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Effect of dietary protein, morning protein, and egg intake on cardiometabolic outcomes at different ages

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EFFECT OF DIETARY PROTEIN, MORNING PROTEIN, AND EGG INTAKE ON CARDIOMETABOLIC OUTCOMES AT DIFFERENT AGES

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ABSTRACT

The long-term effects of total dietary protein and individual food sources of dietary protein on cardiometabolic risk are not clearly understood. The effects of the amount consumed and the timing of dietary protein intake as well as the contribution of overall dietary patterns on various cardiometabolic outcomes are largely unknown, particularly in children. The objective of this dissertation is to estimate the effects of patterns of dietary protein intake and egg consumption on cardiometabolic risk in adolescents and adults.

Prospective data from two studies were used: the National Growth and Health Study (NGHS) with 2105 pre-adolescent girls followed for 10 years and the Framingham Offspring Study (FOS) with 2054 middle-aged adults followed for

12 years. Diet was assessed in both cohorts via 3-day diet records. NGHS outcomes included %body fat, %truncal fat, waist circumference, body mass index, skeletal muscle mass (SMM), fasting glucose (FG), insulin resistance, blood pressure (BP), and lipids. FOS outcomes included FG and BP. Multivariable models including analysis of covariance, logistic regression, and Cox proportional hazards models were used.

Girls consuming ≥75 g/day of total protein (vs. less) had less body fat ($p<0.0001$) and more SMM ($p<0.0001$) by late adolescence. Girls consuming more morning protein had the highest total protein intakes, perhaps contributing to the observed beneficial effects of morning protein on body composition. Consumption of ≥3.5 eggs/week in 9-17 year-old girls was associated with lower %body fat ($p=0.019$) and higher %SMM ($p=0.026$) by later adolescence. There was no evidence that higher egg intake was detrimental to any cardiometabolic outcomes. Girls who consumed more eggs (ages 9-17 years) in combination with more fiber, fruits/vegetables, or physical activity led to statistically significant 43-58% reduced risks of becoming overweight by late adolescence. Adults who consumed ≥5 eggs/week had lower FG ($p=0.0004$) and systolic BP ($p=0.0284$) over

time. Higher egg intakes were associated with a 27% lower risk of IFG or T2D (95% CI:0.51-1.04) and 30% lower risk of HBP (95% CI:0.52-0.96).

In summary, total protein intake, including regular egg consumption, has no adverse effects on cardiometabolic risk in adolescence or adults and may benefit body composition over time.

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LIST OF ABBREVIATIONS

AM……..……..Morning

ANCOVA……...…….Analysis of Covariance

BIA…….Bioelectrical Impedance Analyzer

BMI……...……..Body Mass Index

CCK……...……...Cholecystokinin

CDC……..Centers for Disease Control

CHOL……...Cholesterol

CM……...Centimeters

CMR……Cardiometabolic Risk

CVD……...……..Cardiovascular Disease

DASH……...…..Dietary Approaches to Stop Hypertension

DBP……...…….Diastolic Blood Pressure

DL……...Deciliter

EO…….Eating Occasion

EQ……..Equivalent

EPIC……European Prospective Investigation into Cancer and Nutrition

FFQ……..…...Food Frequency Questionnaire

FOS……..…….Framingham Offspring Study

FREQ……..……..Frequent

FV……..…….Fruits and Vegetables

FNSV……...…Fruits and Non-Starchy Vegetables

G……...……..Grams

GLP-1……...…….Glucagon-Like Peptide-1

HAQ……Habitual Activity Questionnaire

HbAlc…….glycated Hemoglobin

HBP……...……High Blood Pressure

HDL-C……...……...…….High Density Lipoprotein-Cholesterol

HELENA……..Healthy Lifestyle in Europe by Nutrition in Adolescence

HOMA-IR……..Homeostasis Model Assessment of Insulin Resistance

HR……...……...Hazard Ratio

HRS……Hours

HPFS……..……...Health Professionals Follow-Up Study

IFG……...Impaired Fasting Glucose

IGF-1……...…….Insulin like Growth Factor 1

INFREQ……...…….....Infrequent

INTERMIT……...……...Intermittent

KCALS……...……..Kilocalories

KG……...……Kilograms

LBM…….Lean Body Mass

LDL-C……...……..Low Density Lipoprotein-Cholesterol

M……...…….Meters

MET……...…Metabolic Equivalent of Task

MFP…….Meat, Fish, Poultry

MG……...……..Milligrams

MM HG……...……Millimeters of Mercury

MPS……...Muscle Protein Synthesis

mTOR……mammalian Target of Rapamycin

mTORc1……mammalian Target of Rapamycin Complex 1

NDS……...……Nutrition Data System

NGHS…...National Heart, Lung, and Blood Institute's Growth and Health Study

NHANES……...……National Health and Nutrition Examination Survey

NHS……..……..Nurses' Health Study

NHS II……...……..Nurses' Health Study II

PA……Physical Activity

PRO……..Protein

PYY3-36…….Peptide YY3-36

P-YRS……..…….....……Person-Years

RR……...Relative Risk

SAS…….....Statistical Analysis System

SBP……...….....Systolic Blood Pressure

SD……...…….Standard Deviation

SE……...…….Standard Error

SES……...……Socioeconomic Status

SKIP……...……..Skipper

SMM……Skeletal Muscle Mass

SoFAAs…….Solid Fats/Alcoholic beverages/Added sugars

T2DM……...…….Type 2 Diabetes Mellitus

TSP…….....Teaspoon

US…….....United States

USDA……...….United States Department of Agriculture

WC……...Waist Circumference

WK……...……Week

WT-ADJ……...……Weight-adjusted

YRS……...……...Years

CHAPTER 1: INTRODUCTION

1.1 PREVALENCE OF CARDIOVASCULAR RISK FACTORS IN ADULTS

Cardiovascular disease (CVD) continues to be the leading cause of death in the United States (US).[1] The prevalence of risk factors for CVD such as hypertension, dyslipidemia, and diabetes mellitus, remain high. Approximately 33% of adults ≥ 20 years of age have high blood pressure, 43% have a total cholesterol ≥ 200 mg/dL,[2] 38% have prediabetes, and 14% have diabetes.[3] The prevalence of these metabolic conditions varies with age, with a higher prevalence noted in older individuals compared to younger individuals. For instance, approximately 6% of women and 9% of men ages 20 to 34 have high blood pressure, while greater than 75% of women and men have high blood pressure at ages ≥ 75 years.[2] Prevention of these CVD risk factors is important, since early development of these conditions increases lifetime risks of death from CVD.[4]

1.2 CARDIOMETABOLIC RISK IN CHILDREN

CVD risk factors are already identifiable in childhood. For instance, in the National Health and Nutrition Examination Surveys (NHANES) 2011-2012 data, approximately 20% of children ages 8 to 17 had a high total cholesterol (≥ 200

mg/dL), low high-density lipoprotein cholesterol (HDL-C, < 40 mg/dL), or a high non-HDL-C (≥ 145 mg/dL), while 11% have either high or borderline high blood pressure.[5] In NHANES 2005-2010, approximately 27% of boys and 11% of girls had a fasting glucose ≥ 100 mg/dL.[6]

CVD risk parameters such as lipid concentrations [total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglycerides] and blood pressure are worse in obese children compared with normal weight children.[7] Unfortunately, childhood obesity continues to be a public health challenge in the US, with recent statistics suggesting that approximately one third of 2 to 19 year-olds are overweight or obese.[8] Waist circumference, a simple surrogate marker of abdominal fat mass,[9] has risen similarly over the past 25 years. Mean waist circumference in boys has risen by 5.3% and in girls by 8.7% between the 1988-1994 and 2003-2004 NHANES surveys.[10] Currently, approximately 19% of children ages 2 to 18 years have abdominal obesity.[11]

While waist circumference and BMI have been utilized extensively in the research literature as proxies for central and total adiposity, they have some limitations. For instance, waist circumference measurements include both

subcutaneous abdominal adipose tissue and intra-abdominal adipose tissue, consequently waist circumference cannot determine the individual contributions of these fat depots to abdominal girth.[9] In the case of BMI, this measurement does not differentiate between lean mass and fat mass. Furthermore, while BMI is highly correlated with percentage of body fat, this correlation is driven by the tendency for very low-BMI individuals to have a low percent body fat and individuals with a high BMI to have a higher percent body fat. On the other hand, individuals in the middle range may have very different fat percentages, resulting in the potential for misclassification of overweight with BMI.[12] Therefore, there is a need for more studies to include measures of body composition, such as assessment of fat and skeletal mass, when evaluating the effects of obesity on health outcomes.

1.3 METABOLIC CONDITONS TRACK WITH AGE

There is evidence that childhood obesity leads to adult obesity. For example, one systematic review consistently found evidence across 25 studies that overweight and obese youth are at an increased risk for becoming overweight or obese adults.[13] Furthermore, obese children often continue to progress in the severity of obesity. This was evident in the National Longitudinal Study of Adolescent

Health cohort, where nearly 40% of males and 51% of females with an adolescent BMI ≥95th percentile for age and sex had a BMI ≥ 40 (class III obese) by their early thirties compared with less than 5% of normal weight adolescents.[14]

Blood pressure, lipids, and glucose also appear to track from adolescence to adulthood, with the development of abnormal metabolic conditions during childhood increasing the likelihood of developing subclinical or clinical atherosclerosis in adulthood.[15-17] For example, LDL-C and systolic blood pressure during childhood have been shown to be predictive of carotid artery intima-media thickness[18] and coronary artery calcification[19] in adulthood, which are two surrogate measures of atherosclerosis. This may translate into a reduction in quality of life, increased risk of CVD (including coronary heart disease), and earlier mortality.[20-23] Early prevention, ideally in childhood, could be a key strategy to reducing risks associated with these metabolic disorders.

1.4 DIET AS A RISK FACTOR FOR CARDIOMETABOLIC DESEASES

Since dietary habits are known to affect multiple cardiovascular factors including blood pressure, lipids, and glucose,[2] and diet intake tracks from childhood to adulthood,[24] modification of diet early in life is one approach to reducing CVD

risk later in life. In fact, the leading cause of morbidity and mortality in the US is a suboptimal diet, as characterized by insufficient fruits, vegetables, whole grains, and nuts, as well as too many sugar-sweetened beverages and processed meat.[25] Optimal amounts of these foods favorably impacts cardiometabolic outcomes[26] and comprise healthy dietary patterns advocated by both the Dietary Guidelines Advisory Committee and the American Heart Association for reducing cardiovascular risk (e.g. Dietary Approaches to Stop Hypertension, DASH).[27,28]

In the original DASH diet study, individuals were randomized to one of three groups: a typical American diet (i.e. the control diet), a diet high in fruits and vegetables, and the DASH diet. The DASH diet added low-fat dairy to the high fruits and vegetable diet; it was designed to be rich in potassium, calcium, magnesium, and fiber and included higher intakes of whole grains, poultry, and fish. This resulted in the DASH participants consuming 18% of total energy intake from dietary protein compared to ≤ 15% in the other two groups. Although the fruit and vegetable intervention by itself significantly reduced blood pressures compared with the control diet, further reductions were achieved with the DASH diet.[29] Furthermore, secondary analyses of the original

DASH study found that participants in the DASH intervention group had significantly lower total and LDL-C at the end of the study, while there were no significant lipid changes in the fruit and vegetable group.[30] These results suggest that the combination of diet changes comprising the DASH dietary pattern, including higher amounts of protein, resulted in the noted health benefits.[31]

1.5 PROTEIN CONSUMPTION INFLUENCES CARDIOMETABOLIC RISK

Considering the high prevalence of cardiovascular risk factors in the population,[2] dietary interventions that target numerous metabolic pathways and outcomes would be the most beneficial in reducing risk for CVD. Dietary protein intake is one such potentially modifiable risk factor for CVD that impacts a number of relevant pathways. These include satiety,[32] muscle protein synthesis (MPS),[33,34] nitric oxide synthesis,[35] glucose metabolism,[36-38] fatty acid metabolism,[39-41] and direct effects on body composition.[41,42]

1.5.1 Dietary Protein Affects Weight-Related Outcomes in Adults

Dietary protein may increase satiety and reduce food intake by releasing anorexigenic gut peptides such as cholecystokinin (CCK) and peptide YY, by inhibiting anabolic neuronal signaling (decreasing neuropeptide Y mRNA levels)

and activating catabolic signaling (pro-opiomelanocortin neurons producing α-melanocyte-stimulating hormone) in the hypothalamus via a phosphorylated mammalian target of rapamycin (mTOR) and phosphorylated AMP-activated protein kinase-dependent mechanism.[43] In addition, protein intake may diminish brain reward mechanisms, thus decreasing hunger via acute effects on satiety and food intake, and ultimately energy balance.[44]

While some studies suggest higher protein intakes may benefit weight loss[45,46] and assist with weight maintenance,[46-48] many long-term randomized clinical trials have found no effects of macronutrient content on weight loss.[49-51] Dietary adherence is a major problem in weight loss studies, leading some researchers to speculate that dietary compliance may explain some of the discrepant results between studies. Some dietary protein interventions have shown that weight loss occurs among those who adhere to a high-protein diet, while those who fail to adhere failed to lose weight.[45] One possible beneficial effect of a diet high in protein is that a high-protein diet may minimize muscle protein breakdown during weight loss.[52] A conventional low-fat, high carbohydrate diet can result in 30-40% of weight loss resulting from loss of lean body mass (LBM), while a higher protein diet may reduce the LBM loss to <15%.[53] This preservation of fat-

free mass, may also reduce the weight loss-induced decrease in energy expenditure,[54] thus promoting maintenance of weight loss.

Other questions remain regarding dietary protein. Some studies where participants maintained weight suggest the higher protein diet group had greater reductions in fat mass and waist circumference and tended to increase fat free mass ($p=0.05$) compared with the control group, while others question these results.[55] Another important question that remains is whether higher protein diets enhance satiety and reduce overall food intake throughout the day.[56] These acute satiety studies have been criticized because they often focus on meal-specific protein consumption and not on total daily intake.[57] Furthermore, many of these studies are acute feeding studies often providing only one food item (e.g. beef or whey protein) thus, more mechanistic studies are needed to evaluate satiety in real life settings, where individuals typically consume a variety of foods.[58]

In a few prospective studies of adults, a positive association between dietary protein intake and weight gain has been observed.[59-61] Since most of these studies did not assess LBM or fat mass change, it is not clear whether weight gain was

due to an increase in LBM and/or an increase in fat mass.[62] In one of these studies, dietary protein had no effect on waist circumference, suggesting central fat did not increase during protein-related weight gain.[60] In contrast, one recent publication found greater dietary protein intakes were associated with an increase in body weight and BMI over the course of six years, with most of the increase due to an increase in fat mass.[61]

There are other possible explanations for differences between observational and experimental studies of dietary protein such as differences in subject characteristics. For example, a recent study found better weight maintenance was noted in the high protein consuming group (versus low protein) among those in the cohort whose characteristics (e.g. BMI, waist circumference, other dietary variables) were comparable to those of clinical trial participants.[63] In many observational studies, individuals who consume higher amounts of protein may have other unhealthy behaviors that are difficult to fully adjust for in the statistical analyses.[64] For example, individuals that consume a lot of red meat tend to be obese, exercise less, smoke more and have other less healthy lifestyle habits than individuals who consume less meat.[65] Finally, the fact that many randomized clinical trials are weight loss interventions makes them inherently

different from observational studies which could also explain the observed conflicting results.

While this dissertation will not directly evaluate the effects of dietary protein on body fat in adults, improvements in body composition could indirectly benefit the metabolic outcomes (e.g. hypertension, type 2 diabetes) that will be studied. Further, as will be discussed in a later section, the data evaluating the effects of dietary protein on body composition in late childhood and adolescence is extremely limited. Therefore, the information in adults could provide insight into interpreting the data generated from this dissertation.

1.5.2 Dietary Protein Affects Lean Body Mass

Skeletal muscle is a major disposal site for free fatty acids and glucose disposal thus, skeletal muscle can directly affect metabolic health.[42] In addition, MPS and muscle protein breakdown are principally responsible for resting energy expenditure, so an increase in skeletal muscle mass could increase total daily energy expenditure.[66] This may help with body weight loss and weight loss maintenance, resulting in indirect benefits to metabolic health. Consequently, maintaining skeletal muscle mass with the ingestion of dietary protein, which

increases MPS via mammalian target of rapamycin complex 1 (mTORc1) signaling and decreases muscle breakdown, could become critical for overall health.[67]

Overall, there is a clear need for a more thorough understanding of the effects of dietary protein on skeletal muscle mass. Part of the issue is that skeletal muscle changes are influenced by both MPS and breakdown, thus changes in MPS may not predict long-term changes in muscle mass.[68] Unfortunately, breakdown is often not assessed due to being invasive and technically difficult to ascertain, so clarity could come from studies evaluating both components of protein turnover.[69] Furthermore, our understanding of the differential effects of protein source, amount, and timing of intake is lacking on overall skeletal muscle mass as is a better understanding of the macronutrient composition of the diet and the overall matrix in which the protein is consumed.

There is a great need for studies of protein quality (e.g. animal-based proteins versus plant-based proteins) and its effects on skeletal muscle outcomes, since leucine, which is typically higher in animal-protein versus plant protein, is particularly important to MPS.[69] In addition, the role of dietary protein on

strength, which may impact functional outcomes needs to be explored. Finally, as previously mentioned, there is a need to use more protein-rich foods in the interventional studies, since much of the earlier work used isolated amino acids cocktails or supplements.[69] The information from this dissertation work will help to guide the development of future intervention trials.

1.5.3 Dietary Protein Influences BMI and Body Composition in Children

In contrast to the many adult studies, much less is known about the effects of dietary protein on BMI and body composition in children. Much of the literature evaluates dietary protein during the first 24 months of life.[70] In contrast, there is a paucity of literature on the effects of pre-adolescent and adolescent protein intake on BMI and body composition, with very few longitudinal studies.

Observations showing that breastfed infants grow at a slower rate, have a lower insulin like growth factor 1 (IGF-1),[71] and have a lower percentage of intake coming from protein (i.e. about 0.5 grams per kilogram per day less[72]) compared to formula fed infants has led to the idea that differences in hormonal levels and growth patterns is caused by the differences in the protein intake between breastfed and formula fed infants.[71] This idea is called the "early protein

hypothesis", and suggests that higher protein intakes increase plasma concentrations of insulin-releasing amino acids, leading to higher secretion of insulin and IGF-1 levels, resulting in enhanced weight gain and adipogenic activity.[73]

However, a recent randomized clinical trial found, which compared infants fed high protein formula (2.7 grams/100 kilocalories) to low protein formula (1.8 grams/100 kilocalories), which is similar to protein amount in breastmilk (1.5 g/100 kilocalories), found no significant differences in insulin and IGF-1 concentrations during first 12 months. There was also no differences in body fat between the two groups, although length and head circumference were lower in infants consuming the low protein formula.[74]

In the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-sectional study, the highest tertile of protein intake was associated with lower percent body fat, but not BMI in adolescents 12.5 to 17.5 years of age.[75] In a prospective study, researchers found that higher protein intake in 8-10 year old girls, but not boys was associated with less fat gain and an increase in LBM.[76] In a longitudinal study evaluating animal and plant protein intake during puberty on

body composition in young adulthood (18-25 years), researchers found animal intake during puberty was related to higher fat-free mass index (lean mass in kilograms/height in meters^2) in young adulthood in both men and women, while higher animal protein intake was linked with lower fat mass index (fat mass in kilograms/height in meters^2) in men, but not women. Plant protein was not associated with body composition in either men or women.[77] Finally, in a sub-study of the DiOGenes randomized controlled trial evaluating the *ad libitum* effects of dietary protein and glycemic index on CVD risk factors in overweight children, researchers found that the high protein diet group had a smaller waist circumference at the end of six months, despite no apparent change in body weight or BMI.[78]

More research is clearly needed in late childhood and adolescence to elucidate the role of dietary protein on BMI and body composition changes as well as the potential mechanisms for those changes. This is particularly true since dietary protein appears to increase obesity risk during infancy,[70] while it may be beneficial to body composition during adulthood.[42,45,46] Furthermore, whereas there is some evidence that dietary protein is more satiating than other macronutrients in children, very little is actually known on the effects of protein

on food intake in children and adolescents. In particular, this research needs to account for critical periods of growth, such as puberty, where there is some evidence that appetite hormones could be influenced by other hormonal changes occurring during this time.[79]

1.5.4 Protein Influences Blood Pressure

A recent meta-analysis of randomized clinical trials showed that dietary protein compared with carbohydrate intake is associated with blood pressure lowering effects in adults.[80] In the case of long-term studies, the evidence is less clear, with many,[81-83] but not all prospective studies in adults,[84] suggesting that protein intake is inversely associated with blood pressure; some believe that plant protein may be driving this relationship.[81] However, a recent systematic review of the impact of dietary protein on blood pressure in children, found the evidence from intervention and observational studies are insufficient to form meaningful conclusions.[85]

Potential blood pressure lowering mechanisms involving protein include arginine induced vasodilation with arginine serving as a precursor for nitric oxide production,[35,86,87] and the central and peripherally mediated effects of

taurine.[88] Furthermore, the angiotensin converting enzyme inhibiting effect of animal based peptides such as lactotripeptides,[89] as well as other mechanisms (e.g. binding of peptides to opioid receptors) attributed to animal-based proteins have been implicated in lowering blood pressure.[90,91] Finally, since most of the randomized clinical trials have exchanged carbohydrates for protein,[92] some have suggested it is the lower carbohydrates rather than the higher protein intake that reduces blood pressure.[93]

1.5.5 Protein Affects Glucose Metabolism

Prospective adult studies evaluating the effects of protein and protein-rich food sources (e.g. red meat and dairy) on risk of type 2 diabetes mellitus (T2DM) are conflicting, potentially due to how protein was evaluated.[94-98] For instance, when protein sources are analyzed as part of the so-called Western diet[41] or 'processed diet'[99] that is low in fruits, vegetables and whole grains,[100,101] the overall diet is often found to detrimental. Thus, it is unclear whether protein itself is harmful or whether other foods in the overall diet pattern may be linked with risk of T2DM. Another possibility is that the absence of healthy foods in the food matrix may be responsible for glucose dysregulation. Many studies of diet patterns fail to

account completely for other dietary and non-dietary variables in the statistical models.[64]

Randomized clinical trials in normal and overweight adults, as well as with individuals with T2DM suggest increased protein and reduced carbohydrates reduces postprandial glucose response, promotes insulin secretion, and decreases glycated hemoglobin (HbAlc).[41,102] However, a recent systematic review concluded that among studies with <20% loss to follow-up, there was no significant effect of higher protein diets on HbAlc, fasting glucose, or fasting insulin.[103] There is a much greater agreement that a diet higher in dietary protein is one way individuals can lose weight, which will lead to lower fasting glucose and HbAlc, thus reducing risk for risk for T2DM.[52]

Future research should evaluate the impact of protein intake in children on glucose metabolism, since the limited results to date report contrasting findings.[85] Future studies should examine total protein as well as food source, since protein-rich foods may differentially affect glucose metabolism.[36] In particular, more research is needed during adolescence, a time where insulin resistance is a

normal physiological event, with insulin resistance increasing during puberty and then returning to baseline by the end of puberty.[79]

While the benefits for higher protein diets could be attributed to a reduction in carbohydrates (or fats) or to weight loss due to energy restriction,[41] some evidence exists that protein plays a direct role on glucose metabolism. Proposed mechanisms include protein functioning as a substitute for high glycemic index carbohydrate foods, as well as serving as a substrate for gluconeogenesis.[104,105] Although dietary protein was recently found to contribute minimally to glucose production in healthy individuals.[106] Furthermore, protein stimulates the production of insulin from pancreatic beta-cells, thus improving clearance of glucose from the blood and enhancing use of glucose by the peripheral tissues.[102]

1.5.6 Protein Influences Lipids

In a recent meta-analysis of trials in non-diabetic adults, higher protein diets improved HDL-C, but had non-statistically significant effects on total cholesterol and LDL-C.[107] This association between higher-protein diets and higher HDL-C has also been observed amongst free-living adults.[108] For triglycerides, one meta-analysis found an improvement in triglycerides,[103] while another found no effects

in studies at least one year in duration.[107] In children, the extremely limited data from higher quality studies suggests that dietary protein has no effect on lipids.[85] Overall, these results suggest that dietary protein may affect some aspects of lipid metabolism, although it is unclear if the effects are attributable to dietary protein alone or to concurrent reductions in dietary fats, carbohydrates or both.[109]

Some studies of protein-rich foods such as eggs,[110-112] soy,[40,113], lean meat[114,115], and low fat dairy[116,117] have demonstrated beneficial effects on lipids. Mechanisms vary by food source, but animal studies suggest these could include increased hepatic bile acid synthesis, enhanced fecal sterol excretion, reduced hepatic *de novo* cholesterol biosynthesis, and reduced cholesterol uptake resulting from decreased LDL receptor activity.[109]

There is a need to evaluate the impact of the overall food matrix, including macro- and micronutrients and other bioactive compounds with respect to effects on serum lipid levels.[31] Commonly consumed protein foods contribute to nutrient intake and diet quality, thus improving overall health.[118] On the other hand, increased protein could be accompanied by increases in less desirable nutrients (e.g. certain saturated fats) or foods (e.g. processed meat) depending on

food choices.[31,96,119] For instance, one randomized crossover study found that consuming a diet high in beef and low in saturated fat lowered plasma triglycerides, as well as total cholesterol, LDL-C, and non-HDL-C. In contrast, consuming a diet high in both beef and saturated fat only lowered triglycerides with no significant changes in other lipids.[120] Consequently, results could vary depending on food (e.g. meat low vs. high in saturated fat) choices.

The direct effects of protein and related foods will likely vary depending on the types of food being replaced by higher protein intakes. A review by Richter et al concludes that the non-protein composition of the diet in various studies of protein and cardiovascular risk is a likely explanation for widely variable about the effects of animal and plant proteins in the diet.[31] In addition, since healthy and unhealthy behaviors track within individuals, residual confounding by such unknown factors could be responsible for different results in different population subgroups.[64]

1.6 TIMING OF PROTEIN INTAKE MAY IMPACT HEALTH OUTCOMES

There are several factors that need to be considered when evaluating meal pattern research (eating patterns at the level of the meal, e.g. breakfast).[121] For

example, meals have been defined in different ways in the literature (e.g. participant-identified, by time of day, use of a neutral term), which can affect the nutritional intake of various eating occasions (EO).[121] Consequently, the lack of consensus for meal definitions such as breakfast [122] may create different results in the amount of food consumed and total energy intake amongst various studies,[123] resulting in heterogeneity amongst the findings. Furthermore, results could also be influenced by the sociocultural and value-laden terms used to identify an EO, such as amount and quality of food.[124] For instance, this could lead to one individual identifying an EO as breakfast and another identifying the same EO as a snack.[121] These issues need to be considered when evaluating literature on the effects of breakfast on the etiology of chronic disease.

1.6.1 Effects of Morning Protein Intake on Food Intake and Adiposity

Numerous studies have evaluated the effect of breakfast skipping on weight-related outcomes. Generally, the epidemiological literature, which is primarily comprised of cross-sectional and some prospective studies, suggests that breakfast consumption has a beneficial effect on weight-related outcomes,[125] with the proposed mechanism being that breakfast consumption increases satiety, thus decreasing energy intake throughout the day.[126] The few acute clinical

studies, generally suggest that breakfast favorably affects subjective appetite (e.g. sensations of hunger, desire to eat), with mixed results on food intake.[125] In contrast, breakfast does not independently appear to affect weight loss in randomized clinical trials ≥12 weeks.[127-129] In fact, recent publications have suggested that the proposed favorable benefits of breakfast on weight-related outcomes exceeds the evidence.[130,131]

Some authors have suggested caution with interpretation of the limited number of randomized clinical trials because the authors have not considered energy contribution of breakfast in the analysis, which may influence weight related outcomes.[132] For example, consuming a higher amount of food intake earlier in the day may lead to greater weight loss on a calorie-restriction diet compared to when more food intake is consumed later in the day,[133,134] with the weight loss indirectly improving other metabolic outcomes. Although this line of research needs to consider that evidence exists that breakfasts are often underreported potentially due to the selective underreporting of foods that are high in fat or added sugar (e.g. muffins, donuts, pancakes with syrup).[135]

Another limitation of the randomized clinical studies investigating the effects of breakfast on weight-related outcomes is that the studies did not control for the macronutrient composition of the morning meal (e.g. amount of dietary protein).[132] This could be particularly problematic in small studies where macronutrient differences exist at baseline between groups, since short-term research suggests that consuming protein in the morning may have beneficial effects on satiety. For example, in individuals consuming an energy restricted diet of 0.8 grams per kilogram per day of dietary protein, intake of an additional 0.6 grams/kilogram of protein (+0.6 gram/kilogram) at the first meal of the day (versus the same amount added to lunch or dinner) led to greater self-reported fullness over the course of 15 hours.[136] The acute benefits of dietary protein consumption in the morning were also noted in a randomized study of premenopausal women, in which women consuming higher protein breakfast meals (30 grams and 39 grams) had higher satiety ratings and consumed less food at lunch and less total energy intake during the day compared with an isocaloric low protein (3 grams) breakfast.[137] Among adolescents, a higher protein breakfast (35 grams versus 13 grams) promoted greater reductions in food motivation/reward areas of the brain, resulting in reduced subsequent meal

intake, although no differences were noted in subjective assessments of hunger or fullness between the two breakfasts.[138-140]

Randomized clinical trials with longer follow-up periods are extremely limited. A high-protein breakfast (35 grams) compared to a normal protein breakfast (13 grams) resulted in less daily energy intake ($p=0.06$) and a non-significant reduction in fat mass in breakfast skipping adolescents in a 12 week study.[141] In contrast, a three-month randomized clinical study, which manipulated energy (33% of total daily intake was consumed at breakfast) and protein content, found that consuming a high protein breakfast compared to a normal protein breakfast, did not produce differences in weight loss or body composition in individuals with T2DM.[142] Heterogeneity of the study populations and designs could be influencing the inconsistent results.

One proposed mechanism for the benefits of consuming protein in the morning relates to the amount of protein consumed at an EO. Some authors suggest 25-30 grams of protein per meal may be needed to improve satiety and consequently influence body weight management.[45] This idea of a certain threshold may be supported by findings where food intake was reduced when >30 grams of

protein was consumed.[137-140] In some studies, less than 25-30 grams of protein consumed at breakfast does not appear to reduce subsequent meal food intake, though it does appear to improve to improve subjective assessments of satiety.[143,144] This could mean that when the protein intervention is expressed as a percentage of total kilocalories or when participants are on a calorie-restricted diet, the absolute amount of protein may be too little to stimulate beneficial effects of satiety and food intake.[53] Perhaps this is why participants on reduced kilocalorie diets, consuming either a higher protein breakfast (22% protein) or a lower protein breakfast (15% protein), had no significant differences in weight or body composition after three months.[142]

Besides absolute amount of grams of dietary protein consumed at breakfast, researchers should consider other key factors when designing future studies investigating the connection between morning dietary protein intake and weight-related outcomes. Much of the protein intake at breakfast research involves breakfast skippers, which could be influencing the results since the act of changing behaviors could affect appetite. Therefore, there is a need to consider customary breakfast habits when designing and evaluating these research studies.[125] Furthermore, food form (e.g. liquid vs. solid) may be influencing the

results, since individuals consuming solid protein-rich food at breakfast had greater reductions in perceived appetite and fewer calories consumed at the subsequent meal compared to those consuming a liquid protein-rich breakfast.[145] Finally, much of the above research evaluating the role of dietary protein in the morning on satiety and fat mass, involves eggs.[136-138,140,146] Since the protein texture, volume, palatability, and energy density are known to affect satiation and satiety,[147] there is a need to evaluate these outcomes with other protein-rich foods.

1.6.2 Effects of Morning Protein Consumption on Lean Body Mass

Recent publications have recommended looking beyond the quantity of protein consumed, and focusing protein recommendations on the amount consumed at each EO needed to offset muscle loss that occurs with aging.[53,57,148,149] This concept of protein distribution originated from studies demonstrating 25-30 grams of protein intake was needed to achieve maximal MPS.[150-152] However, some authors have suggested that more than 30 gram of dietary protein may be important, particularly in offsetting protein breakdown and there is a need for studies to evaluate both MPS and breakdown.[153,154]

Cross-sectional studies of protein intake have found that older individuals (≥ 60 years of age) consuming >25 grams at one meal had more appendicular skeletal mass than those consuming < 25 grams at each meal,[155] while more frequent consumption of ≥ 30 grams of protein in adults (50-85 years old) was associated with greater knee extensor strength and leg lean mass.[156] Furthermore, non-frail older adults compared to frail and pre-frail adults are significantly more likely to consume a more even protein distribution over the day.[157] These cross-sectional studies are supported by a short-term intervention study demonstrating MPS was optimal when protein was spread across meals (e.g. 30 grams of protein at each meal).[158]

In contrast, one randomized clinical trial determined that evenly distributed protein intakes across meals was less effective in stimulating MPS than was consuming a bolus containing 80% of total protein in one meal.[159] Similarly, another randomized clinical study found that spreading dietary protein intake evenly across meals was not more beneficial than unevenly distributed intake.[160] One possible explanation for these results is that when protein is spread out across the day, there is not a sufficient bolus of protein to maximally stimulate MPS at any given time. Although, since much of the research focuses on MPS,

more research clearly needs to be done to fully understand protein turnover (e.g. both MPS and muscle breakdown).

This protein distribution research has led some researchers to speculate that protein consumption at breakfast has the potential to offset LBM losses by reversing the catabolic state that occurs during sleep.[105] Since protein consumption is lowest at the morning meal,[148] increasing protein content of the morning meal could be a key target for intervention by replenishing body proteins after an overnight fast.[53] Therefore there is a need for future research investigating protein distribution, as well as a need for research on meal specific effects of protein based foods as they could possibly provide a targeted intervention.

More research on the effects of protein timing on LBM in children and young adults are needed. In individuals less than 30 years of age, there is a high metabolic priority for structural development of bone and muscle driven by anabolic hormones (e.g. insulin, growth hormones, IGF-1, and steroid hormones). This physiological environment, with insulin serving as the dominant signal for mTORc1 activation in children, appears to enable

appropriate bone and muscle growth in children and young adults regardless of protein distribution. However, after approximately 30 years of age, most studies in adults suggest that insulin does not stimulate MPS, thus dietary protein and physical activity become the key factors limiting optimal protein turnover.[53,161] These physiological differences could mean that while the 25-30 grams of dietary protein at EO may be needed to stimulate MPS in adults,[162] that amount of dietary protein may not be important for MPS in children or young adults.

Similar to adults, neonatal animal studies suggest mTORC1 signaling is activated for brief period of times. Thus, frequent meals with adequate amounts of protein have been suggested for optimal mTORC1 signaling, protein synthesis, and growth.[163,164] Evidence supporting this concept is limited to neonatal animal models suggesting that bolus intake of dietary protein (specifically leucine) compared to a continuous intake of dietary protein enhances MPS and lean growth.[165,166] Much more work needs to be done to determine the importance of protein distribution in children on LBM.

1.6.3 Effects of Morning Protein Intake on Blood Pressure

In addition to body composition, breakfast may influence other metabolic outcomes. In the cross-sectional HELENA study, skipping breakfast was associated with higher systolic and diastolic blood pressures in boys, but not girls.[167] In one cross-sectional study, individuals who consumed highest amounts of energy earlier in the day had a 30% lower risk of hypertension compared with those having the lowest energy intakes; individuals with the highest energy intakes in the late evening tended to have a higher risk of developing hypertension after ten years of follow-up.[168] Yet another study involving young adults from NHANES data collected from 1999-2006, found that consumption of ready-to-eat cereal in the morning was associated with lower systolic blood pressure and lower risk of elevated blood pressure compared to young adults who skipped breakfast or consumed another type of breakfast.[169] Since ready-to-eat cereal consumption is closely associated with milk consumption, it may be that a higher dairy intake is responsible for the observed effects on blood pressure.

While a mechanism linking breakfast eating per se and blood pressure is unclear, one recent study suggested that skipping breakfast may lead to prolonged daily cortisol elevations.[170] In sum, current data suggest that the timing of food intake

and/or the amount of energy consumed at specific times may impact the risk for vascular diseases[171] although the evidence linking breakfast and blood pressure is very limited.

As previously mentioned, research suggests that higher total protein intake in the diet may be beneficial to lowering blood pressure.[80] Thus, consuming more protein-rich foods in the morning could improve blood pressure due to the dietary protein content. Furthermore, consumption of protein-rich foods could improve the overall nutritional quality of the diet, thereby improving cardiometabolic health. For example, eggs, which are a commonly consumed breakfast item, are an important source of dietary protein (approximately 6 grams per medium egg), folate, fat-soluble vitamins, and mono-and polyunsaturated fatty acids.[172] In addition, eggs are good sources of choline and the antioxidants, lutein and zeaxanthin.[173] This suggests that protein-rich foods, which are nutrient-dense, have the potential to impact multiple cardiometabolic pathways, including blood pressure.

1.6.4 Effects of Morning Protein Consumption on Glucose Metabolism

A number of studies have investigated the effect of breakfast consumption on glucose metabolism. Prospective studies suggest regular consumption of breakfast is associated with a lower risk of T2DM.[174,175] In addition, several acute experimental studies have demonstrated a favorable effect of breakfast on glucose metabolism. Specifically, among individuals with T2DM, the postprandial rise in glucose after lunch and dinner is lower when breakfast is consumed.[176,177] Skipping breakfast is also associated with lower glucagon-like peptide-1 (GLP-1) response, delayed insulin peak, lower concentrations of plasma insulin and C-peptide, and elevated free fatty acids after lunch and dinner in individuals with T2DM.[177,178]

These findings may also extend to normal-weight, non-diabetic individuals. One randomized cross-over study found that male habitual breakfast consumers, who skip breakfast, have higher glucose, insulin, and free-fatty acids, as well as lower GLP-1 after a pre-load compared to individuals that did consume breakfast.[179] In a more recent randomized cross-over study of males and females, which included both habitual breakfast consumers and skippers, researchers also found similar results when participants consumed a carbohydrate-rich breakfast compared to breakfast skipping.[180] Future research should account for frequency

of breakfast consumption and/or skipping since long-term effects are most likely linked with habitual breakfast consumption patterns.[181] Overall, research suggests that the morning meal may have a major impact on glucose metabolism, although longer term experimental studies are needed to confirm the acute research findings and longer-term observational studies are needed to explore the impact of habitual breakfast eating/skipping on risk of impaired fasting glucose and T2DM.

As previously mentioned, skipping breakfast compared to consuming breakfast, creates a second-meal phenomenon, where glucose control during a subsequent meal worsens.[176] Some authors have taken this investigation further to evaluate the role of breakfast macronutrient composition on glucose metabolism. In a randomized crossover study for seven days, consumption of a high-protein breakfast (35% protein) compared to a high-carbohydrate breakfast (15% protein) led to a lower post-breakfast glucose and induced greater insulin and glucose-dependent insulinotropic peptide response to subsequent lunch meal in T2DM.[182] In healthy pre-menopausal women, ingestion of a high protein breakfast (either 30 grams or 39 grams) compared to a low protein breakfast (3 grams of protein) resulted in a non-significant decrease in postprandial glucose and a significant

increase in postprandial insulin.[137] Furthermore, a small randomized parallel-arm study found a statistically significant reduction in postprandial glucose fluctuations and a non-significant reduction in 24-hour area glucose variability when overweight adolescents, who typically skipped breakfast, consumed a high protein breakfast (35 grams) compared to a normal protein breakfast (13 grams) for 12 weeks.[183]

While there is some evidence for a beneficial effect of protein consumption in the morning on glucose metabolism, it is important to note that few studies consider habitual breakfast intake and this could be impacting the results. For example, in a three day cross-over study where habitual breakfast skippers and habitual breakfast eaters skipped breakfast or consumed either a normal protein (12 grams) or a high protein breakfast (32 grams), the results varied based on habitual breakfast consumption. Specifically, the habitual breakfast skippers had higher afternoon and daily glucose responses with consumption of the high protein breakfast compared to breakfast skipping. On the other hand, in habitual breakfast consumers, the high protein breakfast reduced glucose area under the curve compared to the normal protein breakfasts.[184]

1.6.5 Effects of Morning Protein Intake on Lipids

Some authors have suggested that breakfast may impact lipid levels by helping to spread food intake across the day, resulting in reduced postprandial insulin response, thus leading to lower total cholesterol, LDL-C, and triglycerides.[126] Furthermore, breakfast consumers are more likely to have increased consumption of nutrients (e.g. dietary fiber),[185,186] a better overall diet quality,[187] and healthier lifestyle habits (e.g. more physical activity),[188] which could reduce CVD risk factors. However, the data supporting a direct role of breakfast on lipids is limited. In a cross-sectional study, researchers found that skipping breakfast compared to those that regularly consumed breakfast was associated with higher LDL-C, total cholesterol to HDL-C, and LDL-C to HDL-C in males, but no such differences were noted amongst girls.[167] In contrast, a cross-sectional study of children in Cyprus found that girls who consumed breakfast had a tendency to have a higher HDL-C, a lower LDL-C, and a significantly lower serum triglycerides compared with breakfast skippers, while no differences were found for boys.[189] Furthermore, a prospective study found that adults who were classified as breakfast skippers during childhood and adulthood had a higher total cholesterol and LDL-C compared to those who ate breakfast at both time points.[190]

Randomized clinical trial data are also inconsistent. One cross-over study found that omitting breakfast resulted in higher fasting total cholesterol and LDL-C in healthy lean women after two weeks,[191] while a six week randomized clinical trial had no significant effects on lipid outcomes.[192] Overall, as with blood pressure, there is limited evidence to support a role of breakfast consumption on impacting lipid outcomes. In particular, additional research is needed investigating the effects of consuming dietary protein in the morning on lipid outcomes, where there are essentially no studies.

1.7 EGG INTAKE MAY IMPACT BODY FAT AND CARDIOMETABOLIC RISK

For years, recommendations to limit dietary cholesterol to prevent CVD have been a fundamental component of diet policy in the United States, with the belief that restricting dietary cholesterol, lowers plasma cholesterol levels and reduces risk of CVD.[193] Since eggs (including eggs in mixed dishes) are the primary source of dietary cholesterol,[194] containing approximately 185 milligrams of cholesterol per large egg,[195] they have been targeted as a key strategy for reducing dietary cholesterol intake. These recommendations were based on

several lines of evidence including animal studies in which supraphysiologic doses of dietary cholesterol were found to induce atherosclerosis[196] and clinical trials suggesting a dose-response relationship between consumption of dietary cholesterol and increases in total and LDL-C.[197]

More recently, however, the 2013 American Heart Association/ American College of Cardiology lifestyle management guideline on reducing cardiovascular risk determined the data are insufficient to determine the effects of dietary cholesterol on LDL-C.[28] Furthermore, the 2015 Dietary Guidelines Advisory Committee found no appreciable relationship between dietary and serum cholesterol at the current levels of intake.[27,198] Unfortunately after years of diet policy recommending reducing dietary cholesterol intake (including egg consumption), egg intake may have become linked with unhealthy behaviors, since individuals consuming a higher intake of eggs, may have been less likely to follow other healthy dietary and lifestyle advice in general.[199]

1.7.1 Effects of Eggs on Adiposity

Several cross-sectional studies have evaluated egg intake on body fat-related outcomes. Researchers evaluating NHANES data found individuals who

consumed eggs and meat for breakfast, had the highest daily energy intake and had a higher BMI compared to individuals who consumed ready-to-eat cereal, cooked cereal, or quick breads for breakfast.[100] In more recent NHANES data, researchers found that adults classified into one of two egg breakfast patterns (eggs/grain/meat/poultry/fish and meat/poultry/fish/grain/eggs) had the highest breakfast energy and total energy intakes, BMI, waist circumference, and percentage of individuals classified as overweight or obese.[200] The NHANES findings in children were very similar.[201] Furthermore, children and adults consuming the egg patterns also consumed the most saturated fat, solid fat, and cholesterol, as well as the lowest dietary fiber intake at breakfast and throughout the day.[200,201] Since egg consumption is linked with other related diet patterns, it is possible that the observed effects of eggs on health outcomes may be confounded by other dietary as well as non-dietary factors. In fact, a recent publication suggested that the relation of egg consumption with adiposity may vary depending on the other food groups included in the statistical analyses, with the authors finding that the positive association between egg consumption and BMI disappeared after adjustment for dietary confounders.[202]

Some prospective studies have evaluated the effect of egg intake on weight gain. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, researchers found no association between egg consumption and 2-year weight gain in adults.[203] A recent publication in adolescents had similar findings, with egg intake not being associated with excess weight gain after three years of follow-up.[204] Finally, a more recent study found inconsistent effects of egg intake on weight gain amongst three prospective cohorts—the Nurses' Health Study (NHS), Nurses' Health Study II (NHSII), and the Health Professionals Follow-up Study (HPFS). In NHSII, each 1-serving increase in egg intake/day led to a 0.5 kilogram decrease in weight every four years, while in the NHS and HPFS, no such effects were found.[205] The authors further stratified by glycemic load and found that every 1-serving increase of eggs combined with ≥ 10 unit increase in glycemic load over four years was associated with 0.28 kg weight gain; in contrast, every 1-serving increase of eggs combined with a decrease in glycemic load (< 10 units) over four years was associated with a 0.79 kg weight loss.

Short-term randomized clinical studies provide some mechanistic evidence for how egg intake could favorably impact body composition. In one particular cross-over study, researchers found overweight women consuming an egg

breakfast compared to an isocaloric bagel breakfast, reported enhanced satiety, as well as lowered energy intake at lunch, throughout the rest of the day, and up to lunch the next day.[206] Similar results were found in a cross-over study involving normal weight men; in this study an egg-based breakfast resulted in greater feelings of satiety, reduced energy intake at lunch and evening meal, and a significantly lower daily caloric intake compared to an isocaloric cereal or croissant breakfast.[207] It was shown in one study that egg breakfasts lowered postprandial glucose, insulin, and ghrelin area under the curves compared to an isocaloric bagel breakfast.[208] Further, protein is a stronger secretagogue of GLP-1 and CCK than fat and carbohydrate, suggesting the dietary protein in eggs could influence satiety via gut hormones.[79]

In contrast, a study comparing an egg breakfast (8.5 grams of protein) with an isocaloric bagel breakfast (5.5 grams of protein) in children (age 4 to 6 years) and adolescents (age 14 to 17 years) found no significant differences in visual analog scales of satiety and lunch energy intake, while the bagel breakfast led to a significant decrease in ghrelin 30 minutes after breakfast.[209] In a seven-day cross-over study involving overweight participants, comparing isocaloric breakfasts of similar macronutrient content (i.e., both had 19.8 grams of protein), researchers

found the egg breakfast on day 7 significantly increased peptide YY3-36 (PYY3-36) compared to the cereal breakfast. However, the two breakfasts had similar effects on satiety, subsequent lunch energy intake, and 3-hour postprandial ghrelin area under the curve.[210] These small differences in these study outcomes could be due to the similar amounts of protein consumed with the egg breakfasts compared to the control breakfasts.[211]

Randomized clinical studies evaluating effects of egg intake on adiposity outcomes and conducted for a longer period of time are inconclusive. In an eight-week study involving overweight men and women on reduced-calorie diets, consumption of eggs for breakfast compared to a bagel breakfast resulted in significantly greater weight loss (-2.63 vs. -1.59 kilograms) and reduction in BMI (-0.95 vs. -0.59), and a greater (non-statistically significant) reduction in waist circumference and percent body fat..[212] In contrast, other studies have found no statistically significant effects on weight loss[213,214] or fat mass.[214]

1.7.2 Effects of Eggs on Lean Body Mass

Since eggs are a high-quality protein, as evident by their high digestibility and essential amino acid profile, and are a rich source of leucine, they may be

particularly beneficial to skeletal health.[215] For example, one study involving young men found that ingesting whole egg protein after resistance training stimulated MPS in a dose-dependent manner.[216] In a small, 12-week study, researchers found that consuming an egg-based breakfast (two eggs per day) combined with a resistance training program, resulted in a non-statistically significant increase in fat-free mass and non-statistically significant greater strength gain in bench press and squat weight after 12 weeks compared to the bagel breakfast group.[214] Overall, studies with more participants followed for longer periods of time are needed to determine the effects of egg intake on lean body mass.

1.7.3 Effects of Eggs on Blood Pressure

There has been some research evaluating the impact of eggs on blood pressure. One cross-sectional study[202] and three short-term randomized clinical studies in healthy and high-risk individuals[217-219] suggest that consuming two eggs per day is not detrimental to blood pressure. However, long-term studies are needed. In animal studies, a number of egg-derived peptides have been linked with possible antihypertensive properties, perhaps by inhibiting the activity of angiotensin-converting enzyme.[220] Also, the arginine content in eggs could induce

vasodilation, as a result of acting as a substrate for nitric oxide synthesis, thus lowering blood pressure.[92] Consequently, there is a need to evaluate the effects of egg consumption on blood pressure in prospective studies.

1.7.4 Evidence Linking Eggs to Glucose Metabolism

The epidemiologic literature on egg consumption and glucose-related outcomes is inconsistent, with some authors suggesting the variability in results could be due to such factors as differences in study populations and methods (e.g. control for potential dietary confounders).[221] For instance, in a study of men and women ≥ 65 years of age, consuming eggs almost every day (assessed by food frequency questionnaire) led to a higher adjusted mean fasting glucose compared to non-egg consumers after a mean follow-up of 11.3 years (100.6 ± 3.5 vs. 99.5 ± 3.5, $p<0.001$) although the small difference in means could easily be explained by uncontrolled confounding.[222] On the other hand, more recent publications found either no association of egg intake and incident diabetes in individuals followed for 7 to 14 years [223,224] or found that men with the highest intake of eggs had a 45% lower risk of developing T2DM during an average follow-up of 19 years.[225] Two meta-analyses of a small number of studies suggested that egg consumption may be associated with the development of T2DM[226,227] and CVD risk associated

with egg intake may be more pronounced in those with T2DM.[226] although the individual study results were inconsistent and the meta-analyses found that substantial heterogeneity may affect the validity of the pooled results. Finally, a recent meta-analysis of prospective studies found no relation of egg intake to T2DM risk, but a modest increase when restricted to US based studies, leading researchers to speculate that confounding by dietary intake is driving the increase risk for diabetes in the US studies.[228]

The randomized clinical trial literature suggests that eggs are not detrimental to glucose metabolism. Several short-term randomized clinical trials found that consuming two to three eggs per day for 12 weeks as part of an energy-restricted diet[219,229,230] had no negative effects on blood glucose in either males or females. Another study of subjects with T2DM noted an improvement in HbAlc and fasting glucose associated with consuming two eggs per day as part of an energy-restricted diet for 12 weeks.[230]

In addition to the lack of evidence from clinical trials linking egg intake with abnormal glucose metabolism, the weak evidence associating a nutrient in eggs to abnormal glucose metabolism also raises questions about a direct link between

eggs and risk of abnormal glucose. While some research has found an association between dietary cholesterol and risk for gestational and T2DM,[231,232] other research does not show an association between dietary cholesterol intake and the development of T2DM.[222] In fact, one study found women consuming the highest amount of dietary cholesterol intake had a non-statistically significant 23% lower odds of T2DM risk compared with the control group.[233] Overall, given the current evidence, dietary cholesterol seems an unlikely biological mechanism to underlie the association between egg intake and risk of T2DM.[234]

The nutrient composition of eggs could explain the beneficial effects on glucose-related outcomes in some studies. For instance, the protein content of eggs may play a role in glucose metabolism by serving as a substitute for carbohydrates with a higher glycemic load, as a substrate for gluconeogenesis, or by promoting insulin secretion from pancreatic β –cells.[102] Further, egg yolks are a rich source of the carotenoids lutein and zeaxanthin,[235] which have been associated with lower 2-hour post-load glucose and fasting insulin in a cross-sectional analysis,[236] potentially due to their role in modulating inflammation via inhibition of nuclear factor κB and by modifying oxidative stress via interaction with the nuclear factor erythroid 2-related factor 2 pathway.[237] Finally, other micronutrients such

as vitamin D may play important roles in glucose metabolism by improving pancreatic β-cell function through both direct and indirect effects on insulin secretion, improvement in insulin action, and reduction in systemic inflammation.[238]

1.7.5 Evidence Linking Eggs to Lipid Outcomes

Recent randomized clinical trials have investigated the effect of egg consumption on lipids. In studies with participants maintaining weight, or in weight loss studies where the background diet is often controlled, consuming two to three eggs per day either was non-detrimental to LDL-C[217] or LDL-C and HDL-C only increased in participants who are sensitive to the dietary cholesterol in eggs (i.e. hyper-responders). Approximately 25% of individuals are classified as hyper-responders to dietary cholesterol because they are unable to maintain cholesterol homeostasis by suppressing synthesis or decreasing absorption in the small intestine,[239] while hypo-responders experience little or no alterations in serum cholesterol as a result of consuming dietary cholesterol.[173,240,241] It is important to note that while LDL-C may increase in hyper-responders, the LDL-C to HDL-C ratio, which is a valuable marker of CVD risk, is often maintained.[242] These beneficial effects have been seen in both healthy populations, as well as in

populations at higher risk for CVD (e.g. individuals with obesity and metabolic syndrome).[234] Further, when the lipoprotein particle sizes were evaluated, egg intake promoted an increase in both LDL and HDL particles.[173] Finally, in regards to triglycerides, consumption of two to three eggs per day for six to twelve weeks did not adversely affect triglycerides in healthy[217] or hyperlipidemic adults.[243] Collectively, the literature suggests that egg consumption does not adversely affect lipids.

There are several factors to consider when evaluating the current literature. While numerous studies exist using eggs as the exposure to test the effects on lipid outcomes, many of the earlier studies controlled all nutrients except for dietary cholesterol, so these are really dietary cholesterol challenges and not studies evaluating the entire nutritional content of eggs.[244] Furthermore, since dietary cholesterol intake tends to be associated with high saturated fat intake or other dietary patterns associated with low fruit and vegetable intake,[193] there is a need to consider dietary factors in the epidemiological analyses. To date, few studies have accounted for these dietary factors, which highlights a need for more controlled, prospective studies to determine the impact on eggs *per se* on lipid outcomes.[199] Finally, by focusing only on egg consumption and not

evaluating eggs in the context of the overall diet, researchers could be missing key interactions with other dietary factors, such as the fact that eggs co-consumed with raw vegetables can enhance carotenoid and vitamin E absorption.[245,246]

1.8 SUMMARY OF THE EVIDENCE

In the case of the epidemiological literature evaluating the effects of eggs on cardiometabolic outcomes, many of the inconsistencies could be the result of inherent limitations of observational studies. For example, much of the literature relies upon food frequency questionnaires to assess diet. This is problematic because food frequency questionnaires seldom capture eggs in mixed dishes, often do not differentiate between consumption of the entire egg or just the egg white, do not account for method of preparation (e.g. boiled vs. fried), and do not allow for the evaluation of the timing of intake.[247] Furthermore, to determine the impact of eggs on cardiovascular risk more accurately, other dietary factors need to be considered in the analysis, particularly since egg consumers have the tendency to consume higher amounts of saturated fat and less fiber.[200,201] Lack of adjustment for these potential dietary confounders could result in biased estimates of effect in some studies.

There is a need to look at the entire nutritional content of eggs the impact of egg intake *per se* on cardiometabolic outcomes.[199] In the case of the nutritional content of eggs, much of the focus has been on dietary cholesterol. However, it is important to note that eggs contain numerous other nutrients that may positively (e.g. lutein, zeaxanthin, egg-derived peptides)[172,220] or negatively influence (e.g. trimethylamine N-oxide) CVD risk.[248,249]

The effects of eggs on health outcomes must be evaluated in the context of the overall diet. Previous studies evaluating eggs as part of dietary patterns using data-derived patterns (e.g. factor analysis), and some of these studies suggest that eggs are detrimental to cardiometabolic outcomes.[99,201,250] I propose that these effects are confounded by other dietary factors[202] and related foods and nutrients (e.g., intakes of fiber, whole grain, fruits, vegetables, B-vitamins, anti-oxidant vitamins).[193] As a result, researchers are unable to ascertain whether the increased risk is due to egg consumption or the low intake of important nutrients impacting cardiovascular risk. Therefore, eggs should potentially be evaluated in the context of a healthy diet to try to tease out some of these effects.

Finally, egg consumption at meals, particularly the morning meal, need to be addressed. There is some speculation in the literature that protein intake in the morning (often assessed with eggs) may be directly or indirectly (via effects on body composition) beneficial to cardiometabolic outcomes.[136-139,143,183] However, little evidence supports this idea. Furthermore, much of the research involves individuals who typically skip breakfast, so it is unclear if the beneficial effects are due to the morning protein or the result of consuming a breakfast. Thus, future research needs to account for typical morning eating patterns. Finally, questions remain if the benefits seen with morning protein intake are related to an increase in protein intake. Therefore, this dissertation will evaluate the effects of total and morning protein intake on cardiometabolic outcomes.

1.9 OBJECTIVES OF THE DISSERTATION

The objectives of this dissertation are as follows:

1. To estimate the effects of total and morning protein intake on measures of adiposity (percent body fat, percent truncal skinfolds, BMI, waist circumference), percent skeletal muscle mass, and cardiometabolic outcomes (glucose, homeostatic model assessment of insulin resistance,

systolic blood pressure, diastolic blood pressure, total cholesterol, non-HDL-C, LDL-C, LDL-C to HDL-C ratio, triglycerides) in late adolescence.

2. To estimate the effects of daily egg intake, alone and in the context of other diet patterns, on adiposity measures (percent body fat, percent truncal skinfolds, BMI, waist circumference), percent skeletal muscle mass, and cardiometabolic outcomes (glucose, homeostatic model assessment of insulin resistance, systolic blood pressure, diastolic blood pressure, total cholesterol, non-HDL-C, LDL-C, LDL-C to HDL-C ratio, triglycerides) in late adolescence.
3. To examine the impact of egg consumption, alone and in the context of other diet patterns, on fasting glucose and blood pressure among adults in the Framingham Offspring Study (FOS).

CHAPTER 2: EFFECTS OF TOTAL AND MORNING PROTEIN INTAKE ON BODY COMPOSITION AND CARDIOMETABOLIC RISK FACTORS IN LATE ADOLESCENCE

2.1 BACKGROUND

Obesity and cardiometabolic risk factors during childhood increases the likelihood of early cardiovascular disease (CVD).[2] Intake of dietary protein is one potentially modifiable factor that may reduce cardiometabolic risk either directly or indirectly via effects on body composition.[80,102,109,251] A recent review reported that adults consuming a higher-protein diet had greater fat mass loss and preservation of lean mass, as well as greater reductions in triglycerides and blood pressure compared with those consuming a lower-protein diet.[45] In later childhood and adolescence, prospective data on the effects of dietary protein on body composition and cardiometabolic outcomes are sparse with recent systematic reviews of the evidence finding the data insufficient to draw conclusions.[70,85] More research is clearly needed in this under-studied group to clarify the role of protein on these outcomes.

In addition to the need to further investigate the overall association between dietary protein and body composition and cardiometabolic outcomes, questions have been raised about how the distribution and timing of protein intake affects these same outcomes. In recent years, several researchers have suggested that protein recommendations should focus on a targeted amount of protein to be consumed at each meal, as opposed to the current recommendations that focus on requirements as grams of protein per kilogram (kg) of body weight.[69,162] Support for this idea comes from several lines of evidence: acute mechanistic studies suggesting a dietary protein threshold is needed to maximize protein synthesis (MPS) and satiety,[57,148] awareness that the human body has a limited ability to store dietary protein,[252] and data demonstrating that activation of the mammalian target of rapamycin complex 1 (mTORC1) occurs for a limited time.[163]

This evidence has led some researchers to speculate that consuming more protein in the morning would be particularly beneficial,[53,57] since protein consumption in the morning is generally lower than at other meals in both children and adults.[253,254] Further evidence supporting the importance of morning protein intake comes from a study showing that dietary protein consumed at breakfast

led to greater initial and sustained satiety compared to dietary protein consumed at lunch or dinner.[136] Moreover, increasing morning protein intake has been speculated to counteract the effects of fasting during sleep, thus helping to maintain metabolic lean tissue.[53]

Questions remain, however, about whether it is the amount of protein consumed in the morning or is it the consumption of breakfast rather than skipping breakfast that matters.[138,141] Some studies of children have included both breakfast skippers and non-skippers in the determination of breakfast protein effects. Since regular breakfast consumption has been associated with lower body fat and metabolic risk in longitudinal studies,[255,256] future long-term studies need to account for usual morning eating patterns when evaluating different types of breakfasts on the effects on these outcomes. Finally, total protein intake (throughout the day) should be considered in these longitudinal studies since individuals who consume more protein in the morning may consume more overall protein. Thus, it could be overall protein intake rather than morning protein intake that is associated with the beneficial outcomes. Given that short-term clinical trials in adults suggest total daily protein intake has beneficial effects on cardiometabolic outcomes and short-term studies in adolescents

suggest morning protein intake improves satiety, we hypothesized that both total and morning protein intake during early adolescence would be associated with beneficial effects on late adolescent cardiometabolic outcomes. The overall aim of this study was to investigate the effects of total daily protein intake and morning protein intake on late adolescent body composition and cardiovascular risk factors.

2.2 METHODS

2.2.1 Study Population

The current analyses were approved by the Boston University Institutional Review Board and used data from the National Heart, Lung Blood Institute's Growth and Health Study (NGHS). The NGHS enrolled 2,379 black and white girls aged 9-10 years; girls were followed annually for 10 years (until 19-20 years of age) for the development of obesity and cardiometabolic outcomes (e.g. anthropometric measures, blood pressure, lipid and glucose levels). These girls were enrolled from three clinical centers (the University of Cincinnati Children's Hospital Medical Center, the University of California at Berkley, and Westat Inc/Group Health Association in Rockville, MD) to allow for equal numbers of black and white girls from a mix of urban and suburban families.

Girls were included who had a minimum of 6 food records between the ages of 9 and <17 years. For two girls missing dietary data before the age of 17, we substituted data at ages 17.1 and 17.3. The mean number of food records available was 15 (range: 6 to 24 food records). For these girls with at least 6 food records, dietary data were used to create usual morning eating pattern for ages 9 to <17. The selection of the current sample excluded girls missing dietary data or had <8 grams/day of total daily protein intake (n=51). Further exclusions were girls missing the outcome of interest or potential confounders. Specifically, there were 2105 girls for the body composition outcomes, 2104 for blood pressure outcomes, and 1361 girls for the lipid and glucose outcomes.

2.2.2 Dietary Data

Dietary intake was assessed using three-day food records collected on two weekdays and one weekend day during study years 1-5, 7, 8, and 10. Records classified as reliable by a study dietitian were entered into the Nutrition Data System (NDS) of the University of Minnesota's Nutrition Coordinating Center using standard protocols.[257] This allowed the calculation of nutrient intakes including dietary protein. Boston University researchers linked the NDS food

codes with those from the United States Department of Agriculture's (USDA) MyPyramid Equivalents Database, version 2.0 for USDA Food Codes.[258] This allowed the determination of the girls' food (e.g. fruits, vegetables, grains) intake patterns.

For these analyses dietary protein intake was expressed as weight-adjusted protein intake (gram of protein per day) to provide a measure of protein intake that was uncorrelated with body weight. Specifically, this method involves obtaining residuals from a linear regression model with an individual's weight as the independent variable and the individual's protein intake as the dependent variable. Since dietary protein intake is highly correlated with body weight, using the weight-adjusted protein residuals allows the variation due to protein intake to be evaluated directly.

2.2.3 Measurement of Body Composition and Cardiometabolic Risk Factors

Outcome data used for these analyses were derived from data collected at the end of follow-up (ages 17-20 years). A bioelectrical impedance analyzer (BIA) was used to assess total body fat using validated race-specific equations to take into account differences in bone length and content. Specifically, resistance and

reactance measures from BIA were used to calculate percent body fat for whites and blacks separately from predictive models of lean body mass (LBM) developed from a separate validation sample of 126 white and black girls, ages 6 to 17 with dual energy x-ray anthropometry measurements.[259]

Black girls race-specific LBM equation = -8.78 + 0.78 * (height^2/resistance) + 0.1 * reactance + 0.18 * weight

White girls race-specific LBM equation = 1.07 + 0.37 * (height^2/resistance) + -0.17 * (triceps skinfold) + 0.47 * weight

Skeletal muscle mass (SMM) in kilograms was estimated using the BIA-based equation of Janssen et al that was validated in whites, Hispanics, and blacks: [260]

SMM (kg) = [height (centimeter)^2 /BIA-resist (ohm) * 0.401) + (sex * 3.825) + (age * -0.071)] + 5.102

where sex= 0, since this sample includes only girls.

Percent of total fat from truncal fat was estimated using skinfold measurements from the suprailiac and subscapular sites following Cameron's method.[261] Measurement of waist circumference was assessed in duplicate at the narrowest part of the torso between the iliac crest and the ribs. Finally, height was taken with a portable stadiometer, while girls were weighed in kilograms on a Health-o-meter electronic scale in a hospital gown or a large t-shirt. Height and weight measurements were used to calculate BMI as kg/m^2.

Several cardiometabolic factors were considered for these analyses including blood pressure, glucose, insulin resistance, and lipids (total cholesterol, non-high-density lipoprotein cholesterol [non-HDL-C], low-density lipoprotein cholesterol [LDL-C], LDL-C:HDL-C ratio, and triglycerides). Blood pressure was assessed following a standardized protocol of three measurements taken with a 30-second rest between each using a sphygmomanometer (Baum Desktop Model, V-Lok Cuffs). Fasting glucose and lipids were analyzed at a Johns Hopkins laboratory that is a part of the Center for Disease Control's Lipid Standardization Program.[262] The Cholesterol CHOD-PAP method (Boehringer-Mannheim Diagnostics) was used to assess total cholesterol and HDL-C, while triglycerides were determined enzymatically (Abbott A-Gent Triglycerides Reagent Set). LDL-

C was calculated using a modified Friedewald equation: [LDL-C] = [total cholesterol] – [HDL-C] – [triglycerides]/6.5.[263] Non-HDL-C was estimated as the difference between total cholesterol and HDL-C. Finally, homeostasis model assessment (HOMA-IR) was used to assess insulin resistance.[264]

2.2.4 Assessment of Potential Confounders

A number of potential confounding variables were considered for these analyses including race, socioeconomic status (SES), age (at time of diet), as well as variables that averaged information across the baseline period (ages 9 to <17): height, percent body fat as estimated by BIA, physical activity, television/video viewing time, fiber, percent energy from macronutrients, and other dietary factors. Race was self-classified as white or black, while SES was categorized as low, moderate or high based on household income and education. Physical activity was assessed at baseline and in years 3, 5, and 7-10 using the validated *Habitual Activity Questionnaire* (HAQ),[265] which calculates a score by multiplying an estimate of the metabolic equivalent for each reported activity by the weekly frequency and number of weeks per year doing the activity. Usual number of hours spent watching television or video games was determined annually by

means of a questionnaire in which girls completed the number of hours watched each day.

2.2.5 Statistical Analysis

Average food and nutrient intakes were calculated from diet records collected between the ages of 9 and 17; sensitivity analyses were performed to determine cutoff values for intake categories. Total protein intake from diet records was classified as follows: <55 grams/day, 55-<65 grams/day, 65-<75 grams/day, and ≥75 grams/day.

Morning EOs included food and/or beverage consumption between 4:00 and 11:00 am on weekdays and between 4:00 am and noon on weekends. Because some subjects tended to eat small amounts of food repeatedly throughout the day, with some EOs being separated from others by only short amounts of time, we chose to consolidate those EOs that occurred within 60 minutes of one another. To do this, we first identified the EO with the highest amount of protein consumed during the morning hours, and then combined all intakes within 60 minutes of that EO. This was referred to as a consolidated EO. The next step involved identifying the morning EO with the next highest intake of protein and

similarly consolidating intakes occurring within 60 minutes of that EO; the maximum amount of consolidated EOs in the morning was four. Since less than two percent of all days had multiple morning EOs, we chose to consolidate all morning protein intake. Dietary protein intake in the morning was classified into the following categories: <8 grams, 8-<12 grams, 12-<15 grams, and ≥ 15 grams.

Analysis of covariance models were used to calculate adjusted mean percent SMM, percent body fat, percent truncal fat, waist circumference, BMI, and cardiometabolic outcomes (e.g. blood pressure, fasting glucose, HOMA-IR, and lipids) in later adolescence (17-20 years of age) according to category of intake of total protein and morning protein.

Categories for morning eating patterns were as follows: frequently skipped morning EOs, consumed morning EOs intermittently, and frequently consumed a morning EO. These morning EOs were further subdivided based on protein intake of morning EOs.

For the next analysis, we classified each girl according to the frequency with which she skipped morning meals. We began by examining each day's intake to

determine whether the girls consumed a morning meal on that day. If a girl had no consolidated EOs during the morning time period on a given day or if she consumed less than 100 kilocalories and less than one serving in each of the USDA's five major food groups on that morning, she was classified as having no morning EO. Girls who consumed <100 kilocalories during but who did had one or more servings in one of the USDA food groups (e.g., a small plum) were considered to have a morning EO.

To then classify subjects according to the frequency with which she skipped morning meals, it was necessary to combine information across all diet record days. Girls with no morning EOs 40% of the time or more were considered to "frequently skip morning meals". Those who skipped <25% of the time were considered to be infrequent morning meal skippers (or "frequent morning meal eaters"). The remaining girls (who had no morning EO 25-<40% of the time) were considered intermittent morning meal skippers. Finally, within each of the above categories, girls were further classified on the basis of the protein content of their morning EOs. All morning EOs with <15 grams of protein were considered low-protein morning EOs, while consumption of ≥15 grams of protein was considered a high protein morning EO. Girls who consumed <15 grams protein

at morning meals <50% of the time when they consumed a morning EO were considered to consume low protein morning EOs while girls who consumed ≥15 grams protein ≥50% of the time when consuming morning EOs were considered to consume high protein EOs.

Analysis of covariance models were used to calculate adjusted mean percent SMM, percent body fat, percent truncal fat, waist circumference, BMI, and cardiometabolic outcomes (e.g. blood pressure, fasting glucose, HOMA-IR, and lipids) in later adolescence (17-20 years of age). All analyses were conducted using Statistical Analysis Systems software package version 9.4 (SAS Institute, Cary, NC, USA).

2.3 RESULTS

Table 2.1 shows the characteristics of the girls according to usual daily weight-adjusted protein intake at ages 9 to <17 years of age. Girls with a higher protein intakes were taller and had a lower BMI. In addition, higher protein intake was associated with a higher percentage of kilocalories from fat, but a lower percentage of kilocalories from solid fats, alcohols, and added sugars (SOFAAs). Girls with a higher protein intake also watched more television and videos and

consumed more fruits and vegetables, dairy, and dietary fiber. Finally, race-specific differences in protein intake were apparent in that 55.8% of white girls consumed <65 g/day of dietary protein compared with 48.1% of black girls.

Table 2.1. Characteristics of girls in the National Growth and Health Study according to baseline daily protein intake

	Daily protein intake (weight-adjusted protein residuals)				
	<55 g	55-<65 g	65-<75 g	≥75 g	
	n= 474	n=615	n=520	n=496	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*P*-value
Baseline characteristics					
Age (yrs)	10.0 ± 0.5	10.0 ± 0.6	10.0 ± 0.6	10.1 ± 0.5	*0.0059*
Height (cm)	140.6 ± 7.7	140.8 ± 7.6	141.4 ± 7.2	142.5 ± 8.1	*0.0003*
Body mass index (kg/m^2)	18.8 ± 3.8	18.6 ± 3.8	18.4 ± 3.7	18.1 ± 3.7	*0.0365*
Activity score (METS)[1]	19.3 ± 9.9	19.5 ± 8.9	20.1 ± 10.7	20.2 ± 11.2	*0.4284*
Television/video (hrs/day)[1]	4.4 ± 1.9	4.5 ± 2.1	4.5 ± 2.1	5.1 ± 2.2	*<0.0001*
Mean dietary intakes[1]					
Energy intake (kcals/day)	1519 ± 209	1767 ± 226	1947 ± 234	2294 ± 315	*<0.0001*
Protein (grams)	48.8 ± 4.7	60.3 ± 2.9	69.5 ± 2.9	85.8 ± 9.3	*<0.0001*
Protein (grams/kg)	1.0 ± 0.2	1.2 ± 0.3	1.4 ± 0.3	1.7 ± 0.4	*<0.0001*
% energy from protein	13.0 ± 1.7	13.9 ± 1.7	14.5 ± 1.7	15.1 ± 1.7	*<0.0001*
% energy from carbohydrate	54.1 ± 4.6	52.3 ± 4.7	51.2 ± 4.3	49.1 ± 4.3	*<0.0001*
% energy from fat	33.9 ± 3.9	34.9 ± 4.0	35.3 ± 3.7	36.6 ± 3.7	*<0.0001*
% energy from SOFAAS	42.4 ± 5.4	41.6 ± 5.1	41.0 ± 5.1	40.7 ± 4.7	*<0.0001*
% energy from saturated fat	12.3 ± 1.7	12.8 ± 1.6	13.0 ± 1.6	13.5 ± 1.6	*<0.0001*
FV (cup equivalents/day)	2.8 ± 1.1	3.2 ± 1.1	3.5 ± 1.2	4.0 ± 1.3	*<0.0001*
Dairy (cup equivalents/day)	1.2 ± 0.5	1.6 ± 0.5	1.8 ± 0.7	2.1 ± 0.8	*<0.0001*
Fiber (grams/day)	9.4 ± 2.4	10.8 ± 2.5	12.0 ± 2.8	13.7 ± 3.2	*<0.0001*
Added sugars (tsp eq/day)	20.0 ± 6.8	21.8 ± 7.4	22.9 ± 7.2	25.3 ± 8.3	*<0.0001*
Race (row %)					
White	25.3	30.5	25.3	19.0	*<0.0001*
Black	20.0	28.1	24.2	27.8	
Socioeconomic status (row %)					
Low	19.5	27.8	24.7	28.0	*0.0731*
Mid	24.5	30.5	23.6	21.3	
High	22.0	28.5	26.0	23.4	

[1]Mean values from ages 9-<17 years

Adjusted mean body composition values at the end of follow-up (ages 17-20 years of age) according to daily protein intakes are presented in Table 2.2. After adjusting for those factors found to be confounders (race, SES, baseline height, television/video viewing time, and percent kilocalories from carbohydrates), percent SMM increased with increasing amounts of daily protein intake. In addition, percent body fat, percent truncal fat, waist circumference, and BMI showed statistically significant decreases with higher intakes of protein.

Table 2.2. Adiposity and body fat-related outcomes at 17-20 years of age according to average daily protein intake

Daily wt-adj. pro intake, age 9-17 yrs	*n*	Body fat (%) *Mean ± SE*[1]	Truncal fat (%) *Mean ± SE*[1]	WC (cm) *Mean ± SE*[1]	BMI (kg/m^2) *Mean ± SE*[1]	SMM (%) *Mean ± SE*[1]
<55 g	474	32.0 ± 0.30	36.2 ± 0.88	76.6 ± 0.55	25.6 ± 0.29	33.1 ± 0.23
55-<65 g	615	31.8 ± 0.26	34.2 ± 0.75	76.3 ± 0.47	25.3 ± 0.24	33.1 ± 0.20
65-<75 g	520	30.5 ± 0.28	32.1 ± 0.81	75.5 ± 0.51	24.9 ± 0.26	34.0 ± 0.21
≥ 75 g	496	30.0 ± 0.30	30.1 ± 0.86	74.5 ± 0.54	24.2 ± 0.28	34.5 ± 0.23
P-trend		*<0.0001*	*<0.0001*	*0.0042*	*0.0004*	*<0.0001*

[1]Adjusted for race, SES, baseline height, television/video viewing, % energy from carbohydrates.

Mean cardiometabolic outcomes according to daily weight-adjusted protein intakes are presented in Tables 2.3, 2.4, and 2.5. Higher protein intake was

associated with lower DBP, but not SBP. To determine whether the effects of protein on BP were a result of intermediate effects of body fat, % body fat (from BIA) was added to model 2; here, the effects of protein intake on DBP appeared attenuated. In Tables 2.4 and 2.5, the effects of dietary protein were inconsistent across categories of intake. Since the highest protein intakes tended to be associated with lower lipid levels, we conclude that there is no evidence for an adverse effect of dietary protein on these outcomes.

Table 2.3. Systolic and diastolic blood pressures at 17-20 years of age according to average daily protein intake

		Model 1[1]		**Model 2-Adding % Body Fat as Causal Intermediate**[2]	
Daily wt-adj pro intake, age 9-17 yrs	*n*	SBP (mmHg) *Mean ± SE*	DBP (mmHg) *Mean ± SE*	SBP (mmHg) *Mean ± SE*	DBP (mmHg) *Mean ± SE*
<55 g	474	109.4 ± 0.37	66.1 ± 0.36	109.2 ± 0.36	66.0 ± 0.36
55-<65 g	614	109.0 ± 0.32	65.4 ± 0.31	108.8 ± 0.31	65.3 ± 0.31
65-<75 g	520	108.6 ± 0.34	65.1 ± 0.36	108.8 ± 0.33	65.2 ± 0.34
≥ 75 g	496	109.0 ± 0.37	65.1 ± 0.36	109.3 ± 0.36	65.2 ± 0.36
P-trend		*0.3264*	*0.0403*	*0.7308*	*0.1481*

[1]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, % energy from carbohydrates.
[2]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, % energy from carbohydrates, % body fat (assessed by BIA).

Table 2.4. Fasting glucose and HOMA-IR at 17-20 years of age according to average daily protein intake

		Model 1[1]		Model 2-Adding % body fat as Causal Intermediate[2]	
Daily wt-adj pro intake, age 9-17 yrs	*n*	Glucose (mg/dL) *Mean ± SE*	HOMA-IR *Mean ± SE*	Glucose (mg/dL) *Mean ± SE*	HOMA-IR *Mean ± SE*
<55 g	304	86.7 ± 0.84	2.49 ± 0.19	86.4 ± 0.82	2.36 ± 0.18
55-<65 g	387	87.4 ± 0.69	2.59 ± 0.16	87.2 ± 0.68	2.48 ± 0.15
65-<75 g	345	89.1 ± 0.73	2.67 ± 0.16	89.3 ± 0.71	2.73 ± 0.16
≥ 75 g	325	87.9 ± 0.83	2.39 ± 0.19	88.4 ± 0.82	2.56 ± 0.18
P-trend		*0.1711*	*0.8241*	*0.0311*	*0.3031*

[1]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, FV, % energy from carbohydrates.
[2]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, FV, % energy from carbohydrates, % body fat (assessed by BIA).

Table 2.5. Lipid outcomes at 17-20 years of age according to daily protein intake

				Model 1[1]		
Dietary wt-adj pro intake, age 9-17 yrs	*n*	Total chol (mg/dL) *Mean ± SE*	Non-HDL-C (mg/dL) *Mean ± SE*	LDL-C (mg/dL) *Mean ± SE*	LDL: HDL ratio *Mean ± SE*	Log triglycerides *Mean ± SE*
<55 g	304	166.3 ± 1.88	111.8 ± 1.88	99.2 ± 1.73	1.94 ± 0.05	4.30 ± 0.02
55-<65 g	387	163.7 ± 1.62	110.6 ± 1.62	98.1 ± 1.49	1.95 ± 0.04	4.30 ± 0.02
65-<75 g	345	166.1 ± 1.72	113.2 ± 1.72	100.7 ± 1.58	2.00 ± 0.04	4.30 ± 0.02
≥ 75 g	325	160.9 ± 1.85	107.0 ± 1.85	95.0 ± 1.70	1.86 ± 0.05	4.28 ± 0.02
P-trend		*0.1320*	*0.2021*	*0.2305*	*0.3872*	*0.5334*
		Model 2-Adding % body fat as Causal Intermediate[2]				
<55 g	304	165.4 ± 1.86	110.6 ± 1.83	98.2 ± 1.69	1.91 ± 0.04	4.29 ± 0.02
55-<65 g	387	163.1 ± 1.60	109.7 ± 1.58	97.3 ± 1.46	1.92 ± 004	4.29 ± 0.02
65-<75 g	345	166.5 ± 1.69	113.7 ± 1.67	101.1 ± 1.54	2.01 ± 0.04	4.30 ± 0.02
≥ 75 g	325	162.1 ± 1.83	108.7 ± 1.80	96.3 ± 1.67	1.90 ± 0.04	4.30 ± 0.02
P-trend		*0.4960*	*0.9196*	*0.9059*	*0.6328*	*0.7105*

[1]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, % energy from carbohydrates.
[2]Adjusted for race, SES, age (at time of diet assessment), baseline height, television/video viewing, % energy from carbohydrates, % body fat (assessed by BIA).

Beginning with Table 2.6, the effects of morning protein intake rather than total protein intake are shown. Similar to total protein intakes, girls who typically consumed more morning protein also consumed more total fruits and vegetables, dairy, and dietary fiber compared to lower protein morning consumers. Black girls were more likely to be in the highest morning protein intake category than whites. Furthermore, the highest morning protein intake group had the lowest BMI and they watched the most amount of television/videos.

Table 2.6. Characteristics of girls in the National Growth and Health Study according to baseline morning protein intake

	Morning protein intake (weight-adjusted protein residual)				
	<8 g	8-<12 g	12-<15 g	≥15 g	
	n= 519	n=743	n=409	n=434	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*P*-value
Baseline characteristics					
Age (yrs)	10.1 ± 0.6	10.0 ± 0.6	10.0 ± 0.6	10.0 ± 0.5	*0.0100*
Height (cm)	141.4 ± 7.6	141.0 ± 7.5	141.1 ± 7.8	141.9 ± 7.9	*0.3082*
Body mass index (kg/m^2)	18.8 ± 3.8	18.5 ± 3.8	18.5 ± 3.9	18.1 ± 3.5	*0.0385*
Activity score (METS)[1]	19.3 ± 9.5	19.7 ± 9.9	19.8 ± 10.4	20.5 ± 11.0	*0.3495*
Television/video (hrs/day)[1]	4.7 ± 2.0	4.4 ± 2.0	4.7 ± 2.1	4.8 ± 2.3	*0.0008*
Mean dietary intakes[1]					

Energy intake (kcals/day)	1724 ± 324	1815 ± 318	1929 ± 335	2131 ± 394	*<0.0001*
Protein (grams)	58.7 ± 12.1	63.2 ± 11.8	68.9 ± 13.4	76.8 ± 13.9	*<0.0001*
Protein (grams/kg)	1.2 ± 0.3	1.3 ± 0.4	1.4 ± 0.4	1.6 ± 0.4	*<0.0001*
% energy from protein	13.7 ± 1.9	14.0 ± 1.9	14.4 ± 1.8	14.5 ± 1.7	*<0.0001*
% energy from carbohydrate	52.0 ± 4.8	52.0 ± 4.8	51.6 ± 4.7	50.8 ± 4.9	*0.0003*
% energy from fat	35.2 ± 3.8	35.0 ± 3.9	35.1 ± 3.9	35.6 ± 4.2	*0.0460*
% energy from SOFAAS	42.5 ± 5.0	41.4 ± 5.2	41.0 ± 5.0	40.5 ± 5.0	*<0.0001*
% energy from saturated fat	12.8 ± 1.6	12.8 ± 1.7	12.9 ± 1.6	13.1 ± 1.7	*0.0104*
FV (cup equivalents/day)	3.2 ± 1.2	3.3 ± 1.2	3.4 ± 1.2	3.7 ± 1.3	*<0.0001*
Dairy (cup equivalents/day)	1.3 ± 0.5	1.6 ± 0.7	1.9 ± 0.7	2.0 ± 0.8	*<0.0001*
Fiber (grams/day)	10.3 ± 2.7	11.1 ± 2.8	11.9 ± 3.1	13.2 ± 3.5	*<0.0001*
Added sugars (tsp eq/day)	22.1 ± 7.5	21.9 ± 7.6	22.4 ± 7.1	24.1 ± 8.3	*<0.0001*
Race (row %)					
White	24.5	38.8	19.3	17.5	*0.0010*
Black	24.8	32.1	19.5	23.6	
Socioeconomic status (row %)					
Low	21.2	32.0	18.9	28.0	*0.0010*
Mid	26.5	35.9	19.3	18.3	
High	24.7	36.8	19.9	18.6	

[1]Mean values from ages 9-<17 years

Adjusted mean body composition values according to usual morning weight-adjusted dietary protein intake are shown in Table 2.7. Higher morning protein intake was associated with higher percent SMM and lower percent body fat (from BIA). Adding total protein to the models attenuated the results, which suggests that some of the effects of morning protein intake on the body fat measures are attributable to higher overall intake.

Table 2.7. Adiposity and body fat-related outcomes at 17-20 years of age according to average morning protein intake

				Model 1[1]		
AM wt-adj pro intake, age 9-17 yrs	*n*	Body fat (%) *Mean ± SE*	Truncal fat (%) *Mean ± SE*	WC (cm) *Mean ± SE*	BMI (kg/m^2) *Mean ± SE*	SMM (%) *Mean ± SE*
<8 g	519	31.5 ± 0.28	33.9 ± 0.82	75.8 ± 0.51	25.3 ± 0.26	33.4 ± 0.21
8-<12 g	743	31.2 ± 0.24	33.0 ± 0.68	75.6 ± 0.43	25.0 ± 0.22	33.6 ± 0.18
12-<15 g	409	30.9 ± 0.32	33.6 ± 0.92	76.3 ± 0.57	25.2 ± 0.30	33.8 ± 0.24
≥ 15 g	434	30.7 ± 0.31	32.1 ± 0.90	75.4 ± 0.56	24.6 ± 0.29	33.9 ± 0.24
P-trend		*0.0327*	*0.2319*	*0.9396*	*0.1571*	*0.0771*
				Model 2[2]		
<8 g	519	31.4 ± 0.28	33.6 ± 0.81	75.7 ± 0.51	25.2 ± 0.26	33.4 ± 0.21
8-<12 g	743	31.1 ± 0.23	32.8 ± 0.68	75.5 ± 0.43	24.9 ± 0.22	33.7 ± 0.18
12-<15 g	409	30.9 ± 0.32	33.7 ± 0.91	76.4 ± 0.57	25.3 ± 0.30	33.7 ± 0.24
≥ 15 g	434	30.9 ± 0.31	32.7 ± 0.90	75.7 ± 0.56	24.8 ± 0.29	33.8 ± 0.23
P-trend		*0.1881*	*0.6913*	*0.6246*	*0.4067*	*0.3236*

[1]Adjusted for race, SES, television/video viewing, % energy from carbohydrates.
[2]Adjusted for race, SES, television/video viewing, % energy from carbohydrates, non-morning protein intake.

The independent and combined effects of total weight-adjusted dietary protein, morning weight-adjusted protein, and physical activity on mean adiposity and SMM are presented in Table 2.8. Those girls who consumed higher amounts of total protein, regardless of morning protein intake, had lower body fat measures and higher % SMM compared with girls who had lower intakes of total protein. The middle section of the table explores the combined effects of total protein and physical activity. Here, there appears to be effect modification of total protein

intake by physical activity. For example, girls with higher total protein intakes alone (with lower physical activity) had a mean % body fat of 30.8% compared with girls who had lower intakes of total protein (with lower physical activity levels), who had 32.0% body fat. In contrast, girls who had higher total protein and who were also physically active 29.5% body fat. Similar synergistic effects were seen for % truncal fat, waist circumference, BMI, and %SMM. In the bottom section of this table, results were less consistent. Higher morning protein intake alone was of little benefit but those who had higher morning protein intakes combined with higher amounts of physical activity had a lower % body fat (from BIA or truncal fat) and higher percent SMM.

Table 2.8. Modification of effects of total protein and morning protein intakes on mean body-fat-related measures at 17-20 years of age[1]

Pro & PA patterns (age 9-17 yrs)[1]	*n*	**Body fat (%)** *Mean ± SE*	*P-value*	**Truncal fat (%)** *Mean ± SE*	*P-value*	**WC (cm)** *Mean ± SE*	*P-value*	**BMI (kg/m²)** *Mean ± SE*	*P-value*	**SMM (%)** *Mean ± SE*	*P-value*
AM pro / Total pro											
Lower/Lower	829	31.9 ± 0.23	-	34.7 ± 0.65	-	76.2 ± 0.41	-	25.3 ± 0.21	-	33.2 ± 0.17	-
Higher/Lower	260	32.1 ± 0.40	0.6224	36.0 ± 1.16	0.3136	77.2 ± 0.73	0.1978	25.7 ± 0.38	0.3896	32.9 ± 0.30	0.3612
Lower/Higher	433	30.4 ± 0.31	0.0002	30.9 ± 0.90	0.0009	74.7 ± 0.57	0.0437	24.7 ± 0.29	0.0696	34.1 ± 0.24	0.0016
Higher/Higher	583	30.2 ± 0.27	<0.0001	31.3 ± 0.78	0.0011	75.2 ± 0.49	0.1502	24.6 ± 0.25	0.0180	34.3 ± 0.20	<0.0001
Total pro / PA											
Lower/Lower	628	32.0 ± 0.26	-	35.6 ± 0.75	-	76.6 ± 0.47	-	25.4 ± 0.24	-	33.1 ± 0.20	-
Higher/Lower	585	30.8 ± 0.27	0.0016	32.6 ± 0.79	0.0068	75.6 ± 0.49	0.1426	24.8 ± 0.26	0.0721	33.9 ± 0.21	0.0097
Lower/Higher	461	31.8 ± 0.31	0.6040	34.2 ± 0.89	0.2322	76.2 ± 0.56	0.6179	25.4 ± 0.29	0.9926	33.1 ± 0.23	0.9674
Higher/Higher	431	29.5 ± 0.31	<0.0001	29.3 ± 0.90	<0.0001	74.3 ± 0.56	0.0028	24.4 ± 0.29	0.0056	34.7 ± 0.24	<0.0001
AM pro / PA											
Lower/Lower	718	31.5 ± 0.24	-	33.9 ± 0.70	-	75.9 ± 0.44	-	25.1 ± 0.23	-	33.5 ± 0.18	-
Higher/Lower	495	31.3 ± 0.29	0.6096	34.6 ± 0.84	0.5583	76.4 ± 0.53	0.3964	25.2 ± 0.27	0.8175	33.5 ± 0.22	0.9990
Lower/Higher	544	31.1 ± 0.28	0.2557	32.6 ± 0.81	0.2095	75.4 ± 0.51	0.4740	25.1 ± 0.26	0.9567	33.6 ± 0.21	0.6415
Higher/Higher	348	30.0 ± 0.34	0.0004	30.4 ± 1.00	0.0044	75.1 ± 0.62	0.3157	24.6 ± 0.32	0.1760	34.4 ± 0.26	0.0055

[1]Adjusted for race, SES, baseline height, television/video viewing, % energy from carbohydrates.

[2]Lower vs. high morning weight-adjusted protein intake = <12 vs. ≥ 12 g/day; Lower vs. higher weight-adjusted total protein intake = <65 vs. ≥ 65 g/day; Lower vs. higher physical activity = <20 vs. ≥ 20 METS/week.

Table 2.9 shows the characteristics of girls according to the frequency of morning meal skipping. Black girls were 2.5 times more likely to be frequent meal skippers than white girls. Girls who typically had a morning EO (infrequent meal skippers) reported consuming more energy during the day but were also more active and had a lower BMI than frequent morning meal skippers and intermittent skippers. In addition, frequent meal skippers reported consuming fewer calories (240 kilocalories) during the morning hours on the days when they reported a morning EO than did the infrequent meal skippers (who consumed an average of 434 kilocalories during the morning

Table 2.9. Characteristics of girls in the National Growth and Health Study according to frequency of morning meal skipping

	Frequency of Morning Meal Skipping			
	Frequent	Intermittent	Infrequent	
	n= 441	n=495	n=1169	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*P*-value
Baseline characteristics				
Age (yrs)	10.2 ± 0.6	10.0 ± 0.5	10.0 ± 0.6	*<0.0001*
Height (cm)	142.9 ± 7.6	141.7 ± 7.4	140.5 ± 7.7	*<0.0001*
Body mass index (kg/m^2)	19.4 ± 4.1	18.9 ± 3.9	18.0 ± 3.4	*<0.0001*
Activity score (METS)[1]	17.8 ± 9.0	18.6 ± 9.4	21.1 ± 10.7	*<0.0001*
Television/video (hrs/day)[1]	5.3 ± 1.9	5.0 ± 2.0	4.2 ± 2.1	*<0.0001*
Mean dietary intakes[1]				

Energy intake (kcals/day)	1790 ± 369	1859 ± 379	1923 ± 358	*<0.0001*
Protein (grams)	63.2 ± 13.8	64.3 ± 14.8	67.8 ± 13.8	*<0.0001*
Protein (grams/kg)	1.2 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	*<0.0001*
% energy from protein	14.2 ± 2.0	13.9 ± 1.8	14.2 ± 1.8	*0.0026*
% energy from carbohydrate	50.1 ± 4.9	51.4 ± 4.6	52.4 ± 4.8	*<0.0001*
% energy from fat	36.5 ± 3.8	35.7 ± 3.5	34.5 ± 4.0	*<0.0001*
% energy from SOFAAS	42.6 ± 4.8	42.3 ± 4.9	40.6 ± 5.2	*<0.0001*
% energy from saturated fat	13.2 ± 1.6	13.0 ± 1.6	12.7 ± 1.7	*<0.0001*
FV (cup equivalents/day)	3.1 ± 1.2	3.2 ± 1.2	3.5 ± 1.3	*<0.0001*
Dairy (cup equivalents/day)	1.4 ± 0.6	1.5 ± 0.6	1.9 ± 0.7	*<0.0001*
Fiber (grams/day)	10.3 ± 2.8	11.0 ± 3.0	12.1 ± 3.2	*<0.0001*
Added sugars (tsp eq/day)	22.2 ± 8.2	22.9 ± 7.7	22.4 ± 7.5	*0.2548*
Mean AM dietary intakes[1]				
Energy intakes (kcals/AM)	240 ± 95	338 ± 109	434 ± 139	*<0.0001*
Protein (grams/AM)	7.3 ± 3.3	10.3 ± 3.8	13.5 ± 5.0	*<0.0001*
Eggs (oz equivalents/AM)	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	*<0.0001*
FV (cup equivalents/AM)	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	*<0.0001*
Dairy (cup equivalents/AM)	0.3 ± 0.2	0.4 ± 0.2	0.6 ± 0.3	*<0.0001*
Grains (oz equivalents/AM	0.8 ± 0.4	1.2 ± 0.5	1.7 ± 0.6	*<0.0001*
Fiber (grams/day)	1.3 ± 0.7	1.9 ± 0.8	2.7 ± 1.2	*<0.0001*
Added sugars (tsp eq/AM)	2.9 ± 1.9	3.9 ± 2.2	4.8 ± 2.4	*<0.0001*
Race (row %)				
White	11.9	20.0	68.1	*<0.0001*
Black	29.3	26.8	43.9	
Socioeconomic status (row %)				
Low	25.1	23.9	51.0	*<0.0001*
Mid	25.1	23.8	51.2	
High	13.0	23.0	64.0	

[1]Mean values from ages 9-<17 years

In Table 2.10, the association between the frequency of morning meal skipping and mean percent SMM, percent body fat, percent truncal fat, waist circumference, and BMI are examined. Girls who had regular morning EOs (infrequent skippers) had higher SMM and lower percent body fat, percent truncal fat, waist circumference, and BMI than those who frequently or intermittently skipped morning EOs.

Tables 2.11, 2.12, and 2.13 explore the effects of morning eating pattern on cardiometabolic risk factors. Overall, consuming a morning meal was not associated with blood pressure, fasting glucose, or fasting lipids. There was a tendency for girls who regularly ate during the morning hours to have a lower HOMA-IR compared to those girls who frequently skipped morning EOs.

Table 2.10. Adiposity and SMM outcomes at 17-20 years of age according to usual morning eating pattern

AM eating patterns, age 9-17 yrs	*n*	Body fat (%) *Mean ±SE*[1]	p-value	Truncal fat (%) *Mean ± SE*[1]	p-value	WC (cm) *Mean ± SE*[1]	p-value	BMI (kg/m²) *Mean ± SE*[1]	p-value	SMM (%) *Mean ±SE*[1]	p-value
Frequent skipper	441	32.0 ± 0.31	-	35.6 ± 0.91	-	77.0 ± 0.57	-	25.8 ± 0.29	-	33.0 ± 0.24	-
Intermit	495	31.4 ± 0.29	0.1759	34.2 ± 0.84	0.2705	76.5 ± 0.53	0.5476	25.4 ± 0.27	0.3317	33.3 ± 0.22	0.2590
Infreq skipper	1169	30.6 ± 0.19	0.0002	31.8 ± 0.56	0.0004	75.0 ± 0.35	0.0039	24.6 ± 0.18	0.0007	34.0 ± 0.15	0.0001
P-trend		*<0.0001*		*0.0002*		*0.0016*		*0.0003*		*<0.0001*	

[1]Adjusted for race, % energy from protein, television/video viewing.

Table 2.11. Systolic and diastolic blood pressure at 17-20 years of age according to morning meal skipping pattern

		Model 1[1]				**Model 2[2]**			
AM eating patterns, Age 9-17 yrs	*n*	SBP (mmHg) *Mean ± SE*	p-value	DBP (mmHg) *Mean ± SE*	p-value	SBP (mmHg) *Mean ± SE*	p-value	DBP (mmHg) *Mean ± SE*	p-value
Frequent skipper	441	109.3 ± 0.39	-	65.5 ± 0.38	-	109.1 ± 0.38	-	65.4 ± 0.38	-
Intermittent	495	109.2 ± 0.36	0.8307	65.1 ± 0.35	0.4387	109.1 ± 0.35	0.9915	65.1 ± 0.35	0.5028
Infrequent skipper	1168	108.8 ± 0.24	0.3383	65.5 ± 0.23	0.9946	108.9 ± 0.23	0.7908	65.6 ± 0.23	0.7382
P-trend		*0.2989*		*0.8394*		*0.7591*		*0.5728*	

[1]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein.
[2]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein, % body fat (assessed by BIA).

Table 2.12. Glucose and HOMA-IR at 17-20 years of age according to usual morning meal skipping pattern

		Model 1[1]				**Model 2[2]**			
AM eating patterns, Ages 9-17 yrs	*n*	Glucose (mg/dL) *Mean ± SE*	p-value	HOMA-IR *Mean ± SE*	p-value	Glucose (mg/dL) *Mean ± SE*	p-value	HOMA-IR *Mean ± SE*	p-value
Frequent skipper	290	88.7 ± 0.82	-	2.77 ± 0.18	-	88.3 ± 0.81	-	2.64 ± 0.18	-
Intermittent	327	87.5 ± 0.75	0.2687	2.70 ± 0.17	0.7919	87.3 ± 0.73	0.3408	2.64 ± 0.16	0.9941
Infrequent skipper	744	87.6 ± 0.50	0.2986	2.38 ± 0.11	0.0878	87.8 ± 0.50	0.6224	2.46 ± 0.11	0.3986
P-trend		*0.3707*		*0.0602*		*0.7541*		*0.3349*	

[1]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein.
[2]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein, % body fat (assessed by BIA).

Table 2.13. Lipid outcomes at 17-20 years of age according to usual morning eating pattern

						Model 1[1]					
		Total chol (mg/dL)		Non-HDL (mg/dL)		LDL (mg/dL)		LDL to HDL ratio		Log triglycerides	
AM eating patterns, age 9-17 yrs	*n*	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ±SE*	p-value
Frequent skipper	290	165.5 ± 1.94	-	112.2 ± 1.94	-	99.7 ± 1.79	-	1.98 ± 0.05	-	4.31 ± 0.02	-
Intermittent	327	163.2 ± 1.78	0.3781	109.1 ± 1.77	0.2271	97.1 ± 1.63	0.2656	1.91 ± 0.04	0.2884	4.28 ± 0.02	0.3334
Infrequent skipper	744	164.2 ± 1.20	0.5627	110.7 ± 1.20	0.5183	98.2 ± 1.11	0.4801	1.93 ± 0.03	0.3579	4.30 ± 0.02	0.7497
P-trend		*0.6682*		*0.6751*		*0.6080*		*0.4560*		*0.9129*	
						Model 2[2]					
Frequent skipper	290	164.8 ± 1.92	-	111.2 ± 1.89	-	98.8 ± 1.75	-	1.95 ± 0.05	-	4.30 ± 0.02	-
Intermittent	327	162.9 ± 1.75	0.4624	108.6 ± 1.72	0.3115	96.6 ± 1.60	0.3522	1.90 ± 0.04	0.3495	4.27 ± 0.02	0.4305
Infrequent skipper	744	164.6 ± 1.19	0.9478	111.4 ± 1.17	0.9282	98.7 ± 1.08	0.9708	1.95 ± 0.03	0.9224	4.31 ± 0.02	0.7420
P-trend		*0.9070*		*0.7141*		*0.8410*		*0.8944*		*0.5615*	

[1]Adjusted for race, age (at time of diet assessment), % energy from protein and television/video viewing.
[2]Adjusted for race, age (at time of diet assessment), % energy from protein and television/video viewing, % body fat (assessed by BIA)

For the final set of tables, the groups of girls who were frequent, intermittent, or infrequent morning EO skippers were further subdivided according to their protein intake classified as lower vs. higher protein consumers during the morning hours using a cutoff value of 15 grams of protein. These analyses yield 6 patterns of intake (3 meal skipping categories x 2 levels of protein). Characteristics of the girls in each of the six categories are shown in Table 2.14 Girls who infrequently skipped morning EOs, with the morning EO typically being higher in protein, consumed the most calories during the day and had the highest intakes of fruits and vegetables, dairy, and fiber. The lowest protein intakes were found in girls who frequently skipped morning meals and who, when they did eat morning meals, tended to consume low-protein foods.

Table 2.14. Characteristics of girls in the National Growth and Health Study according to independent and combined effects of baseline morning meal skipping habits and morning protein intake

	Morning eating patterns						
	Freq skip & Low AM pro n= 309 *Mean ± SD*	Freq skip & Hi AM pro n= 132 *Mean ± SD*	Intermit & Low AM pro n= 356 *Mean ± SD*	Intermit & Hi AM pro n= 139 *Mean ± SD*	Infreq skip & Low AM pro n= 852 *Mean ± SD*	Infreq skip & Hi AM pro n= 317 *Mean ± SD*	*P*-value
Baseline characteristics							
Age (yrs)	10.2 ± 0.5	10.2 ± 0.6	10.0 ± 0.5	10.1 ± 0.5	10.0 ± 0.6	10.0 ± 0.6	*<0.0001*
Height (cm)	142.7 ± 7.6	143.4 ± 7.7	141.3 ± 7.1	142.7 ± 8.1	140.1 ± 7.5	141.7 ± 8.2	*<0.0001*
Body mass index (kg/m^2)	19.4 ± 4.0	19.2 ± 4.4	18.7 ± 3.6	19.5 ± 4.4	17.9 ± 3.4	18.3 ± 3.7	*<0.0001*
Activity score (METS)[1]	17.5 ± 8.5	18.3 ± 10.0	18.7 ± 9.6	18.3 ± 8.6	21.4 ± 10.7	20.2 ± 10.6	*<0.0001*
Television/video (hrs/day)[1]	5.1 ± 1.9	5.6 ± 1.8	4.7 ± 2.0	5.7 ± 2.0	4.0 ± 2.0	4.9 ± 2.2	*<0.0001*
Mean dietary intakes[1]							
Energy intake (kcals/day)	1752 ± 349	1879 ± 400	1788 ± 322	2040 ± 448	1859 ± 333	2094 ± 369	*<0.0001*
Protein (grams)	61.0 ± 12.6	68.3 ± 15.1	61.3 ± 12.8	71.9 ± 16.9	64.9 ± 12.7	75.4 ± 13.8	*<0.0001*
Protein (grams/kilogram)	1.2 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.4 ± 0.5	1.4 ± 0.4	1.5 ± 0.4	*<0.0001*
% energy from protein	14.0 ± 2.0	14.6 ± 2.0	13.8 ± 1.8	14.1 ± 1.8	14.1 ± 1.8	14.5 ± 1.7	*<0.0001*
% energy from carbohydrate	50.5 ± 4.9	49.2 ± 4.7	51.7 ± 4.4	50.4 ± 4.9	52.9 ± 4.6	51.0 ± 5.0	*<0.0001*
% energy from fat	36.3 ± 3.9	37.0 ± 3.6	35.5 ± 3.4	36.3 ± 3.6	34.1 ±3.8	35.5 ± 4.3	*<0.0001*
% energy from SOFAAS	42.7 ± 4.9	42.4 ± 4.6	42.3 ± 5.0	42.4 ± 4.7	40.5 ± 5.2	40.8 ± 5.2	*<0.0001*
% energy from saturated fat	13.1 ± 1.7	13.5 ± 1.6	12.9 ± 1.6	13.3 ± 1.6	12.6 ± 1.6	13.1 ± 1.7	*<0.0001*
FV (cup equivalents/day)	3.1 ± 1.1	3.2 ± 1.4	3.2 ± 1.1	3.4 ± 1.3	3.5 ± 1.3	3.6 ± 1.3	*<0.0001*
Dairy (cup equivalents/day)	1.3 ± 0.5	1.5 ± 0.7	1.5 ± 0.6	1.7 ± 0.7	1.8 ± 0.7	2.0 ± 0.8	*<0.0001*
Fiber (grams/day)	10.1 ± 2.7	10.7 ± 2.9	10.6 ± 2.7	12.0 ± 3.4	11.9 ± 3.0	12.8 ± 3.4	*<0.0001*
Added sugars (tsp eq/day)	21.9 ± 8.0	22.7 ± 8.6	22.3 ± 7.1	24.7 ± 8.9	21.9 ± 7.2	23.8 ± 7.9	*<0.0001*

Mean AM dietary intakes[1]							
Energy intakes (kcals/AM)	219 ± 81	288 ± 106	299 ± 80	436 ± 110	393 ± 111	545 ± 147	*<0.0001*
Protein (grams/AM)	6.4 ± 2.6	9.5 ± 3.5	8.8 ± 2.5	14.3 ± 3.5	11.7 ± 3.7	18.4 ± 4.8	*<0.0001*
Eggs (oz equivalents/AM)	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.3	*<0.0001*
FV (cup equivalents/AM)	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.3	*<0.0001*
Dairy (cup equivalents/AM)	0.2 ± 0.2	0.3 ± 0.3	0.4 ± 0.2	0.6 ± 0.3	0.5 ± 0.3	0.8 ± 0.4	*<0.0001*
Grains (oz equivalents/AM	0.8 ± 0.4	1.0 ± 0.5	1.1 ± 0.4	1.5 ± 0.5	1.5 ± 0.5	2.0 ± 0.7	*<0.0001*
Fiber (grams/day)	1.2 ± 0.7	1.5 ± 0.8	1.7 ± 0.7	2.4 ± 1.0	2.5 ± 1.1	3.2 ± 1.3	*<0.0001*
Added sugars (tsp eq/AM)	2.7 ± 1.7	3.3 ± 2.3	3.5 ± 1.9	4.8 ± 2.5	4.5 ± 2.2	5.5 ± 2.9	*<0.0001*
Race (row %)							
White	9.8%	2.2%	16.8%	3.3%	55.0%	13.0%	*<0.0001*
Black	19.3%	10.1%	17.1%	9.7%	27.0%	17.0%	
Socioeconomic status (row %)							
Low	17.6%	7.5%	14.5%	9.3%	29.1%	22.0%	*<0.0001*
Mid	16.4%	8.7%	16.9%	6.9%	37.5%	13.7%	
High	10.5%	2.5%	18.6%	4.4%	51.8%	12.2%	

[1]Mean values from ages 9-<17 years

In Table 2.15, measures of adiposity and SMM at the end of follow-up (ages 17-20 years) according to the same six morning eating patterns are shown. Overall, girls who infrequently skipped morning meals had higher SMM and lower % body fat, % truncal fat, waist circumference, and BMI compared to those who frequently skipped or intermittently ate a morning meal. While frequently consuming a morning meal was beneficial, it does not appear here that higher protein content was of greater benefit. In fact those with lower protein intakes appeared to have slightly less adiposity at the end of follow-up.

Finally, Tables 2.16-2.19 show the cardiometabolic outcomes according to these same six categories of morning eating patterns. Generally, morning eating patterns when further stratified by dietary protein intake had no effect on blood pressure. Further, there were no consistent effects of the protein content of the morning eating pattern on fasting glucose, insulin resistance, or lipid levels.

Table 2.15. Adiposity and body fat-related outcomes at 17-20 years of age according to independent and combined effects of baseline morning meal skipping habits and morning protein intake

		Body fat (%)		Truncal fat (%)		WC (cm)		BMI (kg/m²)		SMM (%)	
AM eating patterns, age 9-17 yrs	*n*	*Mean ± SE*[1]	p-value	*Mean ± SE*[1]	p-value	*Mean ± SE*[1]	p-value	*Mean ± SE*[1]	p-value	*Mean ±SE*[1]	p-value
Freq skip & Low AM pro	309	32.2 ± 0.37	-	36.0 ± 1.07	-	77.1 ± 0.68	-	25.9 ± 0.35	-	32.9 ± 0.29	-
Freq skip & Hi AM pro	132	31.8 ± 0.57	0.5283	34.8 ± 1.66	0.5619	76.8 ± 1.05	0.7830	25.6 ± 0.53	0.6159	33.1 ± 0.44	0.5862
Intermit & Low AM pro	356	31.3 ± 0.34	0.0983	33.4 ± 0.99	0.0734	75.8 ± 0.63	0.1700	25.1 ± 0.32	0.1001	33.5 ± 0.26	0.1183
Intermit & Hi AM pro	139	31.8 ± 0.55	0.5586	36.5 ± 1.60	0.7791	78.2 ± 1.01	0.3713	26.1 ± 0.52	0.6906	33.0 ± 0.42	0.8492
Infreq & low AM pro	852	30.6 ± 0.23	0.0003	31.6 ± 0.66	0.0006	74.7 ± 0.42	0.0031	24.6 ± 0.22	0.0015	34.1 ± 0.17	0.0002
Infreq & hi AM pro	317	30.8 ± 0.36	0.0076	32.2 ± 1.06	0.0121	75.6 ± 0.68	0.1077	24.6 ± 0.34	0.0095	33.8 ± 0.28	0.0148
P-trend		*0.0002*		*0.0139*		*0.0089*		*0.0009*		*0.0002*	

[1]Adjusted for race, % energy from protein, television/video viewing.

Table 2.16. Blood pressure at 17-20 years of age according to usual morning eating pattern

		Model 1[1]				**Model 2**[2]			
AM eating patterns, age 9-17 yrs	*n*	SBP (mmHg) *Mean ± SE*	p-value	DBP (mmHg) *Mean ± SE*	p-value	SBP (mmHg) *Mean ± SE*	p-value	DBP (mmHg) *Mean ± SE*	p-value
Freq skip & Low AM pro	309	109.3 ± 0.46	-	65.4 ± 0.45	-	109.1 ± 0.45	-	65.3 ± 0.45	-
Freq skip & Hi AM pro	132	109.3 ± 0.70	0.9532	65.8 ± 0.68	0.6099	109.1 ± 0.68	0.9464	65.7 ± 0.68	0.5655
Intermit & Low AM pro	356	108.9 ± 0.42	0.4777	65.4 ± 0.41	0.9960	108.8 ± 0.41	0.6853	65.4 ± 0.41	0.8800
Intermit & Hi AM pro	139	110.0 ± 0.68	0.4258	64.4 ± 0.66	0.2367	109.7 ± 0.66	0.3912	64.3 ± 0.66	0.2403
Infreq & low AM pro	852	108.8 ± 0.28	0.3612	65.5 ± 0.27	0.7650	108.9 ± 0.27	0.8200	65.6 ± 0.27	0.5219
Infreq & hi AM pro	316	108.9 ± 0.46	0.5488	65.4 ± 0.45	0.9563	109.0 ± 0.44	0.8915	65.4 ± 0.44	0.7757
P-trend		*0.4251*		*0.9519*		*0.9007*		*0.6977*	

[1]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein.
[2]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein, % body fat (assessed by BIA).

Table 2.17. Glucose and HOMA-IR at 17-20 years of age according to usual morning eating pattern

		Model 1[1]				**Model 2[2]**			
AM eating patterns, age 9-17 yrs	*n*	Glucose (mg/dL) *Mean ± SE*	p-value	HOMA-IR *Mean ± SE*	p-value	Glucose (mg/dL) *Mean ± SE*	p-value	HOMA-IR *Mean ± SE*	p-value
Freq skip & Low AM pro	208	88.0 ± 0.95	-	2.46 ± 0.21	-	87.7 ± 0.94	-	2.36 ± 0.21	-
Freq skip & Hi AM pro	82	90.5 ± 1.50	0.1622	3.54 ± 0.34	0.0066	90.0 ± 1.48	0.1962	3.36 ± 0.33	0.0088
Intermit & Low AM pro	237	86.8 ± 0.88	0.3557	2.58 ± 0.20	0.6788	86.8 ± 0.86	0.4460	2.56 ± 0.19	0.4700
Intermit & Hi AM pro	90	89.1 ± 1.43	0.5182	3.00 ± 0.32	0.1664	88.7 ± 1.41	0.5658	2.84 ± 0.31	0.1919
Infreq & Low AM pro	552	87.1 ± 0.59	0.4127	2.38 ± 0.13	0.7527	87.3 ± 0.59	0.6970	2.46 ± 0.13	0.6841
Infreq & Hi AM pro	192	89.2 ± 1.00	0.4015	2.37 ± 0.23	0.7714	89.4 ± 0.99	0.2416	2.43 ± 0.22	0.8156
P-trend		*0.8546*		*0.1798*		*0.7353*		*0.5994*	

[1]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein.
[2]Adjusted for race, SES, age (at time of diet assessment), television/video viewing, % energy from protein, % body fat (assessed by BIA).

Table 2.18. Lipid outcomes at 17-20 years of age according to usual morning eating pattern (Model 1)

		Model 1[1]									
		Total chol (mg/dL)		Non-HDL (mg/dL)		LDL (mg/dL)		LDL to HDL ratio		Log triglycerides	
AM eating patterns, age 9-17 yrs	*n*	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ±SE*	p-value
Freq skip & Low AM pro	208	167.0 ± 2.26	-	113.9 ± 2.26	-	101.3 ± 2.08	-	2.01 ± 0.06	-	4.31 ± 0.03	-
Freq skip & Hi AM pro	82	161.4 ± 3.6	0.1758	107.9 ± 3.57	0.1530	95.5 ± 3.29	0.1372	1.91 ± 0.09	0.3068	4.30 ± 0.05	0.8607
Intermit & Low AM pro	237	164.9 ± 2.09	0.4763	110.4 ± 2.08	0.2563	98.5 ± 1.92	0.3248	1.93 ± 0.05	0.2954	4.26 ± 0.03	0.1992
Intermit & Hi AM pro	90	158.9 ± 3.40	0.0463	105.7 ± 3.40	0.0458	93.3 ± 3.13	0.0332	1.85 ± 0.08	0.1022	4.32 ± 0.04	0.9245
Infreq & Low AM pro	552	165.0 ± 1.41	0.4514	111.6 ± 1.41	0.4047	99.0 ± 1.29	0.3708	1.95 ± 0.03	0.3278	4.30 ± 0.02	0.7298
Infreq & hi AM pro	192	161.9 ± 2.38	0.1234	108.4 ± 2.38	0.0998	95.9 ± 2.19	0.0831	1.89 ± 0.06	0.1276	4.30 ± 0.03	0.7392
P-trend		*0.3285*		*0.3433*		*0.2783*		*0.2629*		*0.9940*	

[1]Adjusted for race, age (at time of diet assessment), % energy from protein and television/video viewing.

Table 2.19. Lipid outcomes at 17-20 years of age according to usual morning eating pattern (Model 2)

		Model 2[1]									
		Total chol (mg/dL)		Non-HDL (mg/dL)		LDL (mg/dL)		LDL to HDL ratio		Log triglycerides	
AM eating patterns, age 9-17 yrs	*n*	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ± SE*	p-value	*Mean ±SE*	p-value
Freq skip & Low AM pro	208	166.4 ± 2.24	-	112.9 ± 2.20	-	100.4 ± 2.04	-	1.99 ± 0.05	-	4.30 ± 0.03	-
Freq skip & Hi AM pro	82	160.3 ± 3.53	0.1359	106.3 ± 3.48	0.1009	94.2 ± 3.22	0.0935	1.86 ± 0.08	0.2105	4.28 ± 0.04	0.7438
Intermit & Low AM pro	237	164.7 ± 2.06	0.5789	110.2 ± 2.03	0.3542	98.3 ± 1.88	0.4304	1.93 ± 0.05	0.4165	4.26 ± 0.03	0.2696
Intermit & Hi AM pro	90	158.0 ± 3.36	0.0361	104.4 ± 3.31	0.0305	92.1 ± 3.07	0.0226	1.81 ± 0.08	0.0691	4.30 ± 0.04	0.9992
Infreq & Low AM pro	552	165.5 ± 1.39	0.7321	112.3 ± 1.37	0.8021	99.6 ± 1.27	0.7165	1.97 ± 0.03	0.7417	4.31 ± 0.02	0.8680
Infreq & Hi AM pro	192	162.3 ± 2.35	0.2100	108.9 ± 2.32	0.2114	96.4 ± 2.14	0.1707	1.90 ± 0.06	0.2805	4.30 ± 0.03	0.9693
P-trend		*0.6109*		*0.7817*		*0.6347*		*0.7131*		*0.5494*	

[1]Adjusted for race, age (at time of diet assessment), % energy from protein and television/video viewing, % body fat (assessed by BIA)

2.4 DISCUSSION

In these analyses, protein intake ages 9 to <17 in girls was associated with increased percent SMM and lower percent body fat, percent truncal fat, waist circumference, and BMI at the end of follow-up (ages 17-20). Consuming higher amounts of morning protein was also associated with higher SMM and lower body fat in later adolescence, although this effect appeared to be in part explained by total daily protein intake. Moreover, when examining the effects of usual morning eating patterns (e.g. frequently skipping, intermittently skipping, or infrequently skipping morning EOs) on body fat outcomes, the results suggest that those who regularly consumed morning meals had higher levels of SMM and a lower % body fat, % truncal fat, waist circumference, and BMI. When these three morning eating patterns were stratified by protein content (lower vs. higher), there was no apparent effect modification of the eating pattern by protein content. Finally, girls who were more physically active and who consumed more protein in the morning hours, and especially those who consumed more total protein had even greater benefits to body composition measures. Overall, these results suggest that the total amount

of protein consumed is more important than morning protein intake on body composition outcomes.

The effects of dietary protein on cardiometabolic outcomes were also examined. Higher dietary protein intake was associated with lower DBP; this effect was mediated by body fat. In contrast, when body fat was added to the glucose models, the effect was strengthened. No significant effects of dietary protein was noted on lipid outcomes. Finally, typically consuming a morning meal did not significantly affect metabolic outcomes.

Our study provides evidence that higher intakes of dietary protein intake ages 9 to <17 is associated with increased SMM and lower body fat, thus improving overall body composition during adolescence; this study adds to the limited literature on this topic in this age group. Similarly, another prospective study found that higher protein intake in 8-10 year old girls was associated with less fat gain and an increase in LBM after 6 years of follow-up; no beneficial effects of dietary protein on body composition

was noted in boys.[76] In another prospective study evaluating the effects of animal and plant protein intake during puberty (girls 9-14 years; boys 10-15 years) on body composition in young adulthood (18-25 years), researchers found animal protein intake was related to higher fat-free mass index (lean mass in kilograms/height in meters^2) in both males and females, while higher animal protein intake was linked with lower fat mass index (fat mass in kilograms/height in meters^2) in men, but not women. Plant protein was not associated with body composition in either men or women.[77] Finally, in a sub-study of the DiOGenes randomized controlled trial evaluating the *ad libitum* effects of dietary protein and glycemic index on cardiovascular risk factors in overweight children (ages 5-18 years), researchers found that the high protein diet group had a smaller waist circumference at the end of six months, despite no apparent change in body weight or BMI.[78]

Recently, some authors have suggested that consuming a certain amount of protein per meal (e.g. 25-30 grams of protein) may improve satiety and stimulate MPS, consequently enhancing body weight management.[45] Since

protein consumption at breakfast is particular low compared to other meals,[253,254] increasing the amount of protein in the morning has been a proposed target. There is a limited about of evidence supporting this hypothesis. For example, a few short-term studies suggest that increasing morning protein intake improves body composition. In a randomized cross-over study involving nine overweight men, researchers found increasing protein intake at breakfast led to greater initial and sustained feelings of fullness compared to increasing protein at other meals.[136] Short-term studies comparing breakfast skipping, a lower protein breakfast (13 grams of protein), and a higher protein breakfast (35 grams of protein) in breakfast skipping adolescent girls found improved satiety, showed greater reductions in food motivation/reward areas of the brain, a reduction in subsequent meal intake, and a reduction in fat mass in the higher protein breakfast group compared to the breakfast skippers.[138,141] While our study provided some evidence to support a specific beneficial effect of morning protein on body composition, it may be that the effects of protein intake during the morning hours would have been even greater

if more girls had consumed higher levels of morning protein than we found in the highest intake category (15 grams or more of protein).

The evidence supporting the idea that higher morning protein intake on improved musculoskeletal health by counteracting the effects of fasting during sleep when lean tissues many be degraded has been extrapolated from studies in adults evaluating protein distribution across the day.[53] Randomized clinical trials comparing evenly distributed protein intakes (e.g. 25-30 g of protein at each meal) to an unequal protein intake have inconsistently found that evenly distributed protein intake is associated with increased muscle protein synthesis (MPS).[158,160] Furthermore, the long-term evidence linking consumption of evenly distributed protein intake to higher lean mass is limited to older adults (aged 67-84 years) followed for two years.[266] Since adults are physiologically different than adolescents, results may differ by age group. Thus, more studies need to be conducted in a younger population.

Our results, which evaluated the effects of morning dietary protein intake in an adolescent population, found higher amounts of protein (≥ 15 vs. 8 grams of protein) in the morning was associated with lower body fat and a trend toward higher SMM in late adolescence. While morning protein intake was associated with beneficial outcomes on some body composition outcomes, it is important to note that the effects were attenuated when daily protein was added to the models suggesting that it was daily protein intake driving the favorable body composition results, and not morning protein intake. However, this may be true in other studies as well in that higher intake of protein during the morning may simply increase the overall intake throughout the day. EOs in adolescent girls, who typically consumed a morning EO, with results showing that regularly consuming a morning EO was beneficial on body composition outcomes. This was irrespective of amount of morning dietary protein consumed and is consistent with other results from prospective studies in children and adolescents suggesting a possible protective role of breakfast consumption or other behaviors associated with breakfast consumption in preventing excess adiposity.[255]

These analyses also evaluated the effects of total dietary protein on cardiometabolic outcomes. Our results showed that higher daily protein intake was associated with lower DBP, but not lower SBP in adolescents. These results were partially attenuated when body fat was added to the models, suggesting that the beneficial effects of dietary protein on body composition may explain some of the subsequent effects on blood pressure. Since some forms of dietary protein (e.g. foods with higher amounts of leucine) have been shown to have beneficial effects on blood pressure, it is possible that the weaker effects seen in the current study are a result of the broad mix of protein-source foods. Our results add to the limited inconsistent data evaluating the effects of dietary protein and blood pressure in children, suggesting part of the inconsistencies could be due to researchers sometimes including measures of obesity (e.g. body fat) in the statistical models.[85]

The effects of dietary protein on glucose and HOMA-IR were also investigated. Consistent with the findings of a recent systematic review

that compared increased-protein diets versus standard-protein diets in overweight children and adolescents,[267] we did not see a significant difference in glucose or HOMA-IR between girls with a higher versus lower protein intake. Although, once body fat was added to the model, results suggested dietary protein was detrimental to blood glucose. These results are in line with the adult epidemiological literature, which have shown an association between dietary protein intake and an increased risk of type 2 diabetes,[95,268,269] but differ from the adult randomized clinical trial data, which often demonstrate a benefit of dietary protein on fasting glucose.[270-272] Overall, these results demonstrates a need to further evaluate the effects of dietary protein on glucose metabolism both with and without adjustment for adiposity.

Lipids was the final cardiovascular risk factor evaluated. Although, there is evidence that dietary protein may exert lipid lowering properties through a variety of mechanisms including increasing hepatic bile acid synthesis and decreasing hepatic *de novo* cholesterol biosynthesis,[109] we found no significant trends in our adolescent population. Our results were

similar with several randomized clinical trials in adolescents showing no significant difference of dietary protein intake on lipids.[273-275]

These analyses also investigated the effects of morning protein intake on cardiometabolic outcomes. Despite some speculation that morning protein intake may reduce glucose and insulin responses by displacing glucose-generating carbohydrates from the meal[276] or may positively influence cardiometabolic outcomes by increasing nutrient dense foods in the diet, thus improving overall diet quality,[118] we found no evidence to support this idea. Lack of support for this idea included results, where we found no significant differences in cardiometabolic outcomes between girls frequently consuming a low (<15 grams of protein) compared to a high protein (≥ 15 gram of protein) morning EO.

Several proposed mechanisms could explain the beneficial effects of dietary protein on body composition. This includes evidence linking dietary protein to the release of anorexigenic gut peptides such as cholecystokinin and peptide YY. Furthermore, data shows that dietary

protein can inhibit anabolic neuronal signaling (decreasing neuropeptide Y mRNA levels) and activate catabolic signaling (pro-opiomelanocortin neurons producing α-melanocyte-stimulating hormone) in the hypothalamus via a phosphorylated mammalian target of rapamycin (mTOR) and phosphorylated AMP-activated protein kinase-dependent mechanism, leading to increase satiety and a reduction in food intake.[43] Protein intake may also diminish brain reward mechanisms, thus decreasing hunger via acute effects on satiety and food intake, and ultimately energy balance.[44] Finally, consuming dietary protein increases MPS via mTOR signaling and decreases muscle breakdown, thus improving overall muscle mass.[67]

A majority of the mechanistic data supporting the benefits of morning protein consumption on skeletal mass comes from studies in adults which have demonstrated a protein threshold, particularly leucine, is needed for achieving maximal mTOR signaling and MPS.[162] In contrast to adults, animal studies in neonates suggest that both insulin and leucine demonstrate a dose-response of mTORC1 signaling and protein synthesis,

with an additive effect when consumed together.[166] This ability of rapidly rising circulating leucine and insulin to activate protein translation decreases with development,[163] with only leucine having the capability to produce a postprandial initiation signal and anabolic response in adulthood.[277] This could mean that children do not need to consume a certain amount of protein at breakfast, in order to achieve optimal growth; total protein intake may be more important to skeletal mass outcomes.

The mechanistic data supporting the benefits of higher morning protein intake on satiety and adiposity is extremely limited. As previously mentioned, a higher protein (35 grams of protein) breakfast compared to either a normal protein (13 grams of protein) breakfast or breakfast skipping has been associated with improved satiety, greater reductions in food motivation/reward areas of the brain, and a reduction in subsequent meal intake.[137,138] Specifically reaching a certain protein threshold of higher protein intake (e.g. 30 grams) has been suggested to elicit these favorable effects. A recent review paper comparing isocaloric meals suggested that 30 grams of dietary protein elicited greater perceived post-prandial two

hour fullness compared to the 15 grams to 25 grams of dietary protein.[57] Further, several studies have utilized the idea of targeting a minimum of 30 grams of protein at each meal and have found greater weight and fat loss compared to the control group, although it is difficult to determine if some of the benefits were due to the protein distribution (and higher protein intake) or the simultaneous reduction in carbohydrates.[278-280] Our data suggests that the increase in total protein consumption is actually driving these favorable results on fat mass, and not just morning protein intake. Although, it is important to note, that our results could also potentially be due to the limited intake of morning protein among the girls.

Research supports a role of physical activity influencing protein metabolism, which could explain the benefits we observed in body composition outcomes when higher amounts of protein were combined with higher amounts of physical activity. The underlying basis for an anabolic state from both dietary protein ingestion and physical activity is MPS stimulation via activation of mTOR.[151] Thus, combining physical

activity with dietary protein results in an additive effects on MPS, reducing the amount of protein needed to achieve MPS, leading to an increase in net protein balance.[152,216,281] Since this effect last for at least 24 hours, a recent review suggested that consuming adequate dietary protein may be more important than protein timing of a factor on the long-term synergistic effects of protein and exercise.[282] This could possibly explain why a stronger affect was observed on SMM when higher total protein was combined with higher amounts of physical activity compared to when higher amounts of morning protein was combined with higher amounts of physical activity. Finally, it is important to note that this increase in SMM, could have resulted in an increase in energy expenditure, thus leading to the improvement in the body fat outcomes.[54]

These analyses were subject to a number of limitations. Common to other epidemiologic studies, our dietary data was obtained by self-report and subject to potential error. Children and adolescents in particular have trouble accurately reporting dietary intake due to difficulties estimating portion sizes and being unfamiliar with recipes and preparation methods.

Another limitation was the use of 15 grams of protein as the cut-point for high protein in the morning because too few girls regularly consumed higher than this amount, which is lower than the amount proposed in the literature for creating metabolic benefits.[57,162] In addition, too few girls ate multiple morning EOs, so we were unable to investigate if the outcomes differed when protein was consumed at one morning EO compared to if protein was consumed at multiple EOs. Finally, quite a few girls were missing glucose, HOMA-IR, and lipid measurements, so we had a reduced number of girls for these outcomes.

A number of strengths balanced out the limitations. Importantly, we were able to use multiple years of food records. This allowed us to evaluate if the acute studies of morning protein intake translated into long-term outcomes in adolescents. Furthermore, we were able to take an average of dietary intake from ages 9 to <17, which reduced the variability around unusual dietary intakes. In addition, we were able to account for typical morning eating patterns in the analyses, by categorizing girls by usual morning eating patterns (e.g. typically skips morning EOs, intermittently

eats morning EOs, etc.). Finally, there were repeated assessments of adiposity and cardiometabolic outcomes, as well as potential confounders.

2.5 CONCLUSION

These results suggest that total protein is associated with lower levels of adiposity and higher levels of SMM during later adolescence. Furthermore, morning protein intake appears beneficial on these outcomes as a result of being associated with a higher total protein intake. Given the high prevalence of obesity in the adolescent population, future dietary recommendations should take into account the beneficial effects of dietary protein on body composition in adolescents.

The effects of dietary protein on cardiometabolic outcomes appear to be inconsistent. Part of the inconsistencies depends whether measures of obesity (e.g. body fat) are added to the statistical models. This highlights the importance of future research presenting this data both with and without measures of obesity in the models and could explain some of the inconsistencies of the effects of dietary protein on cardiometabolic

outcomes in the literature. Furthermore, research should also consider the effects of specific foods on cardiometabolic effects, as protein-based foods (e.g. milk, yogurt, processed meats, and eggs) can have widely divergent effects on cardiometabolic outcomes. Thus, future research should evaluate the effects of these protein-based foods on these outcomes.

CHAPTER 3: EFFECTS OF EGG INTAKE ON BODY COMPOSITION AND CARDIOMETABOLIC OUTCOMES IN ADOLESCENTS

3.1 BACKGROUND

The childhood obesity epidemic continues to be a major public health problem in the United States, with currently 17% of youth classified as obese.[8] In comparing obese children with those of normal weight, it has been found that elevated body mass index (BMI) is associated with higher blood pressure, lipids, and fasting glucose.[7] Since evidence suggests that early cardiovascular risk parameters (including BMI) track into adulthood, there is a need to identify the determinants of early cardiometabolic risk factors in younger populations.[16,17] Poor diet is one such factor linked to excess adiposity and abnormal metabolic outcomes in children and adolescents.[6]

Research investigating the effects of dietary protein consumption on obesity and body composition in children is sparse. In some studies, higher dietary protein intake during infancy has been associated with a

higher BMI in childhood,[70] although the mechanism of such an effect is unclear.[283] In children five years of age and older, there is some evidence that higher protein intake may be associated with a smaller waist circumference,[78] although data linking dietary protein with body fat and fat free mass is generally mixed.[76,77,284]

Due to the protein content, researchers have suggested that eggs may regulate body weight by increasing satiety and reducing food intake.[207,208,285] Furthermore, egg protein appears to stimulate muscle protein synthesis (MPS) in a dose-dependent manner, potentially resulting in greater lean muscle mass and higher resting energy expenditure.[216,286] However, questions remain whether the short-term effects of egg consumption on satiety and MPS translate into long-term outcomes, such as changes in body fat and skeletal muscle mass, particularly in children. A randomized clinical trial in adults linked morning egg consumption (versus bagel consumption) to weight loss when consuming an energy-restricted diet,[212] while the limited number of prospective cohort studies has found no association between egg intake and two year weight gain in

adults or three year weight gain in children.[203,204] In adults, however, there is some evidence that an increase in egg intake over the course of four years along with a decrease in glycemic load is associated with weight loss.[205] This suggests dietary quality may impact the effects of egg-related eating patterns on body weight and body fat outcomes.

There are essentially no longitudinal studies examining the effects of egg intake on cardiometabolic outcomes among adolescents. In adults, egg intake may directly impact cardiometabolic risk factors such as impaired glucose metabolism although data are not conclusive. While a recent meta-analysis of prospective studies found no association of egg intake with risk for diabetes in the overall population, the authors noted a higher diabetes risk with ≥ 3 eggs per week in studies from the United States, while studies from outside the United States (e.g. Europe and Japan) had a 15% nonsignificant lower risk of diabetes.[228] Randomized clinical trials including those with individuals with type 2 diabetes have found no detrimental effects of egg consumption on fasting glucose.[219,230] Since few epidemiological studies have adjusted for dietary confounders potentially

leading to these conflicting results,[221] there is a need for more carefully designed longitudinal studies to understand the independent impact of eggs on glucose metabolism.

Other cardiometabolic outcomes that are potentially impacted by egg consumption include blood pressure and lipid metabolism. For instance, while animal studies suggest that a number of egg-derived peptides exhibit angiotensin I converting enzyme inhibitory activity,[287] short-term clinical studies in adults have not supported the potential blood pressure lowering properties of eggs.[217,218] Interestingly, in one of the few studies that have examined the cardiometabolic effects of eggs in younger populations, it was found that children eating two eggs daily for 30 days lowered their diastolic blood pressure, maintained their LDL to HDL ratio, and generated less atherogenic LDL-C.[288]

Overall, there is a need to evaluate the effects of egg intake on cardiometabolic outcomes (e.g. glucose, blood pressure, and lipids) particularly in a younger population. Furthermore, eggs should be

examined in the context of a healthy diet since egg intake is often associated with higher intakes of saturated fat and low intakes of dietary fiber, which can be detrimental to cardiovascular health.[289] Given the evidence, we hypothesized that egg consumption in early adolescence, particularly as part of a healthy eating pattern, will be associated with beneficial effects on body composition and no adverse effects on cardiometabolic outcomes in late adolescence. Therefore, the objective of this study was to estimate the effects of usual egg intake, alone and in combination with healthier patterns of diet and physical activity, on late adolescent body composition and cardiometabolic outcomes.

3.2 METHODS

3.2.1 Study Population

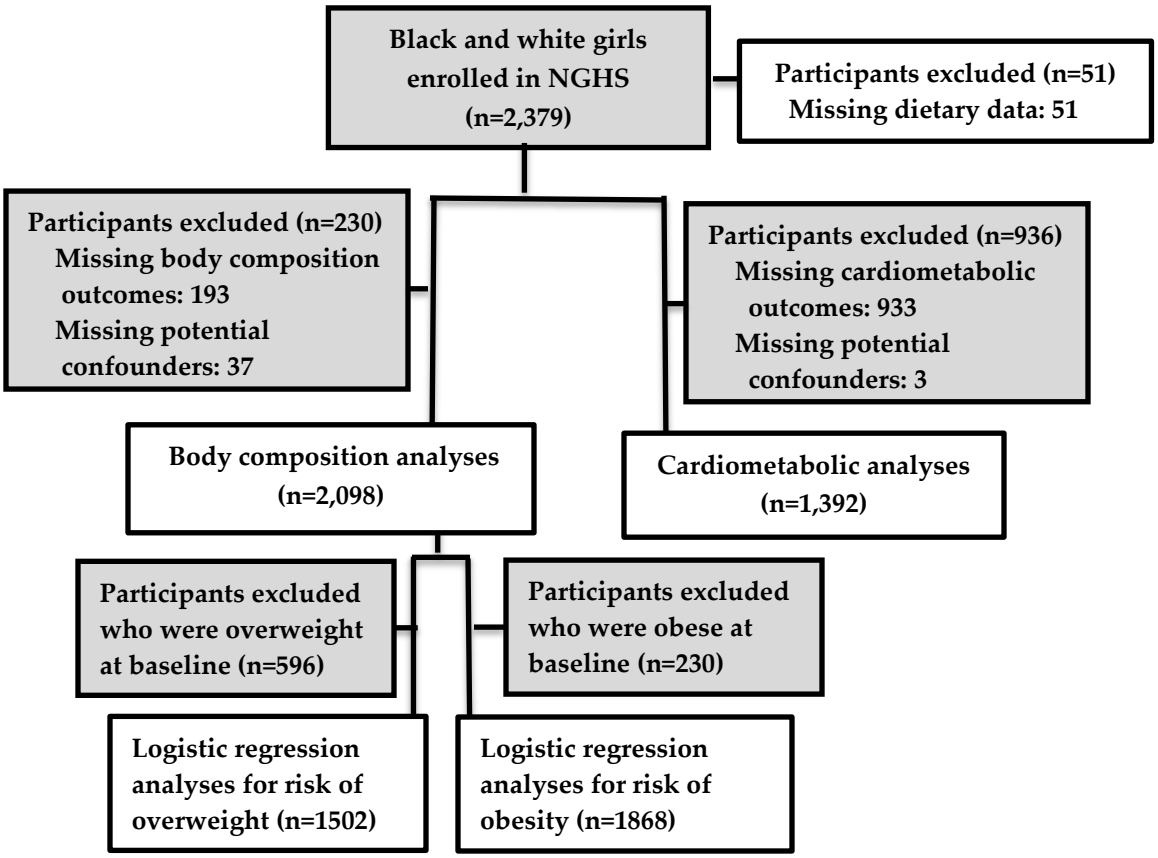
These analyses included data from 2,379 black and white girls enrolled in the National Heart, Lung, and Blood Institute's Growth and Health Study (NGHS). Participants were 9-10 years of age at enrollment and were followed annually for 10 years (until 19-20 years of age). They were recruited from three clinical centers (the University of Cincinnati

Children's Hospital Medical Center, the University of California at Berkley, and Westat Inc./Group Health Association in Rockville, MD) in order to obtain equal numbers of black and white girls from a representative sample of urban and suburban families. Participants were assessed annually for biracial differences in the development of obesity and other cardiometabolic risk (CMR) factors.

The selection for the current sample is shown in Figure 3.1. For these analyses, girls missing dietary data were excluded (n=51). For two girls missing dietary data before the age of 17, we substituted data at ages 17.1 and 17.3. Further exclusions were specific to the outcomes being examined. For the body fat analyses, girls with missing adiposity outcomes or potential confounders of interest for these outcomes (e.g., height, physical activity, television/video watching) were excluded (n=230), yielding a sample of 2098 girls. Additionally, girls who were overweight at baseline, defined as having a BMI at or greater than the 85th percentile of girls the same age from the Centers for Disease Control (CDC) growth charts, were excluded (n = 596) from the analyses

examining the risk becoming overweight during adolescence, while those at or greater than the 95th percentile of girls the same age from the CDC growth charts were excluded (n = 290) from analyses estimating the risk of developing obesity.[290] For the cardiometabolic analyses, girls with missing data for any of the cardiometabolic outcomes or potential confounders of interest were excluded (n=936), yielding a sample of 1392 girls.

Figure 3.1. Flow Diagram of Selection of Study Sample



3.2.2 Dietary Data

Diet was assessed by three day food records (two weekdays and one weekend day) collected during eight of the 10 exam years (study years 1-5, 7, 8, and 10). Girls were instructed by a study dietitian on the use of standard household measures to estimate portion sizes. If needed, girls obtained additional information on recipes, brands, and other details from a parent. Upon completion of a food record, a study dietitian reviewed the dietary data to determine reliability based on standard rules. Records deemed reliable were entered into the Nutrition Data System (NDS) of the University of Minnesota using standardized protocols.[257] Since NDS is updated regularly to reflect changes in food composition, as well as foods currently in the marketplace, the nutrients were derived using the NDS nutrient database appropriate to the year of the data collection.

Researchers from Boston University linked the food codes from NDS to those in the United States Department of Agriculture's (USDA) "Pyramid Serving Database for USDA Survey Food Codes, Version 2".[258] This allowed the determination of the participant's dietary intake for eggs and other foods. Eggs and other foods in this data set include whole foods, composite foods, and those

derived from mixed dishes. For example, total egg intake includes whole eggs, eggs in a mixed dish (e.g., quiche), and eggs in baked goods. Since eggs are a common food ingredient, only 7 girls had no egg intake. All dietary data collected between the ages of 9 and 17 were averaged to enhance precision of estimated dietary intake.

3.2.3 Measurement of Body Composition and CMR Factors

All outcome data were collected at the end of follow-up (ages 17-20 years). Total body fat was assessed with a bioelectrical impedance analyzer (BIA), using race-specific equations to estimate percent fat mass due to racial differences in bone content and length.[259] These race-specific equations were developed and validated in an NGHS ancillary study using dual-energy X-ray absorptiometry as the gold standard.[259]

Black girls race-specific LBM equation = -8.78 + 0.78 * (height^2/resistance) + 0.1 * reactance + 0.18 * weight

White girls race-specific LBM equation = 1.07 + 0.37 * (height^2/resistance) + -0.17 * (triceps skinfold) + 0.47 * weight

Skeletal muscle mass (SMM) in kilograms (kg) was calculated using the BIA-based equation of Janssen et al:

SMM (kg) = [height (centimeters)^2 /BIA-resist (ohm) * 0.401) + (sex * 3.825)+(age * -0.071)] + 5.102

where sex= 0, since this sample includes only girls.

The Janssen equations was developed and cross-validated against whole body SMM determined by magnetic resonance imaging in a sample of 388 men and women varying in age (18-86), BMI [16-48 weight in kilograms divided by height in meters squared (kg/m^2)], and race (including blacks and whites).[260] Skinfold measurements at the subscapular and suprailiac sites were used to determine truncal fat using Cameron's method (percent of total fat from truncal fat).[261] Waist circumference was measured in duplicate at the narrowest part of the torso between the ribs and iliac crest.

Height and weight were also measured in duplicate. Height was assessed with a portable stadiometer, while girls were weighed on a Health-o-meter electronic scale in a large t-shirt or a hospital gown. If the two height measures differed by

more than 0.5 centimeters or if the two weight measures differed by more than 0.3 kilograms, then a third measurement was taken. BMI was calculated as kg/m^2.

Cardiometabolic factors considered included blood pressure, fasting glucose, lipids [total cholesterol, low-density lipoprotein cholesterol (LDL-C), LDL-C to high-density lipoprotein cholesterol (LDL: HDL ratio), non-HDL-C, triglycerides], and insulin resistance. Blood pressure was assessed with a V-Lok Cuff mercury sphygmomanometer (Baum Desktop Model) using a standardized protocol, with three measurements taken with a 30-second rest between each. Fasting lipids and glucose were analyzed at a Johns Hopkins laboratory that was a part of the Centers for Disease Control and Prevention's Lipid Standardization Program.[262] Total cholesterol and HDL-C were assessed with the Cholesterol CHOD-PAP method (Boehringer-Mannheim Diagnostics), while triglycerides were ascertained enzymatically (Abbott A-Gent Triglycerides Reagent Set). LDL-C was determined using a modified Friedewald equation: [LDL-C] = [total cholesterol] – [HDL-C] – [triglycerides]/6.5.[263] Both LDL-C and HDL-C were used to calculate the LDL to HDL ratio, which some have suggested is a better predictor of cardiovascular disease risk than LDL-C alone.[291] Non-HDL-C, an

aggregate measure of all the atherogenic particles (very low-density lipoprotein, intermediate-density lipoprotein, chylomicron remnants, lipoprotein (a), and LDL-C),[292] was estimated as the difference between total cholesterol and HDL-C. Finally, homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin resistance.[264]

3.2.4 Assessment of Potential Confounders

A number of potential confounders were explored in these analyses including race (self-described as black or white), socioeconomic status (classified as low, moderate, or high) age, physical activity, television/video watching, height, BMI, waist circumference, percent of energy from carbohydrates, and other dietary factors. Physical activity and television/video watching was self-reported; the validated Health Activity Questionnaire was used to assess usual physical activity,[265] while television/video watching was estimated by means of a questionnaire in which girls reported the usual number of hours watched each day. Final analyses included factors that led to confounding as measured by a ≥ 5% change in estimated effects. For the body composition analyses, the final models included race, percent energy from carbohydrates, and baseline height, while the final models for the cardiometabolic analyses included race, age (at

time of diet assessment), baseline height, and percent of energy from carbohydrates.

3.2.5 Statistical Analysis

All cut-points were determined through sensitivity analyses to reflect the distributions of the data while optimizing analytic power. For analyses related to the various body composition measures, mean egg consumption during early to mid-adolescence (ages 9-<17) was categorized as follows: <0.5 eggs per week (n=271), 0.5-<3.5 eggs per week (n=1474), and ≥3.5 eggs per week (n=353). For the analyses examining risk of becoming overweight or obese in late adolescence, egg intake was dichotomized using cutoff values of <0.5 versus ≥ 0.5 eggs per week. For analyses of cardiometabolic outcomes, the categories of mean egg intake from ages 9-<17 were <1.0 egg per week (n=361), 1.0-3.0 eggs per week (n=703), and ≥ 3 eggs per week (n=328). Finally, when considering cardiometabolic effects within a healthy pattern, egg intake was dichotomized using the cut-off values of <3 versus ≥3 eggs per week.

Healthy patterns were characterized by the intake of selected foods and nutrients considered to be markers of a healthy diet (fiber, dairy, fruits, and vegetables).

The effects of egg consumption within a healthy dietary pattern on both body composition and cardiometabolic outcomes were measured by combining the dichotomized egg categories described above and the following dichotomized healthy diet indicators: fiber, < 12 versus ≥ 12 grams per day; dairy, <2 versus ≥2 cups per day; fruit and vegetables, <1.75 versus ≥ 1.75 cup equivalents per day.

The effects of egg consumption on body composition and cardiometabolic outcomes within patterns of usual physical activity were also estimated. For these analyses, egg intake was dichotomized as described above and then combined with levels of physical activity dichotomized as <20 versus ≥ 20 metabolic equivalent score (METS) per week.

Analysis of covariance models were used to calculate adjusted mean body fat, truncal fat, waist circumference, BMI, SMM, and cardiometabolic outcomes (e.g. fasting glucose, HOMA-IR, blood pressure, and lipids) in later adolescence (17-20 years of age). Multiple logistic regression analyses were used to estimate adjusted relative risk of being overweight at the end of follow-up (17-20 years of age) using the BMI cutoff of ≥ 25 kg/m^2. All analyses were completed using Statistical Analysis Systems software, version 9.4 (SAS Institute, Cary, NC).

3.3 RESULTS

Table 3.1 shows the characteristics of the girls according to their usual intake of eggs between 9 and 17 years of age. Girls who consumed more eggs per week were slightly older and watched more television than girls in the other groups. Their diets were characterized by higher intakes of energy, protein, and fat. They also had lower intakes of carbohydrates and energy-adjusted fiber compared to girls consuming the fewest eggs per week.

Table 3.1. Characteristics of girls in the National Growth and Health Study according to egg consumption

	Weekly number of eggs consumed			
	0 to <0.5	**0.5 to <3.5**	**≥3.5**	
	n= 271	n=1474	n=353	
	Mean (SD)	*Mean (SD)*	*Mean (SD)*	*P*-value
Baseline characteristics				
Age (yrs)	10.0 ± 0.5	10.0 ± 0.6	10.1 ± 0.6	*0.0484*
Height (cm)	141.2 ± 7.5	141.0 ± 7.5	142.9 ± 8.4	*0.0002*
Body mass index (kg/m^2)	18.7 ± 3.8	18.4 ± 3.7	18.7 ± 3.9	*0.2475*
Age at menarche (years)	12.3 ± 1.3	12.4 ± 1.2	12.1 ± 1.2	*0.0023*
Activity score (METS)[1]	20.0 ± 10.1	19.8 ± 9.9	19.8 ± 11.1	*0.9418*
Television/video (hrs/day)[1]	4.5 ± 2.0	4.5 ± 2.1	5.1 ± 2.0	*<0.0001*
Mean dietary intakes[1]				
Energy intake (kcals/day)	1701.8 ± 332.0	1870.6 ± 350.6	2057.2 ± 402.0	*<0.0001*
% energy from protein	14.1 ± 2.0	14.0 ± 1.8	14.5 ± 1.8	*<0.0001*
% energy from carbohydrate	53.3 ± 5.1	52.0 ± 4.7	49.2 ± 4.4	*<0.0001*
% energy from fat	33.6 ± 4.0	35.0 ± 3.8	37.2 ± 3.6	*<0.0001*
% energy from saturated fat	12.4 ± 1.8	12.9 ± 1.6	13.4 ± 1.6	*<0.0001*
Dairy (cup equivalents/day)	1.7 ± 0.8	1.7 ± 0.7	1.7 ± 0.7	*0.4068*
Fiber (grams/day)	10.5 ± 3.1	11.5 ± 3.0	12.1 ± 3.3	*<0.0001*
Fiber (grams/1,000 kcals)	6.2 ± 1.4	6.2 ± 1.4	5.9 ± 1.2	*0.0014*

FV (cup eq/day)	3.1 ± 1.2	3.4 ± 1.2	3.7 ± 1.3	*<0.0001*
Race (%)				
White	56.8	50.9	19.8	*<0.0001*
Black	43.2	49.1	70.2	
Socioeconomic status (%)				
Low	19.2	22.2	28.6	*0.0269*
Mid	46.9	42.3	41.6	
High	33.9	35.5	29.8	

[1]Mean values from ages 9-<17 years

Table 3.2, examines the association between mean egg intake at 9-<17 years of age and mean percent body fat, percent truncal fat, waist circumference, BMI, and percent SMM at the end of adolescence. Results suggest higher egg intake has beneficial effects on these outcomes. In particular, consuming ≥ 3.5 eggs per week was associated with a lower percent body fat compared with to those consuming the fewest eggs (<0.5 eggs per week) (p=0.0190). Individuals with a higher egg intake also had a tendency to have lower truncal fat compared with those in the lowest egg intake category (p=0.0836). Lastly, SMM increased from 33.0% to 34.0% with increasing intake of eggs (p=0.0262).

Table 3.2. Adiposity and SMM outcomes at 17-20 years of age according to average weekly egg intake

Egg intake/wk, age 9-17 yrs	*n*	**Body fat (%)** *Mean ± SE*[1]	**Truncal fat (%)** *Mean ± SE*[1]	**WC (cm)** *Mean ± SE*[1]	**BMI (kg/m²)** *Mean ± SE*[1]	**SMM (%)** *Mean ± SE*[1]
<0.5	271	31.8 ± 0.40	34.3 ± 1.16	76.4 ± 0.72	25.4 ± 0.37	33.0 ± 0.30
0.5 to <3.5	1474	31.1 ± 0.17	33.3 ± 0.49	75.7 ± 0.31	25.0 ± 0.16	33.7 ± 0.13
≥ 3.5	353	30.6 ± 0.36	31.6 ± 1.03	75.3 ± 0.64	24.7 ± 0.33	34.0 ± 0.27
P-trend		*0.0190*	*0.0836*	*0.2750*	*0.2165*	*0.0262*

[1]Adjusted for race, % energy from carbohydrates, and baseline height.

Table 3.3 explores whether the effects of egg consumption on these anthropometric outcomes were modified by a healthy lifestyle, by cross-classifying egg intake individually with other foods and nutrients (dairy, fiber, fruits and vegetables), and physical activity. In these analyses, girls with a higher egg intake or a healthier lifestyle had lower levels of body fat, a smaller waist circumference, and a lower BMI. However, the lowest percent total body fat, percent truncal fat, waist circumference, and BMI as well as the highest percent SMM was generally found among girls who had *both* higher egg intakes and a healthier lifestyle.

Table 3.3. Effects of egg intake as a part of healthy patterns on mean adiposity and SMM outcomes at 17-20 years of age[1]

Eating pattern		**Body fat (%)**		**Truncal fat (%)**		**WC (cm)**		**BMI (kg/m²)**		**SMM (%)**	
(age 9-17 yrs)	*n*	*Mean ± SE*	*P-value*	*Mean ± SE*	*P-value*	*Mean ± SE*	*P-value*	*Mean ± SE*	*P-value*	*Mean ± SE*	*P-value*
Eggs/Fiber											
Lower/Lower	197	32.3 ± 0.46	-	35.2 ± 1.35	-	77.0 ± 0.84	-	25.7 ± 0.43	-	32.6 ± 0.35	-
Moderate/Lower	928	31.5 ± 0.21	0.0946	34.3 ± 0.62	0.5276	76.0 ± 0.39	0.2566	25.2 ± 0.20	0.3330	33.5 ± 0.16	0.0325
Higher/Lower	179	31.4 ± 0.49	0.1649	34.0 ± 1.44	0.5409	76.7 ± 0.90	0.7675	25.6 ± 0.46	0.8519	33.3 ± 0.38	0.1726
Lower/Higher	74	30.4 ± 0.76	0.0268	31.4 ± 2.21	0.1317	74.5 ± 1.38	0.1128	24.4 ± 0.71	0.1360	34.2 ± 0.58	0.0165
Moderate/Higher	546	30.5 ± 0.28	0.0005	31.5 ± 0.83	0.0175	75.2 ± 0.51	0.0544	24.7 ± 0.27	0.0456	34.1 ± 0.22	0.0003
Higher/Higher	174	29.8 ± 0.50	0.0003	29.5 ± 1.44	0.0039	74.0 ± 0.90	0.0150	24.0 ± 0.46	0.0079	34.5 ± 0.38	0.0002
Eggs/Dairy											
Lower/Lower	199	32.0 ± 0.46	-	33.9 ± 1.35	-	76.2 ± 0.84	-	25.3 ± 0.43	-	32.9 ± 0.35	-
Moderate/Lower	1027	31.3 ± 0.20	0.1691	33.7 ± 0.60	0.8993	75.7 ± 0.37	0.5835	25.1 ± 0.19	0.6104	33.5 ± 0.16	0.1221
Higher/Lower	254	31.0 ± 0.42	0.0977	32.5 ± 1.22	0.4433	75.8 ± 0.76	0.7268	25.0 ± 0.39	0.5717	33.7 ± 0.32	0.1216
Lower/Higher	72	31.3 ± 0.77	0.4395	35.3 ± 2.24	0.5929	76.8 ± 1.40	0.7168	25.4 ± 0.72	0.9036	33.3 ± 0.59	0.6002
Moderate/Higher	447	30.6 ± 0.32	0.0144	32.3 ± 0.93	0.3334	75.6 ± 0.58	0.5481	24.8 ± 0.30	0.3494	34.0 ± 0.24	0.0114
Higher/Higher	99	29.6 ± 0.66	0.0034	29.6 ± 1.91	0.0697	74.0 ± 1.19	0.1298	24.1 ± 0.61	0.1079	34.6 ± 0.50	0.0068
Eggs/FV											
Lower/Lower	156	32.6 ± 0.52	-	36.3 ± 1.51	-	77.6 ± 0.94	-	26.1 ± 0.49	-	32.4 ± 0.40	-
Moderate/Lower	669	31.6 ± 0.25	0.0738	34.9 ± 0.74	0.3983	76.2 ± 0.46	0.1948	25.3 ± 0.24	0.1884	33.4 ± 0.19	0.0292
Higher/Lower	136	31.4 ± 0.56	0.1134	33.2 ± 1.64	0.1716	76.3 ± 1.02	0.3691	25.5 ± 0.53	0.4106	33.4 ± 0.43	0.0955
Lower/Higher	115	30.7 ± 0.61	0.0144	31.4 ± 1.77	0.0346	74.7 ± 1.10	0.0502	24.4 ± 0.57	0.0251	33.9 ± 0.46	0.0115
Moderate/Higher	805	30.7 ± 0.23	0.0006	32.0 ± 0.68	0.0089	75.3 ± 0.42	0.0258	24.7 ± 0.22	0.0135	34.0 ± 0.18	0.0003
Higher/Higher	217	30.1 ± 0.45	0.0002	30.9 ± 1.30	0.0069	74.8 ± 0.81	0.0254	24.3 ± 0.42	0.0079	34.3 ± 0.34	0.0003
Eggs/PA											
Lower/Lower	149	32.5 ± 0.53	-	36.2 ± 1.55	-	77.4 ± 0.96	-	25.8 ± 0.50	-	32.5 ± 0.40	-
Moderate/Lower	846	31.5 ± 0.22	0.0711	34.6 ± 0.65	0.3387	76.2 ± 0.40	0.2354	25.2 ± 0.21	0.2592	33.5 ± 0.17	0.0351
Higher/Lower	208	30.9 ± 0.46	0.0178	32.5 ± 1.34	0.0748	75.6 ± 0.84	0.1528	24.8 ± 0.43	0.1151	33.8 ± 0.35	0.0175
Lower/Higher	122	30.9 ± 0.59	0.0436	31.8 ± 1.72	0.0578	75.1 ± 1.08	0.1025	24.8 ± 0.56	0.1771	33.7 ± 0.45	0.0597
Moderate/Higher	628	30.6 ± 0.26	0.0011	31.5 ± 0.76	0.0070	75.0 ± 0.48	0.0266	24.8 ± 0.25	0.0629	34.0 ± 0.20	0.0012

Higher/Higher	145	30.3 ± 0.54	0.0029	30.8 ± 1.58	0.0147	75.1 ± 0.98	0.0897	24.8 ± 0.51	0.1450	34.1 ± 0.41	0.0065

[1] Lower egg intake = <0.5 per week, moderate egg intake = 0.5 to <3.5 eggs per week, higher egg intake = ≥ 3.5 eggs per week; Lower vs. higher fiber = <12 vs. ≥12 grams/day; Lower vs. higher dairy = <2 vs. ≥2 servings/day; Lower vs. higher fruit and vegetable = <1.75 vs. ≥1.75 cup equivalents/day; Lower vs. higher physical activity = <20 METS vs. ≥ 20 METS per week.
[2]Adjusted for race, % energy from carbohydrates, and baseline height.

The results of the logistic regression analyses examining the effects of egg intake on the risk of becoming overweight or obese in late adolescence are shown in table 3.4. After adjusting for race, percent of energy from carbohydrates, and baseline height, girls who consumed 0.5 or more eggs per week had a non-statistically significant 30% reduction in risk of becoming overweight and a 21% reduced risk of being obese by late adolescence.

Table 3.4. Effect of egg consumption on risk of overweight and obesity at 17-20 years of age

Egg intake/week, age 9-17 yrs	**Overweight (≥ 25 kg/m²)**			**Obesity (≥ 30 kg/m²)**		
	n	*Cases*	*RR (95% CI)*[1]	*n*	*Cases*	*RR (95% CI)*[1]
<0.5	200	38	1.00	242	18	1.00
≥ 0.5	1302	203	0.70 (0.47-1.04)	1566	106	0.79 (0.47 – 1.33)

[1]Adjusted for race, % energy from carbohydrates, and baseline height.

Table 3.5 shows the results of the multiple logistic regression analyses examining the effects of egg consumption combined with indicators of healthy dietary and activity patterns on risk of being overweight in late adolescence. Overall, healthier patterns that included egg intake were linked with a lower risk of

becoming overweight. In particular, participants who consumed more fiber or fruits and vegetables or who were more physically active, and who also consumed eggs had a statistically significant 43-58% reduced risk of overweight at the end of adolescence.

Table 3.5. Effect of healthy patterns on risk of overweight in girls ≥ 17 years of age

	Overweight (BMI ≥ 25 kg/m²)		
Eating pattern (9-17 yrs)[1]	*n*	*Cases*	*RR (95% CI)*[2]
Eggs/Fiber			
Lower/Lower	139	29	1.00
Lower/Higher	61	9	0.76 (0.33-1.73)
Higher/Lower	765	137	0.75 (0.47-1.18)
Higher/Higher	537	66	0.53 (0.32-0.86)
Eggs/Dairy			
Lower/Lower	143	27	1.00
Lower/Higher	57	11	1.18 (0.53-2.61)
Higher/Lower	879	137	0.69 (0.44-1.10)
Higher/Higher	423	66	0.83 (0.50-1.38)
Eggs/FV			
Lower/Lower	113	28	1.00
Lower/Higher	87	10	0.44 (0.20-0.97)
Higher/Lower	558	109	0.68 (0.42-1.10)
Higher/Higher	744	94	0.42 (0.26-0.69)
Eggs/PA			
Lower/Lower	100	24	1.00
Lower/Higher	100	14	0.60 (0.29-1.26)
Higher/Lower	724	118	0.56 (0.34-0.93)
Higher/Higher	578	85	0.57 (0.34-0.96)

[1] Lower vs. higher egg intake = <0.5 per week vs. ≥ 0.5 eggs per week; Lower vs. higher fiber intake = <12 vs. ≥12 grams per day; Lower vs. higher dairy intake = <2 vs. ≥2 servings per day; Lower vs. higher fruit and vegetable intake = <1.75 vs. ≥1.75 cup equivalents per day; Lower vs. higher physical activity = <20 METS per week vs. ≥20 METS per week.
[2]Adjusted for race, % energy from carbohydrates, and baseline height.

Beginning in table 3.6, we explored the effects of usual egg intake on cardiometabolic risk. The smaller sample size for these analyses necessitated a change to the referent group (from <0.5 egg/week to <1.0 egg/week) and higher egg consumers group (from ≥3.5 eggs/week to 3.0 eggs/week) to derive more stable estimates of effect. Girls who consumed less than one egg per week were less likely to watch television and tended to report lower energy intakes. Those with an intake of three or more eggs per week had a higher percentage of energy from protein and fat as well as higher intake of fruits and vegetables. They also had lower intakes of carbohydrates and energy-adjusted fiber compared to girls consuming less than one egg per week.

Table 3.6. Characteristics of girls in the National Growth and Health Study with cardiometabolic data according to average weekly egg intake

	Weekly number of eggs consumed			
	0 to <1.0	**1.0 to <3.0**	**≥3.0**	
	n=361	n=703	n=328	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*P*-value
Baseline characteristics				
Age (years)	10.0 ± 0.6	10.0 ± 0.6	10.1 ± 0.6	*0.4989*
Height (cm)	141.0 ± 7.2	141.3 ± 7.7	143.1 ± 8.6	*0.0004*
Body mass index (kg/m^2)	18.5 ± 3.5	18.6 ± 4.0	18.9 ± 4.0	*0.3150*
Activity score (METS)[1]	20.4 ± 10.4	19.5 ± 9.5	19.2 ± 10.1	*0.2196*
Television/video (hrs/day)[1]	4.3 ± 1.9	4.5 ± 2.2	5.1 ± 2.0	*<0.0001*
Mean dietary intakes[1]				
Energy intake (kcals/day)	1751.0 ± 318.1	1886.2 ± 348.5	2051.3 ± 390.8	*<0.0001*
% energy from protein	14.0 ± 2.0	14.0 ± 1.8	14.5 ± 1.8	*<0.0001*

% energy from carbohydrate	53.3 ± 4.9	51.9 ± 4.7	49.4 ± 4.7	*<0.0001*
% energy from fat	33.7 ± 4.1	35.1 ± 3.8	37.0 ± 3.8	*<0.0001*
% energy from saturated fat	12.5 ± 1.8	12.9 ± 1.6	13.3 ± 1.6	*<0.0001*
Dairy (cup eq/day)	1.7 ± 0.8	1.7 ± 0.7	1.6 ± 0.7	*0.3763*
Fiber (grams/day)	11.0 ± 3.2	11.6 ± 3.0	12.1 ± 3.2	*<0.0001*
Fiber (grams/1,000 kcals)	6.3 ± 1.5	6.2 ± 1.3	5.9 ± 1.3	*0.0015*
FV (cup eq/day)	3.2 ± 1.2	3.4 ± 1.2	3.7 ± 1.3	*<0.0001*
Race (%)				
White	58.7%	49.2%	28.1%	*<0.0001*
Black	41.3%	50.8%	71.9%	
Socioeconomic status (%)				
Low	15.5%	20.9%	27.7%	*0.0007*
Mid	45.2%	40.5%	42.7%	
High	39.3%	38.6%	29.6%	

[1]Mean values from ages 9-<17 years

Tables 3.7 and 3.8 show the associations between egg consumption at 9-<17 years of age and mean levels of glucose, HOMA-IR, blood pressure, and lipids in late adolescence. Overall, there were no adverse effects of egg consumption on these outcomes. For total cholesterol, there was an inverse linear trend with egg intake and similar but borderline inverse effects for non-HDL and LDL-cholesterol.

Table 3.7. Glucose, HOMA-IR, and blood pressure at 17-20 years of age according to average weekly egg intake

Egg intake/wk, age 9-17 years	*n*	Glucose (mg/dL) *Mean ± SE*[1]	HOMA-IR *Mean ± SE*[1]	SBP (mmHg) *Mean ± SE*[1]	DBP (mmHg) *Mean ± SE*[1]
<1.0	361	88.0 ± 0.72	2.61 ± 0.16	108.4 ± 0.41	65.5 ± 0.41
1.0 to <3.0	703	87.6 ± 0.51	2.58 ± 0.12	109.2 ± 0.29	65.8 ± 0.29
≥ 3.0	328	88.1 ± 0.77	2.50 ± 0.17	109.1 ± 0.44	65.2 ± 0.43
P-trend		*0.9048*	*0.6671*	*0.2110*	*0.5752*

[1]Adjusted for race, age (at time of diet assessment), % energy from carbohydrates, and baseline height.

Table 3.8. Lipid outcomes at 17-20 years of age according to average weekly egg intake

Egg intake/wk, age 9-17 yrs	*n*	Total chol (mg/dL) *Mean ± SE*[1]	Non-HDL-C (mg/dL) *Mean ± SE*[1]	LDL-C (mg/dL) *Mean ± SE*[1]	LDL: HDL ratio *Mean ± SE*[1]	Log triglycerides *Mean ± SE*[1]
<1.0	361	166.4 ± 1.70	112.5 ± 1.70	99.7 ± 1.56	1.98 ± 0.04	4.31 ± 0.02
1.0 to <3.0	703	164.4 ± 1.19	111.0 ± 1.19	98.8 ± 1.10	1.95 ± 0.03	4.29 ± 0.02
≥ 3.0	328	161.3 ± 1.81	107.9 ± 1.81	95.5 ± 1.67	1.89 ± 0.04	4.29 ± 0.02
P-trend		*0.0481*	*0.0787*	*0.0775*	*0.1672*	*0.4572*

[1]Adjusted for race, age (at time of diet assessment), % energy from carbohydrates, and baseline height.

Table 3.9 examines the effects of eggs combined with indicators of healthy dietary and physical activity patterns on mean glucose, HOMA-IR, and blood pressure. After cross-classifying dichotomous egg intake with other markers of a healthy diet pattern (also dichotomized), it is apparent that there is no adverse effect of egg consumption in these girls. There appears to be a trend toward a beneficial effect of egg intake among those who consumed more fruits and vegetables or fiber on diastolic blood pressures. Finally, girls who were more

physically active who also consumed more eggs had a non-statistically significant reduction in insulin resistance.

Table 3.9. Effects of egg as a part of healthy patterns on mean glucose, HOMA-IR, and blood pressure at 17-20 years of age

Eating pattern[1]		Glucose (mg/dL)		HOMA-IR		SBP (mmHg)		DBP (mmHg)	
(age 9-17 years)	*n*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*
Eggs/Fiber									
Lower/Lower	682	87.3 ± 0.51	-	2.64 ± 0.12	-	109.1 ± 0.30	-	66.0 ± 0.29	-
Lower/Higher	382	88.7 ± 0.71	0.1182	2.50 ± 0.16	0.4744	108.7 ± 0.41	0.4389	65.0 ± 0.40	0.0439
Higher/Lower	175	88.0 ± 1.05	0.5173	2.81 ± 0.24	0.5073	109.6 ± 0.60	0.4549	65.6 ± 0.59	0.5262
Higher/Higher	153	88.1 ± 1.10	0.4907	2.17 ± 0.25	0.0888	108.6 ± 0.63	0.4575	64.8 ± 0.62	0.0649
Eggs/Dairy									
Lower/Lower	746	87.9 ± 0.50	-	2.62 ± 0.11	-	109.2 ± 0.29	-	65.7 ± 0.28	-
Lower/Higher	318	87.4 ± 0.80	0.6163	2.52 ± 0.18	0.6707	108.4 ± 0.46	0.1497	65.6 ± 0.45	0.9386
Higher/Lower	249	88.0 ± 0.89	0.8994	2.54 ± 0.20	0.7311	109.4 ± 0.51	0.7308	65.2 ± 0.50	0.3788
Higher/Higher	79	88.6 ± 1.53	0.6770	2.40 ± 0.35	0.5558	108.3 ± 0.88	0.3550	65.1 ± 0.86	0.4893
Eggs/FV									
Lower/Lower	503	87.2 ± 0.60	-	2.59 ± 0.14	-	109.0 ± 0.35	-	66.2 ± 0.34	-
Lower/Higher	561	88.2 ± 0.58	0.2361	2.59 ± 0.13	0.9855	109.0 ± 0.34	0.9972	65.2 ± 0.33	0.0384
Higher/Lower	124	87.1 ± 1.24	0.9404	2.53 ± 0.28	0.8562	108.7 ± 0.71	0.7665	65.5 ± 0.70	0.3830
Higher/Higher	204	88.7 ± 0.96	0.2073	2.48 ± 0.22	0.6712	109.3 ± 0.55	0.6035	65.0 ± 0.54	0.0638
Eggs/PA									
Lower/Lower	596	87.9 ± 0.55	-	2.71 ± 0.13	-	109.3 ± 0.32	-	65.9 ± 0.31	-
Lower/Higher	468	87.6 ± 0.64	0.6951	2.42 ± 0.15	0.1286	108.6 ± 0.37	0.1586	65.4 ± 0.36	0.3959
Higher/Lower	199	88.4 ± 0.98	0.6502	2.74 ± 0.22	0.9081	108.8 ± 0.57	0.5053	65.0 ± 0.56	0.2032
Higher/Higher	129	87.8 ± 1.19	0.9386	2.17 ± 0.27	0.0695	109.6 ± 0.68	0.6833	65.4 ± 0.67	0.5052

[1] Lower vs. higher egg intake = <3 per week vs. ≥ 3 eggs per week; Lower vs. higher fiber intake = <12 vs. ≥12 grams per day; Lower vs. higher dairy intake = <2 vs. ≥2 servings per day; Lower vs. higher fruit and vegetable intake = <1.75 vs. ≥1.75 cup equivalents per day; Lower vs. higher physical activity = <20 METS per week vs. ≥20 METS per week.[2]Adjusted for race, age (at time of diet assessment), % energy from carbohydrates, and baseline height

Table 3.10. Effects of egg as a part of healthy patterns on mean lipids at 17-20 years of age

Eating pattern[1]		Total chol (mg/dL)		Non-HDL-C (mg/dL)		LDL-C (mg/dL)		LDL: HDL ratio		Log triglycerides	
(age 9-17 yrs)	*n*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*	*Mean ± SE*[2]	*P-value*
Eggs/Fiber											
Lower/Lower	682	166.4 ± 1.21	-	112.9 ± 1.21	-	100.3 ± 1.11	-	1.99 ± 0.03	-	4.31 ± 0.02	-
Lower/Higher	382	162.4 ± 1.68	0.0498	108.7 ± 1.67	0.0416	96.7 ± 1.54	0.0606	1.90 ± 0.04	0.0852	4.27 ± 0.02	0.1343
Higher/Lower	175	162.7 ± 2.47	0.1785	109.7 ± 2.47	0.2384	97.0 ± 2.27	0.1853	1.93 ± 0.06	0.3888	4.31 ± 0.03	0.8896
Higher/Higher	153	160.3 ± 2.58	0.0322	106.5 ± 2.59	0.0241	94.3 ± 2.37	0.0217	1.85 ± 0.06	0.0525	4.28 ± 0.03	0.4373
Eggs/Dairy											
Lower/Lower	746	166.2 ± 1.17	-	112.6 ± 1.17	-	100.1 ± 1.08	-	1.97 ± 0.03	-	4.30 ± 0.02	-
Lower/Higher	318	162.0 ± 1.88	0.0611	108.7 ± 1.89	0.0876	96.5 ± 1.74	0.0786	1.92 ± 0.05	0.3937	4.30 ± 0.02	0.9845
Higher/Lower	249	162.8 ± 2.09	0.1526	109.1 ± 2.09	0.1473	96.5 ± 1.93	0.1047	1.89 ± 0.05	0.1945	4.30 ± 0.03	0.9162
Higher/Higher	79	157.8 ± 3.59	0.0265	105.1 ± 3.59	0.0503	93.0 ± 3.30	0.0413	1.87 ± 0.09	0.3048	4.29 ± 0.05	0.8618
Eggs/FV											
Lower/Lower	503	166.1 ± 1.42	-	112.8 ± 1.42	-	100.4 ± 1.30	-	2.00 ± 0.03	-	4.30 ± 0.02	-
Lower/Higher	561	164.1 ± 1.37	0.3150	110.2 ± 1.37	0.1868	97.8 ± 1.27	0.1617	1.92 ± 0.03	0.0938	4.29 ± 0.02	0.8669
Higher/Lower	124	163.3 ± 2.91	0.3952	110.0 ± 2.90	0.3831	96.6 ± 2.68	0.1946	1.92 ± 0.07	0.3230	4.37 ± 0.04	0.0753
Higher/Higher	204	160.4 ± 2.26	0.0329	106.9 ± 2.26	0.0265	95.0 ± 2.08	0.0294	1.87 ± 0.06	0.0541	4.25 ± 0.03	0.1185
Eggs/PA											
Lower/Lower	596	166.1 ± 1.30	-	112.1 ± 1.30	-	99.7 ± 1.19	-	1.95 ± 0.03	-	4.30 ± 0.02	-
Lower/Higher	468	163.6 ± 1.51	0.2104	110.7 ± 1.51	0.5047	98.3 ± 1.39	0.4629	1.97 ± 0.04	0.6088	4.29 ± 0.02	0.8812
Higher/Lower	199	161.4 ± 2.32	0.0730	107.0 ± 2.32	0.0570	94.4 ± 2.13	0.0325	1.83 ± 0.06	0.0703	4.29 ± 0.03	0.8438
Higher/Higher	129	161.7 ± 2.80	0.1568	109.5 ± 2.81	0.4111	97.2 ± 2.58	0.3923	1.97 ± 0.07	0.7483	4.29 ± 0.04	0.9058

[1] Lower vs. higher egg intake = <3 per week vs. ≥ 3 eggs per week; Lower vs. higher fiber intake = <12 vs. ≥12 grams per day; Lower vs. higher dairy intake = <2 vs. ≥2 servings per day; Lower vs. higher fruit and vegetable intake = <1.75 vs. ≥1.75 cup equivalents per day; Lower vs. higher physical activity = <20 METS per week vs. ≥20 METS per week. [2]Adjusted for race, age (at time of diet assessment), % energy from carbohydrates, and baseline height.

Table 3.10 shows that participants who consumed more eggs combined with higher fiber, dairy, or fruits and vegetables had the lowest mean total cholesterol, non-HDL-C, and LDL-C. For example, girls who consumed higher dairy and had higher egg intakes had a mean LDL-C that was 7.1 mg/dL lower than the referent group (p=0.0413) and a total cholesterol that was 8.4 mg/dL lower than the referent group (p=0.0265). Finally, there was no effect of these egg-related diet patterns on triglyceride levels.

3.4 DISCUSSION

These results suggest that intake of ≥ 3.5 eggs per week, particularly when consumed as part of a healthy diet, was associated with lower levels of body fat and increased SMM during later adolescence. In addition, higher egg intake when combined with higher amounts of fiber or fruits and vegetables was associated with lower body fat, truncal fat, waist circumference, and BMI and increased levels of SMM. Girls who consumed more eggs in combination with higher amounts of fiber, fruits or vegetables, or who were more physically active had 43-58% reductions in risk of overweight in late adolescence.

These results provide no evidence that consuming 3 or more eggs per week is detrimental to cardiometabolic outcomes during adolescence. In fact, when eggs were consumed with higher amounts of fiber, dairy, or fruits and vegetables, girls had a significantly lower LDL-C, non-HDL-C, and total cholesterol compared to the referent group and tended to have lower diastolic blood pressure levels as well.

The long-term effects of egg consumption on body weight have been virtually unexamined in children and only infrequently studied in adults. In the European Prospective Investigation in Cancer (EPIC) and Nutrition-Potsdam cohort, for each 100 gram per day increase in egg intake, there was a 65% non-statistically significant increase in weight gain (≥ 2 kilograms) in men and a 44% non-statistically significant increase in weight gain in women over two years. Besides consuming more eggs, however, men and women who gained weight over the two year period were more likely to consume more processed meat, sweets, and soft

drinks compared to weight maintainers, raising concerns about the lack of adjustment for potential dietary confounders in this study.[203]

Confounding by other diet and lifestyle factors is a common problem in studies of individual foods and nutrients and such confounding could explain the conflicting results between studies. In one study, researchers found a positive association between egg intake and BMI and waist circumference. However, after adjusting the two egg eating patterns [egg/meat, poultry, fish (MPF)/grains/vegetables and egg/MPF/grains] for other dietary factors (e.g. fast food consumption) and diabetes medications, these relations disappeared.[202]

Further evidence of the importance of examining other dietary factors comes from a longitudinal study involving three cohorts (Nurses' Health Study, Nurses' Health Study II, and Health Professionals Follow-up Study). In this particular study, researchers found that the effects of egg intake on 4-year weight gain differed based on whether glycemic load simultaneously increased or decreased. For instance, if glycemic load

increased, then each 1-serving per day of eggs was associated with a 0.61 pound increase in weight. On the other hand, if glycemic load decreased, then each 1-serving per day of eggs was associated with a 1.75 pound decrease in weight.[205] Our results, which accounted for potential dietary confounders, was one of the first prospective studies to confirm the favorable effects of eggs intake on body fat during adolescence.

Short-term clinical trial data suggest that egg intake may play a role in maintaining a healthy body weight through its effects on satiety. Specifically, several randomized cross-over studies in adults have found that a breakfast containing eggs compared to an isocaloric breakfast increases satiety and leads to reduced food intake at the subsequent meal, with no compensation occurring later in the day for the reduction in caloric intake.[206-208] In an eight week, randomized clinical trial, researchers found that overweight adults consuming a breakfast with two eggs while on an energy restricted diet lost more weight and tended to have a greater reduction in waist circumference compared to those consuming a bagel for breakfast. However, there were similar reductions in percent body fat

from baseline between the egg and bagel groups.[212] This absence of an observed effect of egg intake on percent body fat in short-term studies (5-12 weeks)[212,293-295] may be due to the short duration of these studies combined with measurement error making it difficult to detect smaller changes in body fat assessed by either BIA or dual X-ray absorptiometry.

A small protein differential between the egg and control groups or the fact that the egg-related meal provided a fairly low amount of protein could also be impacting these results. Consumption of dietary protein improves satiety and reduces food intake at subsequent meals as a result of releasing anorexigenic gut peptides such as cholecystokinin, peptide YY, and glucagon-like peptide-1.[43] In addition, protein intake may diminish brain reward mechanisms, thus decreasing hunger and leading to a reduction in eating.[44] Further, the essential amino acid leucine, which is particularly high in egg protein,[286] has been shown to modulate food intake via mammalian target of rapamycin and AMP-activated protein kinase-dependent mechanisms in the hypothalamus.[147] Overall, this

information suggests dietary protein related mechanisms are linked with the beneficial effects on adiposity measures.

The protein content in eggs may have influenced the higher percent SMM noted in girls consuming ≥3.5 eggs per week compared to those consuming <0.5 eggs per week. Dietary protein stimulates MPS and reduces muscle protein breakdown.[296] Thus, dietary protein can positively influence muscle mass.[151] Evidence related to egg protein in particular is limited to data suggesting young men exhibited a dose-response in MPS associated with egg protein feeding.[216] While changes in MPS do not necessarily predict changes in lean mass,[68] higher MPS could lead to greater SMM, which is associated with higher resting energy expenditure.[286] Consequently, the higher SMM noted with ≥3.5 eggs per week could be another explanation for the lower body fat in girls coming higher intakes of eggs.

Previous studies evaluating the effects of eggs on adiposity in the context of the overall diet commonly use data-derived dietary patterns. This

approach often results in eggs being classified as part of a Western diet or processed food pattern, which makes it difficult to separate the effects of eggs from the other unhealthy eating patterns associated with egg intake.[289] Consequently, these results from data-derived dietary patterns often suggest that consumption of eggs is linked with a higher risk of obesity and abdominal obesity.[99,297,298]

In contrast with these data-derived approaches, our current study demonstrates that egg intake in combination with consumption of other healthy foods and nutrients had beneficial effects on measures of adiposity. In a recent review, it was suggested that fruits and vegetables may have a protective effect on childhood obesity due to the low energy density (i.e. high water and fiber content) of these foods.[299,300] Fiber in particular is likely involved in the link between plant foods and obesity, although the data in children are limited.[301] Potential mechanisms underlying the observed inverse association between dietary fiber and body weight regulation include gastric distention, delaying gastric emptying, and effects on gut hormones.[302]

Dairy was inversely associated with risk of childhood overweight/obesity in a recent meta-analysis of prospective studies. Specifically, for each one serving increase in dairy consumption per day, there was a 0.65% non-statistically significant reduction (p=0.07) in percentage of body fat.[303] The underlying mechanisms by which dairy may benefit adiposity are incompletely understood,[304] but could relate to protein and leucine content,[43,44,147] since dairy is a good source of both.[286] In addition, there is some evidence from animal studies that the calcium found in dairy sources, may inhibit lipogenesis and promote lipolysis. Finally, some authors have speculated that consuming dairy may offset the consumption of more energy-dense beverages (e.g. sugar sweetened beverages), thus assisting with energy balance.[305]

There may also be a synergistic effect between egg intake and physical activity. Studies relying on objective measures of physical activity (e.g. accelerometers) have found activity, as a result of an increase in energy expenditure, to be inversely associated with adiposity.[306,307] For example, a

previous paper involving the NGHS cohort found that active girls (compared to moderately active or inactive girls) had a lower BMI and sum of skinfold thickness throughout the duration of the ten year study.[308]

There are several possible mechanisms to explain the effects seen on SMM. Dairy has a high content of branch chain amino acids, especially leucine, which is thought to be the most potent postprandial stimulator of MPS.[164,309] Furthermore, it has been well established that physical activity stimulates MPS.[310] Physical activity, especially resistance training, has also been shown to sensitize skeletal muscle for up to 24 hours to the anabolic properties of protein ingestion, increasing the amplitude and duration of MPS. Consequently, exercising prior to ingesting protein, allows for greater use of dietary protein for de novo MPS.[151,164] Finally, while we found that both fiber and fruits and vegetables were associated with higher levels of SMM, there is no evidence that these foods directly benefit SMM. Therefore, it may be that these foods are indicator of other healthy lifestyle patterns that are positively influencing SMM.

Our cardiometabolic results add to the one known study evaluating egg intake and blood pressure and lipid outcomes in children.[288] The lack of an adverse effect noted from egg intake on fasting glucose supports the adult randomized clinical trial results[219,230] and a recent meta-analysis which concluded that there was no association between egg consumption and risk of diabetes.[228] Furthermore, our finding of no relation between eggs and HOMA-IR in a prospective cohort is similar to adult cross-sectional data,[224] suggesting that eggs do not adversely affect insulin resistance.

In contrast with our data, two earlier meta-analyses suggested a link between egg consumption and risk of diabetes,[226,227] although these studies have been criticized for failure to account for dietary confounders.[221] Since public health campaigns for years had recommended limited dietary cholesterol and specifically eggs due to their high dietary cholesterol content, individuals with high intakes of eggs may represent those who ignored public health advice; such individuals may have been more likely to have other unhealthy dietary patterns as well.[199] Consequently,

incomplete control for confounding by other diet and lifestyle factors could explain the very different conclusions.

No association was observed between egg consumption and blood pressure in these analyses. Our results differed from a study in children in which consuming two eggs per day led to a reduction in diastolic blood pressure after four weeks of the intervention; no significant difference was noted with systolic blood pressure.[288] Although, our results are in agreement with the data from short-term randomized clinical trials in healthy and unhealthy adults which suggest no link between egg intake and blood pressure levels.[217,218,293]

Our findings are consistent with other literature evaluating the effects of egg consumption on total cholesterol, LDL-C, LDL to HDL ratio, and triglycerides. Epidemiological research examining the link between egg intake and total cholesterol suggest either no association or an inverse association.[311] In a recent review, researchers concluded that there were no adverse effects of egg consumption on total cholesterol and triglycerides

in healthy and hyperlipidemic adults.[197] Furthermore, randomized clinical trials with different populations have found that the dietary cholesterol in eggs increases both LDL-C and HDL-C in people susceptible to plasma cholesterol increases, without changing the LDL to HDL ratio.[173] This includes evidence from the pediatric population, where researchers found no change in LDL-C to HDL-C ratio or triglycerides in children randomized to either 2 eggs per day or the equivalent amount of egg whites for 30 days.[288]

The literature evaluating the effects of egg intake on non-HDL-C is extremely limited. One study found that consuming 4 eggs per day for four weeks increased non-HDL-C but only in insulin-sensitive adults, not insulin resistant, normal-weight individuals or insulin resistant, obese individuals.[312] In another study, adults on an energy-restricted diet had statistically significant decreases in non-HDL-C regardless if they consumed two eggs or 100 grams of lean protein per day[230]

As previously mentioned, research evaluating the effects of eating patterns containing eggs on various outcomes (e.g. cardiometabolic) often use data-derived methods.[99,313] These patterns include a low intake of fruits and vegetables or whole grains, making it difficult to determine if the increase in risk for cardiovascular risk factors is related to eggs or to the lack of these foods in the diet that promote cardiometabolic benefits. Consequently, in order to avoid this limitation, we evaluated eggs in combination with foods typically associated with a healthy diet (e.g. fruits and vegetables and dairy).[26]

There are a number of possible mechanisms by which egg in combination with other health foods could benefit lipid levels as seen in this study. Mechanisms involving dairy include milkfat-related increases in HDL-C, dietary calcium effects on total cholesterol and LDL-C, perhaps by increasing fecal fat excretion, and whey protein-related inhibition of angiotensin converting enzyme leading to a decrease in endogenous fat production and lower total cholesterol and LDL-C.[314] The experimental evidence linking consumption of fruits and vegetables to lipid outcomes is

limited.[315,316] However, the beneficial effects of fruits and vegetables on lipids may be the result of the dietary fiber content reducing the absorption of dietary fat and cholesterol, increasing bile acid excretion, and producing short chain fatty acids which have been shown to inhibit cholesterol synthesis.[302] Finally, the beneficial effects of dairy, fruits and vegetables, and fiber on body composition may explain subsequent favorable effects on lipids.[300,302,314]

This study has several strengths including the prospective study design with ten years of follow-up and the numerous three-day food records that allowed for a more precise estimate of dietary intake. In addition, there were repeated assessments of adiposity and cardiometabolic outcomes, as well as potential confounders. On the other hand, there are several limitations for this particular study. Since dietary intake in observational studies is self-reported, it is always subject to some degree of misreporting and this report could vary by weight status. Further, while we were able to comprehensively evaluate the effects of egg intake on adiposity and cardiometabolic outcomes, quite a few of the girls were missing

cardiometabolic outcome lab values, thus reducing the study population for these outcomes. Finally, there were low levels of intake of certain foods in this population, including eggs. The low intake among girls was probably a reflection of the significant decline in egg consumption among adults and children in the United States from the mid-1960s to the early 1990s.[317]

3.5 CONCLUSION

These results suggest that usual egg intake, particularly when consumed as part of a healthy diet, is associated with lower levels of adiposity and higher levels of SMM during later adolescence. Furthermore, there was no evidence that egg intake was linked to adverse effects on cardiometabolic outcomes. Therefore, these results provide no evidence that eggs should be avoided.

CHAPTER 4: EGG INTAKE IS ASSOCIATED WITH LOWER RISKS OF IMPAIRED FASTING GLUCOSE AND HIGH BLOOD PRESSURE IN FRAMINGHAM OFFSPRING STUDY ADULTS

4.1 BACKGROUND

Cardiovascular disease (CVD), the leading cause of death in the United States, is responsible for approximately one in four deaths.[318] Elevated glucose, type 2 diabetes, and high blood pressure (HBP) are all important determinants of CVD risk.[319] Diet is a potentially modifiable determinant of these cardiometabolic risk factors. For decades, it has been suggested that eggs may increase CVD risk, primarily as a result of their dietary cholesterol content.[320,321] However, in recent years, the belief that dietary cholesterol increases risk of CVD has been increasingly questioned.[197,322]

The effects of egg consumption on glucose metabolism and other markers of cardiometabolic risk are not definitively known. Three recent meta-analyses had inconsistent findings, with one suggesting egg intake was associated with incident type 2 diabetes,[323] while the other two found no

association between egg consumption and risk of type 2 diabetes.[228,324] Short-term randomized clinical trials also demonstrated no adverse effect of egg consumption on glucose metabolism, including studies evaluating egg consumption in individuals with type 2 diabetes.[219,229,230] Some authors have speculated the inconsistent findings in the epidemiological literature could be due to methods (e.g. control for potential dietary confounders) and differences in study populations.[199,221] Further evidence questioning a direct link between egg consumption and risk of type 2 diabetes comes from a lack of strong evidence for a biological mechanism supporting this link. While some research has found an association between dietary cholesterol and risk of type 2 diabetes,[231] other research does not show an association or suggests an inverse association exists between dietary cholesterol and type 2 diabetes.[222,233] Therefore, there is a need for more controlled, prospective studies evaluating the effects of egg intake on risk of type 2 diabetes.

There has been some research evaluating the impact of eggs on blood pressure in human studies. One cross-sectional study[202] and three short-

term randomized clinical studies in healthy and high-risk individuals[217-219] suggest that consuming two eggs per day is not detrimental to blood pressure. However, long-term studies are needed. In animal studies, a number of egg-derived peptides have been linked to possible antihypertensive properties, perhaps by inhibiting the activity of angiotensin-converting enzyme.[90,220] Consequently, there is a need to evaluate the effects of egg consumption on blood pressure in prospective studies.

As a result of many years of dietary recommendations in the United States limiting dietary cholesterol in order to reduce CVD risk, and consequently eggs due to their high cholesterol content,[311] it is possible that egg consumption may have become associated with less healthy behaviors. Individuals consuming higher amounts of eggs may have been less likely to follow other healthy dietary and lifestyle advice in general. Consequently, egg consumption may be inversely associated with healthy eating patterns such as intakes of fruits and vegetables or whole grains.[193] Therefore, observational studies of the impact of egg consumption on

health outcomes must consider other food intake and dietary pattern. Given the evidence, we hypothesize that egg consumption, particularly in the context of healthy patterns, does not detrimentally affect fasting glucose or blood pressure. The overall goal of this study is to examine the impact of egg consumption, alone and in the context of other eating patterns, on fasting glucose and blood pressure among adults in the Framingham Offspring Study (FOS).

4.2 METHODS

4.2.1 Study Population

These analyses were approved by the Boston University Institutional Review Board and use data from the prospective Framingham Offspring Study. The study began in 1971 with 5,124 offspring and spouses of the original Framingham Heart Study cohort.[325] Subjects are assessed approximately every four years for the development of CVD as well as CVD risk factors, including fasting blood glucose and blood pressure. Diet was assessed using three-day diet records during the third (starting in 1984) and the fifth (starting in 1991) examination cycles. For individuals

with complete dietary data at exams three and five, mean egg intake was estimated, although the third exam functioned as the baseline exam. If individuals only had dietary intake at exam three or five, then egg intake from that exam was used. For these analyses, exam visit three provided baseline data for most subjects; for those missing dietary data at exam three, exam visit five was treated as the baseline visit.

Figure 4.1. Flow diagram of selection of study sample

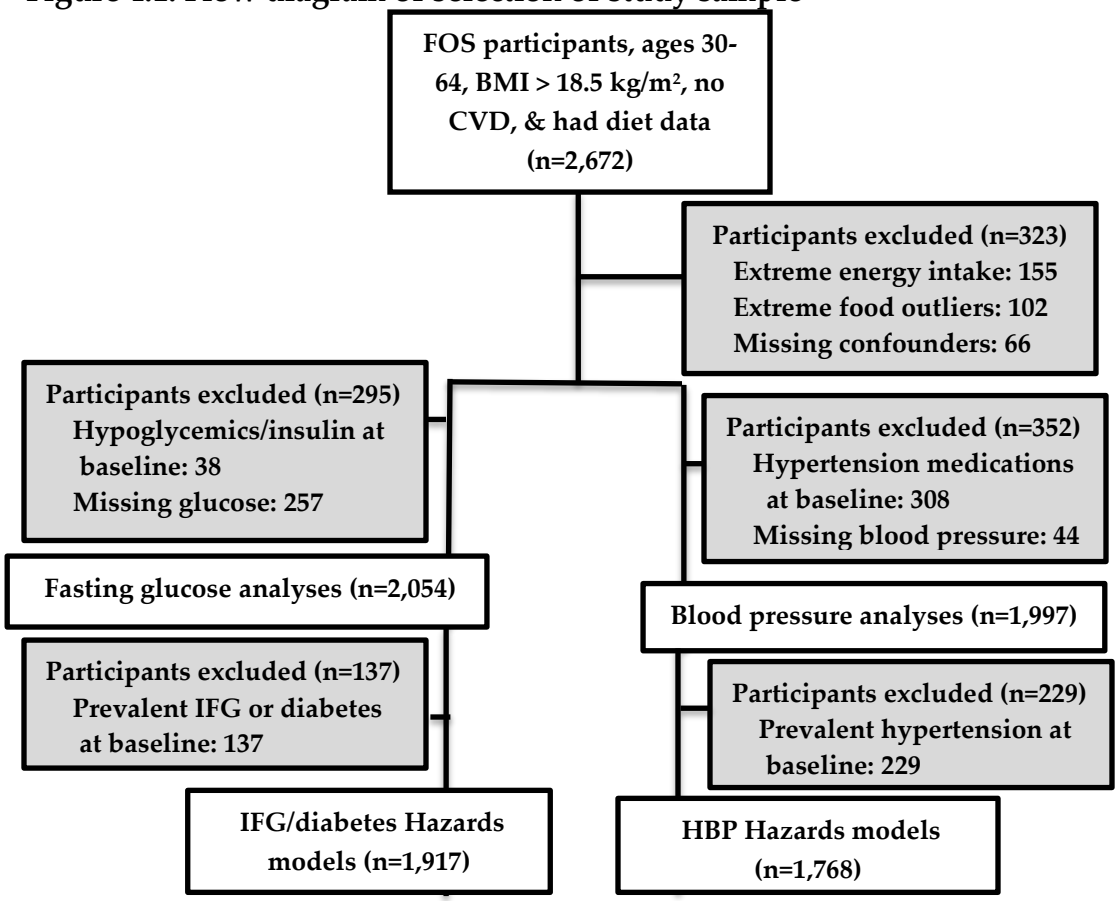


Figure 4.1 shows a flow diagram of the selection of the study sample. The baseline data set included adults, ages 30 to 64 years with a BMI greater than 18.5 kg/m^2, who were free of CVD and who had available dietary record data (n=2672). Men who reported an energy intake of <1,200 kcals or >4,000 kcals per day and women reporting <1,000 kcals or >3,500 kcals per day (n=155) were excluded as were extreme outliers for other food groups (e.g., >20% of total kcals per day from alcohol, >35 eggs per week) (n=102). Those with missing data for potential confounders of interest (n=66) were also excluded.

Finally, exclusions were made for fasting glucose and blood pressure analyses. For fasting glucose, individuals taking oral hypoglycemic medications or insulin at baseline (n=38) or with missing fasting glucose measures (n=257) were excluded, leaving a sample of 2,054 men and women for the assessment of egg intake on follow-up fasting glucose. Additionally, individuals with impaired fasting glucose (IFG) or diabetes (n=137) at baseline (even though not taking hypoglycemic medications) were leaving 1,917 for analyses with IFG or diabetes as the outcome variable. Subjects taking hypertension medications at baseline (n=308) or

missing blood pressure data (n=44) were excluded, leaving 1,997 men and women for the analysis of adjusted mean follow-up blood pressure levels. An additional 229 individuals with hypertension were excluded from analyses where blood pressure is the outcome.

4.2.2 Dietary Assessment

At exams three and five, participants were instructed by a trained nutritionist in the completion of three-day (two weekdays and one weekend day) food records. Approximately 70% of participants completed these food records, resulting in roughly 16,000 days of dietary data. Food record data were entered into Nutrient Data System (NDS), developed at the University of Minnesota, in accordance with a standardized protocol to calculate mean intakes of macro- and micronutrients.[257] Researchers from Boston University linked the underlying food codes from NDS to United States Department of Agriculture (USDA) Pyramid food serving data for eggs and other food groups using previously described methods.[326] Eggs and other food servings in this data set include both whole foods, composite foods, and those derived from mixed dishes. For example, total egg intake includes

whole eggs, eggs in baked goods, and eggs in a mixed dish (e.g., quiche). Since eggs area common food ingredient, only 46 individuals had no egg intake. The above linkage of dietary record data with USDA Food Pyramid serving data also allowed for the calculation of Healthy Eating Index scores for each subject.[327]

4.2.3 Main Outcome Measures

Blood specimens were collected in a standardized manner at each examination following a 12-hour fast and then stored at -80 degrees centigrade. Measurement and analysis of fasting glucose has been previously described.[328] Extreme values of fasting glucose for nine subjects were truncated to 200 mg/dL to eliminate the influence of these outliers. Mean systolic and diastolic blood pressures were the average of two visit-specific measurements taken with a mercury sphygmomanometer after participants sat quietly for five minutes. Additional analyses explored the risk of incident IFG/type 2 diabetes and HBP. For the IFG/type 2 diabetes outcome, subjects developing either IFG or type 2 diabetes were combined to enhance statistical power. IFG was defined as fasting glucose ≥110 mg/dL due to this cutoff conferring a higher risk for metabolic and/or

cardiovascular risk abnormalities compared to a fasting glucose ≥100 mg/dL,[329] while incident diabetes was defined as a fasting glucose ≥126 mg/dL or taking a glucose-lowering medication. Incident HBP was defined as any of the following: mean systolic blood pressure ≥140 mmHg or mean diastolic blood pressure ≥90 mmHg at two consecutive exams, mean systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥95 mmHg at a single exam, or use of antihypertensive medication.

4.2.4 Potential Confounders

There were a number of potential confounders considered. For the glucose and systolic blood pressure analyses, age, sex, dietary fiber, and baseline body mass index (BMI) or waist circumference (WC) were included in the final models. For the risk of developing diabetes and high blood pressure analyses, age, sex, baseline BMI, and consumption of solid fats/alcoholic beverages/added sugars (SoFAAs) were included in the final models. WC was measured starting at exam visit 4 and rounded to the next lowest ¼ inch; for those missing WC data at exam three, exam visit four data was used for the baseline data. Other potential confounders explored (but not included in the final models since they changed the effect estimates less

than 5%) were education level, physical activity index, cigarette smoking, implausible dietary intake, energy, sodium, and potassium intake, Healthy Eating Index-2005 score and other dietary factors. The physical activity index, a modification of the original method reported by Kannel,[330] was a composite score calculated from the self-reported number of hours spent participating in moderate and vigorous activities. Implausible dietary intake was calculated as a ratio of reported energy intake to estimated energy requirement.

4.2.5 Statistical Analyses

Cut-points were determined through sensitivity analyses to reflect the observed patterns in the data while optimizing analytic power. Egg consumption was categorized as follows: <0.5 eggs per week (n=353), 0.5-<5 eggs per week (n=1329), and ≥5 eggs per week (n=372). For eating pattern analyses, egg consumption and other food intakes were dichotomized and the following cutoff values were used: eggs, <2.5 versus ≥2.5 per week; total dairy, <1.75 versus ≥1.75 cups equivalents per day; total fish, <7 versus ≥7 ounces per week; whole grains, <0.5 versus ≥0.5

ounce equivalents per day; fiber, <15 versus ≥15 grams per day; fruits and nonstarchy vegetables, <3 versus ≥3 cup equivalents per day.

Analysis of covariance (ANCOVA) was used to calculate adjusted mean fasting glucose and blood pressure levels after four years of follow-up across categories of egg consumption as well as eggs combined with healthy eating patterns. For individuals with exam three dietary data, outcomes for ANCOVA analyses were assessed approximately four years later at exam four, while outcomes were assessed at exam six for individuals with only exam five dietary data. For the fasting glucose and blood pressure analyses, BMI and WC was assessed both in stratified analyses and as a potential causal intermediate. Additionally, all analyses were stratified by sex. For any subjects who began glucose or blood pressure-lowering medications during the follow-up period, baseline measurement values were substituted for follow-up values.

Cox proportional hazard's models were used to estimate the hazard ratio (HR) and 95% confidence intervals (CI) for long-term risk of IFG/diabetes and HBP according to egg intake. Follow-up for incident IFG/type 2

diabetes and incident HBP started at the time of baseline egg assessment and continued until the first of the following: incident IFG/type 2 diabetes (for IFG/type 2 diabetes analyses) or incident HBP (for HBP analyses), loss to follow-up, end of follow-up (through the end of exam 7), or death. In addition, dichotomous egg consumption was cross-classified with intakes of other foods including dairy, total fish, whole grains, dietary fiber, and fruit and non-starchy vegetables to determine the effect of egg consumption on fasting glucose, IFG, blood pressure, and HBP in the context of a healthy eating pattern. All analyses were performed using Statistical Analysis Systems software, version 9.4 (SAS Institute, Cary, NC).

4.3 RESULTS

Table 4.1 shows the baseline characteristics of participants according to their usual egg intake per week adjusted for sex. Individuals, who consumed less than 0.5 eggs per week, were more likely to be female and they tended to consume a higher percentage of total energy from protein, carbohydrates, and SoFAAs ($p < 0.0001$, for all). Those with an intake of five or more eggs per week had a higher BMI and the lowest intakes of

fruits and vegetables as well as higher intakes of fat and saturated fat. Finally, those with consumption of five or more eggs per week had higher dietary cholesterol intake.

Table 4.1. Baseline characteristics of subjects in the Framingham Offspring Study according to egg consumption[1]

	Weekly number of eggs consumed			
	0 to 0.5	**0.5 to <5**	**≥5**	***P*-trend**
Subjects, n	353	1329	372	
Age, years	49.6 ± 0.47	48.6 ± 0.24	48.1 ± 0.46	*0.0728*
Height, cm	169 ± 0.33	169 ± 0.18	169 ± 0.33	*0.1775*
BMI, kg/m^2	26.2 ± 0.23	26.0 ± 0.12	26.8 ± 0.23	*0.0031*
Male, n (%)	133 (37.7%)	568 (42.7%)	223 (60.0%)	*<0.0001*
Smoker, n (%)	79 (22.4%)	285 (21.4%)	98 (26.3%)	*0.14*
More than high school[2], n (%)	178 (57.2%)	754 (63.2%)	201 (61.1%)	*0.15*
Systolic blood pressure, mmHg	124.1 ± 0.84	122.4 ± 0.43	122.1 ± 0.82	*0.1458*
Diastolic blood pressure, mmHg	78.4 ± 0.49	77.8 ± 0.25	77.8 ± 0.48	*0.5266*
Glucose, mg/dL	95.4 ± 0.71	92.5 ± 0.36	92.7 ± 0.69	*0.0014*
Physical activity index	12.7 ± 0.42	12.4 ± 0.22	13.0 ± 0.41	*0.2956*
Energy intake, kcals/d	1722 ± 24.1	1919 ± 12.4	2074 ± 23.6	*<0.0001*
Dietary cholesterol, mg	173 ± 4.19	242 ± 2.16	402 ± 4.11	*<0.0001*
Protein, % of energy	17.7 ± 0.17	16.8 ± 0.09	16.4 ± 0.17	*<0.0001*
Carbohydrate, % of energy	47.4 ± 0.42	46.2 ± 0.22	43.9 ± 0.41	*<0.0001*
Fat, % of energy	32.9 ± 0.34	35.1 ± 0.18	38.0 ± 0.33	*<0.0001*
Saturated fat, % of energy	10.9 ± 0.15	12.0 ± 0.08	13.3 ± 0.15	*<0.0001*
SoFAAs, % energy	12.5 ± 0.28	10.3 ± 0.14	8.7 ± 0.27	*<0.0001*
FNSV, cup equivalents/day	2.6 ± 0.07	2.6 ± 0.04	2.3 ± 0.07	*0.0060*
Whole grains, ounce equivalents/day	0.6 ± 0.04	0.6 ± 0.02	0.5 ± 0.04	*0.1691*
Dairy, cup equivalents/day	1.3 ± 0.05	1.4 ± 0.02	1.4 ± 0.05	*0.1628*
Dietary fiber, grams/day	16.1 ± 0.32	16.1 ± 0.16	15.5 ± 0.31	*0.1632*

[1]Data are adjusted means ± standard error, unless otherwise noted. All means are adjusted for sex.
[2]Subjects missing education data were included in analysis by use of dummy variables

Table 4.2. Effects of egg intake on fasting glucose and blood pressure after four years of follow up, stratifying by baseline BMI and by sex[1]

	All subjects		BMI < 25 kg/m²		BMI ≥ 25 kg/m²		Women		Men	
Egg intake/wk	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE
					Fasting glucose (mg/dL)					
<0.5	353	96.6 ± 0.73	157	90.9 ± 0.72	196	101.0 ± 1.20	220	94.2 ± 0.90	133	99.5 ± 1.21
0.5 to <5	1329	93.2 ± 0.38	610	88.7 ± 0.37	719	96.4 ± 0.63	761	90.6 ± 0.49	568	96.4 ± 0.59
≥ 5	372	92.9 ± 0.72	132	89.3 ± 0.79	240	96.5 ± 1.09	149	90.4 ± 1.10	223	96.0 ± 0.94
P-trend		*0.0004*		*0.10*		*0.0098*		*0.0025*		*0.0450*
					Systolic blood pressure (mmHg)					
<0.5	354	125.7 ± 0.78	164	119.4 ± 1.13	190	131.1 ± 1.09	223	122.7 ± 1.01	131	129.9 ± 1.20
0.5 to <5	1269	123.6 ± 0.41	618	118.6 ± 0.58	651	127.8 ± 0.59	743	121.6 ± 0.55	526	126.1 ± 0.60
≥ 5	374	123.3 ± 0.76	143	120.0 ± 1.21	231	127.0 ± 0.99	147	121.3 ± 1.25	227	125.8 ± 0.91
P-trend		*0.0284*		*0.76*		*0.0071*		*0.34*		*0.0173*
					Diastolic blood pressure (mmHg)					
<0.5	354	78.6 ± 0.47	164	74.3 ± 0.69	190	82.3 ± 0.65	223	75.8 ± 0.61	131	82.4 ± 0.75
0.5 to <5	1269	77.6 ± 0.25	618	74.5 ± 0.35	651	80.1 ± 0.35	743	75.4 ± 0.33	526	80.3 ± 0.37
≥ 5	374	77.6 ± 0.46	143	75.0 ± 0.74	231	80.2 ± 0.60	147	74.9 ± 0.75	227	80.7 ± 0.57
P-trend		*0.12*		*0.51*		*0.0279*		*0.38*		*0.15*

[1]All subjects' means are adjusted for age, sex, dietary fiber, and BMI. BMI stratified means are adjusted for age, sex, and dietary fiber. Sex stratified means are adjusted for age, dietary fiber, and BMI.

Table 4.2 shows that consuming five or more eggs per week was associated with a fasting glucose concentration at follow-up that was 3.7 mg/dL lower than that of subjects consuming less than 0.5 eggs per week ($p=0.0004$) after adjusting for age, sex, dietary fiber, and BMI. These effects were stronger among overweight (4.5 mg/dL difference) than normal weight individuals (1.6 mg/dL difference). While men generally had higher fasting glucose levels, higher egg intakes were associated with lower glucose levels in men and women. Egg consumption was also inversely associated with both systolic and diastolic blood pressures in overweight individuals and with systolic blood pressure in men but not women.

Table 4.3 shows the effects of egg intake on fasting glucose and blood pressure after adjusting for age, sex, dietary fiber, and WC. In contrast to the previous table, Table 4.3 shows consumption of five or more eggs per week is not significantly associated with lower fasting glucose ($p=0.0934$). Effects were stronger among those without abdominal obesity compared to those with abdominal obesity. There were no significant differences

noted in blood pressure when results were stratified by abdominal obesity.

Table 4.3. Effects of egg intake on fasting glucose and blood pressure after four years of follow up, stratifying by baseline waist circumference

	All subjects[1]		No abdominal obesity[2]		Abdominal obesity[3]	
Egg intake/week	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**
			Fasting glucose (mg/dL)			
<0.5	353	100.0 ± 1.20	255	93.4 ± 0.64	98	104.5 ± 2.03
0.5 to <5	1329	95.4 ± 0.80	967	90.8 ± 0.33	362	98.7 ± 1.04
≥ 5	372	97.4 ± 1.34	233	89.6 ± 0.68	139	100.6 ± 1.69
P-trend		*0.0934*		*<0.0001*		*0.2457*
			Systolic blood pressure (mmHg)			
<0.5	354	125.6 ± 0.77	261	123.1 ± 0.91	93	133.0 ± 1.54
0.5 to <5	1269	123.7 ± 0.41	952	121.0 ± 0.48	317	130.6 ± 0.83
≥ 5	374	123.4 ± 0.76	248	121.0 ± 0.94	126	129.5 ± 1.33
P-trend		*0.0379*		*0.1011*		*0.1020*
			Diastolic blood pressure (mmHg)			
<0.5	354	78.6 ± 0.47	261	77.5 ± 0.56	93	82.0 ± 0.93
0.5 to <5	1269	77.6 ± 0.25	952	76.2 ± 0.29	317	81.3 ± 0.50
≥ 5	374	77.5 ± 0.46	248	76.3 ± 0.58	126	81.0 ± 0.80
P-trend		*0.1148*		*0.1359*		*0.4624*

[1]All subjects' means are adjusted for age, sex, dietary fiber, and waist circumference (WC).

[2]No abdominal obesity = WC < 35 inches for females and < 40 inches males. WC stratified means are adjusted for age, sex, and dietary fiber.

[3]Abdominal obesity = WC ≥ 35 inches for females and ≥ 40 inches for males

The effects of eggs as part of eating patterns on mean fasting glucose and blood pressure levels are shown in Table 4.4. In these analyses, the beneficial effects of eggs on blood glucose were strengthened when consumed with more dairy, total fish, fruits and nonstarchy vegetables, and dietary fiber. Participants with higher egg intakes combined with more dietary fiber also had lower systolic ($p = 0.0195$) and diastolic ($p = 0.0201$) blood pressures compared with the referent group (lower egg and lower dietary fiber intakes). Lower egg intake and higher fiber intake was also associated with lower fasting blood glucose compared to the referent group. Individuals with higher egg intake and higher dairy intake, had non-statistically significant reductions in both systolic and diastolic blood pressures compared to individuals with lower intakes of both.

Table 4.4. Effects of egg intake as part of healthy diet patterns on mean fasting glucose and blood pressure levels after four years of follow up[1]

Baseline diet pattern[2]	Fasting glucose (mg/dL)			SBP (mmHg)			DBP (mmHg)		
	n	Mean ± SE	*P value*	n	Mean ± SE	*P value*	n	Mean ± SE	*P value*
Eggs/Dairy									
Lower/Lower	876	94.2 ± 0.47	-	841	124.3 ± 0.51	-	841	78.1 ± 0.31	-
Lower/Higher	294	94.1 ± 0.80	0.9154	287	124.5 ± 0.86	0.8574	287	77.2 ± 0.52	0.1427
Higher/Lower	621	93.7 ± 0.55	0.4693	608	123.7 ± 0.59	0.4646	608	77.8 ± 0.36	0.5196
Higher/Higher	263	91.9 ± 0.86	0.0203	261	122.5 ± 0.91	0.0793	261	77.0 ± 0.56	0.0784
Eggs/Total fish									
Lower/Lower	592	94.6 ± 0.57	-	582	124.4 ± 0.61	-	582	77.9 ± 0.37	-
Lower/Higher	578	93.7 ± 0.57	0.2221	546	124.3 ± 0.62	0.9680	546	77.9 ± 0.38	0.9755
Higher/Lower	461	94.0 ± 0.64	0.4424	470	123.4 ± 0.67	0.3046	470	77.3 ± 0.41	0.2954
Higher/Higher	423	92.2 ± 0.67	0.0063	399	123.3 ± 0.73	0.2545	399	77.9 ± 0.45	0.9935
Eggs/Whole grains									
Lower/Lower	656	94.7 ± 0.54	-	626	124.9 ± 0.58	-	626	78.2 ± 0.36	-
Lower/Higher	514	93.5 ± 0.61	0.1279	502	123.6 ± 0.65	0.1396	502	77.5 ± 0.40	0.2483
Higher/Lower	516	93.2 ± 0.61	0.0762	501	123.1 ± 0.65	0.0331	501	77.2 ± 0.40	0.0851
Higher/Higher	368	93.0 ± 0.72	0.0591	368	123.8 ± 0.76	0.2377	368	78.0 ± 0.47	0.8443
Eggs/Fiber									
Lower/Lower	765	94.9 ± 0.57	-	737	125.0 ± 0.54	-	737	78.5 ± 0.33	-
Lower/Higher	405	92.9 ± 0.58	0.0136	391	123.1 ± 0.74	0.0364	391	76.8 ± 0.45	0.0018
Higher/Lower	585	93.3 ± 0.67	0.0351	574	123.7 ± 0.61	0.1135	574	77.8 ± 0.37	0.1917
Higher/Higher	299	92.9 ± 0.65	0.0367	295	122.7 ± 0.85	0.0195	295	77.1 ± 0.52	0.0201
Eggs/ FNSV[3]									
Lower/Lower	786	94.4 ± 0.49	-	769	124.7 ± 0.53	-	769	78.1 ± 0.32	-
Lower/Higher	384	93.5 ± 0.70	0.2779	359	123.6 ± 0.77	0.2437	359	77.4 ± 0.47	0.2536
Higher/Lower	634	93.4 ± 0.55	0.1610	631	123.5 ± 0.58	0.1360	631	77.6 ± 0.36	0.3121
Higher/Higher	250	92.4 ± 0.87	0.0448	238	122.9 ± 0.95	0.1060	238	77.5 ± 0.58	0.3697

[1]All means are adjusted for age, sex, and BMI.
[2]Lower vs. higher egg intake = <2.5 vs. ≥2.5 eggs/week; Lower vs. higher dairy intake = <1.75 vs. ≥1.75 servings/day; Lower vs. higher total fish intake = <7 vs. ≥7 ounces/week; Lower vs. higher whole grain intake = <0.5 vs. ≥0.5 ounce equivalents/day; Lower vs. higher fiber intake = <15 vs. ≥15 grams/day; Lower vs. higher fruit and non-starchy vegetables = <3 vs. ≥3 cup equivalents/day.
[3]FNSV, fruit non-starchy vegetables.

Occurrence of IFG/type 2 diabetes and HBP by egg intake are shown in Table 4.5. Participants consuming five or more eggs per week (vs. <0.5 eggs/week) had a 28% lower risk of incident IFG/diabetes (95% CI: 0.51-1.03) during the follow-up period (mean time = 11.2 years); women in particular appeared to benefit from egg intake, with a 37% non-statistically significant reduction in risk of IFG/type 2 diabetes. In regards to incident HBP, individuals in the highest category of egg intake had a 32% reduction in risk (CI: 0.50-0.93) when followed for a mean of 10.9 years compared with the referent group. These effects of egg intake on high blood pressure were stronger in men than women.

Table 4.5. Occurrence of impaired fasting glucose and high blood pressure associated with egg intake, stratifying by sex[1]

Egg intake/week	n	PY	Cases	Incidence rate/1000 PY	HR (95% CI)
All subjects					
IFG/Type 2 diabetes					
<0.5	316	3364.7	60	0.01783	1.00
0.5 to <5	1254	14318.8	219	0.01530	0.74 (0.55, 0.98)
≥ 5	347	3779.4	74	0.01958	0.72 (0.51, 1.03)
High blood pressure					
<0.5	303	3082.6	93	0.03017	1.00
0.5 to <5	1142	12629.0	337	0.02669	0.90 (0.71, 1.14)
≥ 5	323	3511.0	78	0.02222	0.68 (0.50, 0.93)
Women					
IFG/Type 2 diabetes					
<0.5	204	2155.6	33	0.01531	1.00
0.5 to <5	727	8478.0	81	0.00955	0.56 (0.37, 0.84)
≥ 5	143	1529.7	24	0.01569	0.63 (0.36, 1.10)
High blood pressure					
<0.5	199	2096.1	51	0.02433	1.00
0.5 to <5	685	7645.0	192	0.02512	1.08 (0.79, 1.48)
≥ 5	135	1500.7	28	0.01866	0.68 (0.42, 1.09)
Men					
IFG/Type 2 diabetes					
<0.5	112	1209.2	27	0.02233	1.00
0.5 to <5	527	5840.7	138	0.02363	0.97 (0.64, 1.48)
≥ 5	204	2249.7	50	0.02223	0.87 (0.54, 1.41)
High blood pressure					
<0.5	104	986.5	42	0.04258	1.00
0.5 to <5	457	4984.0	145	0.02909	0.71 (0.50, 1.00)
≥ 5	188	2010.3	50	0.02487	0.62 (0.40, 0.94)

[1]Models for all subjects are adjusted for age, sex, solid fats/alcoholic beverages/added sugars (SoFAAs), and BMI. Sex is not included in the models stratifying by sex. Impaired fasting glucose is defined as ≥110 mg/dL and/or a type 2 diabetes diagnosis. High blood pressure is defined as mean systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at two consecutive exams, or mean systolic blood pressure ≥160mmHg or diastolic blood pressure ≥95 at a single exam, or use of antihypertensive medication. IFG, impaired fasting glucose; PY, person-years.

Table 4.6 shows the effects of egg-related eating patterns on risk of incident IFG/type 2 diabetes and HBP. Eating patterns that included eggs generally resulted in lower risk of IFG/type 2 diabetes. In particular, eating patterns that included eggs as well as higher amounts of fiber, fish, and whole grains resulted in a statistically significant 29-31% reduction in risk of IFG/type 2 diabetes. Patterns that included higher intakes of eggs in combination with more fish, fiber, and fruits and non-starchy vegetables resulted in a 27-29% lower risk in developing HBP. Table 4.7 shows that the effects of eggs along with higher amounts of fiber were particularly beneficial on IFG/type 2 diabetes in women, while the effects of all egg-related eating patterns on risk of HBP were stronger in men than in women.

Table 4.6. Effects of egg-related diet patterns on risks of impaired fasting glucose and high blood pressure[1]

	IFG / Type 2 diabetes		High Blood Pressure	
Baseline diet pattern[2]	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**
Eggs/Dairy				
Lower/Lower	813	1.00	740	1.00
Lower/Higher	274	1.42 (1.04, 1.93)	254	0.92 (0.70, 1.20)
Higher/Lower	579	1.05 (0.82, 1.35)	542	0.81 (0.66, 1.00)
Higher/Higher	251	0.88 (0.62, 1.23)	232	0.75 (0.56, 1.00)
Eggs/Total fish				
Lower/Lower	548	1.00	507	1.00
Lower/Higher	539	0.78 (0.59, 1.04)	487	0.94 (0.75, 1.17)
Higher/Lower	431	0.90 (0.67, 1.20)	412	0.84 (0.65, 1.08)
Higher/Higher	399	0.71 (0.52, 0.95)	362	0.73 (0.56, 0.94)
Eggs/Whole grains				
Lower/Lower	604	1.00	552	1.00
Lower/Higher	483	0.93 (0.70, 1.24)	442	0.90 (0.72, 1.14)
Higher/Lower	479	1.01 (0.77, 1.32)	444	0.70 (0.55, 0.89)
Higher/Higher	351	0.71 (0.51, 0.98)	330	0.87 (0.68, 1.12)
Eggs/Fiber				
Lower/Lower	563	1.00	523	1.00
Lower/Higher	524	0.71 (0.52, 0.98)	471	0.85 (0.66, 1.08)
Higher/Lower	393	0.88 (0.68, 1.12)	374	0.79 (0.64, 0.98)
Higher/Higher	437	0.69 (0.50, 0.97)	400	0.71 (0.56, 0.94)
Eggs/ FNSV				
Lower/Lower	733	1.00	680	1.00
Lower/Higher	354	0.73 (0.53, 1.01)	314	0.82 (0.64, 1.05)
Higher/Lower	593	0.84 (0.66, 1.08)	568	0.77 (0.63, 0.95)
Higher/Higher	237	0.77 (0.55, 1.08)	206	0.72 (0.53, 0.98)

[1]Models are adjusted for age, sex, and BMI. Impaired fasting glucose (IFG) is defined as ≥110 mg/dL and/or a type 2 diabetes diagnosis. High blood pressure is defined as mean systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg at two consecutive exams, or mean systolic blood pressure ≥160mmHg or diastolic blood pressure ≥95 at a single exam, or use of antihypertensive medication.

[2]Lower vs. higher egg intake = <2.5 vs. ≥2.5 eggs/week; Lower vs. higher dairy intake = <1.75 vs. ≥1.75 servings/day; Lower vs. higher total fish intake = <7 vs. ≥7 ounces/week; Lower vs. higher whole grain intake = <0.5 vs. ≥0.5 ounce equivalents/day; Lower vs. higher fiber intake = <15 vs. ≥15 g/day; Lower vs. higher fruit and non-starchy vegetables (FNSV) = <3 vs. ≥3 cup equivalents/day.

Table 4.7 Effects of egg-related diet patterns on risks of IFG and HBP, stratified by sex[1]

	IFG / Type 2 diabetes				High Blood Pressure			
	Women		Men		Women		Men	
Baseline Diet pattern[2]	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**	**n**	**HR (95% CI)**
Eggs/Dairy								
Lower/Lower	532	1.00	281	1.00	493	1.00	247	1.00
Lower/Higher	143	1.26 (0.76, 2.09)	131	1.48 (0.98, 2.20)	141	0.89 (0.61, 1.30)	113	0.94 (0.64, 1.39)
Higher/Lower	311	0.91 (0.61, 1.34)	268	1.12 (0.81, 1.56)	302	0.85 (0.64, 1.12)	240	0.76 (0.56, 1.04)
Higher/Higher	88	0.72 (0.36, 1.41)	163	0.91 (0.61, 1.37)	83	0.86 (0.54, 1.37)	149	0.69 (0.48, 1.01)
Eggs/Total fish								
Lower/Lower	357	1.00	191	1.00	340	1.00	167	1.00
Lower/Higher	318	0.99 (0.64, 1.52)	221	0.65 (0.45, 0.96)	294	0.97 (0.72, 1.31)	193	0.88 (0.62, 1.24)
Higher/Lower	211	0.99 (0.62, 1.60)	220	0.81 (0.56, 1.17)	210	0.92 (0.66, 1.30)	202	0.76 (0.53, 1.09)
Higher/Higher	188	0.67 (0.41, 1.11)	211	0.67 (0.46, 0.98)	175	0.80 (0.56, 1.13)	187	0.64 (0.44, 0.93)
Eggs/Whole grains								
Lower/Lower	389	1.00	215	1.00	364	1.00	188	1.00
Lower/Higher	286	0.66 (0.42, 1.05)	197	1.24 (0.85, 1.82)	270	0.76 (0.55, 1.03)	172	1.12 (0.79, 1.59)
Higher/Lower	244	0.73 (0.47, 1.12)	235	1.27 (0.88, 1.8)	239	0.69 (0.50, 0.95)	205	0.72 (0.50, 1.04)
Higher/Higher	155	0.67 (0.40, 1.12)	196	0.76 (0.50, 1.16)	146	0.91 (0.64, 1.30)	184	0.86 (0.60, 1.23)
Eggs/Fiber								
Lower/Lower	405	1.00	158	1.00	391	1.00	132	1.00
Lower/Higher	270	0.53 (0.33, 0.87)	254	1.13 (0.76, 1.67)	243	0.87 (0.62, 1.21)	228	0.83 (0.58, 1.20)
Higher/Lower	240	0.75 (0.48, 1.14)	153	1.18 (0.77, 1.81)	240	0.83 (0.62, 1.12)	134	0.75 (0.55, 1.02)
Higher/Higher	159	0.58 (0.35, 0.96)	278	0.89 (0.60, 1.32)	145	0.83 (0.56, 1.25)	255	0.61 (0.41, 0.91)
Eggs/ FNSV								
Lower/Lower	462	1.00	271	1.00	444	1.00	236	1.00
Lower/Higher	213	0.57 (0.34, 0.94)	141	0.87 (0.57, 1.31)	190	0.81 (0.57, 1.13)	124	0.83 (0.57, 1.19)
Higher/Lower	290	0.65 (0.43, 0.98)	303	0.95 (0.69, 1.30)	292	0.76 (0.57, 1.01)	276	0.77 (0.57, 1.04)

Higher/Higher	109	0.84 (0.49, 1.45)	128	0.72 (0.46, 1.12)	93	1.02 (0.67, 1.55)	113	0.53 (0.34, 0.84)

[1]Models are adjusted for age and BMI. Impaired fasting glucose (IFG) is defined as ≥110 mg/dL and/or a type 2 diabetes diagnosis. High blood pressure is defined as mean systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥90 mmHg at two consecutive exams, or mean systolic blood pressure ≥160mmHg or diastolic blood pressure ≥95 at a single exam, or use of antihypertensive medication.
[2]Lower vs. higher egg intake = <2.5 vs. ≥2.5 eggs/week; Lower vs. higher dairy intake = <1.75 vs. ≥1.75 servings/day; Lower vs. higher total fish intake = <7 vs. ≥7 ounces/week; Lower vs. higher whole grain intake = <0.5 vs. ≥0.5 ounce equivalents/day; Lower vs. higher fiber intake = <15 vs. ≥15 grams/day; Lower vs. higher fruit and non-starchy vegetables (FNSV) = <3 vs. ≥3 cup equivalents/day.

4.4 DISCUSSION

In our study, consuming five or more eggs per week had no detrimental effect on fasting glucose or blood pressure over four years of follow up in a healthy adult population. In fact, higher egg intake was associated with a modest reduction in fasting glucose and systolic blood pressure. When outcomes were stratified by baseline BMI, the higher-risk overweight individuals benefitted the most from egg consumption. The beneficial effect of egg intake on mean fasting glucose and risk of developing IFG or type 2 diabetes was generally stronger when combined with higher intakes of other healthy foods including total fish, whole grains, fiber, and fruits and non-starchy vegetables after adjusting for SoFAAs.

Consuming five or more eggs per week was associated with lower systolic and diastolic blood pressure among overweight subjects and as well as a reduction in risk of HBP. Higher egg consumption in those consuming higher amounts of

fish, fiber, and fruits and non-starchy vegetables was also linked to substantial reductions in long-term risk for incident HBP. Overall, these results add support to previous short-term randomized clinical trials suggesting that egg consumption does not adversely affect these important cardiovascular risk factors[217,219,230] and may in fact be beneficial to fasting glucose and blood pressure levels, particularly when consumed as part of an overall healthy eating pattern.

The epidemiologic literature on egg consumption and glucose-related outcomes is inconsistent, with some authors suggesting the variability in results could be due to such factors as differences in study populations and methods (e.g. control for potential dietary confounders).[221] For instance, in a study of men and women ≥ 65 years of age, consuming eggs almost every day (assessed by food frequency questionnaire) was associated with a higher adjusted mean fasting glucose compared to non-egg consumers after a mean follow-up of 11.3 years (100.6 ± 3.5 vs. 99.5 ± 3.5, $p<0.001$) although the authors acknowledged that these were not clinically meaningful differences.[222] Our results are similar to the findings from the Kuopio Ischaemic Heart Disease Risk Factor Study, which found that men ages 42 to 60 years of age who had the highest egg intakes (>45 grams per day) assessed by food records had the lowest fasting glucose and a 45% lower risk of

developing diabetes over approximately 20 years of follow-up.[225] In contrast, our results differed from two recent meta-analyses, which found an increased risk of type 2 diabetes with egg intake assessed by food frequency questionnaires in studies from the United States (US); no association appeared to exist in non-US studies.[228,323]

Several short-term randomized clinical trials found that consuming two to three eggs per day for 12 weeks as part of an energy-restricted diet[219,229,230] had no negative effects on blood glucose in either males or females. Another study of subjects with type 2 diabetes noted an improvement in hemoglobin Alc and fasting glucose associated with consuming two eggs per day as part of an energy-restricted diet for 12 weeks,[230] while other evidence suggests that CVD risk associated with egg intake may be more pronounced in those with type 2 diabetes.[226]

Previous studies evaluating eggs as part of dietary patterns using data-derived patterns (e.g. factor analysis) suggests eggs are detrimental to glucose metabolism.[99,250] Since eggs are often linked with less healthy eating patterns such as high intake of saturated fat or lower intakes of whole grains, fruits, and

vegetables, resulting in lower intake of nutrients associated with cardiovascular health such as dietary fiber, B vitamins, and antioxidants,[193] it is often difficult to separate out these effects of eggs from their associated eating patterns.[202] As a result, researchers are unable to ascertain whether the increased risk is due to egg consumption or the low intake of important nutrients impacting cardiovascular risk. In our analysis, egg consumption was evaluated in combination with eating patterns (e.g. higher amounts of eggs combined with higher amounts of whole grains). In contrast to previous research, we found the beneficial effects of eggs on fasting glucose were strengthened when eggs were combined with higher intakes of other healthy foods such as dairy, fish, fruits and non-starchy vegetables or higher intakes of dietary fiber.

The nutrient composition of eggs could explain the beneficial effects on glucose-related outcomes in some studies. For instance, the protein content of eggs may possibly play a role in glucose metabolism by serving as a substitute for carbohydrates with a higher glycemic load, as a substrate for gluconeogenesis, or by promoting insulin secretion from pancreatic β –cells.[102] Further, egg yolks are a rich source of the carotenoids lutein and zeaxanthin,[235] which have been associated with lower 2-hour post-load glucose and fasting insulin in a cross-

sectional analysis,[236] potentially due to their role in modulating inflammation via inhibition of nuclear factor κB and by modifying oxidative stress via interaction with the nuclear factor erythroid 2-related factor 2 pathway.[237] Finally, eggs are one of the few food sources of vitamin D,[331] which may play an important role in glucose metabolism by improving pancreatic β-cell function through both direct and indirect effects on insulin secretion, improvement in insulin action, and reduction in systemic inflammation.[238]

A number of possible mechanisms could explain the synergistic effects of eggs and other eating patterns on glucose metabolism. Dietary fiber, particularly cereal fiber (found in high amounts in whole grains), has been shown to reduce the risk of type 2 diabetes in a meta-analysis of prospective studies; this beneficial effect was attributed to the delay of gastric emptying, thereby reducing postprandial glucose response.[332] Furthermore, fruits, vegetables (particularly green leafy vegetables), and whole grains have also been associated with lower risk for type 2 diabetes,[333,334] perhaps as a result of antioxidant nutrients reducing inflammation and oxidative stress.[237,335,336] In some studies, dairy has been inversely related to risk of type 2 diabetes [94] potentially due to effects of vitamin D and calcium on improving insulin action and section, or factors in milk fat or

dairy proteins, which regulate glycemic response and insulin sensitivity.[238,314] Finally, fish may reduce diabetes risk due to the dietary protein content. Purported beneficial mechanisms of fish protein on glucose metabolism include decreased postprandial insulin secretion and improved insulin sensitivity when consuming fish protein compared to other dietary proteins.[36]

The favorable effects of eggs on blood pressure may be attributed to a number of egg-derived peptides that have possible antihypertensive properties, often through the inhibition of the angiotensin-converting enzyme.[220] Also, the arginine content in eggs could induce vasodilation, as a result of acting as a substrate for nitric oxide synthesis, thus lowering blood pressure.[92]

In this study, high fiber intake combined with egg consumption was particularly beneficial to blood pressure. This potential benefit of dietary fiber on blood pressure has been noted in several meta-analyses of randomized clinical trials, particularly in those with hypertension.[337,338] Proposed mechanisms through which fiber intake might affect blood pressure are unclear, but some possibilities in the literature include its beneficial effects on insulin sensitivity and body weight regulation.[302]

This study has several important strengths. The three-day records provided detailed estimates of individual food intake collected in a standardized fashion. Use of the food records instead of a food frequency questionnaire may have specifically enabled a more accurate assessment of egg intake. Furthermore, the wealth of dietary data also enabled us to examine the independent effects of egg intake on cardiovascular risk factors as well as the effects of eggs combined with other dietary factors. Finally, a number of potential confounders were systematically collected in the Framingham Study, thus enhancing the validity of the results.

This study also had several limitations. The dietary data is self-reported and is potentially subject to both random error and reporting bias. In addition, out of the 5,124 participants enrolled in FOS, only 3,284 (64%) provided dietary records and these food records may not have captured typical dietary intake. Furthermore, these dietary records were only collected at baseline. Finally, the range of egg intake was limited, which could be the result of individuals following the diet policy at the time to reduce their egg intake. As a result, our egg intake categories were set to optimize power.

4.5 CONCLUSION

This prospective study suggests that consuming five or more eggs per week does not adversely affect glucose or blood pressure related outcomes. In fact, moderate intakes of eggs may have beneficial effects on blood glucose and long-term risk of IFG or diabetes. Further, moderate intakes of eggs were linked to lower systolic blood pressure and a significantly reduced risk for developing incident HBP. Overall, these results provide no evidence to restrict egg intake to reduce the risk of elevated glucose or high blood pressure in healthy adults. Rather, moderate amounts of eggs may reduce risk for impaired fasting glucose and hypertension when consumed as part of healthy eating patterns.

CHAPTER 5: GENERAL DISCUSSION

The epidemic of overweight and obesity in children and adults continues to be a major public health problem in the United States.[8] Elevated body mass index (BMI) has been linked to abnormal glucose metabolism, as well as elevated lipids and blood pressure, increasing risk for the development of cardiovascular disease (CVD) at younger ages.[2,4] Since cardiometabolic conditions (including BMI) track with age, reducing these modifiable CVD risk factors earlier in life is critical for optimizing health across the lifespan.[15,16] Diet is one such modifiable factor that may impact cardiovascular risk.[28,339]

Dietary protein intake has been linked to a number of cardiometabolic outcomes. While high protein intake during infancy appears to increase risk for childhood obesity, the literature on the effects of dietary protein consumption during late childhood and adolescence on adiposity and other cardiometabolic outcomes is inconclusive due to the limited data.[85,340] In adult studies, there appear to be contrasting findings between the epidemiological research and randomized clinical trials. Randomized clinical trials often suggest that dietary protein and protein-rich foods are beneficial to body composition and cardiometabolic outcomes,[45] while some epidemiological studies often suggest that dietary

protein (particularly animal protein) and protein-based foods are detrimental to these outcomes.[60,63,65,268]

There are several possible explanations for the discrepant findings between the epidemiological literature and randomized clinical trial data. One possibility could be the lack of adjustment of potential dietary confounders in the observational studies. For example, egg consumption is linked with a higher intake of saturated fat and lower intakes of dietary fiber, which can adversely affect cardiovascular health.[289] Consequently, lack of adjustment for these dietary confounders makes it difficult to determine if the detrimental effects of this protein based food noted in some papers is due to egg consumption or to the lack of other foods or nutrients in the diet that are beneficial to cardiometabolic outcomes.[221] Furthermore, a majority of the epidemiological literature use weight as an outcome, not body composition, so it is unclear if the increase in weight is due to fat or muscle.[65] These factors suggest a need for more carefully designed prospective studies to evaluate the effects of dietary protein and protein-rich foods on adiposity and other cardiometabolic outcomes.

There is also some speculation in the literature that consuming protein-rich foods, such as eggs, in the morning is beneficial on adiposity and cardiometabolic outcomes. This idea is based on data from adult studies where consumption of 25 to 30 grams of dietary protein, often as isolated protein sources such as whey or beef, maximized muscle protein synthesis.[148] Since there is no ability to store protein, researchers have suggested that adequate amounts should be consumed at multiple meals.[253] Furthermore, since protein intake in the morning is particularly low,[252] may be particularly satiating[187] and may help overcome the negative protein balance that occurs during sleep,[105] it is thought that increasing protein intake in the morning is particularly important to the improvement of body composition outcomes. Furthermore, morning protein intake may directly or indirectly as a result of effects on body composition, benefit cardiometabolic outcomes.[276] Currently, there is little evidence to support the benefits of morning protein intake, particularly in children and adolescents where there are few studies investigating this topic. Since adults have a different hormonal profile than adolescents,[53] more research is needed to understand the impact of morning protein intake during adolescence on adiposity and cardiometabolic outcomes.

The few previous studies in children and adolescents had several limitations. One key limitation is that these studies were all short-term, so it is unclear if the short-term beneficial effects will translate to long-term beneficial outcomes. Furthermore, many of these studies focused on satiety and subsequent food intake, with one evaluating body fat, so it is unknown if increasing morning protein intake during adolescence improves other cardiometabolic outcomes. In addition, a majority of the studies enrolled children who typically skipped breakfast. Therefore, it is unknown if the act of consuming breakfast (and not the dietary protein) influenced the results.[138,139,141] Finally, none of the studies accounted for total protein intake, so further research is needed to determine if the higher protein intake at breakfast was beneficial or was it potentially due to the favorable effects of increasing total protein intake seen in this population.[76-78]

The first study in this dissertation evaluated the effects of consuming dietary protein (both total and morning protein) ages 9-17 on cardiometabolic outcomes in late adolescence to address the gaps in the literature. Our results found that higher protein intake ages 9 to 17 years of age in girls was associated with increased percent SMM and lower percent body fat, truncal fat, waist circumference, and BMI at the end of follow-up (ages 17-20). While we found

that higher morning protein intake (≥ 15 vs. 8 grams of protein) was associated with higher SMM and lower body fat at the end of follow-up, these effects appeared partially explained by total protein intake. Further, when we evaluated morning protein intake (low vs. high) by typical morning eating patterns 9 to 17 years of age (e.g. typically skips morning eating occasions, intermittently skips morning eating occasions, or frequently consumes morning eating occasions), body composition outcomes were similar within the low versus high subcategories of each morning eating pattern. These results suggested that total protein intake was more important to body composition outcomes than morning protein intake.

This study also evaluated total and morning protein intake on cardiometabolic outcomes in girls ages 9 to 17 years of age. Overall, higher amounts of total protein intake were not beneficial on cardiometabolic outcomes at the end of follow-up. In fact, there was an indication of an adverse effect on fasting glucose. Finally, morning protein intake did not appear to benefit or adversely affect cardiometabolic outcomes.

This study adds several novel findings to the literature. One key finding is that higher protein intake between the ages of 9 to 17 increases SMM and decreases body fat, which adds to the extremely limited literature on the effects of dietary protein in this age group. Furthermore, our research studied the effects of morning protein intake from foods on adiposity and cardiometabolic outcomes in children. This is one of the first to our knowledge that addressed this particular question in children utilizing a prospective dataset, since previous studies were short-term. Ultimately, our information on body fat and SMM suggest that these outcomes could be used as important end-points to determine optimal protein intakes in adolescents, since there are essentially no studies in the literature determining protein requirements in children.[341]

In order to determine optimal protein requirements in adolescents, future research needs to evaluate other factors. Since our research results only involved girls, and there are known hormonal differences between girls and boys during puberty, resulting in boys acquiring more muscle mass at a faster rate than girls, future research should also evaluate the long-term effects of dietary protein in boys.[342] In addition, protein distribution across the day in this age group also needs to be assessed since it is the underlying idea behind morning protein

intake. This will help to further determine if protein requirements should stay as daily recommendations or if they should be meal-specific as some researchers have proposed.[148,162] Finally, longer randomized clinical trials should be conducted to test if ≥15 grams of protein in the morning (controlling for total protein) is beneficial to body composition outcomes. In particular, dose response studies evaluating 15, 20, 25, and 30 grams of dietary protein in the morning over longer-term are needed to be studied in a randomized clinical setting due to the difficulty of finding these higher ranges in a general adolescent population.

This study also added to the limited data in the literature of the effects of dietary protein intake in children on cardiometabolic outcomes.[85] Our results, suggested that body fat influenced outcomes. Consequently, future studies should account for body size when determining the effects of dietary protein intake on these outcomes. This could include evaluating these effects with protein expressed as grams per kilogram or grams per ideal body weight. Finally, these studies should include bigger sample size since quite a few girls in our prospective dataset were missing glucose and lipids and evaluate an at risk population of children (e.g. overweight children) to determine if consuming higher amounts of dietary protein had similar effects on cardiometabolic outcomes as our study.

The second study for this dissertation evaluated the effects eggs, a protein-rich food, on adiposity and various cardiometabolic outcomes in children. Using prospective data from the National Growth and Health Study (NGHS), we found that consumption of ≥ 3.5 eggs per week ages 9 to 17 was associated with lower percent body fat and a higher percent skeletal muscle during late adolescence after accounting for various potential confounders (including dietary confounders). These effects were stronger when girls were consuming a higher intake of eggs combined with healthier eating patterns. Furthermore, the risk for becoming overweight decreased when more eggs were consumed with higher intakes of fiber and fruits and vegetables. Finally, there was no evidence of a detrimental effect on egg consumption on cardiometabolic outcomes.

This study adds important longitudinal evidence to the literature that eggs are not detrimental to cardiometabolic outcomes in adolescents. As a result of using the NGHS dataset, which includes diet records for 8 of the 10 years, we were able to provide a more precise estimate of the effects of egg intake on adiposity and cardiometabolic outcomes in late adolescence, where the data is particularly sparse. Furthermore, we were able to provide novel findings that egg intake may

be beneficial on body composition outcomes potentially as a result of its protein content. Future research should include the evaluation of other protein foods on these outcomes, since protein foods can vary widely in their composition of fatty acids, micronutrients, other nutrients, and preparation and processing methods which all influence cardiometabolic risk.[343]

There are also some limitations in the current literature evaluating protein-rich foods such as eggs on cardiometabolic outcomes in adults. Studies in adults evaluating the effects of egg intake on increasing risk of type 2 diabetes (T2DM) or CVD often do not account adjust for dietary factors in the statistical models.[221] Furthermore, almost all the prospective studies evaluating the effects of egg consumption on risk of T2DM, use food frequency questionnaires,[228] which are often unable able to capture eggs in mixed dishes or baked items, resulting in an inaccurate estimation of egg intake. Finally, in light of recent data suggesting a benefit of egg-derived peptides on hypertension in animal models, long-term research is needed in humans to evaluate the effects of egg intake on blood pressure.[90] Therefore, the third and final study included in this dissertation investigated the effects of egg consumption on risk of T2DM and hypertension in

adults using the Framingham Offspring Study. These relations were studied independently and in the context of other healthy eating patterns.

Our study, which utilized diet records, found that consuming five or more eggs per week did not adversely affect fasting glucose or blood pressure in a healthy adult population. In fact, higher intake of eggs was associated with a modest reduction in fasting glucose and systolic blood pressure. Furthermore, there was a non-significant reduction in impaired fasting glucose and a significant reduction in risk of developing high blood pressure when consuming ≥5 eggs per week. These results were strengthened when combined with healthier eating patterns.

This study adds several novel findings to the literature. First, this study supports previous research suggesting eggs are not detrimental to glucose metabolism[219,225,230] and may in fact be beneficial, especially when consumed as part of healthy eating patterns. This is important since foods are not consumed in isolation; they are consumed as part of an overall diet. Further, this is the first study to our knowledge to prospectively study egg consumption on risk of high blood pressure. While our findings support the animal studies, further research

is needed to confirm our data that egg consumption reduces risk of developing high blood pressure.

Overall, our results call for future studies to investigate other protein based foods on cardiometabolic outcomes, particularly in children, where the data is extremely limited. Protein foods, such as eggs, are expressed as ounce-equivalent by the United States Department of Agriculture, with individual protein foods differing in digestion and absorption kinetics as well as amino acid profiles.[346] This makes it difficult to compare across protein sources. Since protein ounce equivalents are more similar in kilocalories as opposed to amount of protein, and these foods contain other important nutrients,[118,347] more research needs to be conducted studying the effects of different protein foods (e.g. animal vs. plant protein foods) on these outcomes. This is particularly true in children where the data is sparse.

There is clearly a need to carefully address potential confounders, particularly dietary confounders in epidemiological studies evaluating protein-based foods. This may help explain some of the discrepant findings between the clinical trial data and the epidemiological literature. Consequently, this may lead to the

modification of recommendations such as the caution associated with consumption of eggs and dietary cholesterol in individuals with T2DM due to an association with an increased risk of CVD. Since clinical studies have failed to show a link between increased dietary cholesterol consumption and adverse effects on glucose metabolism, residual confounding could be the reason for the association in epidemiological studies.[234]

In addition, further research is needed to determine the protein requirements children. For example, the Recommended Dietary Allowance for children ages 14 to 18 is based on adult estimates of requirements derived from nitrogen balance studies.[344] Furthermore, researchers have recently suggested that protein distribution and timing are factors that influence requirements, suggesting a need for meal-based protein recommendations to achieve optimal health.[53,148,253] Our longitudinal data in adolescents is the first step to moving towards evaluating the importance of meal-specific protein recommendations.

There is quite a bit of speculation in the literature on the benefits of protein timing in the literature, with little data to support this idea. In particular, there is a need to investigate these concepts in randomized clinical trials, particularly

involving mix meals, extending beyond several hours since short term outcomes such as satiety and muscle protein synthesis do not necessarily translate into long term outcomes such as body fat and lean mass. Ideally, these studies could be conducted using data that includes body composition assessments, as well as amino acids. Leucine has received much attention in the literature and more research should investigate the importance of this particular essential amino acid on the benefits of protein timing.[162] Finally, more research is needed on this topic with better assessments of physical activity (e.g. accelerometers) since physical activity impacts an individual's response to dietary protein consumption and the current literature often uses a questionnaire to assess physical activity.[151,345]

Finally, an important contribution of this dissertation is the observation that egg intakes on cardiometabolic outcomes in children and adults were modified by markers of a healthy diet (e.g. Dietary Approaches to Stop Hypertension eating pattern score, fiber, and fruits and vegetables). For example, the beneficial effects of egg consumption on body composition in adolescents, and fasting glucose and blood pressure in adults were consistently strengthened when combined with healthier eating patterns. These results support the most recent report from the 2015 *Dietary Guidelines Advisory Committee*, which emphasized a need to evaluate

the overall dietary pattern as opposed to just focusing on individual foods that decrease risk for chronic disease.[27]

In conclusion, our analyses provide longitudinal data supporting the beneficial effects of dietary protein on body composition in late adolescence. Furthermore, while there is speculation that morning protein intake is particularly beneficial to body composition, our research suggests that these effects may be partially explained by total protein intake. Moreover, regularly consuming eggs, a protein-rich food, has no adverse effect on cardiometabolic risk and may benefit body composition and related risk factors. In conclusion, the overall message of this dissertation is that total protein intake, including regular egg consumption, does not adversely affect cardiometabolic risk and may benefit body composition and cardiovascular risk factors. Future studies need to examine the longitudinal effects of other protein-based foods in carefully designed prospective studies, particularly evaluating these foods in the context of other eating patterns.

LIST OF JOURNAL ABBREVIATIONS

Acta Physiol (Oxf)	Acta Physiologica
Adv Exp Med Biol	Advances in Experimental Medicine and Biology
Adv Nutr	Advances in Nutrition
Am Heart J	American Heart Journal
Am J Cardiovasc Dis	American Journal of Cardiovascular Disease
Am J Clin Nutr	American Journal of Clinical Nutrition
Am J Epidemiol	American Journal of Epidemiology
Am J Hum Biol	American Journal of Human Biology : The Official Journal of the Human Biology Council
Am J Lifestyle Med	American Journal of Lifestyle Medicine
Am J Physiol Endocrinol Metab	American Journal of Physiology. Endocrinology and Metabolism
Am J Physiol Regul Integr Comp Physiol	American Journal of Physiology. Regulatory, Integrative and Comparative Physiology
Am J Public Health	American Journal of Public Health
Ann Intern Med	Annals of Internal Medicine
Annu Rev Nutr	Annual Review of Nutrition

Appl Physiol Nutr Metab	Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition et Metabolisme
Arch Intern Med	Archives of Internal Medicine
Arterioscler Thromb Vasc Biol	Arteriosclerosis, Thrombosis, and Vascular Biology
Biochim Biophys Acta	Biochimica et Biophysica Acta
BMC Med	BioMed Central Medicine
BMJ	British Medical Journal
BMJ open	British Medical Journal Open
Br J Clin Pharmacol	British Journal of Clinical Pharmacology
Br J Nutr	The British Journal of Nutrition
Clin Chem	Clinical Chemistry
Clin Exp Hypertens	Clinical and Experimental Hypertension
Clin Interv Aging	Clinical Interventions in Aging
Clin Lab Med	Clinics in Laboratory Medicine
Clin Lipidol	Clinical Lipidology
Clin Nutr	Clinical Nutrition
Crit Rev Food Sci Nutr	Critical Reviews in Food Science and Nutrition

Curr Atheroscler Rep	Current Atherosclerosis Reports
Curr Opin Clin Nutr Metab Care	Current Opinion in Clinical Nutrition and Metabolic Care
Curr Opin Lipidol	Current Opinion in Lipidology
Diabetes Metab Syndr Obes	Diabetes, Metabolic Syndrome and Obesity : Targets and Therapy
Eur J Clin Nutr	European Journal of Clinical Nutrition
Eur J Nutr	European Journal of Nutrition
Exerc Sport Sci Rev	Exercise and Sport Sciences Reviews
Food Funct	Food and Function
Food Nutr Bull	Food and Nutrition Bulletin
Food Nutr Res	Food and Nutrition Research
Health Aff (Millwood)	Health Affairs
Horm Res Paediatr	Hormone Research in Peadiatrics
Int J Cardiol	International Journal of Cardiology
Int J Food Sci Nutr	International Journal of Food Sciences and Nutrition
Int J Obes (Lond)	International Journal of Obesity
Int J Pediatr Endocr	International Journal of Pediatric Endocrinology

Int J Pediatr Obes	International Journal of Pediatric Obesity
J Acad Nutr Diet	Journal of the Academy of Nutrition and Dietetics
JAMA	Journal of the American Medical Association
JAMA Intern Med	Journal of the American Medical Association Internal Medicine
JAMA Pediatr	Journal of the American Medical Association Pediatrics
J Am Coll Cardiol	Journal of the American College of Cardiology
J Am Coll Nutr	Journal of the American College of Nutrition
J Am Diet Assoc	Journal of the American Dietetic Association
J Appl Physiol	Journal of Applied Physiology
J Clin Endocrinol Metab	Journal of Clinical Endocrinology and Metabolism
J Clin Lipidol	Journal of Clinical Lipidology
J Dairy Sci	Journal of Dairy Science
J Diabetes Complications	Journal of Diabetes and its Complications
J Hypertens	Journal of Hypertension

J Lipids	Journal of Lipids
J Nutr	Journal of Nutrition
J Nutr Educ Behav	Journal of Nutrition Education and Behavior
J Pediatr	Journal of Pediatrics
J Physiol	Journal of Physiology
Meat Sci	Meat Science
Med Sci Sports Exerc	Medicine and Science in Sports and Exercise
Metabolism	Metabolism: Clinical and Experimental
NCHS data brief	National Center for Health Statistics Data Brief
N Engl J Med	New England Journal of Medicine
Nutr J	Nutrition Journal
Nutr Metab (Lond)	Nutrition and Metabolism
Nutr Metab Cardiovasc Dis	Nutrition, Metabolism and Cardiovascular Diseases
Nutr Res	Nutrition Research
Nutr Res Rev	Nutrition Research Reviews
Nutr Rev	Nutrition Reviews

Obes Rev	Obesity Reviews : An Official Journal of the International Association for the Study of Obesity
Pediatr Obes	Pediatric Obesity
Physiol Behav	Physiology and Behavior
PLoS One	Public Library of Science One
Proc Nutr Soc	Proceedings of the Nutrition Society
Public Health Nutr	Public Health Nutrition

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CURRICULUM VITAE

Melanie Marie Mott, MS, RD
Year of birth: 1980
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EDUCATION

2017	Ph.D. Nutrition and Metabolism, Boston University School of Medicine, Boston MA; "Effect of Dietary Protein, Morning Protein, and Egg Intake on Cardiometabolic Outcomes at Different Ages"
2006	Dietetic Internship, Beth Israel Deaconess Medical Center, Boston MA; *Registered Dietitian (RD): #953215*
2005	Didactic Program in Dietetics, Ohio University, Athens, OH
2003	M.S. Athletic Training Education, Ohio University, Athens, OH
2002	B.S. Athletic Training (major), Nutrition (minor), East Carolina University, Greenville, NC

RESEARCH EXPERIENCE

1/2013-present	Ph.D. Candidate, Section of Preventive Medicine and Epidemiology, under the supervision of Dr. Lynn Moore, Boston University School of Medicine, Boston, MA
6/2012-1/2015	Research Assistant, Section of Preventive Medicine and Epidemiology, under the supervision of Andrea Coviello, Boston University School of Medicine, Boston, MA

6/2007-7/2011	Clinical Research Coordinator, Whitaker Cardiovascular Institute, under the supervision of Noyan Gocke, Boston University School of Medicine, Boston, MA
8/2006-5/2007	Clinical Research Coordinator, Department of Endocrinology, under the supervision of Shalender Bhasin, Boston Medical Center, Boston, MA

TEACHING EXPERIENCE

Fall 2016, Fall 2015 Spring 2015	Teaching Assistant, NU 620 "Clinical Nutrition Research", Boston University School of Medicine, Boston MA
Spring 2011, Fall 2011 Fall 2010, Fall 2009	Clinical Instructor, HS 410 "Human Physiology Internship", Boston University, Boston, MA
Spring 2005 (3 sections), Fall 2004 (2 sections)	Laboratory Instructor, HCFN 222L "Food Science Lab", Ohio University, Athens, OH
Winter 2005 (3 sections)	Laboratory Instructor, HCFN 120L "Meal Management Lab", Ohio University, Athens, OH

PROFESSIONAL MEMBERSHIPS & SERVICE

2005-present	Massachusetts Academy of Nutrition and Dietetics: Awards Chair (2014-present), Treasurer-elect/Treasurer (2010-2012), Nominating Committee (2009-2010), Annual Nutrition Convention & Exposition Poster Chair (2008-2012)
2005-present	The Academy of Nutrition and Dietetics' Research Dietetic Practice Group: Chief Editor of *The Digest*

	(2010-2011), Co-Editor of *The Digest* (2008-2010), Conference Coordinator for *The Digest* (2008)
2003-present	Academy of Nutrition and Dietetics (AND): Academy FNCE®2016 State Volunteer Committee (2016), Career Website Committee (2009-2010), New Member Value Committee (2007-2009), Student Representative for Student Council Advisory Committee (2005-2006)
2003-2005	Mid-Ohio Valley Dietetic Association: Continuing Professional Instruction Chair/Chair-Elect (2003-2005)

REFEREED PUBLICATIONS

1. **Mott MM**, Bradlee ML, Singer MR, Moore LL. Egg intake is associated with lower risks of impaired fasting glucose and high blood pressure in Framingham Offspring Study adults. *In Progress.*
2. **Mott MM**, Simonetti J, Coviello AD. Race-ethnic disparities in the prevalence and diagnosis of obesity in premenopausal women from a high risk, diverse urban population. *Under Review.*
3. **Mott MM**, Singer MR, Bradlee ML, Daniels SR, Moore LL. Egg intake as part of a DASH eating pattern is linked with lower body fat in later adolescence. *FASEB J*. 2016, 30. S1154.14.
4. **Mott MM** and Coviello AD. Race-ethnic disparities in reproductive dysfunction in women with and without polycystic ovary syndrome. *Curr Trends Endocinol.* 2016, 8. pp. 11-20.
5. Jones EJ, **Mott MM**, Coviello AD. Oral Presenter: Cardiovascular risk factors are underdiagnosed in women with a history of gestational diabetes. *J Cardiovasc Nurs.* 2016, 31. pp. 9-10.
6. Ferreira S, **Mott M**, Singer M, Bradlee ML, Daniels S, Moore L. Waist size in 9-11 year-old black and white girls predicts development of cardiometabolic risk over ten years. *FASEB J*. 2015, 29. S381.2.

7. **Mott MM**, Kitos NR, Coviello AD. Practice patterns in screening for metabolic disease in women with PCOS of diverse race-ethnic backgrounds. *Endocr Pract.* 2014, 20. pp. 855-63.

8. McDonnell ME, Ganley-Leal LM, Meta A, Bigornia SJ, **Mott M**, Rehman Q, Farb MG, Hess DT, Joseph L, Gokce N, Apovian CM, McDonnell. B lymphocytes in human subcutaneous adipose crown-like structures. *Obesity*. 2012, 20. pp. 1372-8.

9. Bigornia S, Farb MG, **Mott M**, Joseph L, Hess D, Apovian CM, Vita JA, Gokce N. Relation of depot-specific adipose inflammation to insulin resistance in human obesity. *Nutr Diabetes.* 2012, 2. pp. 1-6.

10. Farb MG**,** Ganley-Leal L**, Mott M**, Liang YM, Widlansky M, Bigornia SJ, Fiscale AJ, Apovian CM, Carmine B, Hess DT, Vita JA, Gokce N. Visceral fat impairs arteriolar function in human obesity. *Arterioscler Thromb Vasc Biol.* 2012, 32. pp. 467-473.

11. Farb MG, Bigornia S, **Mott M,** Tanriverdi K, Morin KM, Freeman JE, Joseph L, Hess DT, Apovian CM, Vita JA, Gokce N. Reduced adipose tissue inflammation represents an intermediate cardiometabolic phenotype in obesity. *JACC*. 2011, 58. pp. 232-237.

12. Gauthier MS, O'Brien EL, Bigornia S, **Mott M**, Cacicedo JM, Xu XJ, Gokce N, Apovian C, Ruderman N. Decreased AMP-activated protein kinase activity is associated with increased inflammation in visceral adipose tissue and with whole-body insulin resistance in morbidly obese humans. *Biochem Biophys Res Commun*. 2011, 404. pp. 382-387.

13. Hamburg NM, **Mott M**, Bigornia S, Vita J, Gokce N. Maladaptive arterial remodeling in severe obesity is reversed with weight loss. *Vasc Med.* 2010, 15. pp. 215-222.

14. Bigornia S, **Mott MM**, Hess D, Apovian C, McDonnell M, Duess MA, Kluge M, Fiscale A, Vita J, Gokce N. Long-term successful weight loss improves vascular endothelial function in severely obese individuals. *Obesity.* 2010, 18. pp. 754-759.

15. Apovian CM, Bigornia S, **Mott M**, Meyers MR, Ulloor J, Gagua M, McDonnell M, Hess D, Joseph L, Gokce N. Adipose macrophage infiltration is associated with insulin resistance and vascular

endothelial dysfunction in obese subjects. *Arterioscler Thromb Vasc Biol*. 2008, 28. pp. 1654-1659.

INVITED PRESENTATIONS

1. **Mott MM.** Disparities in screening rates for metabolic disease in women with PCOS from a high risk urban population. The Endocrine Society's Minority Affairs Community Forum. June 2013. San Francisco, CA.

ABSTRACT PRESENTATIONS

1. **Mott MM**, Singer MR, Bradlee ML, Daniels SR, Moore LL. Egg intake as part of a DASH eating pattern is linked with lower body fat in later adolescence. Experimental Biology. April 2016. San Diego, CA. *Selected for the Emerging Leader in Nutrition Science Poster Competition.*
2. Jones EJ, **Mott MM**, Coviello AD. Racial-ethnic disparities in cardiovascular risk in women with a history of gestational diabetes. Preventive Cardiovascular Nurses Association's Annual Symposium. April 2015. Anaheim, CA. *Selected for a moderated poster session.*
3. Jones EJ, **Mott MM**, Coviello AD. Cardiovascular risk factors are under-diagnosed in women with a history of gestational diabetes. Preventive Cardiovascular Nurses Association's Annual Symposium. April 2015. Anaheim, CA. *Selected as one of two outstanding abstracts for oral presentation*.
4. Ferreira SE, **Mott M**, Singer MR, Bradlee ML, Daniels SR, Moore LL. Waist size in 9-11 year-old black and white girls predicts development of cardiometabolic risk over ten years. Experimental Biology. March 2015. Boston, MA. *Finalist for American Society of Nutrition's 2015 Emerging Leaders in Nutrition Competition & selected for oral presentation.*
5. **Mott MM**, Singer MR, Bradlee ML, Moore LL. Long-term impact of

egg consumption on lipid and glucose levels in healthy adults. Obesity Week. November 2014. Boston, MA.

6. Kitos NR, **Mott MM**, Willard DL, Ursino-Toraldo M, Coviello AD. Race-Ethnic disparities in cardiovascular risk in premenopausal women from a high-risk, urban population. The International Congress on Endocrinology/The Endocrine Society Meeting. June 2014. Chicago, IL. *Selected for Poster Preview Presentation.*
7. Willard DL, **Mott MM**, Kitos NR, Coviello AD. Practice patterns in the diagnosis of PCOS: low testing rates for other disorders with similar clinical presentations. The International Congress on Endocrinology/The Endocrine Society Meeting. June 2014. Chicago, IL.
8. Coviello AD, **Mott MM**, Kitos NK. Premenopausal black women with PCOS are disproportionately affected by reproductive dysfunction compared to other race-ethnic groups. 16th World Congress of Gynecological Endocrinology. March 2014. Firenze, Italy. *Selected for oral presentation.*
9. Glazer NL, Hwang S, **Mott MM**, Ramachandran V, O'Connor G, Coviello AD. Higher cortisol is associated with disordered glucose metabolism in middle age men and women in the Framingham Offspring Study. Obesity Week. November 2013. Atlanta, GA.
10. **Mott MM**, Kitos NR, Simonetti J, Glazer NL, Coviello AD. Obesity is underestimated by health care providers in younger women: racial-ethnic disparities in the diagnosis of obesity. Obesity Week. November 2013. Atlanta, GA. *Diversity Section Abstract Award.*
11. Willard Devina, **Mott MM**, Kitos NR, Glazer NL, Coviello AD. TSH Screening in Women Being Evaluated for Polycystic Ovary Syndrome (PCOS). Evans Days – Boston University Department of Medicine. October 2013. Boston, MA.
12. **Mott MM**, Glazer NL, Coviello A. Testosterone but not DHEAS is positively correlated with liver transaminases in women with PCOS. The Endocrine Society Meeting. June 2013. San Francisco, CA.
13. **Mott MM**, Glazer NL, Coviello A. Disparities in screening rates for metabolic disease in women with PCOS from a high risk

underserved urban population. The Endocrine Society Meeting. June 2013. San Francisco, CA. *Selected for the Presidential Poster Competition*.

14. **Mott MM**, Kitos NR, Glazer NL, Coviello A. Racial-ethnic disparities in the prevalence of obesity and reproductive dysfunction in women with PCOS from a diverse high risk urban population. American Association of Clinical Endocrinologists Annual Scientific and Clinical Congress. May 2013. Phoenix, AZ.
15. **Mott MM**, Glazer NL, Coviello A. Screening practices for metabolic disease in women with PCOS at BUMC. Evans Days – Boston University Department of Medicine. October 2012. Boston, MA.
16. Farb MG, Gangley-Leal L, **Mott M**, Liang YM, Widlansky M, Bigornia SJ, Fiscale AJ, Apovian CM, Carmine B, Hess DT, Vita JA, Gokce N. Arteriolar function in visceral adipose tissue is impaired in human obesity. American Heart Association Scientific Sessions. November 2011. Orlando, FL. *Selected for oral presentation.*
17. Bigornia S, Farb MG, **Mott M**, Joseph L, Hess D, Apovian CM, Vita JA, Gokce N. Association between insulin resistance and macrophage-mediated inflammation in subcutaneous and visceral fat depots in obese individuals. Obesity Society Meeting. October 2010. San Diego, CA.
18. **Mott M**, Bigornia S, Farb MG, Tanriverdi K, Morin K, Freedman J, Joseph L, Apovian CM, Vita JA, Gokce N. Increased waist circumference and triglycerides are linked to adipose tissue inflammation in normal weight individuals. Obesity Society Meeting. October 2010. San Diego, CA.
19. Bigornia S, **Mott M**, Tanriverdi K, Freeman J, Joseph L, Apovian CM, Vita JA, and Gokce N. Reduced adipose tissue inflammation represents an intermediate cardiometabolic phenotype in obesity. American Heart Association Scientific Sessions. November 2009. Orlando, FL.
20. Gauthier MS, O'Brien E, Bigornia S, **Mott MM**, Gokce N, Apovian C, and Ruderman N. Decreased AMP-activated protein kinase is associated with markers of inflammation and infiltration of immune cells in visceral and subcutaneous adipose tissue, and with whole-body insulin resistance in obese patients. The Obesity Society

Meeting. October 2009. Washington, D.C.

21. Bigornia S, **Mott MM**, Tanriverdi K, Freedman J, McDonnell M, Apovian CM, and Gokce N. Directional weight change modulates adipose tissue inflammatory phenotype and metabolic syndrome components. The Obesity Society Meeting. October 2009. Washington, D.C.
22. Rubin D, Liang MY, Bigornia S, **Mott M,** Gokce N, Nikolajczyk B, Apovian C, Gangley-Leal L, and McDonnell M. Human B cell toll-like expression in insulin resistance. The American Diabetes Association Scientific Meeting. June 2009. New Orleans, LA.
23. Bigornia S, **Mott M**, Tanriverdi K, Morin K, Apovian C, Mcdonnell M, Hess D, Freedman J, and Gokce N. Hyperinsulinemia is associated with proatherogenic adipose gene expression in obese subjects. The Obesity Society Meeting. October 2008. Phoenix, AZ.
24. **Mott M**, Bigornia S, Tanriverdi K, Morin K, Apovian C, Vita J, Freedman J, and Gokce N. Downregulation of adipose mitochondrial genes is associated with impaired glycemic control in obese subjects. The Obesity Society Meeting. October 2008. Phoenix, AZ.
25. Hamburg N, **Mott M**, Bigornia S, Apovian CA, Hess D, Vita JA, and Gokce N. Arterial remodeling in obesity is reversed with weight loss. Society for Vascular Medicine Meeting. May 2008. Minneapolis, Minnesota.
26. **Mott MM**, Bigornia S, Meyers MR, Joseph L, McDonnell M, Hess D, Apovian C, and Gokce N. Adipose macrophage infiltration is associated with proinflammatory gene expression and systemic vascular endothelial dysfunction in obese subjects. Evans Days – Boston University Department of Medicine. November 2007. Boston, MA.
27. Bigornia S, **Mott MM**, Meyers M, Vita J, Apovian C, Hess D, and Gokce N. Long-term weight loss improves vascular endothelial function in the extremely obese. North American Association for the Study of Obesity Meeting. October 2007. New Orleans, LA.
28. List EO, Berryman DE, Kohn DT, Palmer AP, **Mott MM,** Valente A,

Alosio W, and Kopchick JJ. Reversal of diet-induced obesity and diabetes in mice: Comparison of a low fat verses low carbohydrate diet. The Endocrine Society Meeting. June 2005. San Diego, CA.

29. Palmer AP, Berryman, DE, List EO, Kohn DT, **Mott MM**, Gosney E, and Kopchick JJ. Macrophage infiltration in adipose tissue: Influence on diet and weight loss. The Endocrine Society Meeting. June 2005. San Diego, CA.
30. **Mott MM,** Berryman DE, List EO, Kohn DT, Palmer AP, and Kopchick JJ. Comparison of adiposity and adipocytokines profiles in mice fed low fat, low carbohydrate, or high fat diets. The Endocrine Society Meeting. June 2005. San Diego, CA.