	P-yrs	CVD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	111941	573	512	1.00	-	1.00	-
0.5-<1/d	167657	801	478	0.93	(0.83-1.03)	0.91	(0.82-1.02)
1-<2/d	294401	1319	448	0.88	(0.80-0.98)	0.88	(0.79-0.98)
2-<6/d	187694	938	500	0.98	(0.87-1.11)	0.97	(0.86-1.09)
<i>P for linear trend³</i>				0.5437		0.6691	
Per one serving/day				1.01	(0.97-1.06)	1.01	(0.97-1.06)
				HPFS			
				Multivariable		No Diet Update	
Dairy Intake	P-yrs	CVD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	31914	263	824	1.00	-	1.00	-
0.5-<1/d	51527	434	842	1.00	(0.85-1.16)	1.01	(0.86-1.19)
1-<2/d	79041	695	879	0.99	(0.85-1.15)	1.04	(0.89-1.21)
2-<6/d	46204	398	861	0.89	(0.75-1.05)	0.93	(0.78-1.10)
<i>P for linear trend³</i>				0.0832		0.2187	
Per one serving/day				0.95	(0.89-1.01)	0.96	(0.91-1.02)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, and fiber.

²Diet not updated upon report of CABG, angina, or high cholesterol.

³Linear trend across total dairy intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable. d=day; p-yrs=person years.

Finally, we examined the effects of total dairy intake and CVD risk as shown in

Table 4.11. Overall, there was little effect of total dairy intake on CVD risk among

these hypertensive men and women. Men who reported consuming 2-<6 s/d had

a non-statistically significant 11% (95% CI: 0.75-1.05) lower CVD risk compared

to men with the lowest dairy intake (<0.5 s/d). A weak J-shaped association was

observed for women with HBP, with the lowest CVD risk found in those reporting an intake of 1-<2 servings per day.

Table 4.12 High total dairy intake combined with a high DASH diet score is associated with a lower risk of CVD in women and men

Dairy, DASH score	P-yrs	CVD Cases	NHS		Multivariable		No Diet Update	
			I /100K p-yrs	HR ²	95% CI	HR ³	95% CI	
<1/d, <22	184373	901	489	1.00	-	1.00	-	
<1/d, ≥22	95226	473	497	0.95	(0.85-1.07)	0.92	(0.82-1.04)	
1-<6/d, <22	271653	1284	473	0.97	(0.88-1.06)	0.97	(0.89-1.06)	
1-<6/d, ≥22	210441	973	462	0.88	(0.79-0.97)	0.86	(0.77-0.95)	

Dairy, DASH score	P-yrs	CVD Cases	HPFS		Multivariable		No Diet Update	
			I /100K p-yrs	HR ²	95% CI	HR ³	95% CI	
<1/d, <22	50984	420	824	1.00	-	1.00	-	
<1/d, ≥22	32456	277	853	0.90	(0.76-1.05)	0.86	(0.73-1.02)	
1-<6/d, <22	70098	623	889	1.00	(0.88-1.14)	1.02	(0.90-1.16)	
1-<6/d, ≥22	55147	470	852	0.84	(0.73-0.97)	0.85	(0.73-0.98)	

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, and *trans* fat.

²Diet not updated upon report of CABG, angina, or high cholesterol.

d=day; p-yrs=person years.

The combined effects of total dairy intake and adherence to the DASH diet is shown in Table 4.12. Both men and women who consumed one or more servings of dairy per day and had a higher DASH score (≥22) had the lowest risk of developing CVD. Among hypertensive women, the risk of CVD was 12% lower

(95% CI: 0.79-0.97) than that of subjects in the referent group while for men, the risk of CVD in this same category was 16% lower (95% CI: 0.73-0.97). For both men and women, there was evidence of effect modification (more than additive) of dairy intake by the DASH score.

4.6 DISCUSSION

In these analyses, higher yogurt consumption (≥ 2 s/week) among hypertensive women and men in the NHS and HPFS was associated with a reduced risk of total CVD (17% and 18% lower risks, respectively), CHD (26% and 18% lower risks, respectively), and stroke (6% and 19% lower risks, respectively). These beneficial effects were also observed when higher yogurt intakes were combined with the DASH diet score, a healthy diet that has been linked with BP-lowering effects.

Observational studies examining the effect of yogurt intake on risk of CVD among hypertensives have not been published. To our knowledge, this is the first and largest prospective study to estimate the independent effect of yogurt intake on risk of CVD among those with HBP. Several randomized controlled feeding trials have recruited hypertensive individuals to assess the effects of fermented milk products on CVD risk factors¹⁸⁸⁻¹⁹⁰. Jauhianen et al conducted a randomized,

placebo-controlled, double-blind, parallel group trial with 89 hypertensive subjects; they observed that treatment with 200 ml/d of *Lactobacillus helveticus*-fermented milk containing 1.2 mg of IPP/100 g and 1.3 mg of VPP/100 g for the first 12 weeks and increasing to 5.8 mg IPP/100 g and 6.6 mg VPP/100 g for the final 12 weeks had beneficial effects on the augmentation index (a measure of arterial stiffness) compared to those who had *Lactobacillus helveticus*-fermented milk without IPP or VPP¹⁸⁹.

Aortic stiffness has been shown to be an independent predictor of coronary events in a longitudinal study of over 1,000 hypertensive French adults (mean age=51 years) followed for six years²⁰⁸. A similar randomized, double-blind placebo-controlled, parallel feeding trial of 25 Japanese hypertensives male subjects (mean age=54 years) found an improvement of endothelial function after ingestion of 1.25 g of casein hydrolysate powder containing 3.42 mg of VPP and 3.87 mg of IPP per day for 1 week¹⁹⁰. Another randomized crossover clinical trial on 70 Caucasian adults with pre-hypertension or hypertension examined the effects of daily consumption of 15 mg IPP (consumed in capsules of milk protein hydrolysate twice per day for four weeks) without dairy-related minerals and found that IPP led to 3.8 mm Hg reductions in SBP and 2.3 mm Hg reductions in DBP among subjects with stage 1 hypertension²⁰⁹. Taken together, these

randomized trials in hypertensive subjects showed a beneficial effect of the casein-derived tripeptides IPP and VPP on blood pressure and other CVD risk factors.

These tripeptides could play a potential role in platelet aggregation and clot formation via their inhibition of the angiotensin converting enzyme (ACE) in the renin-angiotensin system (RAS)¹⁵⁴, one of the major BP regulators in the body. Inhibition of ACE, leads to lower concentrations of angiotensin II, a potent vasoconstrictor¹⁵⁴. Angiotensin II has also been shown to increase intracellular calcium concentration in vascular smooth muscle cells (VSMC) and pH in platelets from hypertensive patients²¹⁰, which may be associated with enhanced platelet aggregation²¹¹. When platelets aggregate, they release different growth factors that take part in the development of atherosclerosis by promoting VSMC proliferation²¹². Platelets from hypertensive patients produce more reactive oxygen species (ROS)^{213,214}. ROS enhance platelet activity by reducing the bioavailability of nitric oxide (NO)²¹³, an important vasodilator²¹⁵ and inhibitor of platelet aggregation^{216,217}. Fermented dairy-derived peptides have been shown to improve vascular response in vitro possibly by the stimulation of NO release in spontaneously hypertensive rats (SHR)¹⁵². Thus, biopeptides from fermented

dairy have been linked to different platelet signaling pathways (such as RAS) and may play a role in hypertension-associated CVD.

Several prospective studies among those without HBP observed an inverse association of yogurt intake on markers of CVD^{28,218}. A 5-year prospective study of 1,080 Caucasian women >70 years old in Australia showed that those who consumed >100 g/d (> 0.4 c/d) of yogurt had significantly less common carotid artery intima-media thickness²⁸, a predictor of stroke and MI^{219,220}, compared with those who consumed <100 g/d of yogurt. The investigators did not observe comparable beneficial effects with other dairy foods, suggesting that yogurt has unique effects on CVD risk factors²⁸. A 4-year case-control study in 885 Italian patients observed a 45% lower odds of acute MI among those who consumed 7 or more cups of yogurt per week compared with non-yogurt consumers.²¹⁸ Similar to the Australian cohort, the effects of yogurt were greater than those for milk (OR: 0.78) and cheese (OR: 0.77)²¹⁸.

No long-term observational studies have examined the effect of dairy intake on CVD risk among individuals with prevalent HBP. Studies of dairy intake in general, irrespective of HBP status, suggest that higher dairy intake is associated

with a lower CVD risk^{198,221}. A meta-analysis of total dairy intake found an inverse association with risk of stroke that may be due to the BP-lowering effects of dairy.²²² These findings are in agreement with our analyses in which we saw that increased dairy intake was linked with lower risks of HBP and stroke in the NHS (Chapter 3). Overall, there is a moderate amount of evidence that links dairy intake with a beneficial effect on risk of CVD.

When high yogurt intakes were combined with a high DASH score, we observed a greater beneficial effect compared with the independent effects of yogurt or the DASH eating pattern alone in both cohorts. The independent effect of a DASH diet intervention was observed in the ENCORE (Exercise and Nutrition interventions for CardiOvasculaR hEalth) study, where investigators recruited overweight (BMI between 25-40 kg/m²) middle-aged (mean age=52 years) hypertensive men and women²²³. They found that those randomized to the DASH diet intervention had lower BP, brachial artery flow-mediated dilation, baroreflex sensitivity, and left ventricular mass after a 4-month study period compared with those consuming a typical American diet²²³. Increased left ventricular mass predicts left ventricular hypertrophy, a structural consequence of HBP and one of the strongest known predictors of cardiovascular morbidity

and mortality among hypertensives²²⁴, independent of other CVD risk factors. Sensitivity of the baroreflex system is an early consequence of HBP²²⁵ and may reflect reduced viscoelastic properties of the vascular wall, which contains the baroafferent stretch receptors owing to arterial stiffness and atherosclerosis^{226,227}. The DASH diet intervention in the ENCORE study improved BRS by 33%²²³, perhaps as a result of reduced vascular stiffness among hypertensives²²⁸. An analysis of a subset of the original DASH trial with stage 1 hypertension²²⁹ found that approximately 75% of the 131 hypertensive participants in the DASH intervention arm experienced a BP-lowering effect compared to only a quarter or half of the participants in the control and FV groups, respectively, after 24-weeks in the randomized, cross-over, feeding trial²²⁹.

In terms of the biochemical mechanisms of the DASH diet on CVD risk factors, high inorganic nitrates from fruits and leafy vegetables have been implicated as playing a role in the non-enzymatic generation of NO²³⁰. The DASH diet was estimated to have contributed approximately 1200 mg nitrate/d²³¹. A meta-analysis and systematic review of randomized controlled trials in adults demonstrated a significant effect of inorganic nitrate supplementation on SBP (decrease of 4.4 mm Hg) and DBP (decrease of 1.1 mm Hg)²³². In addition to high amounts of inorganic nitrate, the DASH diet promotes increased consumption of

hypotensive minerals such as magnesium, calcium, and potassium^{233,234}. These minerals have been shown to have protective effects on CVD risk factors via their antioxidant capacity to lower oxidative stress²³⁵, inflammatory response and coagulation abnormalities in type 2 diabetics²³⁶, increased sodium excretion in pre-hypertensive adults²³⁷, and sympathetic activation²³⁸ and endothelial function²²³ among adults with HBP.

Yogurt was the only dairy product that had measurable CVD-lowering effects for both hypertensive men and women in our analyses. One reason for this consistency could be the inherent nature of how yogurt is usually eaten (as a food by itself) which may impact the accuracy of the reported intake levels with the FFQ. I propose that for several reasons, yogurt is the dairy product least prone to misreporting. Other dairy subtypes such as milk and cheese are often eaten as a part of mixed dishes (e.g., soups, pizza, sandwiches, casseroles, mixed beverages) and thus, are more susceptible to error in the estimation of intake both from misreporting and from the nature of the food list on the FFQ itself. The validity of the reported intakes of dairy (comparing the FFQ with 7-day food diaries of 173 NHS participants and 127 male health professionals) in these two cohorts was examined in previous analyses. The correlations of reported intakes

of milk with the two methods in NHS and HPFS were 0.81 and 0.88, respectively; those for cheese were 0.57 and 0.59, respectively, and for yogurt were 0.97 and 0.86, respectively).^{126,128} These results support the possibility that yogurt intake may have been reported more accurately and/or completely than other forms of dairy. Bingham et al found lower reported intakes of foods such as cakes, milk, and desserts in under-reporters in a validation study of weighed records, FFQs, and 24-hour recalls against the urinary nitrogen technique.²³⁹ Pryer et al found the largest differences between reporters and low-energy reporters (under-reporters) for foods such as confectionery, biscuits and cakes, and high-fat dairy products (including high-fat milk and cheese).¹⁶⁸ Both of these studies suggest that cheese, milk and milk-containing foods and dishes may be under-reported in a variety of dietary assessment techniques that rely on participant recall. If milk and cheese intakes are legitimately associated with lower CVD risk, then under-reporting of these dietary exposures could lead to underestimation of the beneficial effects on CVD outcomes as a consequence of subjects from the higher-intake groups potentially being misclassified.

It is possible that the intake of dairy was reported with error in this study and that misreporting could differ by type of dairy product (e.g., milk, cheese) and by the actual amount consumed. This is more likely to be a problem here since the

subjects had prevalent HBP, and as health care professionals, the cohort participants may have been aware of their own increased risk of CVD. During the 1980s through the 1990s, there were many public health messages targeted at middle-aged women recommending that they increase their dairy consumption to prevent hip fractures²⁴⁰ and osteoporosis²⁴¹. At the same time, however, messages about dairy intake were conflicting^{194,195} with many clinicians and cardiologists recommending the avoidance of milk and other dairy products to avoid hypothesized adverse effects on weight and CVD risk. The majority of the dairy reportedly consumed by the NHS women was low-fat dairy (64%), and the majority of that (79.5%) was skim or semi-skim milk. The many public health messages combined with the subjects' personal demographic and risk status could have led to biased reporting of the amount and type of dairy consumed by study participants, which could in turn have attenuated the true effects of dairy on CVD risk. Previous evidence suggests that higher milk intakes are inversely associated with risk of CVD among non-hypertensives^{70,186}. If subjects with higher intakes of dairy in this study under-reported their actual intakes, this could have falsely attenuated the effects of dairy on CVD risk (as a result of the inclusion of nurses and health professionals with higher dairy intakes in the referent group).

This study had several strengths including its prospective design and the use of updated repeated measures of diet for the estimation of long-term dietary intakes and repeated measures of potential confounding variables. The sample size allowed us to estimate the overall effects of each dairy subtype among those with HBP. The use of medical records to confirm CHD and stroke was also a strength of the study.

This study is also subject to several limitations. Since yogurt is highly correlated with an overall healthy diet and lifestyle, it is possible that some potential confounder associated with an overall healthy lifestyle was not measured.

Yogurt is a complex dairy product and we were not able to ascertain the effects of specific types of yogurts especially those with different added bacterial strains. The evidence on the effects of specific strains is scarce but studies¹⁴⁸ have shown that different combinations of bacterial strains added to yogurt have unique effects on BP and CVD risk factors. Future studies are needed to assess the effects of different types of yogurt.

Our participants were predominantly Caucasians of European ancestry, which limits the generalizability of our findings. Although this is also a strength in that the potential confounding of race is virtually eliminated from our analyses, our results cannot be applied to other racial or ethnic groups, which may have different risk factors for developing chronic diseases than our cohorts. The number of stroke cases in the HPFS was lacking ample statistical power to provide stable effect estimates in many of our exposures. Therefore, a larger cohort of men with ample statistical power is warranted in order to independently examine the effects of each dairy type on risk of stroke. Another key limitation for these analyses is the lack of participants with high regular yogurt intakes. In order to maximize statistical power, the highest yogurt category for both NHS and HPFS cohorts was limited to 2 or more servings per week, far below the usual dairy recommendation for yogurt and dairy (2-3 servings/day with emphasis on one serving from each type of dairy such as yogurt, cheese, and milk)¹⁶⁷. This limits the interpretability of the estimates for 1 serving/day increase of yogurt as the estimates were extrapolated from 2 or more servings/week.

4.7 CONCLUSION

The results of this study suggest that higher long-term yogurt intakes are associated with lower risks of CVD, CHD, and stroke in hypertensive women and men. We found no adverse effects of consuming milk, cheese, or dairy on risk of CVD. Higher yogurt and total dairy intake in combination with an overall healthy diet as measured by the DASH score was also associated with a lower risk of CVD, CHD, and stroke in both hypertensive men and women. Our findings suggest that incorporation of yogurt into a healthy diet pattern among those with HBP may aid in the secondary prevention of CVD prevention.

Further, these results add to the growing body of evidence showing that there is no basis for avoidance of dairy products in the diet because of its cholesterol and SFA content as intake levels as consumed by [educated, Caucasian, hypertensive] adults in this study do not confer any increased risk. Future studies on the specific types of yogurt and its effects on CVD among those with HBP in populations with larger demographic diversity are warranted.

CHAPTER 5: GENERAL DISCUSSION

High blood pressure (HBP) is a major cause of heart disease, stroke, and renal failure and thereby a major cause of disability and premature death². Major strides have been made over the past several decades in the treatment of high blood pressure but with rising rates of obesity worldwide, the rates of incident high blood pressure have continued to rise. By the age of 55, 54% of adults have HBP and among the obese, rates are even higher². HBP is not just a disease of older adults; the rate of HBP development rises steadily from the adolescent years into older adulthood (≥ 75 years)². Therefore, prevention or delay in the onset of HBP is the key to stemming the rising tide of death and disability from blood pressure-related diseases.

Blood pressure levels are affected by many factors. Genetics, aging, and sex are some of the major non-modifiable risk factors but diet, activity, and smoking have also been shown to be strong behavioral, modifiable determinants of BP². Furthermore, obesity, which is impacted by the same modifiable and non-modifiable risk factors, is an important cause of HBP². It was estimated that as much as 65% of HBP cases are attributable to obesity². Cross-sectional studies indicate that more than 85% of hypertensive individuals are overweight or

obese.⁴⁷ Thus, these modifiable risk factors exert epigenetic effects on likelihood of developing HBP and secondary outcomes such as CVD.

Evidence from systematic reviews^{14,15} and meta-analyses^{16,17} suggest that dietary protein consumption seems to benefit BP. Observational studies, which have been largely cross-sectional, have shown weak beneficial effects of plant proteins in particular on BP.¹⁴ There are few observational studies that have addressed the long-term effects of total protein or animal and plant proteins on BP level or long-term risk of HBP, and results have been conflicting.¹⁸⁻²⁰

The first study for this dissertation addressed the questions associated with long-term effects of total protein as well as animal and plant protein intakes on blood pressure using data from the Framingham Offspring Study. We found that protein intake (from both animal and plant sources) had independent, beneficial effects on mean SBP and DBP, and lowered the long-term risk of incident HBP among middle-aged adults. The effects were even stronger for individuals with a healthy diet as indicated by selected dietary factors such as higher intakes of fruits and vegetables (FV) or fiber. This study adds much-needed long-term evidence that both animal and plant proteins have similar beneficial effects on

risk of HBP. The use of dietary records for determining protein intakes among healthy normotensive adults in this prospective study provides convincing evidence of unbiased effects of protein on HBP risk.

In 1997, a landmark clinical trial testing the impact of a dietary intervention on blood pressure was published. This eating pattern was characterized by higher intakes of fruits and vegetables as well as low-fat dairy, and came to be known by the acronym DASH, or Dietary Approaches to Stop Hypertension⁵⁷. In the DASH trials, the greatest reductions in BP have been seen with the “combined” diet that had both higher intakes of FV and higher intakes of low-fat dairy products rather than a high FV diet alone. Dairy products are an important source of dietary protein and so it has been hypothesized that the protein content of dairy may explain at least some of its beneficial effects on blood pressure.

The 2010 Dietary Guidelines Advisory Committee (DGAC) reviewed the evidence for the association between dairy intake and BP. They concluded that overall, there is a moderate body of evidence pointing to an inverse relationship between the intake of milk and milk products and BP, particularly SBP; the evidence for a reduction in DBP is weaker.²⁴ The DGAC summary suggests that

the evidence is not completely consistent and that differences in results across studies may result from actual differences in effects of different types of dairy products or from residual confounding associated with other dietary factors, and demographic factors.²⁴ Dairy intake has been inversely associated with BP and HBP risk in several prospective studies such as the CARDIA study²⁵, the Women's Health Study²⁶, and the Rotterdam study²⁷. However, some studies such as one in elderly women^{28,29}, the Hoorn Study¹⁹, and the general Dutch population³⁰ found no association between dairy intake and BP. Most studies of dairy intake and BP have combined all types of dairy into one exposure, which could mask differential effects of individual types of dairy (milk, cheese, or yogurt). The nutrient profiles of the different dairy subtypes vary which may be responsible for variable effects of dairy subtypes on BP. Observational studies that specifically evaluate yogurt consumption in relation to BP are scarce but beginning to emerge. A cross-sectional analysis from NHANES 1999-2004 data,⁴⁴ and a few long-term studies from an elderly Mediterranean population in the PREDIMED study¹²⁰ and the Framingham Offspring Study Cohort¹²¹ all linked yogurt intake with a lower risk of HBP. These observational studies did not have ample statistical power to capture effects of usual daily intakes of yogurt. Thus,

we decided to evaluate the effects of usual yogurt intake on HBP risk among a total of 184,231 subjects in three large prospective cohorts.

The second study for this dissertation uses data from two NHS cohorts and the HPFS to examine the effects of different amounts of yogurt consumed and risk of incident HBP among middle-aged and older adults. First, we observed that those with higher intakes of total dairy had a lower risk for developing HBP compared to those with lower dairy intakes in all three cohorts—NHS, NHS II, and HPFS. We then evaluated the specific effects of yogurt consumption, while controlling for other forms of dairy that were also consumed. We found that men and women who consumed yogurt regularly (5 or more servings per week) had lower risks of incident HBP than those consuming higher amounts of milk and cheese. These results support the hypothesis that yogurt may have specific properties that contribute stronger BP-lowering effects than milk and cheese.

In addition to the effects of yogurt on risk of incident HBP, there is some evidence that yogurt and other forms of fermented dairy products may play a hypotensive role in BP control.⁴⁰ A meta-analysis by Dong et al of 13 randomized controlled trials, mostly of 4-8 weeks duration, in both normotensive and

hypertensive adults ages 35 years or older, derived a summary estimate for the beneficial effects of fermented milk of about 2 mm Hg decrease in SBP¹⁴⁸. This decrease in SBP was found to be associated with 7% and 10% lower CHD and stroke mortality risks, respectively¹⁷³. The trials among subjects with prevalent hypertension in the meta-analysis by Dong and colleagues found a 4 mm Hg decrease in SBP associated with fermented milk supplementation¹⁴⁸, suggesting a greater CVD benefit among hypertensives compared with normotensives¹⁷³.

The current hypothesis in terms of yogurt's biochemical effect on BP relates to its modulation of the renin-angiotensin pathway. Specifically, casein derived tripeptides (IPP and VPP) from certain fermented milk products inhibit the rate determining enzyme of RAS³⁶, which slows down vasoconstriction and simultaneously increases vasodilation. This inhibition has been shown in several experimental models characterized by varying concentrations and type(s) of lactic-acid producing bacterial strains³⁷⁻³⁹. Hirota et al showed that fermented milk tripeptides VPP and IPP improved vascular endothelial function independent of its BP-lowering effects in hypertensive subjects in a randomized trial of hypertensive males,¹⁹⁰ suggesting that fermented dairy products may have CVD-lowering capabilities that are independent of its BP-lowering effects.

Since most previous studies of fermented dairy products have been of short duration, there is a need for longer-term, well-controlled prospective studies to evaluate the effects of fermented dairy consumption on CVD risk among individuals with prevalent HBP.

A number of studies of total dairy intake have found that higher consumption is associated with a lower CVD risk^{198,221}. A meta-analysis of total dairy intake found an inverse association with risk of stroke and concluded that this may be due to the BP-lowering effects of dairy²²². Some prospective studies among those without prevalent HBP observed an inverse association of yogurt intake on markers of CVD^{28,218} that were not found in association with other dairy foods such as milk and cheese, which again may suggest that yogurt has unique effects on CVD risk factors²⁸. In contrast, it is also possible that reported yogurt intake may be less susceptible to bias in these historical cohort studies than are other forms of dairy, since dairy consumption was considered by many to be a risk factor for CVD at that time.

Diet patterns are also associated with CVD risk. A recent meta-analysis of longitudinal studies by Salehi et al showed that those with higher adherence to a

DASH-like diet have reduced risks of CVD, CHD, stroke, and heart failure by 20%, 21%, 19% and 29%, respectively²⁴². DASH diet adherence has also been associated with beneficial effects on diabetes, fasting glucose^{243,244}, total cholesterol, and LDL-cholesterol,^{243,245,246} all of which are risk factors for CVD. Thus, the evidence suggests that both diet and HBP may independently promote the development of CVD. Therefore, we hypothesized that yogurt intake may lower the risk of CVD both directly and indirectly (by lowering risk for HBP) and that these effects may be stronger than those found for other forms of dairy.

The final chapter evaluates the effects of yogurt consumption the risk of CVD among adults in the NHS and HPFS cohorts with prevalent HBP. These analyses add important new evidence suggesting that yogurt consumption lowers the risk of CVD in this high-risk population. To our knowledge, our analyses were among the first to analyze the effects of dairy intake on CVD risk among those with prevalent hypertension. The majority of previous studies have focused on estimating effects of dairy intake on risk of incident CVD among those who are healthy and free from HBP. Our results suggest that even among those who have HBP, consuming more yogurt is associated with lower risk for incident CVD,

CHD and stroke alone or in combination with an overall healthy diet as measured by the DASH diet score.

Our longitudinal analyses are among the first to have adequate statistical power to be able to estimate an independent effect of long-term regular yogurt consumption on HBP. The *Dietary Guidelines for Americans* recommend 2-3 servings/day (s/d) of dairy, with an emphasis on low-fat dairy and consuming a variety of dairy sources¹¹⁰. The majority of previous studies lacked sufficient statistical power to estimate the effect of a 1 s/d increase of yogurt, hence yogurt was usually combined with other dairy foods into a total dairy exposure. As a result, the yogurt-specific effects on cardiovascular outcomes have been unknown.¹¹⁵ This dissertation therefore addresses this important gap in the evidence.

Our results call for future studies focusing on different types of yogurt, such as yogurts with varying fat content, Greek yogurt (with twice the amount of protein, the most potent nutrient regulator of BP,¹⁴⁸ but half the carbohydrate content of regular yogurt), and yogurt with added probiotics, to see if there are different effects of specific types of yogurt on BP.

Finally, another important contribution of this dissertation is the observation that the effects of protein, dairy, and yogurt on BP were modified by the overall diet pattern. We found that individuals with a healthier overall eating pattern had even greater benefits from the added intakes of protein and other dairy products. In the NHS and HPFS cohorts, each participant was characterized according to the degree to which their pattern was consistent with that of the DASH eating pattern. We then examined the effects of yogurt consumption among individuals whose diet was more or less consistent with a DASH pattern. In these analyses, we found that the combined effects were approximately additive (i.e., risk reductions for those with higher yogurt intake plus DASH eating pattern = risk reductions for higher yogurt alone + risk reduction for DASH diet pattern alone). These results echo the most recent *Dietary Guidelines for Americans* that emphasize the need to consider the overall diet pattern rather than just focusing on individual foods for lowering risks of chronic disease and improving overall health.

Animal models have found specific biochemical mechanisms by which yogurt components may modulate BP. Our studies indicate that the time is right for randomized controlled trials of humans to examine the mechanisms by which

yogurt consumption impacts BP and CVD risk factors. Future studies should also examine direct effects of probiotics on HBP. It would be very difficult to accurately calculate the amount of probiotics in commercially-produced yogurt as the probiotic concentration is affected by several variable factors such as: handling and transport time shelf-life, refrigeration temperatures, etc. This adds to the complex problem of what type(s) and amounts of probiotics would be feasible to add to commercially produced yogurt in order to have any meaningful public health impact. Although this question seems to be a difficult one to answer and could certainly take many more years of research both in the basic and population-based sciences, our study provides evidence that yogurt consumption (of any type) may have a meaningful public health difference in terms of lowering risk of HBP, one of the major risk factors for CVD.

Our analyses provide longitudinal data supporting beneficial effects of long-term protein, dairy, and yogurt intakes on risk of incident HBP. These results also suggest that yogurt consumption among those with prevalent HBP lowers the risk of myocardial infarction and stroke. Future studies are needed to examine the effects of specific types of yogurt on cardiovascular outcomes as well as the applicability of these results to other racial/ethnic populations. The overall

message of this dissertation is that dairy consumption, especially yogurt, is a beneficial part of a healthy dietary pattern, and regular intake may reduce the necessity of pharmacologic HBP treatment.

APPENDIX

**Table A.1 The beneficial effects of high yogurt intake on incident HBP
(sensitivity analysis)**

				NHS Multivariable		+BMI	
				I /100K			
Yogurt Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk	905176	27012	2984	1.00	-	1.00	-
1-<5/wk	413323	13892	3361	0.96	(0.94-0.98)	0.98	(0.96-1.00)
≥5/wk	28333	680	2400	0.80	(0.74-0.87)	0.86	(0.80-0.93)
<i>P for linear trend⁵</i>				<i><0.0001</i>		<i><0.0001</i>	
Per 1 s/d increase				0.80	(0.75-0.86)	0.87	(0.81-0.93)
				NHS II Multivariable		+BMI	
				I /100K			
Yogurt Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk	799690	15090	1887	1.00	-	1.00	-
1-<5/wk	529542	10311	1947	0.92	(0.89-0.94)	0.95	(0.92-0.97)
≥5/wk	47026	733	1559	0.85	(0.79-0.92)	0.90	(0.84-0.97)
<i>P for linear trend⁵</i>				<i><0.0001</i>		<i><0.0001</i>	
Per 1 s/d increase				0.77	(0.71-0.83)	0.85	(0.79-0.92)
				HPFS Multivariable		+BMI	
				I /100K			
Yogurt Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk	372110	10870	2921	1.00	-	1.00	-
1-<5/wk	107893	3062	2838	0.93	(0.90-0.97)	0.94	(0.90-0.98)
≥5/wk	10123	258	2549	0.95	(0.84-1.08)	1.02	(0.90-1.15)
<i>P for linear trend⁵</i>				<i>0.0170</i>		<i>0.1733</i>	
Per 1 s/d increase				0.86	(0.77-0.97)	0.92	(0.81-1.04)
				META-ANALYSIS Multivariable		+BMI	
				I /100K			
Yogurt Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk	2076976	52972	2550	1.00	-	1.00	-
1-<5/wk	1050758	27265	2595	0.94	(0.93-0.96)	0.96	(0.95-0.98)
≥5/wk	85482	1671	1955	0.85	(0.80-0.89)	0.90	(0.86-0.95)

	<i>P for linear trend</i> ⁴	<0.0001	<0.0001
	<i>P for heterogeneity</i> ⁵	0.2907	0.5740
	<i>I</i> ²	20.4	0.0
Per 1 s/d increase	0.80	(0.76-0.84)	0.87 (0.83-0.91)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, milk, and cheese

² Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, milk, cheese, and BMI

³Linear trend across yogurt intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable.

⁴Test for between-study heterogeneity by using a fixed effects meta-analysis model

Table A.2 Higher yogurt intake combined with higher DASH diet score is linked with a lower risk of incident HBP

Yogurt + DASH score tertiles	P-yrs	Cases	I /100K p-yrs	NHS Multivariable		+BMI	
				HR ¹	95% CI	HR ²	95% CI
<1/wk+T1	321204	10251	3191	1.00	-	1.00	-
<1/wk+T2	376295	10587	2813	0.94	(0.91-0.97)	0.96	(0.93-0.99)
<1/wk+T3	207677	6174	2973	0.86	(0.83-0.89)	0.91	(0.88-0.94)
1-<5/wk+T1	76999	2821	3664	1.00	(0.96-1.04)	1.01	(0.97-1.05)
1-<5/wk+T2	150923	4865	3223	0.90	(0.87-0.93)	0.92	(0.89-0.96)
1-<5/wk+T3	185402	6206	3347	0.84	(0.81-0.87)	0.90	(0.87-0.93)
≥5/wk+T1	2787	84	3014	0.94	(0.76-1.16)	1.01	(0.82-1.26)
≥5/wk+T2	10224	225	2201	0.78	(0.68-0.89)	0.84	(0.73-0.96)
≥5/wk+T3	15323	371	2421	0.68	(0.61-0.75)	0.76	(0.69-0.85)

Yogurt + DASH score tertiles	P-yrs	Cases	I /100K p-yrs	NHS II Multivariable		+BMI	
				HR ¹	95% CI	HR ²	95% CI
<1/wk+T1	341608	7244	2121	1.00	-	1.00	-
<1/wk+T2	269405	5074	1883	0.91	(0.88-0.94)	0.97	(0.94-1.01)
<1/wk+T3	188677	2772	1469	0.71	(0.68-0.74)	0.81	(0.77-0.84)
1-<5/wk+T1	103617	2353	2271	0.93	(0.89-0.98)	0.95	(0.91-1.00)
1-<5/wk+T2	182891	3787	2071	0.85	(0.82-0.88)	0.92	(0.88-0.96)
1-<5/wk+T3	243034	4171	1716	0.71	(0.68-0.74)	0.82	(0.78-0.85)
≥5/wk+T1	4463	78	1748	0.85	(0.68-1.06)	0.91	(0.73-1.13)

≥5/wk+T2	11818	200	1692	0.78	(0.68-0.90)	0.84	(0.73-0.97)
≥5/wk+T3	30745	455	1480	0.69	(0.62-0.76)	0.81	(0.74-0.89)

HPFS

Multivariable

+BMI

Yogurt + DASH

I /100K

score tertiles	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk+T1	144259	4463	3094	1.00	-	1.00	-
<1/wk+T2	124348	3595	2891	0.89	(0.85-0.93)	0.91	(0.87-0.95)
<1/wk+T3	103503	2812	2717	0.82	(0.78-0.86)	0.87	(0.83-0.92)
1-<5/wk+T1	18735	571	3048	0.93	(0.85-1.02)	0.92	(0.85-1.01)
1-<5/wk+T2	36432	1071	2940	0.87	(0.81-0.92)	0.88	(0.82-0.94)
1-<5/wk+T3	52726	1420	2693	0.77	(0.73-0.82)	0.83	(0.78-0.88)
≥5/wk+T1	1063	38	3576	1.17	(0.85-1.61)	1.24	(0.90-1.70)
≥5/wk+T2	2500	65	2600	0.89	(0.69-1.13)	0.95	(0.74-1.21)
≥5/wk+T3	6560	155	2363	0.75	(0.64-0.89)	0.85	(0.72-1.00)

META-ANALYSIS

Multivariable

+BMI

Yogurt + DASH

I /100K

score tertiles	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/wk+T1	807071	21958	2721	1.00	-	1.00	-
<1/wk+T2	770048	19256	2501	0.92	(0.92-0.94)	0.95	(0.93-0.97)
<1/wk+T3	499857	11758	2352	0.81	(0.79-0.83)	0.87	(0.85-0.89)
1-<5/wk+T1	199351	5745	2882	0.96	(0.94-0.99)	0.98	(0.95-1.00)
1-<5/wk+T2	370246	9723	2626	0.88	(0.86-0.90)	0.92	(0.89-0.94)
1-<5/wk+T3	481162	11797	2452	0.78	(0.76-0.80)	0.86	(0.84-0.88)
≥5/wk+T1	8313	200	2406	0.94	(0.82-1.08)	1.01	(0.88-1.16)
≥5/wk+T2	24542	490	1997	0.79	(0.72-0.87)	0.85	(0.78-0.93)
≥5/wk+T3	52628	981	1864	0.69	(0.65-0.74)	0.80	(0.75-0.85)

P for heterogeneity⁴

<0.0001-0.6262

<0.0001-0.6693

I²

0-96.2

0-89.8

¹Adjusted for age, race, physical activity, total protein, energy, HBP family history

²Adjusted for age, race, physical activity, total protein, energy, HBP family history, and BMI

³Test for between-study heterogeneity by using a fixed effects model

Table A.3 BMI has a greater effect on HBP risk than yogurt

			NHS			
			Multivariable			
Yogurt Intake+ BMI	P-yrs	Cases	I /100K p-yrs	HR¹	95% CI	
<1/wk + BMI \geq 30	103217	5861	5678	1.00	-	
<1/wk + BMI: 25-<30	247660	9494	3833	0.68	(0.66-0.70)	
<1/wk + BMI<25	554299	11657	2103	0.41	(0.40-0.43)	
1-<5/wk + BMI \geq 30	47582	2971	6244	0.97	(0.93-1.01)	
1-<5/wk + BMI: 25-<30	120436	5059	4201	0.66	(0.63-0.68)	
1-<5/wk + BMI<25	245305	5862	2390	0.41	(0.39-0.42)	
\geq 5/wk + BMI \geq 30	2106	103	4891	0.85	(0.70-1.03)	
\geq 5/wk + BMI: 25-<30	6502	236	3630	0.64	(0.56-0.73)	
\geq 5/wk + BMI<25	19725	341	1729	0.34	(0.30-0.38)	
			NHS II			
			Multivariable			
Yogurt Intake+ BMI	P-yrs	HBP Cases	I /100K p-yrs	HR¹	95% CI	
<1/wk + BMI \geq 30	133516	5909	4426	1.00	-	
<1/wk + BMI: 25-<30	199728	4748	2377	0.56	(0.54-0.58)	
<1/wk + BMI<25	466445	4433	950	0.26	(0.25-0.27)	
1-<5/wk + BMI \geq 30	83322	3877	4653	0.97	(0.93-1.01)	
1-<5/wk + BMI: 25-<30	140712	3281	2332	0.50	(0.48-0.53)	
1-<5/wk + BMI<25	305508	3153	1032	0.25	(0.24-0.26)	
\geq 5/wk + BMI \geq 30	6420	284	4424	1.03	(0.91-1.16)	
\geq 5/wk + BMI: 25-<30	11572	208	1797	0.45	(0.39-0.51)	
\geq 5/wk + BMI<25	29034	241	830	0.23	(0.20-0.27)	
			HPFS			
			Multivariable			
Yogurt Intake+ BMI	P-yrs	Cases	I /100K p-yrs	HR¹	95% CI	
<1/wk + BMI \geq 30	24786	1240	5003	1.00	-	
<1/wk + BMI: 25-<30	144034	4852	3369	0.70	(0.66-0.74)	
<1/wk + BMI<25	203291	4778	2350	0.50	(0.47-0.54)	
1-<5/wk + BMI \geq 30	7137	356	4988	0.95	(0.85-1.07)	
1-<5/wk + BMI: 25-<30	39782	1307	3285	0.65	(0.60-0.71)	
1-<5/wk + BMI<25	60974	1399	2294	0.47	(0.44-0.51)	
\geq 5/wk + BMI \geq 30	361	23	6367	1.36	(0.90-2.05)	
\geq 5/wk + BMI: 25-<30	2883	85	2948	0.65	(0.52-0.81)	
\geq 5/wk + BMI<25	6878	150	2181	0.51	(0.43-0.60)	

¹Adjusted for age, race, physical activity, energy, HBP family history, intakes of total protein, fruits and vegetables, milk, and cheese

Table A.4 High milk intake is associated with a lower risk of HBP

				NHS			
				Multivariable		+BMI	
				I /100K			
Milk Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<4/wk	603863	17991	2979	1.00	-	1.00	-
4/wk-<1/d	238967	8290	3469	1.00	(0.97-1.03)	1.00	(0.97-1.02)
1-<2/d	334198	10355	3098	0.97	(0.95-1.00)	0.97	(0.95-1.00)
2-<6/d	169805	4948	2914	0.93	(0.90-0.96)	0.96	(0.93-0.99)
<i>P for linear trend[‡]</i>				<0.0001		0.0054	
Per 1 s/d increase				0.97	(0.95-0.98)	0.98	(0.97-0.99)
				NHS II			
				Multivariable		+BMI	
				I /100K			
Milk Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<4/wk	528292	10761	2037	1.00	-	1.00	-
4/wk-<1/d	258533	5365	2075	0.95	(0.91-0.98)	0.95	(0.92-0.98)
1-<2/d	348874	6405	1836	0.92	(0.89-0.95)	0.95	(0.92-0.98)
2-<6/d	240559	3603	1498	0.80	(0.77-0.84)	0.88	(0.84-0.91)
<i>P for linear trend[‡]</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.91	(0.89-0.92)	0.94	(0.93-0.96)
				HPFS			
				Multivariable		+BMI	
				I /100K			
Milk Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<4/wk	232664	6855	2946	1.00	-	1.00	-
4/wk-<1/d	89061	2769	3109	0.96	(0.92-1.01)	0.97	(0.92-1.01)
1-<2/d	97879	2709	2768	0.92	(0.88-0.96)	0.93	(0.89-0.98)
2-<6/d	70522	1857	2633	0.87	(0.82-0.92)	0.89	(0.84-0.94)
<i>P for linear trend[‡]</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.94	(0.92-0.96)	0.95	(0.93-0.97)
META-ANALYSIS							
				Multivariable		+BMI	
				I /100K			
Milk Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<4/wk	1364819	35607	2609	1.00	-	1.00	-
4/wk-<1/d	586561	16424	2800	0.98	(0.96-0.99)	0.98	(0.96-1.00)
1-<2/d	780951	19469	2493	0.95	(0.93-0.96)	0.96	(0.94-0.98)

2-<6/d	480886	10408	2164	0.87	(0.85-0.89)	0.92	(0.90-0.94)
				<i>P for linear trend</i> ⁴	<0.0001		<0.0001
				<i>P for heterogeneity</i> ⁵	<0.0001		0.0024
				<i>I</i> ²	93.9		84.3
Per 1 s/d increase				0.94	(0.93-0.95)	0.96	(0.95-0.97)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and cheese

²Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and cheese, BMI

³Linear trend across milk intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

Table A.5 Increased cheese consumption is inversely associated with risk of HBP in women

				NHS		+BMI	
				Multivariable			
				I /100K			
Cheese Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/week	555905	14218	2558	1.00	-	1.00	-
1-4/week	670158	23229	3466	0.98	(0.96-1.01)	0.97	(0.95-1.00)
5/week-<1/d	94484	3310	3503	0.95	(0.91-0.99)	0.93	(0.89-0.97)
1-4/d	26285	827	3146	0.89	(0.83-0.96)	0.89	(0.83-0.96)
<i>P for linear trend</i> ³				0.0003		<0.0001	
Per 1 s/d increase				0.92	(0.87-0.96)	0.90	(0.86-0.94)
				NHS II		+BMI	
				Multivariable			
				I /100K			
Cheese Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/week	236141	4777	2023	1.00	-	1.00	-
1-4/week	808058	15515	1920	0.98	(0.95-1.01)	0.96	(0.93-1.00)
5/week-<1/d	234890	4400	1873	0.95	(0.91-1.00)	0.93	(0.89-0.98)
1-4/d	97169	1442	1484	0.86	(0.81-0.91)	0.85	(0.79-0.90)
<i>P for linear trend</i> ³				<0.0001		<0.0001	
Per 1 s/d increase				0.88	(0.84-0.93)	0.87	(0.83-0.92)
				HPFS		+BMI	
				Multivariable			

Cheese Intake	P-yrs	Cases	I /100K		HR ¹	95% CI	HR ²	95% CI
			p-yrs					
<1/week	128030	3534	2760		1.00	-	1.00	-
1-4/week	267813	7912	2954		1.02	(0.97-1.06)	0.98	(0.94-1.02)
5/week-<1/d	66819	1962	2936		1.01	(0.95-1.07)	0.95	(0.89-1.00)
1-4/d	27464	782	2847		1.08	(0.99-1.17)	1.00	(0.92-1.09)
<i>P for linear trend³</i>					0.1557		0.3415	
Per 1 s/d increase					1.05	(0.98-1.12)	0.97	(0.90-1.04)

META-ANALYSIS

Cheese Intake	P-yrs	Cases	I /100K		Multivariable		+BMI	
			p-yrs		HR ¹	95% CI	HR ²	95% CI
<1/week	920076	22529	2449		1.00	-	1.00	-
1-4/week	1746029	46656	2672		0.99	(0.97-1.01)	0.97	(0.95-0.99)
5/week-<1/d	396193	9672	2441		0.96	(0.94-0.99)	0.94	(0.91-0.96)
1-4/d	150918	3051	2022		0.92	(0.88-0.96)	0.90	(0.86-0.93)
<i>P for linear trend³</i>					<0.0001		<0.0001	
<i>P for heterogeneity⁴</i>					0.0003		0.0552	
<i>I²</i>					88.1		66.3	
Per 1 s/d increase					0.93	(0.90-0.96)	0.90	(0.88-0.93)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and milk

²Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, yogurt, and milk, BMI

³Linear trend across cheese intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

Table A.6 Low-fat dairy intake is linked with lower risks of HBP in NHS II and HPFS

Low-fat dairy intake (s/d)	P-yrs	Cases	NHS		HR ²	95% CI	
			I /100K	Multivariable			
			p-yrs	HR ¹			
<0.5	554021	15605	2817	1.00	-	1.00	-

0.5-<1.5	538999	17604	3266	1.00	(0.98-1.02)	0.99	(0.97-1.02)
1.5-<2.5	151434	5417	3577	0.97	(0.96-0.98)	0.97	(0.96-0.99)
2.5-<6	102378	2958	2889	1.00	(0.95-1.05)	1.03	(0.98-1.09)
<i>P for linear trend⁴</i>				<0.0001		0.0004	
Per 1 s/d increase				0.96	(0.95-0.98)	0.97	(0.96-0.99)
NHS II							
				Multivariable		+BMI	
Low-fat dairy intake (s/d)	P-yrs	Cases	I /100K p-yrs	HR¹	95% CI	HR²	95% CI
<0.5	345352	6882	1993	1.00	-	1.00	-
0.5-<1.5	602083	11735	1949	0.93	(0.90-0.96)	0.95	(0.92-0.98)
1.5-<2.5	212301	4444	2093	0.89	(0.85-0.92)	0.93	(0.90-0.97)
2.5-<6	216522	3073	1419	0.76	(0.72-0.79)	0.83	(0.80-0.87)
<i>P for linear trend⁴</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.91	(0.89-0.92)	0.94	(0.92-0.95)
HPFS							
				Multivariable		+BMI	
Low-fat dairy intake (s/d)	P-yrs	Cases	I /100K p-yrs	HR¹	95% CI	HR²	95% CI
<0.5	191325	5509	2879	1.00	-	1.00	-
0.5-<1.5	196755	5789	2942	0.95	(0.91-0.98)	0.95	(0.91-0.98)
1.5-<2.5	47413	1517	3200	0.91	(0.86-0.96)	0.92	(0.87-0.98)
2.5-<6	54633	1375	2517	0.85	(0.80-0.91)	0.87	(0.81-0.92)
<i>P for linear trend⁴</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.94	(0.92-0.96)	0.95	(0.93-0.97)
META-ANALYSIS							
				Multivariable		+BMI	
Low-fat dairy intake (s/d)¹	P-yrs	Cases	I /100K py	HR¹	95% CI	HR²	95% CI
<0.5	1090698	27996	2567	1.00	-	1.00	-
0.5-<1.5	1337837	35128	2626	0.97	(0.96-0.99)	0.97	(0.96-0.99)
1.5-<2.5	411148	11378	2767	0.96	(0.95-0.97)	0.97	(0.95-0.98)
2.5-<6	373533	7406	1983	0.86	(0.83-0.88)	0.91	(0.88-0.96)
<i>P for linear trend³</i>				<0.0001		<0.0001	
<i>P for heterogeneity⁴</i>				<0.0001		0.0034	
<i>I²</i>				94.0		83.2	
Per 1 s/d increase				0.94	(0.93-0.95)	0.96	(0.95-0.97)

¹Adjusted for age, race, physical activity, energy intake, HBP family history, and intakes of FV, total protein, and high-fat dairy

²Adjusted for age, race, physical activity, energy intake, HBP family history, intakes of FV, total protein, and high-fat dairy, BMI

³Linear trend across low-fat dairy categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

Table A.7 High-fat dairy consumption is inversely associated with risk of HBP

in NHS and NHS II

				NHS		+BMI	
				Multivariable			
High-fat Dairy intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5	885924	27422	3095	1.00	-	1.00	-
0.5-<1.5	399668	12552	3141	0.97	(0.95-0.99)	0.97	(0.94-0.99)
1.5-<6	61241	1610	2629	0.90	(0.85-0.95)	0.92	(0.87-0.97)
<i>P for linear trend³</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.95	(0.92-0.97)	0.95	(0.92-0.97)
				NHS II		+BMI	
				Multivariable			
High-fat dairy intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5	900424	17829	1980	1.00	-	1.00	-
0.5-<1.5	432059	7647	1770	0.95	(0.92-0.97)	0.94	(0.91-0.97)
1.5-<6	43775	658	1503	0.87	(0.80-0.94)	0.88	(0.81-0.95)
<i>P for linear trend³</i>				<0.0001		<0.0001	
Per 1 s/d increase				0.91	(0.88-0.95)	0.91	(0.88-0.95)
				HPFS		+BMI	
				Multivariable			
High-fat dairy intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5	313244	9236	2949	1.00	-	1.00	-
0.5-<1.5	153357	4304	2807	0.99	(0.96-1.03)	0.96	(0.93-1.00)
1.5-<6	23525	650	2763	0.99	(0.91-1.07)	0.97	(0.89-1.05)
<i>P for linear trend⁴</i>				0.7220		0.2001	
Per 1 s/d increase				0.99	(0.95-1.03)	0.97	(0.94-1.01)
				META-ANALYSIS		+BMI	
				Multivariable			

High-fat dairy intake	I /100K			NHS		+BMI	
	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5	2099592	54487	2595	1.00	-	1.00	-
0.5-<1.5	985084	24503	2487	0.97	(0.95-0.98)	0.96	(0.94-0.97)
1.5-<6	128541	2918	2270	0.91	(0.87-0.94)	0.92	(0.88-0.96)
<i>P for linear trend³</i>				<0.0001		<0.0001	
<i>P for heterogeneity⁴</i>				0.0095		0.0606	
<i>I²</i>				80.0		66.4	
Per 1 s/d increase				0.95	(0.93-0.97)	0.95	(0.93-0.96)

¹Adjusted for age, race, physical activity, FV, total protein, energy intake, low-fat dairy, HBP family history

²Adjusted for age, race, physical activity, FV, total protein, energy intake, low-fat dairy, HBP family history, BMI

³Linear trend across high-fat dairy intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable

⁴Test for between-study heterogeneity by using a fixed effects model

Table A.8 Higher DASH diet scores modifies the beneficial effect of total dairy intake on incident risk of HBP

Total Dairy + DASH score tertiles ¹	P-yrs	Cases	I /100K p-yrs	NHS Multivariable		+BMI	
				HR ²	95% CI	HR ³	95% CI
<1.5/d + T1	275267	9051	3288	1.00	-	1.00	-
<1.5/d + T2	368640	10376	2815	0.92	(0.89-0.95)	0.95	(0.92-0.97)
<1.5/d + T3	227815	6785	2978	0.83	(0.81-0.86)	0.88	(0.85-0.91)
1.5-<3/d + T1	103700	3527	3401	0.94	(0.90-0.98)	0.94	(0.90-0.98)
1.5-<3/d + T2	148257	4645	3133	0.87	(0.83-0.90)	0.89	(0.86-0.92)
1.5-<3/d + T3	141985	4641	3269	0.80	(0.77-0.83)	0.85	(0.82-0.89)
≥3/d + T1	19702	652	3309	0.91	(0.84-0.99)	0.93	(0.86-1.01)
≥3/d + T2	30522	932	3054	0.86	(0.80-0.92)	0.92	(0.85-0.98)
≥3/d + T3	30945	975	3151	0.75	(0.70-0.81)	0.84	(0.78-0.90)
				NHS II Multivariable		+BMI	
Total Dairy Intake+ DASH score ¹	P-yrs	Cases	I /100K p-yrs	HR ²	95% CI	HR ³	95% CI
<1.5/d + T1	215537	4894	2271	1.00	-	1.00	-
<1.5/d + T2	332267	6498	1956	0.94	(0.90-0.98)	1.01	(0.97-1.05)

<1.5/d + T3	147822	2705	1830	0.77	(0.73-0.80)	0.88	(0.84-0.92)
1.5-<3/d + T1	107107	2351	2195	0.94	(0.89-0.98)	0.97	(0.92-1.02)
1.5-<3/d + T2	241556	4446	1841	0.87	(0.83-0.91)	0.95	(0.91-0.99)
1.5-<3/d + T3	144826	2583	1784	0.71	(0.67-0.74)	0.83	(0.79-0.87)
≥3/d + T1	36253	566	1561	0.77	(0.71-0.84)	0.83	(0.76-0.91)
≥3/d + T2	92882	1331	1433	0.75	(0.70-0.80)	0.86	(0.80-0.92)
≥3/d + T3	58008	760	1310	0.59	(0.54-0.63)	0.72	(0.67-0.78)

HPFS

Multivariable

+BMI

Total Dairy Intake+

DASH score¹

I /100K

	P-yrs	Cases	p-yrs	HR²	95% CI	HR³	95% CI
<1.5/d + T1	104074	3236	3109	1.00	-	1.00	-
<1.5/d + T2	101450	3118	3073	0.94	(0.90-0.99)	0.96	(0.92-1.01)
<1.5/d + T3	101501	2712	2672	0.81	(0.77-0.86)	0.87	(0.82-0.92)
1.5-<3/d + T1	39299	1195	3041	0.93	(0.87-1.00)	0.94	(0.87-1.00)
1.5-<3/d + T2	45988	1306	2840	0.84	(0.78-0.89)	0.86	(0.80-0.91)
1.5-<3/d + T3	48303	1262	2613	0.76	(0.71-0.81)	0.82	(0.76-0.87)
≥3/d + T1	16039	485	3024	0.97	(0.88-1.07)	0.96	(0.87-1.06)
≥3/d + T2	16155	442	2736	0.85	(0.77-0.94)	0.87	(0.79-0.97)
≥3/d + T3	17317	434	2506	0.76	(0.69-0.84)	0.82	(0.74-0.91)

META-ANALYSIS

Multivariable

+BMI

Total Dairy Intake+

DASH score¹

I /100K

	P-yrs	Cases	p-yrs	HR²	95% CI	HR³	95% CI
<1.5/d + T1	594878	17181	2888	1.00	-	1.00	-
<1.5/d + T2	802357	19992	2492	0.93	(0.91-0.95)	0.97	(0.95-0.99)
<1.5/d + T3	477138	12202	2557	0.81	(0.79-0.83)	0.88	(0.86-0.90)
1.5-<3/d + T1	250106	7073	2828	0.94	(0.91-0.96)	0.95	(0.92-0.97)
1.5-<3/d + T2	435801	10397	2386	0.86	(0.84-0.88)	0.90	(0.88-0.93)
1.5-<3/d + T3	335114	8486	2532	0.76	(0.74-0.78)	0.84	(0.81-0.86)
≥3/d + T1	71994	1703	2365	0.88	(0.83-0.92)	0.90	(0.86-0.95)
≥3/d + T2	139559	2705	1938	0.81	(0.77-0.84)	0.88	(0.85-0.92)
≥3/d + T3	106270	2169	2041	0.77	(0.73-0.80)	0.79	(0.76-0.83)

P for heterogeneity⁴

<0.0001-0.9966

<0.0001-0.9335

I²

0-87.3

0-76.8

¹Adjusted for age, race, physical activity, total protein, energy intake, HBP family history

²Adjusted for age, race, physical activity, total protein, energy intake, HBP family history, BMI

³Test for between-study heterogeneity by using a fixed effects model

Table A.9 Higher yogurt intake is linked with lower CHD risk in women and men

				NHS			
Yogurt Intake	P-yrs	CHD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ³	95% CI	HR ⁵	95% CI
<1/mo	338759	1052	311	1.00	-	1.00	-
1/mo-<1/wk	156811	366	233	0.85	(0.75-0.96)	0.85	(0.75-0.96)
1-<2/wk	136193	284	209	0.79	(0.69-0.90)	0.83	(0.72-0.94)
≥2/wk	131244	244	186	0.74	(0.64-0.86)	0.76	(0.66-0.88)
<i>P for linear trend⁴</i>				<0.0001		0.0003	
Per one serving/day				0.49	(0.35-0.70)	0.53	(0.37-0.75)
				HPFS			
Yogurt Intake	P-yrs	CHD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ³	95% CI	HR ⁵	95% CI
<1/mo	119120	794	667	1.00	-	1.00	-
1/mo-<1/wk	40850	221	541	0.94	(0.80-1.09)	0.92	(0.79-1.07)
1-<2/wk	27612	145	525	0.89	(0.74-1.07)	0.90	(0.75-1.07)
≥2/wk	21553	107	496	0.82	(0.66-1.01)	0.84	(0.68-1.04)
<i>P for linear trend⁴</i>				0.0439		0.0815	
Per one serving/day				0.61	(0.37-0.99)	0.65	(0.41-1.05)

¹Adjusted for age, race, smoking, energy intake, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, milk and cheese

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across yogurt intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.10 Increased yogurt intake is associated with lower risk of stroke in women and men

				NHS			
Yogurt Intake	P-yrs	Stroke Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI

<1/mo	338825	823	243	1.00	-	1.00	-
1/mo-<1/wk	156784	332	212	0.98	(0.86-1.11)	0.97	(0.85-1.10)
1-<2/wk	136169	277	203	0.91	(0.79-1.05)	0.92	(0.80-1.05)
≥2/wk	131215	253	193	0.94	(0.81-1.09)	0.92	(0.79-1.07)
<i>P for linear trend³</i>				0.3707		0.2450	
Per one serving/day				0.85	(0.60-1.21)	0.81	(0.57-1.15)

HPFS

Yogurt Intake	P-yrs	Stroke Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<1/mo	119242	349	293	1.00	-	1.00	-
1/mo-<1/wk	40927	88	215	0.88	(0.69-1.12)	0.84	(0.66-1.07)
1-<2/wk	27654	41	148	0.59	(0.43-0.83)	0.71	(0.53-0.96)
≥2/wk	21581	45	209	0.81	(0.59-1.12)	0.81	(0.59-1.12)
<i>P for linear trend³</i>				0.0760		0.1085	
Per one serving/day				0.49	(0.22-1.08)	0.53	(0.24-1.15)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, milk and cheese

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across yogurt intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.11 Higher yogurt combined with a higher DASH diet score is linked with a lower risk of CHD in women and men

Yogurt, DASH score	P-yrs	CHD Cases	I /100K p-yrs	NHS Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<2/wk, <25	392084	1067	272	1.00	-	1.00	-
<2/wk, ≥25	239680	635	265	0.90	(0.81-1.00)	0.87	(0.78-0.96)
≥2/wk, <25	54880	95	173	0.75	(0.61-0.93)	0.78	(0.63-0.96)
≥2/wk, ≥25	76364	149	195	0.77	(0.65-0.93)	0.75	(0.62-0.90)

HPFS

Multivariable No Diet Update

Yogurt, DASH score	P-yrs	CHD	I /100K	HR ¹	95% CI	HR ²	95% CI
		Cases	p-yrs				
<1/wk, <25	105527	643	609	1.00	-	1.00	-
<1/wk, ≥25	64231	389	606	0.87	(0.76-1.00)	0.90	(0.78-1.03)
≥1/wk, <25	16500	115	697	1.13	(0.92-1.38)	1.13	(0.93-1.37)
≥1/wk, ≥25	25796	147	570	0.79	(0.65-0.96)	0.79	(0.65-0.96)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol and *trans* fat

²Diet was not updated upon report of CABG, angina, or high cholesterol

Table A.12 Higher yogurt intake combined with a higher DASH score is associated with a lower risk of stroke in women and men

Yogurt, DASH score	P-yrs	NHS					
		STR Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<2/wk, <25	392086	886	226	1.00	-	1.00	-
<2/wk, ≥25	239692	546	228	0.91	(0.81-1.03)	0.89	(0.79-1.00)
≥2/wk, <25	54846	111	202	1.02	(0.83-1.24)	0.99	(0.81-1.21)
≥2/wk, ≥25	76369	142	186	0.84	(0.70-1.02)	0.81	(0.67-0.98)

Yogurt, DASH score	P-yrs	HPFS					
		STR Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<1/wk, <25	105651	265	251	1.00	-	1.00	-
<1/wk, ≥25	64324	165	257	0.85	(0.69-1.05)	0.84	(0.68-1.04)
≥1/wk, <25	16532	40	242	0.93	(0.67-1.31)	0.93	(0.67-1.30)
≥1/wk, ≥25	25817	63	244	0.77	(0.58-1.04)	0.81	(0.61-1.08)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol and *trans* fat

²Diet was not updated upon report of CABG, angina, or high cholesterol

Table A.13 Increased milk consumption is linked with a higher risk of CHD in women but a lower risk of CHD in men

		NHS					
		CHD		Multivariable		No Diet Update	
Milk Intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	296355	700	236	1.00	-	1.00	-
0.5-<2/d	363260	942	259	1.10	(1.00-1.22)	1.09	(0.98-1.21)
2-<5/d	103393	304	294	1.23	(1.06-1.42)	1.19	(1.03-1.38)
<i>P for linear trend³</i>				0.0045		0.0152	
Per one serving/day				1.09	(1.03-1.16)	1.08	(1.01-1.15)

		HPFS					
		CHD		Multivariable		No Diet Update	
Milk Intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	92010	554	602	1.00	-	1.00	-
0.5-<2/d	87682	548	625	0.97	(0.86-1.10)	0.96	(0.85-1.08)
2-<5/d	29443	165	560	0.76	(0.63-0.91)	0.78	(0.65-0.93)
<i>P for linear trend³</i>				0.0044		0.0088	
Per one serving/day				0.90	(0.83-0.97)	0.90	(0.84-0.97)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, yogurt and cheese

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across milk intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.14 Higher milk intake is not associated with risk of stroke

		NHS					
		Stroke		Multivariable		No Diet Update	
Milk Intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	296343	637	215	1.00	-	1.00	-
0.5-<2/d	363239	821	226	0.99	(0.89-1.10)	0.99	(0.88-1.10)
2-<5/d	103410	227	220	0.96	(0.81-1.13)	0.95	(0.80-1.11)
<i>P for linear trend³</i>				0.6076		0.5090	
Per one serving/day				0.98	(0.92-1.05)	0.98	(0.91-1.05)

		HPFS					
		Stroke		Multivariable		No Diet Update	
Milk Intake	P-yrs	Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	92140	229	249	1.00	-	1.00	-

0.5-<2/d	87814	205	233	0.87	(0.71-1.05)	0.88	(0.73-1.07)
2-<5/d	29450	89	302	1.06	(0.82-1.38)	1.07	(0.82-1.39)
<i>P for linear trend³</i>				0.8248		0.7437	
Per one serving/day				1.01	(0.90-1.14)	1.02	(0.91-1.14)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, yogurt and cheese

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across milk intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.15 Higher milk consumption combined with a high DASH diet score is associated with a lower risk of CHD in men

Milk Intake + DASH score ¹	P-yrs	CHD Cases	I /100K p-yrs	NHS		No Diet Update	
				Multivariable HR ²	95% CI	HR ³	95% CI
Milk<1/d+DASH<22	294827	725	246	1.00	-	1.00	-
Milk<1/d+DASH≥22	172085	400	232	0.93	(0.82-1.06)	0.92	(0.81-1.05)
Milk:1-<5/d+DASH<22	161983	462	285	1.13	(1.00-1.28)	1.15	(1.02-1.30)
Milk:1-<5/d+DASH≥22	134113	359	268	1.06	(0.92-1.22)	1.01	(0.88-1.16)
		1946					
Milk Intake + DASH score ¹	P-yrs	CHD Cases	I /100K p-yrs	HPFS		No Diet Update	
				Multivariable HR ²	95% CI	HR ³	95% CI
Milk<1/d+DASH<22	79848	508	636	1.00	-	1.00	-
Milk<1/d+DASH≥22	55358	329	594	0.84	(0.72-0.98)	0.82	(0.71-0.96)
Milk:1-<5/d+DASH<22	41510	234	564	0.81	(0.69-0.95)	0.80	(0.69-0.94)
Milk:1-<5/d+DASH≥22	32419	196	605	0.78	(0.65-0.94)	0.78	(0.65-0.93)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, yogurt, cheese, and *trans* fat

²Diet not updated upon report of CABG, angina, or high cholesterol

Table A.16 Higher milk intake with a high DASH diet score is linked with a lower risk of stroke in men

Milk Intake + DASH score ¹	P-yrs	Stroke Cases	I /100K p-yrs	NHS		No Diet Update	
				Multivariable		No Diet Update	
				HR ²	95% CI	HR ³	95% CI
Milk<1/d+DASH<22	294807	626	212	1.00	-	1.00	-
Milk<1/d+DASH≥22	172058	390	227	1.00	(0.88-1.15)	0.97	(0.85-1.11)
Milk:1-<5/d+DASH<22	161986	372	230	1.07	(0.83-1.22)	1.05	(0.92-1.20)
Milk:1-<5/d+DASH≥22	134141	297	221	0.95	(0.82-1.11)	0.92	(0.79-1.07)

Milk Intake + DASH score ¹	P-yrs	Stroke Cases	I /100K p-yrs	HPFS		No Diet Update	
				Multivariable		No Diet Update	
				HR ²	95% CI	HR ³	95% CI
Milk<1/d+DASH<22	79966	188	235	1.00	-	1.00	-
Milk<1/d+DASH≥22	55440	142	256	0.93	(0.73-1.18)	0.86	(0.68-1.10)
Milk:1-<5/d+DASH<22	41541	113	272	1.06	(0.84-1.35)	1.06	(0.83-1.34)
Milk:1-<5/d+DASH≥22	32457	80	246	0.85	(0.64-1.13)	0.82	(0.62-1.09)

¹Cumulative average of milk intake and DASH score (no dairy) was calculated up to the first of the following events: stroke diagnosis, lost to follow up, death, end of study

²Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, yogurt, cheese, and *trans* fat

³Diet not updated upon report of CABG, angina, or high cholesterol

Table A.17 Higher cheese intake is associated with a modest beneficial effect on CHD risk in women

Cheese Intake	P-yrs	CHD Cases	I /100K p-yrs	NHS		No Diet Update	
				Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d	370105	994	269	1.00	-	1.00	-
0.25-<0.5/d	251940	621	246	0.94	(0.84-1.04)	0.94	(0.84-1.04)
0.5-4/d	140963	331	235	0.92	(0.80-1.05)	0.91	(0.79-1.04)
<i>P for linear trend³</i>				0.1529		0.1178	
Per one serving/day				0.85	(0.68-1.06)	0.84	(0.67-1.05)

HPFS

Multivariable No Diet Update

Cheese Intake	P-yrs	CHD		I /100K			
		Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.25/d	87528	520	594	1.00	-	1.00	-
0.25-<0.5/d	71650	423	590	1.02	(0.89-1.16)	1.04	(0.91-1.19)
0.5-4/d	49958	324	649	1.10	(0.95-1.28)	1.10	(0.94-1.27)
<i>P for linear trend³</i>				0.2143		0.2314	
Per one serving/day				1.15	(0.92-1.43)	1.15	(0.91-1.45)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, yogurt and milk

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across cheese intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.18 Higher cheese intake is inversely associated with risk of stroke in women

Cheese Intake	P-yrs	Stroke Cases	I /100K p-yrs	NHS			
				Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d	370112	829	224	1.00	-	1.00	-
0.25-<0.5/d	251932	559	222	0.93	(0.83-1.04)	0.93	(0.83-1.04)
0.5-4/d	140949	297	211	0.92	(0.79-1.06)	0.91	(0.79-1.05)
<i>P for linear trend⁴</i>				0.1790		0.1416	
Per one serving/day				0.85	(0.67-1.08)	0.84	(0.66-1.06)

Cheese Intake	P-yrs	Stroke Cases	I /100K p-yrs	HPFS			
				Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d	87688	205	234	1.00	-	1.00	-
0.25-<0.5/d	71696	193	269	1.16	(0.95-1.43)	1.17	(0.95-1.43)
0.5-4/d	50020	125	250	1.11	(0.88-1.41)	1.11	(0.87-1.41)
<i>P for linear trend³</i>				0.3117		0.3267	
Per one serving/day				1.19	(0.85-1.68)	1.20	(0.83-1.72)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, fiber, yogurt and milk

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across cheese intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling this variable as a continuous variable

Table A.19 Higher cheese intake combined with a high DASH diet score is associated with a lower risk of CHD in women and men

NHS							
Cheese, DASH score	P-yrs	CHD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	220021	592	269	1.00	-	1.00	-
<0.25/d, ≥22	150084	402	268	0.98	(0.86-1.12)	0.95	(0.83-1.08)
0.25-4/d, <22	236789	595	251	0.96	(0.85-1.09)	0.96	(0.85-1.08)
0.25-4/d, ≥22	156114	357	229	0.85	(0.74-0.99)	0.83	(0.72-0.95)

HPFS							
Cheese, DASH score	P-yrs	CHD Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	47610	289	607	1.00	-	1.00	-
<0.25/d, ≥22	39918	231	579	0.87	(0.73-1.04)	0.85	(0.71-1.02)
0.25-4/d, <22	73748	453	614	1.05	(0.90-1.23)	1.06	(0.91-1.23)
0.25-4/d, ≥22	47860	294	614	0.93	(0.78-1.11)	0.93	(0.78-1.11)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, yogurt, and milk

²Diet not updated upon report of CABG, angina, or high cholesterol

Table A.20 Higher cheese intake with a high DASH diet score is associated with a lower risk of stroke in women

NHS							
Cheese, DASH score	P-yrs	Stroke Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	220019	492	224	1.00	-	1.00	-
<0.25/d, ≥22	150093	337	225	0.94	(0.81-1.09)	0.91	(0.79-1.06)
0.25-4/d, <22	236774	506	214	0.91	(0.80-1.04)	0.91	(0.80-1.03)
0.25-4/d, ≥22	156106	350	224	0.89	(0.89-1.03)	0.86	(0.74-1.00)

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HPFS

Cheese, DASH score	P-yrs	Stroke Cases	I /100K p-yrs	Multivariable		No Diet Update	
				HR ¹	95% CI	HR ²	95% CI
<0.25/d, <22	47705	102	214	1.00	-	1.00	-
<0.25/d, ≥22	39982	103	258	1.03	(0.77-1.37)	0.94	(0.70-1.27)
0.25-4/d, <22	73802	199	270	1.29	(1.01-1.66)	1.26	(0.98-1.61)
0.25-4/d, ≥22	47914	119	248	1.03	(0.78-1.37)	0.98	(0.74-1.29)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, yogurt, milk, and *trans* fat

²Diet not updated upon report of CABG, angina, or high cholesterol

Table A.21 Higher total dairy intake is linked with a lower CHD risk in men

		NHS		Multivariable		No Diet Update	
Dairy Intake	P-yrs	CHD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	112150	317	283	1.00	-	1.00	-
0.5-<1/d	167949	419	249	0.92	(0.79-1.06)	0.91	(0.79-1.06)
1-<2/d	194899	677	230	0.87	(0.75-1.00)	0.88	(0.76-1.01)
2-<6/d	188010	533	284	1.08	(0.92-1.27)	1.07	(0.91-1.26)
<i>P for linear trend³</i>				0.0454		0.0517	
Per one serving/day				1.06	(1.00-1.13)	1.06	(1.00-1.13)

		HPFS		Multivariable		No Diet Update	
Dairy Intake	P-yrs	CHD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	31973	183	569	1.00	-	1.00	-
0.5-<1/d	51640	305	591	1.02	(0.84-1.22)	1.05	(0.87-1.28)
1-<2/d	79213	505	638	1.05	(0.88-1.25)	1.11	(0.93-1.33)
2-<6/d	46309	275	594	0.87	(0.71-1.07)	0.92	(0.75-1.14)
<i>P for linear trend³</i>				0.0679		0.1653	
Per one serving/day				0.94	(0.87-1.00)	0.95	(0.89-1.02)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, and fiber

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across total dairy intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable

Table A.22 Total dairy intake is inversely associated with risk of stroke in women

				NHS			
		Stroke	I /100K	Multivariable		No Diet Update	
Dairy Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	112153	256	228	1.00	-	1.00	-
0.5-<1/d	167938	382	227	0.94	(0.80-1.10)	0.92	(0.78-1.08)
1-<2/d	294855	642	218	0.90	(0.77-1.05)	0.88	(0.75-1.03)
2-<6/d	188047	405	215	0.88	(0.74-1.05)	0.86	(0.72-1.03)
<i>P for linear trend³</i>				0.2106		0.1485	
Per one serving/day				0.96	(0.90-1.02)	0.95	(0.89-1.02)

				HPFS			
		Stroke	I /100K	Multivariable		No Diet Update	
Dairy Intake	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI
<0.5/d	32020	81	253	1.00	-	1.00	-
0.5-<1/d	51717	129	249	0.96	(0.72-1.27)	0.93	(0.70-1.24)
1-<2/d	79317	190	240	0.88	(0.67-1.15)	0.89	(0.67-1.17)
2-<6/d	46349	123	265	0.93	(0.68-1.26)	0.94	(0.69-1.29)
<i>P for linear trend³</i>				0.7054		0.9080	
Per one serving/day				0.98	(0.88-1.09)	0.99	(0.89-1.11)

¹Adjusted for age, race, smoking, energy, family history of MI, physical activity, BMI, anti-hypertensive medication use, and intakes of alcohol, *trans* fat, and fiber

²Diet was not updated upon report of CABG, angina, or high cholesterol

³Linear trend across total dairy intake categories was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable

Table A.23 Higher total dairy intake combined with a high DASH diet score is associated with a lower risk of CHD in women and men

				NHS			
		CHD	I /100K	Multivariable		No Diet Update	
Dairy, DASH score	P-yrs	Cases	p-yrs	HR ¹	95% CI	HR ²	95% CI

<1/d, <22	184691	482	261	1.00	-	1.00	-
<1/d, ≥22	95408	254	266	0.96	(0.82-1.12)	0.93	(0.79-1.09)
1-<6/d, <22	272119	705	259	1.01	(0.89-1.14)	1.02	(0.90-1.15)
1-<6/d, ≥22	210790	505	240	0.88	(0.77-1.01)	0.87	(0.75-1.00)

				HPFS			
				Multivariable		No Diet Update	
Dairy, DASH score	P-yrs	CHD Cases	I /100K p-yrs	HR ¹	95% CI	HR ²	95% CI
<1/d, <22	51092	301	589	1.00	-	1.00	-
<1/d, ≥22	32521	186	572	0.86	(0.71-1.05)	0.85	(0.70-1.03)
1-<6/d, <22	70266	441	628	0.99	(0.85-1.16)	1.02	(0.88-1.19)
1-<6/d, ≥22	55256	339	614	0.87	(0.73-1.03)	0.88	(0.74-1.05)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, and *trans* fat

²Diet not updated upon report of CABG, angina, or high cholesterol

Table A.24 Higher total dairy intake combined with a high DASH diet score is linked with a lower risk of stroke in women and men

				NHS			
				Multivariable		No Diet Update	
Dairy, DASH score	P-yrs	Stroke Cases	I /100K py	HR ¹	95% CI	HR ²	95% CI
<1/d, <22	184688	419	227	1.00	-	1.00	-
<1/d, ≥22	95402	219	230	0.94	(0.79-1.12)	0.93	(0.78-1.10)
1-<6/d, <22	272105	579	213	0.92	(0.81-1.05)	0.92	(0.81-1.05)
1-<6/d, ≥22	210797	468	222	0.88	(0.76-1.02)	0.85	(0.73-0.98)

				HPFS			
				Multivariable		No Diet Update	
Dairy, DASH score	P-yrs	Stroke Cases	I /100K py	HR ¹	95% CI	HR ²	95% CI
<1/d, <22	51169	119	233	1.00	-	1.00	-
<1/d, ≥22	32568	91	279	0.97	(0.73-1.30)	0.90	(0.66-1.21)
1-<6/d, <22	70338	182	259	1.01	(0.79-1.29)	1.01	(0.79-1.29)

1-<6/d, ≥22 55329 131 237 0.78 (0.59-1.03) 0.77 (0.58-1.01)

¹Adjusted for age, race, smoking energy, family history of MI, physical activity, anti-hypertensive medication use, and intakes of alcohol, and *trans* fat

²Diet not updated upon report of CABG, angina, or high cholesterol

LIST OF JOURNAL ABBREVIATIONS

Am J Cardiol	American journal of cardiology
Am J Clin Nutr	The American journal of clinical nutrition
Am J Epidemiol	American journal of epidemiology
Am J Hypertens	American journal of hypertension
Ann Intern Med	Annals of internal medicine
Ann Intern Med	Archives of internal medicine
Arch Med Res	Archives of medical research
BMC medicine	BioMed central medicine
BMJ	British Medical Journal
Clin Exp Pharmacol Physiol	Clinical and experimental pharmacology and physiology
Curr Cardiovasc Risk Rep	Current cardiovascular risk reports
Curr Hypertens Rep	Current hypertension reports
Curr Opin Lipidol	Current opinion in lipidology
Eur J Nutr	European journal of nutrition
Eur J Clin Nutr	European journal of clinical nutrition

FASEB J	FASEB journal : official publication of the Federation of American Societies for Experimental Biology
Int J Dairy Tech	International journal of dairy technology
Int J Obes (Lond)	International journal of obesity
J Hum Hypertens	Journal of human hypertension
J Hypertens	Journal of hypertension
J Nutr	Journal of nutrition
JAMA Association	The journal of the American Medical Association
JRSM cardiovascular disease	The royal society of medicine journals: cardiovascular disease
Kidney Int	Kidney international
N Engl J Med	The New England journal of medicine
Nutr Metab Cardiovasc Dis diseases	Nutrition, metabolism and cardiovascular diseases
Nutr Res	Nutrition research
Nutr Rev	Nutrition reviews
PLoS One	Public Library of Science

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Hypertension (DASH) Trial. *The American journal of clinical nutrition*.
2001;74(1):80-89.

Curriculum Vitae

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ACADEMIC TRAINING

- 2016** Ph.D. Department of Medicine, Nutrition and Metabolism Graduate Program, Boston University School of Medicine, Boston MA; "Dietary Protein, Dairy, Yogurt, and Risk of High Blood Pressure and Subsequent Cardiovascular Disease in Middle-Aged Adults"
- 2009** B.S. Rutgers University School of Environmental and Biological Sciences, New Brunswick NJ; Biochemistry (major), Nutrition (minor)

RESEARCH EXPERIENCE

- 9/2010-5/2016** Ph.D. Candidate, Section of Preventive Medicine and Epidemiology, under the supervision of Dr. Lynn L. Moore, Boston University School of Medicine, Boston MA
- 9/2008-5/2009** Undergraduate Research Assistant, under the supervision of Dr. Peter C. Kahn, Rutgers University, New Brunswick NJ. "Analyzing and organizing known protein-ligand structures for structural studies"
- 5/2008-8/2008** Undergraduate Research Assistant, under the supervision of Dr. Trevor C. Creamer, University of

Kentucky, Lexington KY. "Expressing calmodulin mutants for fluorescent anisotropy structural studies"

INDUSTRY EXPERIENCE

- 7/2009-7/2010** Creative Flavorist Assistant, International Flavors and Fragrances, Dayton NJ
- 9/2008-7/2009** Assistant Flavor Technologist, Flavor Dynamics Inc., South Plainfield NJ
- 6/2007-1/2008** Assistant Research Biologist, Fort Dodge Animal Health, Monmouth Junction NJ

HONORS AND AWARDS

- 2/2015** American Heart Association Council on Epidemiology and Prevention – Minority Travel Grant Award
- 2013-2014** Division of Graduate Biomedical Sciences Endowment Award
- 2012, 2013, 2014, 2015** Graduate Medical Sciences Travel Award
- Fall 2011-Present** Nutrition & Metabolism program representative and Secretary of the Graduate Medical Sciences Student Organization, Department of Medicine, Boston University School of Medicine, Boston MA
- 2009** Magna Cum Laude
- 2009** Theodore Chase Outstanding Scholar in Biochemistry Award

- 9/2008-5/2009 Kappa Theta Epsilon National Cooperative Education Honor Society Member, Rutgers University, New Brunswick NJ.
- 9/2008-5/2009 Phi Beta Kappa Honor Society Member, Rutgers University, New Brunswick NJ.
- 2008-2009 Susan E. Mangianelli Scholarship Award
- 2005-2009 Rutgers University Merit Award Scholarship Award
- 2005-2009 School of Environmental and Biological Sciences Dean's List

TEACHING EXPERIENCE

- Spring 2015** Teaching Assistant, FC 709 "Research Design and Statistical Methods for Biomedical Sciences", Boston University School of Medicine, Boston MA
- Fall 2013, 2014** Teaching Assistant, NU 804 "Topics in Research Design and Statistical Methods", Boston University School of Medicine, Boston MA
- Spring 2014** Teaching Assistant, NU 620 "Clinical Nutrition Research", Boston University School of Medicine, Boston MA
- Spring 2009** Instructor, 11:115:403 "General Biochemistry II", Rutgers University, New Brunswick, NJ
- Fall 2008** Instructor, 11:115:403 "General Biochemistry I", Rutgers University, New Brunswick, NJ

SCIENTIFIC PRESENTATIONS

- 3/2016 Epidemiology and Prevention, Lifestyle and Cardiometabolic Health Scientific Sessions, Baltimore, MD. **“Long-term Yogurt Intake is Associated with a Lower Risk of High Blood Pressure in Middle-aged Nurses and Health Professionals.”** (moderated poster session)
- 3/2015 FASEB Experimental Biology, Boston, MA **“Effects of Dietary Protein on Skeletal Muscle Mass and Sarcopenia Risk in Middle-aged Framingham Adults.”** (poster and oral presentation - Emerging Leaders in Nutrition Science Poster Competition)
- 3/2015 Epidemiology and Prevention, Lifestyle and Cardiometabolic Health Scientific Sessions, Baltimore, MD. **“Dietary Protein Lowers Obesity Risk in Middle-Aged Framingham Offspring Study Adults.”** (moderated poster session)
- 7/2014 Boston Nutrition Obesity Research Center, Boston, MA. **“Beneficial Effects of Dietary Potassium on Adolescent Blood Pressure.”** (poster session competition)
- 3/2014 Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism Scientific Sessions, San Francisco, CA. **“Beneficial Effects of Dietary Potassium on Adolescent Blood Pressure.”** (moderated poster session)
- 11/2013 Boston University Nutrition & Metabolism Student Seminar Series, Boston MA. **“Effects of Dietary Sodium, Potassium, and the Potassium:Sodium Ratio on Adolescent Blood Pressure.”**

- 4/2013 FASEB Experimental Biology, Boston, MA **“Dietary Sodium and Potassium Impact Blood Pressure in Adolescence.”** (poster presentation)
- 9/2012 Obesity Annual Scientific Meeting, San Antonio, TX. **“Differences in Body Fat Explain Beneficial Effects of Dietary Protein on Adolescent Blood Pressure.”** (poster presentation)
- 4/2012 FASEB Experimental Biology, San Diego, CA. **“Dietary Protein and Risk of Elevated Blood Pressure in Adolescent Girls.”**
- 4/2012 FASEB Experimental Biology, San Diego, CA. **“Dietary Protein and Risk of Elevated Blood Pressure in Adolescent Girls.”** (Nutritional Epidemiology and Energy/Macronutrients Research Interest Groups - Student Poster Competition)
- 3/2012 Tufts University Future of Food and Nutrition Graduate Student Research Conference, Boston, MA. **“Dietary protein and EBP Risk in Adolescent Girls.”** (poster presentation competition)
- 10/2012 Boston University School of Medicine Department of Medicine Evans Day Annual Symposium, Boston, MA. **“Dietary Protein and Risk of Elevated Blood Pressure in Adolescent Girls.”** (top ten clinical poster finalist)

PROFESSIONAL MEMBERSHIPS

American Heart Association
 American Society of Nutrition

BIBLIOGRAPHY

1. **Buendia JR**, Bradlee ML, Singer MR, Daniels SR, Moore LL. Longitudinal Effects of Dietary Sodium and Potassium on Blood Pressure in Adolescent Girls. *JAMA Pediatr.* 2015; PMID: 25915457
2. **Buendia JR**, Bradlee ML, Singer MR, Moore LL. Diets higher in protein predict lower high blood pressure risk in Framingham offspring study adults. *Am J Hypertens.* 2014; PMID: 25194158
3. Moore LL, Bradlee ML, Singer MR, Qureshi MR, **Buendia JR**, Daniels SR. A DASH-style eating pattern and risk of elevated blood pressure in adolescent girls. *Br J Nutr.* 2012; PMID: 22243687

COMPUTER SKILLS

SAS, MS Office, Windows and MAC OS applications

FOREIGN LANGUAGES

Tagalog

Native language

French

Basic proficiency and knowledge