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The Relationship of The Mediterranean Diet and the Dietary Approaches to Stop Hypertension (DASH) Style Diet With Cardiometabolic Health

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THE RELATIONSHIP OF THE MEDITERRANEAN DIET AND THE DIETARY APPROACHES TO STOP HYPERTENSION (DASH) STYLE DIET WITH CARDIOMETABOLIC HEALTH

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DEDICATION

This dissertation is dedicated to my wife, HeeJin and kids, Eddie Jiho, Clara Jimin, and Olivia Seyoung who have been a great and everlasting source of support and encouragement in my academic journey. I am truly thankful for having all of you in my life. I also dedicate this dissertation to my mother-in-law and father-in-law who have always given me the solid and substantial help, as well as to my mother and father who have loved me unconditionally and encouraged me faithfully throughout my life.

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ABSTRACT

Introduction: Much evidence shows that the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diet are associated with risk reduction of cardiometabolic disease as well as lower risk of all-cause, cardiovascular disease (CVD), and cancer mortality. A subgroup of obese individuals (metabolically healthy obese (MHO) phenotype) and normal weight individuals (metabolically obese normal weight (MONW) phenotype) have been identified to have different cardiometabolic risks compared with their counterparts in the same category of body mass index (BMI). Scarce evidence exists demonstrating a relationship between adherence to Mediterranean diet or DASH style diets with MHO and MONW phenotypes, or on the role of MHO and MONW phenotypes as important effect modifiers of the relationship between Mediterranean and DASH style diets with mortality risk. In addition, it is unclear how much the adjustment for adiposity modifies or attenuates the association of the Mediterranean diet and DASH style diet with cardiometabolic risk.

Methods: Data from adults aged 20-90 years were analyzed from participants of the National Health and Nutrition Examination Survey III, 1988–1994 with its mortality file linked until December 31, 2011. Mediterranean diet scores (MDS) and DASH index were calculated using food frequency questionnaires and the 24-hr dietary recall data. MHO and MONW individuals were identified using the criterion including high fasting glucose, insulin resistance, blood pressure, triglycerides, C-reactive protein, and low high-density lipoprotein-cholesterol. For aim 1, multiple logistic regression analyses

were conducted to generate odds ratios (ORs) and 95% confidence intervals (CIs) to estimate the associations of tertiles of MDS and DASH index with the presence of MHO and MONW phenotypes. For aim 2, multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% CIs of mortality across the tertiles of MDS and DASH index and 1-SD increment of MDS and DASH index. For aim 3, multiple linear regression analyses were applied using traditional and causal mediation analysis to estimate the regression coefficients between diet score, obesity parameters, and markers for insulin resistance and inflammation.

Results: For aim 1, higher MDS was associated with higher odds of MHO phenotype (tertile 3 vs 1; OR, 2.57 [95% CI, 1.04-6.35]; P trend = 0.04), whereas higher DASH index was associated with lower odds of MONW phenotype (tertile 3 vs 1; OR, 0.59 [95% CI, 0.38-0.93]; P trend = 0.03) only in the younger age group, after adjusting for potential confounders. For aim 2, in MHO individuals, the multivariable-adjusted HR of all-cause mortality in the highest tertile compared to the first tertile of MDS was 0.44 (95% CI, 0.26-0.75; P for trend <0.001). The corresponding HR was 0.23 (95% CI, 0.02-2.10; P for trend = 0.03) for cancer mortality. A 5-point increment in the adherence to MDS was associated with a 43% reduction in the risk of all-cause mortality (HR, 0.59; 95% CI, 0.37-0.94). However, no risk reduction of all-cause, CVD, and cancer mortality was found in MUO phenotype. In MONW individuals, 1-SD increment in the adherence to DASH style diet was significantly associated with 23% reduction in the risk of all-cause mortality (HR, 0.77 [95% CI, 0.66-0.90]), after adjustment for potential confounders. The corresponding HRs for CVD mortality were 0.70 (95% CI, 0.53-0.93). However, no association was observed in MHNW phenotype**.** For aim 3, waist

circumference mediated the association of MDS with log insulin, log HOMA-IR, fasting glucose, post-load glucose, HbA1c, log hs-CRP, white blood cell, and fibrinogen, with proportion of mediation ranging from 14.4% to 42.3%. In addition, the mediated effects of waist circumference were greater than those of BMI consistently in all markers in both traditional and causal mediation analysis. However, no mediation effect by adiposity was observed in the association of DASH style diet with the markers for insulin resistance and inflammation.

Conclusions: Adherence to Mediterranean diet or DASH style diet was associated with MHO and MONW phenotypes only in the younger age group, suggesting that potential dietary intervention to prevent cardiometabolic disease may be different by age group. Adherence to a Mediterranean dietary pattern appears to improve longevity in the MHO phenotype within an obese population, whereas higher DASH index was associated with a lower risk of mortality in MONW adults. Waist circumference mediated the association of the Mediterranean diet with insulin resistance and inflammation to a greater extent than BMI, suggesting that lowering abdominal obesity may be one of the pathways through which the Mediterranean diet reduces insulin resistance and inflammation.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

BMI ..... Body Mass Index

DASH..... Dietary Approaches to Stop Hypertension

DBP.....Diastolic Blood Pressure

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

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

Hs-CRP ..... High-sensitivity C-Reactive Protein

MDS.....Mediterranean Diet Scores

MHNW .....Metabolically Healthy Normal Weight

MHO .....Metabolically Healthy Obese

MONW ..... Metabolically Obese Normal Weight

MUO .....Metabolically Unhealthy Obese

SBP ..... Systolic Blood Pressure

WC ..... Waist Circumference

CHAPTER 1

INTRODUCTION

1.1 Statement of problem

Obesity is associated with an increased risk of type 2 diabetes and cardiovascular disease (CVD) (Guh et al. 2009), but not all obese individuals are equally affected. Thus, even in the same category of body mass index (BMI), a subgroup of obese individuals with normal metabolic characteristics has been identified as "Metabolically Healthy Obese (MHO)", compared with "Metabolically Unhealthy Obese (MUO)" (Kim, Kim, et al. 2014, Primeau et al. 2010, Karelis et al. 2004). In the same context, a subgroup of normal weight individuals who are susceptible to metabolic abnormalities due to their unfavorable body composition has been designated as "Metabolically Obese Normal Weight (MONW)" [also referred to as "Metabolically Unhealthy Normal Weight (MUNW)"], compared with their metabolically normal counterparts with the same normal category of BMI representing "Metabolically Healthy Normal Weight (MHNW)" (Kim, Han, et al. 2014, Choi et al. 2013, Lee et al. 2011, Yajnik and Yudkin 2004, Ruderman, Schneider, and Berchtold 1981). These metabolic phenotypes are determined by complex interaction between gene, environment, and life style characteristics (Teixeira et al. 2015, Samocha-Bonet et al. 2014, Oliveros et al. 2014, Phillips 2013).

It is well known that the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diets are associated with lower risk of cardiometabolic

disease and CVD mortality in both observational and intervention studies (Estruch et al. 2013, Azadbakht 2013, Kastorini et al. 2011, Sofi et al. 2008). Underlying mechanisms for cardiovascular health benefits of Mediterranean diet and DASH style diets are complex, but can be explained by the improvement of metabolic profiles including insulin resistance, lipid profiles, blood pressure, and inflammatory markers (Grosso et al. 2014). Therefore, it is plausible that adherence to Mediterranean diet and DASH style diets might be associated with a metabolically heathy phenotype. However, few studies have investigated the relationship of the Mediterranean diet and DASH style diet with MHO phenotype or MONW phenotype.

The Mediterranean diet and DASH style diets seem to be independently associated with cardiometabolic risk after adjusting for adiposity (Kastorini et al. 2010). However, it is unclear how much the adjustment for adiposity modifies or attenuates the association of the Mediterranean diet and DASH style diet with cardiometabolic risk. Using mediation analysis, the role of adiposity underlying the relationship of the Mediterranean diet and DASH style diet with cardiometabolic risk could be clarified. However, no study has explored the mediator role of adiposity on the relationship of the Mediterranean diet and DASH style diet with cardiometabolic risk.

To address this gap in the literature, we will evaluate the association of the Mediterranean diet and DASH style diet with MHO phenotype, MONW phenotype, and mortality risk, while examining adiposity as a potential mediator, using the National Health and Nutrition Examination Survey (NHANES) III and its linked mortality file.

1.2 Purpose and objectives

In this dissertation, the following specific aims will be examined using the National Health and Nutrition Examination Survey (NHANES) III and its linked mortality file.

Aim 1: Is the adherence to the Mediterranean diet or DASH style diet associated with metabolic health in both normal weight and obese populations?

Hypothesis 1.1: The adherence to the Mediterranean diet or DASH style diet will be positively associated with metabolic health in a representative US population.

Hypothesis 1.2: The adherence to the Mediterranean diet or DASH style diet will be associated to a greater extent with the MHO phenotype in a representative US population.

Hypothesis 1.3: The adherence to the Mediterranean diet or DASH style diet will be associated to a lesser extent with the MONW phenotype in a representative US population.

Aim 2: Does the adherence to the Mediterranean diet or DASH style diet reduce all-cause, CVD, and cancer mortality risk in the individuals with unfavorable metabolic phenotype in both normal weight and obese population?

Hypothesis 2.1: The adherence to the Mediterranean diet or DASH style diet will be associated with lower all-cause, CVD, and cancer mortality risk in the NHANES III follow-up cohort.

Hypothesis 2.2: The association between adherence to the Mediterranean diet or DASH style diet and lower mortality will be modified by MUO and MONW phenotypes in the NHANES III follow-up cohort.

Aim 3: Is adiposity a mediator of the influence of the Mediterranean diet or DASH style diet on the cardiometabolic risk?

Hypothesis 3: The relation of the adherence to the Mediterranean diet or DASH style diet with cardiometabolic risk will be mediated by adiposity in a representative US population.

1.3 Significance and relevance of the dissertation research

Not all obese individuals are at an increased risk of metabolic disease (Karelis et al. 2004). Therefore, consideration of differential characteristics of subgroups of obese individuals may need to be considered in recommending guidelines for disease prevention or early treatment of obesity. Stratification of obese individuals based on their metabolic phenotype may be crucial in determining appropriate obesity management strategies (Primeau et al. 2010). Furthermore, a better understanding of metabolically healthy obese (MHO) phenotype will be helpful to develop targeted preventative strategies and evidence based public health approaches to reduce obesity-related morbidity and mortality, and associated medical costs (Stefan et al. 2008).

A number of meta analyses have documented the association of adherence to the Mediterranean diet and DASH style diets with reduced risk of metabolic syndrome (Kastorini et al. 2011) and type 2 diabetes (Koloverou et al. 2014), in addition to modification of cardiovascular risk factors (Nordmann et al. 2011) and decreased inflammation and improved endothelial function (Schwingshackl and Hoffmann 2014). In addition, a systematic review suggested that there is a possible role of the adherence to Mediterranean diet in preventing overweight and obesity (Buckland, Bach, and Serra-Majem 2008). However, the weakness of previous studies is not considering the

differential characteristics of a subgroup of obese individuals. Only one study found that higher dietary quality is positively associated with MHO phenotype in middle to old aged population, but it was not based on Mediterranean diet and DASH style diet. Additional weaknesses of this study were restricted age range and cross-sectional study design (Phillips et al. 2013). Our study will overcome these weaknesses using a nationally representative data that has both cross-sectional and longitudinal characteristics and a valid definition of metabolically healthy phenotype.

While the inverse association of Mediterranean diet and the DASH style diets with all-cause mortality and cardiovascular mortality is well-known (Sofi et al. 2008), no other studies have explored the role of metabolic health as an important effect modifier of the relationship of Mediterranean diet and the DASH style diets with all-cause and CVD mortality. Consequently, this investigation will reveal how Mediterranean diet and the DASH style diets interact with MHO phenotype and MONW phenotype to predict all-cause and CVD mortality.

Research on methods for mediation analysis is a fast growing field in epidemiology (Richiardi, Bellocco, and Zugna 2013). Mediation analysis helps us to examine the mechanisms underlying an observed relationship between exposure and outcome, and investigate how they relate to a mediator. By exploring the role of adiposity as a mediator, we will be able to better understand the nature of the association of Mediterranean diet or DASH style diet with cardiometabolic risk and cardiovascular death. This dissertation is innovative because this approach has not been used to examine this particular hypothesis. Moreover, causal mediation analysis is a novel approach to determine controlled direct effect, natural direct effect, and natural indirect effect using

counterfactual approaches in which unbiased valid estimates of direct effect and indirect effect can be obtained (VanderWeele 2009).

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CHAPTER 2

BACKGROUND

2.1 Characteristics of Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diet

2.1.1 Mediterranean diet

The Mediterranean diet, representing the dietary pattern in the populations neighboring the Mediterranean Sea, has been documented as a model for healthy eating (Willett et al. 1995), and is characterized by high intake of olive oil (high monounsaturated/saturated fat), legumes, grains (including bread and potatoes), fruits, and vegetables; moderate intake of milk and dairy products, alcohol; and low intake of meat and meat products (Trichopoulou et al. 2003).

It has been reported that adherence to the Mediterranean diet is related to a reduced risk of cardiovascular morbidity and mortality. Meta-analysis of 12 prospective cohort studies with 1,574, 299 subjects followed from three to 18 years showed that an increase in the adherence score was significantly associated with a reduced risk of mortality (pooled relative risk (RR) 0.91, 95% confidence interval (CI) 0.89 to 0.94) and cardiovascular mortality (pooled RR 0.91,0.87 to 0.95) (Sofi et al. 2008). A recently published multicenter trial of 7,447 individuals in Spain showed that both groups assigned to a Mediterranean diet with extra-virgin olive oil and a Mediterranean diet with

nuts had a reduced incidence of major cardiovascular events (HR 0.70, 0.54 to 0.92; 0.75, 0.54 to 0.96, respectively) (Estruch et al. 2013).

The adherence to Mediterranean diet is known to be related to an improvement in cardiovascular risk factors and inflammatory profiles. Meta-analysis of 6 randomized controlled trials (RCTs) comparing Mediterranean to low-fat diets in 2,650 overweight/obese subjects showed that those assigned to a Mediterranean diet had more favorable changes in cardiovascular risk factors and inflammatory markers including BMI, systolic blood pressure (BP), diastolic BP, fasting plasma glucose, total cholesterol, and high sensitivity C-reactive protein (hs-CRP) (Nordmann et al. 2011). In addition, a meta-analysis of 17 RCTs including 2300 individuals showed that high adherence to a Mediterranean diet is related to decreased inflammation and improved endothelial function such as decreased hs-CRP, decreased interleukin-6 (IL-6), decreased intracellular adhesion molecule-1, increased flow mediated dilatation, and increased adiponectin) (Schwingshackl and Hoffmann 2014).

It has been documented that the adherence to Mediterranean diet is related to a reduced risk of metabolic syndrome and type 2 diabetes. A meta-analysis of 50 clinical trials and observational studies including 534,906 individuals showed that the adherence to Mediterranean diet is associated with reduced risk of metabolic syndrome (log hazard ratio (HR): -0.69, -1.24 to -1.16) and its components (Kastorini et al. 2011). In addition, a meta-analysis of 10 clinical trials and prospective cohort studies including 136,846 subjects showed that higher adherence to the Mediterranean diet was associated with a reduced risk of developing type 2 diabetes (combined RR 0.77, 0.66 to 0.89) (Koloverou et al. 2014).

There have been different approaches to evaluate the adherence to Mediterranean diet (Bach et al. 2006). Created by positive or negative scoring of the components, 'Mediterranean Diet Score' (MDS), is the most largely used index since it is easy to apply. Basically a score from 0 to 1 was assigned to the daily intake of each component of Mediterranean Diet in which the sex-specific medians of the sample were used as cut-off points (Costacou et al. 2003). A point was given if the subject's intake was over the sample median for the protective components such as legume, grains, vegetables and fruits below the median for non-protective components such as meat and dairy products. Therefore, MDS ranged 0 for minimal adherence to 8 for maximum adherence if the index had eight components. Usually, a score of 4 or more has been associated with adequate MDP adherence and better health outcomes. Another approaches to calculate the adherence to Mediterranean diet include an adherence index that adds or subtracts standardized components and a "Mediterranean Adequacy Index" based on a ratio between components were used (Bach et al. 2006).

2.1.2 The Dietary Approaches to Stop Hypertension (DASH) style diet

The DASH style diet that was originally developed to prevent hypertension has been reported as a healthy eating pattern (Champagne 2006, Appel et al. 1997). It is characterized by high intake of fruits, vegetables, nuts and legumes, low fat dairy products, and whole grains; and low intake of fat (total/saturated), sodium, sweets, and red meats (Sacks et al. 2001).

It has been reported that the adherence to the DASH style diet is related to a reduced risk of cardiometabolic abnormalities. A meta-analysis of 6 prospective cohort studies with 260,011 subjects showed that the DASH style diet reduced the incidence of

CVDs (RR 0.80, 95%CI, 0.74 to 0.86), CHD (RR 0.79, 0.71 to 0.88), stroke (RR 0.81, 0.72 to 0.92), and heart failure (RR 0.71, 0.58 to 0.88) (Salehi-Abargouei et al. 2013). In addition, a meta-analysis of 9 RCTs showed that the DASH style diet reduced fasting insulin (mean difference -0.15, -0.22 to -0.08; $P < 0.001$) (Shirani, Salehi-Abargouei, and Azadbakht 2013).

There have been different approaches to evaluate the adherence to the DASH diet index based on the composition of food and nutrients (Miller et al. 2013): 1) Mellen's index is a completely nutrient-based DASH index with 9 components in which a better adherence is related to higher levels of protein, fiber, magnesium, calcium, and potassium, or lower levels of total fat, saturated fat, sodium, and cholesterol) (Mellen et al. 2008); 2) Fung's index that is composed of 7 food groups and one nutrient with scoring system of quintile rankings using eating guide developed by the National Heart, Lung and Blood Institute (Fung et al. 2008); 3) Dixon's index that is comprised of 8 food groups and one nutrient evaluating the adherence to the DASH Eating Plan in the 2005 Dietary Guidelines for Americans (Dixon et al. 2007); and 4) Günther's index that is a food-based index with 10 components evaluating the adherence to the DASH Eating Plan in the 2005 Dietary Guidelines for Americans (Gunther et al. 2009).

2.2 Characteristics of MHO phenotype and MONW phenotype

The risk of developing obesity-related complications corresponds to the degree of obesity (Guh et al. 2009). However, the existence of these obesity-related metabolic abnormalities varies extensively among obese individuals (Karelis et al. 2004). In addition, although BMI is widely used as a simple measure of defining obesity and shows

a reliable association with adverse metabolic outcomes, it does not necessarily correlate with adiposity.

Despite being outwardly obese, a subset of obese individuals appears to be protected or more resistant to the development of metabolic abnormalities associated with obesity. These individuals with MHO phenotype, namely benign or uncomplicated obesity demonstrate several favorable metabolic characteristics such as high insulin sensitivity, relatively low visceral fat, and no sign of dyslipidemia or hypertension (Kim, Kim, et al. 2014, Primeau et al. 2010, Karelis, Brochu, and Rabasa-Lhoret 2004). A few studies investigating the effect of lifestyle intervention on MHO phenotype consistently conclude that there is a potential benefit of distinguishing MHO phenotype and MUO phenotype.

In addition, some non-obese subjects with MONW phenotype might be susceptible to insulin resistance, type 2 diabetes and CVD even though they are not outwardly obese (Ruderman, Schneider, and Berchtold 1981). Furthermore, MONW subjects display lower physical activity energy expenditure, a higher level of visceral adiposity, impaired insulin sensitivity, and a more atherogenic lipid profile, which together contribute to an increased risk of cardiometabolic disease (Kim, Han, et al. 2014, Choi et al. 2013, Lee et al. 2011, Yajnik and Yudkin 2004).

Meig et al. (Meigs et al. 2006) proposed two different definitions for ‘metabolically healthy’ phenotype: one is absence of metabolic syndrome components defined by the updated criteria from the Adult Treatment Panel III; the other one is absence of insulin resistance defined by being more than 75 percentile of HOMA-IR (homoeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose

(mg/dl) x fasting insulin (lU/mL)/405).Individuals without metabolically healthy profiles within the same category of BMI were designated as the 'metabolically unhealthy' phenotype. Wildman et al. (Wildman et al. 2008) defined 'metabolically healthy' phenotype as having 0 or 1 component among six cardiometabolic abnormalities. Karelis and Rabasa-Lhoret (Karelis and Rabasa-Lhoret 2008) also proposed a similar definition of 'metabolically healthy' phenotype as having 0 or 1 component among five cardiometabolic abnormalities (table 2.1).

In normal weight population, the individuals who had over the highest tertile of percent body fat were designated as 'Normal Weight Obesity' that was also regarded as metabolically unhealthy phenotype (Romero-Corral et al. 2010).

2.3 Relationship between diet and metabolic health

Several studies have explored the association between dietary factors and metabolic phenotype. However, these reports have been limited to MHO phenotype (Camhi et al. 2015), without considering dietary pattern (Manu et al. 2012), or with specific age groups (Hankinson et al. 2013). A few studies have reported associations in MONW phenotype in non-US populations (Choi et al. 2012), but with limited generalizability (Suliga et al. 2015). Several studies demonstrated that dietary factors are related to metabolic phenotype in both obese and normal weight population (Phillips et al. 2013), but only focused on women (Kimokoti et al. 2014), or single foods such as olive oil (Gutierrez-Repiso et al. 2014).

A study in a multi-ethnic group of obese Americans aged 40–59 years showed that there was no difference in total energy or dietary macro/micro nutrient intake between MHO and MUO (Hankinson et al., 2012). However, there was a limitation of a

small sample size (n=149 for MHO; 19% of obese group) in this study. A study on US adults aged 20-79 years using the NHANES 1999-2004 showed that MHO, MUO, and MHNW males had similar energy intake and diet composition of micronutrients. However, MHO females consumed less fiber (Manu et al., 2012). A study in Ireland of adults aged 45-74 years showed that MHO and MUO individuals had similar total calorie intake, dietary macronutrient composition and dietary quality. However, it was shown that MHO individuals had better compliance with food pyramid recommendations (Phillips et al., 2013). A study on Korean adults > 20 years showed that MONW individuals had higher carbohydrate intake than MHNW individuals (Choi et al., 2012).

2.4 Mediation analysis

In epidemiological studies it is often necessary to unravel the pathways linking an exposure to an outcome (Richiardi, Bellocco, and Zugna 2013). Usually, confounding or mediator variables in epidemiologic studies are controlled by multivariate methods such as multivariable linear or logistic regression, depending on the objectives of the study and/or whether the dependent variable is continuous or dichotomous. Mediation analysis is a statistical procedure that can be used to explain the process underlying the relationship between the exposure and the outcome, and the extent to which this relationship can be mediated by a third variable (Baron and Kenny 1986).

Commonly the purpose of mediation analysis is to determine the total effect of the exposure on the outcome, the effect of the exposure that performs through a given set of mediators of interest (indirect effect: shown as an indirect pathway with a broken arrow in Figure X) and the effect of the exposure unexplained by the same mediators (direct

effect: shown as a direct pathway with a solid arrow in Figure X) (Figure 2.1) (Richiardi, Bellocco, and Zugna 2013).

Baron and Kenny proposed the following criteria to consider mediation: 1) the exposure variable is significantly related to the mediator; 2) the mediator is significantly related to the outcome variable; 3) the exposure variable is significantly related to the outcome variable; and 4) the association between the exposure and outcome variable is no longer significant when the mediator is included in the regression model (Baron and Kenny 1986). However, the third and fourth criteria have been called into question by other researchers.. For the third criteria, the effect of the exposure variable on the outcome variable may not be significant when direct and mediated effects have opposite sign. For the fourth criteria, when mediation is not complete, the path from the exposure to the outcome may still be significant even though the effect of the exposure on the outcome would be decreased (Valeri and Vanderweele 2013).

2.4.1 Traditional approach to mediation analysis

The approach to mediation analysis proposed by Baron and Kenny focused on comparing two regression models in which one model was conditioned on the mediator and the other one was not. The exposure coefficient in the regression models would be interpreted as a direct effect in the model adjusted for the mediator, and as a total effect in the model unadjusted for the mediator (Richiardi, Bellocco, and Zugna 2013). In detail, the first step consists of regressing the outcome on the exposure and confounding factors to estimate the overall effect of exposure. In the next step, the mediator is also controlled to estimate the direct effect of exposure. Finally, the indirect effect by the mediator is estimated by calculating a) the difference of estimates between the coefficient of direct

effect and the coefficient of the overall effect (difference method); or b) the product of the coefficient of the path from exposure to mediator and the coefficient of the path from mediator to outcome (product method) (Baron and Kenny 1986).

In addition, the structural equation modeling (SEM) focused on the path analysis has been used to estimate the direct and indirect effects by modeling covariance and correlation matrices (Valeri and Vanderweele 2013).

2.4.2 Causal or counterfactual approach to mediation analysis

The traditional approach to evaluate mediation tends to produce a bias due to incorrect statistical analysis and study design by not considering mediator-outcome confounding, exposure-mediator interaction, and mediator-outcome confounding affected by the exposure (Richiardi, Bellocco, and Zugna 2013).

As unmeasured exposure-outcome confounders can generate confounding bias of estimates of total causal effects, unmeasured mediator-outcome confounders can generate bias of estimates of direct and indirect effects. This issue is also considered as a collider bias when the mediator is included as a covariate and mediator-outcome confounders were not adjusted simultaneously in a regression model (Figure 2.2) (Cole and Hernan 2002) (VanderWeele 2009).

In the traditional approach to mediation analysis, the direct effect is estimated by conditioning on the mediator. When the estimates of direct effect are different across various levels of the mediator, there should be an interaction between the exposure and the mediator in explaining the outcome. Thus, in the presence of exposure-mediator interaction, it is necessary to introduce another analytical framework to estimate the

effect of an exposure that is not explained by a mediator (Richiardi, Bellocco, and Zugna 2013).

To avoid collider bias, it is crucial to adjust for mediator-outcome confounding in regression models. However, when mediator-outcome confounding is also affected by the exposure, this adjustment will produces improper estimates. Figure X shows that L as an intermediate confounder is both a mediator-outcome confounder and a variable that exists on the direct path from the exposure to the outcome. Adjustment for L in traditional regression models would bias the estimate of the direct effect by blocking the path A → L → Y, which would attenuate the direct effect and subsequently overestimate the indirect effect. Therefore, it is necessary to apply the counterfactual analytical framework in which the confounding effect of L can be adjusted without blocking the corresponding direct path from the exposure A to the outcome Y, and thus controlled direct effect can be obtained (Figure 2.3) (Richiardi, Bellocco, and Zugna 2013).

To overcome these issues, counterfactual approaches as a causal mediation analysis can be made to determine controlled direct effect, natural direct effect, and natural indirect effect shown as the equations below (VanderWeele 2009) (Richiardi, Bellocco, and Zugna 2013).

(1) controlled direct effect (a, a*;m) = Y_{am} - Y_{a*m} : the controlled effect comparing exposure level A = a to A = a* ,with the mediator set to M = m.

(2) natural direct effect (a, a*; a*) = $Y_{aM(a*)}$ - $Y_{a*M(a*)}$: the natural direct effect comparing exposure level A = a to A = a*, with the mediator set to M = M a*.

(3) natural indirect effect (a, a*; a) = $Y_{aM(a)}$ - $Y_{aM(a*)}$: the natural indirect effect comparing the effects M = Ma versus M = Ma*, with the exposure set to A = a. (here,

Y_a : the counterfactual outcome (or potential outcome) Y for each individual under exposure A = a; Y_{a^*} : the counterfactual outcome (or potential outcome) Y for each individual under exposure A = a*; Y_{am} : the counterfactual outcome (or potential outcome) Y for each individual if A were set to a and M to m)

Controlled direct effect is the contrast between the counterfactual outcome if the subject were exposed at A =a and the counterfactual outcome if the same subject were exposed at A = a*, with the mediator set to a fixed value M=m. Natural direct effect is the contrast between the counterfactual outcome if the subject were exposed at A =a and the counterfactual outcome if the same subject were exposed at A = a*, with the mediator assuming whatever value it would have taken at the reference value of the exposure A =a*. Natural indirect effect is the contrast, having set the exposure at level A = a, between the counterfactual outcome if the mediator assumed whatever value it would have taken at a value of the exposure A = a and the counterfactual outcome if the mediator assumed whatever value it would have taken at a reference value of the exposure A = a* (Robins and Greenland 1992, Richiardi, Bellocco, and Zugna 2013).

In addition to identifying the individual effects of natural direct effect, and natural indirect effect, and controlled direct effect, we can estimate the population average effects for each of them. Using this counterfactual framework, unbiased valid estimates of direct effect and indirect effect can be obtained and interpretation of mediation analysis will be improved (Robins and Greenland 1992, Richiardi, Bellocco, and Zugna 2013).

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Table 2.1. Comparison of definitions of metabolic healthy phenotype

	(Wildman et al. 2008)	(Meigs et al. 2006) (A)	(Meigs et al. 2006) (B)	(Karelis and Rabasa-Lhoret 2008)
High fasting glucose ≥ 100 mg/dL or antidiabetic medication use	V	V		
High blood pressure (systolic/diastolic blood pressure ≥130/85 mm Hg) or antihypertensive medication use	V	V		
High triglycerides (≥ 150 mg/dL) or on cholesterol-lowering medication	V	V		V
Low HDL-C (< 40 mg/dL in men or < 50 mg/dL in women) or on cholesterol-lowering medication	V	V		V
High waist circumference (≥102cm for men and ≥88 for women)		V		
High LDL-C (> 100 mg/dL)				V
High hs-CRP (> 90th pct)	V			V
High HOMA-IR (> 90th pct)	V		V (75th pct)	V
Criteria for metabolic health	<2	<3	HOMA-IR <75th pct	<1

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostasis model assessment of insulin resistance.

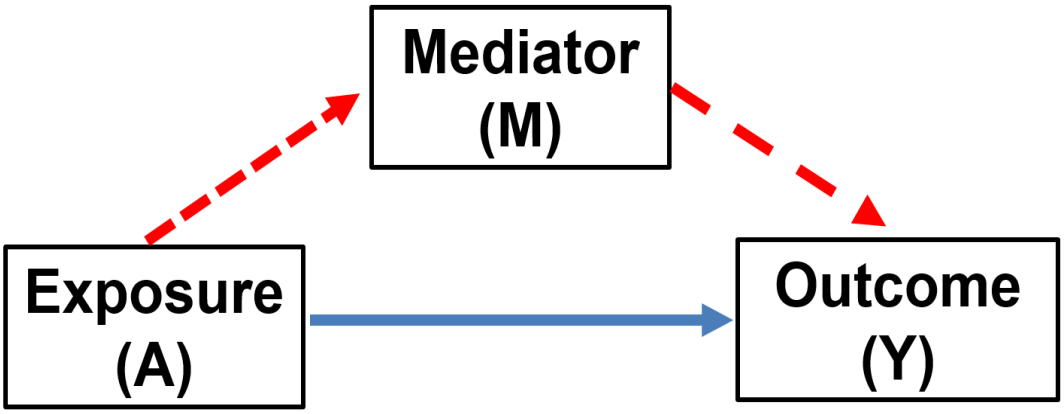

Figure 2.1 Mediation model

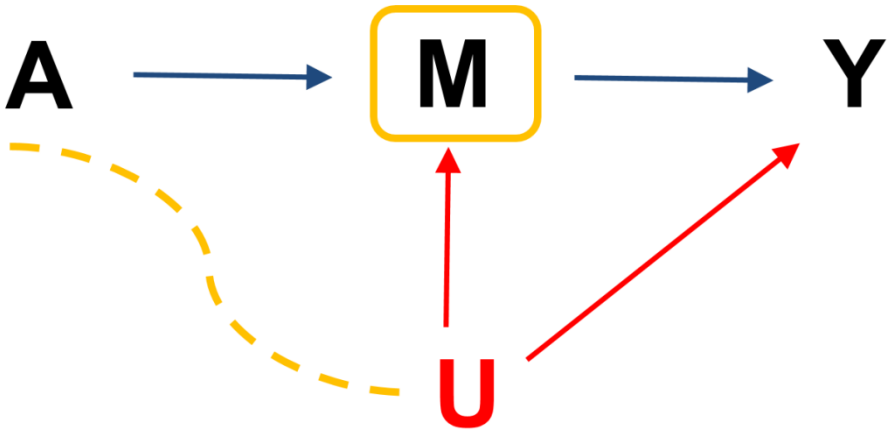

Figure 2.2 Effect of adjusting for a mediator (M) on the estimate of an exposure (A)-outcome (Y) association in the presence of a mediator-outcome confounder (U)

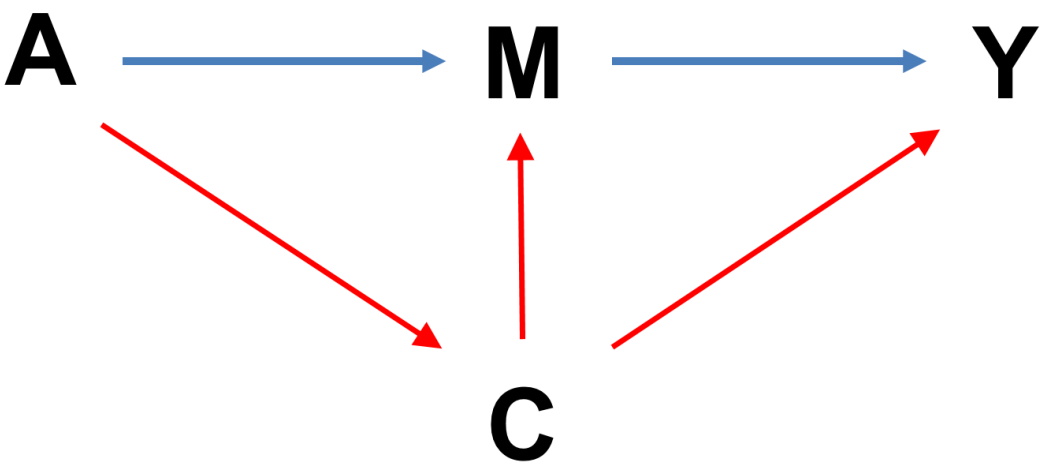

Figure 2.3 Intermediate confounding

C is affected by an exposure (A) and is also a mediator (M)-outcome (Y) confounder.

CHAPTER 3

METHODOLOGY

3.1 Statement of research aims and hypotheses

The overall aim of the present study was to evaluate the association of the Mediterranean diet and DASH style diet with metabolic health in both normal weight and obese populations, and to investigate the differential association of the adherence to MD or DASH style diet with mortality risk reduction according to the metabolic phenotypes in both normal weight and obese populations using the National Health and Nutrition Examination Survey (NHANES) III and its linked mortality file. Furthermore, we examined the role of adiposity as a mediator of the association between adherence to the Mediterranean diet or DASH style diet and cardiometabolic risk factors.

In specific aim #1, we investigated the association of Mediterranean diet and DASH style diet with MHO and MONW phenotypes in a cross-sectional study according to the two age groups exhibiting a substantially different in cardiovascular risk, based on being less than or greater than 45 years in men; before or after menopause in women. In specific aim #2, we assessed whether Mediterranean diet and DASH style diet would have a differential health benefit on mortality risk reduction in MHO individuals and MUO individuals as well as MONW individuals and MHNW individuals in a prospective study design. In specific aim #3, we examined the association of Mediterranean diet and DASH style diet with markers for insulin resistance and inflammation. In addition, we

investigated whether this association would be mediated by BMI and waist circumference, using both approaches in traditional mediation analysis and causal mediation analysis in a cross-sectional study.

3.2 Study population

We used data from the National Health and Nutrition Examination Survey (NHANES) III for the cross-sectional analyses and its mortality file linked until December 31, 2011 for the prospective analyses. The NHANES III was conducted between 1988 and 1994. A complex multi-stage stratified clustered probability sampling scheme was used to achieve a nationally representative sample of the civilian, non-institutionalized US population. Collected data included personal interviews, physical examinations, and laboratory tests.

Among the participants, we included adults aged 20 to 90 years with complete food frequency questionnaires (FFQ), 24-hour diet recalls, cardiometabolic parameters including fasting insulin, glucose, triglycerides, high-density lipoprotein cholesterol (HDL-C), hs-CRP, BP, and BMI for the aims #1 and #2, as well as waist circumference (WC) for the aim #3. Overall, we excluded pregnant or lactating women, those who reported implausible extreme energy intakes (<1st and >99th percentiles of energy intake/d in adults) and those with hs-CRP >10mg/dL, BMI < 18.5 kg/m^2, BMI > 60 kg/m^2. Additional exclusion criteria were different from each specific aim.

To determine the mortality status, all subjects were linked to the National Death Index through 31 December 2011 using probabilistic matching. The underlying cause listed on the death certificate was applied to determine cause of death that was identified using the underlying Cause of Death-113 (UCOD-113) groups (International

Classification of Disease (ICD), 10th revision). Total mortality was defined as deaths with any underlying cause of death; CVD mortality was defined as circulatory-specific deaths (ICD-10: I00-I69); cancer death was defined as cancer-specific deaths (ICD-10: C00-C99). (NHANESIII)

Follow-up for each subject for deaths continued from the date of the examination to the date of death or 31 December 2011. Thus, censoring occurred at the time of death from any cause for the total mortality outcome; or at the time of death for those who died from causes other than CVD for the CVD mortality outcome. Otherwise, the subjects not matched with a record of death were considered to be alive in terms of being right-censored.

3.3 Diet assessment

3.3.1 Assessment of Mediterranean diet

An 81-item food frequency questionnaires (FFQ) and the 24-hr dietary recall data, validated by the Nutrition Methodology Working Group (United States. National Center for Health Statistics. Plan and Operation of the Third National Health and Nutrition Examination Survey, 1988–94. US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention: Hyattsville, MD, Washington, DC, 1994), were used to assess dietary intake. Adherence to the Mediterranean diet was evaluated using the scoring methodology developed by Panagiotakos et al. (Panagiotakos, Pitsavos, and Stefanadis 2006, Panagiotakos et al. 2007). In brief, scores 0 to 5 were assigned for the weekly consumption of food items assumed to be contributing to a Mediterranean dietary pattern, whereas scores on the inverse ordinal scale were assigned for the consumption of food items assumed to be

against the Mediterranean dietary pattern. For instance, the scores assigned to the weekly consumption frequencies of legumes were as follows: no servings, less than 1 serving, 1–2 servings, 3–4 servings, 5–6 servings, and >6 servings were assigned scores of 0, 1, 2, 3, 4 and 5, respectively. Similar score assignments were used for the food items of whole grains, potatoes, fruits, vegetables, fish, and olive oil. Reverse scores were assigned for the components of red meat and products, poultry and full-fat dairy products. For the alcohol consumption, a score of 5 was assigned for consumption of less than 300 ml (36g) of alcohol per day, 0 for no consumption or for consumption of >700 ml (84g) per day. It has been shown that MDS is highly associated with prevalent cardiometabolic diseases, 10-year CVD risk, and inflammation and coagulation markers, in addition to capturing inherent characteristics of Mediterranean dietary pattern (Panagiotakos, Pitsavos, and Stefanadis 2006, Panagiotakos et al. 2007, Carter et al. 2010).

The NHANES III FFQ applied a 1-month reference period without recording portion sizes. Thus, we calculated the MDS, assuming that the number of servings per week were equivalent to the number of times that a food item was consumed per week (Table 3.3). Potatoes were excluded in our MDS assessment, because the way potatoes are prepared in U.S. is quite different from European countries (Fung et al. 2005). The amount of alcohol consumed daily was estimated using the following assumption: 12.8g for 12-oz beer, 11g for 4-oz glass of wine, and 14g for an ounce of liquor based on the questionnaire provided. Additionally, gender-specific cut-offs were applied: a score of 5 was assigned for consumption of less than 28g and 14 g of alcohol per day, 0 for no consumption or for consumption greater than 70g and 28g per day in men and women, respectively, and the cutoffs for subcategories between 0 and 5 were reassigned in even

intervals (Liangpunsakul, Crabb, and Qi 2010). Olive oil consumption was not measured in the NHANES III FFQ. Thus, we approximated olive oil consumption by calculating the ratio of total monounsaturated fatty acid to total saturated fatty acids using the 24-h dietary recall data, then dividing it into the six even intervals. Finally, the possible MDS ranged from 0 to 50, with higher values of this MDS indicating greater adherence to the Mediterranean diet.

3.3.2 Assessment of DASH style diet

The DASH style diets is characterized by high intake of fruits, vegetables, nuts and legumes, low fat dairy products, and whole grains; and low intake of fat (total/saturated), sodium, sweets, and red meats (Sacks et al. 2001). We used the 24-hr dietary recall data to calculate Mellen's index which is one of the established DASH index scores (Miller et al. 2013), because NHANES FFQ did not provide information on servings. Mellen's index, a nutrient-based index with 9 components including saturated fat, total fat, protein, cholesterol, fiber, magnesium, calcium, potassium, and sodium using target nutrient values used in clinical trials (Mellen et al. 2008), applied absolute targets on the basis of a 2100-kcal diet for both men and women. Individuals satisfying the goal for each component received 1 point, those who meet an intermediate goal, defined as the midpoint between the DASH diet goal and the nutrient content of the DASH control diet (Appel et al. 1997) received one-half of a point for the component, and those who meet neither goal received 0 points for the component. Finally, the possible DASH score ranged from 0 to 9, with higher values of this DASH score indicating greater adherence to the DASH style diet.

3.4 Assessment of metabolic health

We determined metabolic health, using the metabolic parameters that were measured under quality control standards of Centers for Disease Control and Prevention. BMI was calculated as kg/m^2; height was measured to the nearest 0.1cm and weight to the nearest 0.01 kg. Subjects were classified according to their BMI as obese (BMI $\geq 30 kg/m^2$) and normal weight (BMI $<25 kg/m^2$). WC was measured at the level of the right iliac crest. BP was averaged over five separate measurements. Glucose was measured in serum, using a modified hexokinase enzymatic method. Serum insulin was measured using a radioimmnuoassay (Pharmacia Diagnostics). HDL-C and triglycerides were measured using a Hitachi 704 analyzer (Boehringer-Mannheim Diagnostics). Serum CRP concentrations were measured by latex-enhanced nephlometry (Department of Laboratory Medicine, Immunology Division, University of Washington, Seattle). Metabolic health was defined when the individual had fewer than two cardiometabolic abnormalities (systolic/diastolic BP $\geq$ 130/85 mm Hg or antihypertensive medication use, triglycerides $\geq$ 150 mg/dL or on cholesterol-lowering medication, fasting glucose $\geq$ 100 mg/dL or antidiabetic medication use, homoeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose (mg/dl) x fasting insulin (lU/mL)/405) > the 90th percentile, hs-CRP > the 90th percentile, and HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on cholesterol-lowering medication (Wildman et al. 2008).

3.5 Assessment of covariates

Potential confounders were included as covariates in multivariable logistic and Cox proportional hazards regression models. Participants' sociodemographic characteristics, medical history, and lifestyle related characteristics were measured during

the personal interview. Sociodemographic variables included age, gender, race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or others), educational attainment (<12 years, 12 years, or >12 years of education), living with spouse, and level of income measured based on poverty income ratio (PIR) which is the ratio of household income to the appropriate poverty threshold (low (PIR ≤ 1.3), middle (1.3<PIR ≤ 3.5), and high (PIR>3.5)). Family history of coronary heart disease (CHD) and parental history of DM were self-reported. Smoking status was categorized as never, former, and current. The amount of alcohol consumed daily was estimated using the above mentioned assumption. Moderate alcohol use was defined as consumption up to 28g and 14 g of alcohol per day in men and women (Liangpunsakul, Crabb, and Qi 2010). Physical activity was categorized based on the recommended levels of physical activity (Haskell et al. 2007). Recommended physical activity was designated as a self-reported leisure time moderate activity (3 ≤ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs ≥ 6) three or more times per week; physical inactivity as no reported leisure time physical activity; and insufficient physical activity as not meeting the criteria for recommended levels of physical activity but not inactive.

3.6 Statistical methods

We compared the baseline characteristics of participants by their tertiles for MDS or DASH index. Continuous variables were presented by mean (SE: standard error) and compared using linear regression analyses. Categorical variables were expressed by percentage with SE and were compared using Rao-Scott $\chi 2$ tests. We used two models according to degrees of adjustment: model 1 adjusted for age, sex, and race or ethnicity;

model 2 further adjusted for education, income, living with spouse, smoking, alcohol intake, physical activity, the presence of family history of CVD, parental history of DM, and total energy intake, and BMI. All analyses were conducted with SAS, version 9.4 (SAS Institute, Inc) to take into account the complex survey design and the sampling weights used in NHANES III. For the subgroup analysis, domain analysis was applied to preserve the complex sampling design in which the entire samples were used for estimating the variance of subpopulations. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

For hypothesis 1.1, we expected that Mediterranean diet or DASH style diet would be favorable risk factors for metabolic health. Thus, we tested for the association of 1) continuous values and 2) the tertiles of MDS or DASH index with metabolic health using multivariable logistic regression analyses. In this model, we expect that the odds of being metabolic health will increase with increasing MDS and DASH index. The logistic regression model is described as follows.

1) $logit\,[\pi(x)] = \alpha + \beta 1 * (MDS, DASH\ index) + \beta 2 * Cov1 + \beta 3 * Cov2 + \cdots$

2) $logit\,[\pi(x)] = \alpha + \beta 1 * Q1 + \beta 2 * Q2 + \beta 3 * Q3 + \beta 4 * Cov1 + \beta 5 * Cov2 + \cdots$

$(supposing\ that\ Q1, Q2, and\ Q3\ are\ tertiles\ of MDS\ or\ DASH\ index)$

For hypothesis 1.2, we tested for the association of 1) continuous values and 2) the tertiles of MDS or DASH index with metabolic health using multivariable logistic regression analyses. In this model, we expected that the odds of being MHO would increase with increasing MDS and DASH index in obese population.

For hypothesis 1.3, we tested for the association of 1) continuous values and 2) the tertiles of MDS or DASH index with metabolic health using multivariable logistic

regression analyses. In this model, we expected that the odds of being MONW would decrease with increasing MDS and DASH index in normal weight population.

For hypothesis 2, cumulative mortality was estimated using Kaplan-Meier methods. Cox proportional hazards (PH) regression models were applied to estimate the hazard ratios (HRs) and 95% CIs for all-cause, CVD and cancer mortality. The proportional hazards assumption of the Cox models was evaluated with log of negative log survival curves based on Kaplan-Meier estimates for MDS tertile group as well as age, gender, and race/ethnicity. For Hypothesis 2.1, we tested the association of MDS and DASH index with all-cause, CVD, and cancer mortality using Cox proportional hazards (PH) regression analysis. In this model, we expected that hazard ratios for total, CVD, and cancer mortality risk would decrease with increasing numbers of MDS and DASH index as follows.

3) $h(t|x) = h0(t) * \exp\{\beta1*(MDS, DASH\ index) + \beta2 * Cov1 + \beta3 * Cov2 + \cdots\}$

4) $h(t|x) = h0(t) * \exp\{\beta1*Q1 + \beta2 * Q2 + \beta3 * Q3 + \beta4 * Cov1 + \beta5 * Cov2 + \ldots\}$ $(supposing\ that\ Q1, Q2, and\ Q3\ are\ tertiles\ of MDS\ or\ DASH\ index)$

For hypothesis 2.2, we tested for interactions of 1) MDS and DASH index with being MHO or not in obese population by including the interaction terms in the Cox PH models based on Satterthwaite adjusted F tests as follows.

5) $h(t|x) = h0(t) * \exp\{\beta1*(MDS, DASH\ index) + \beta2 * (MHO\ or\ not) + \beta3 * (interaction\ terms) + \beta3 * Cov1 + \cdots\}$

6) $h(t|x) = h0(t) * \exp\{\beta1*(MDS, DASH\ index) + \beta2 * (MUNW\ or\ not) + \beta3 * (interaction\ terms) + \beta3 * Cov1 + \cdots\}$

If the coefficient of this interaction terms was significant, we did a stratified analysis according to the status of being MHO or not, using the same model used in 3) or 4). This test also was applied to interactions of MDS and DASH index with being MONW or not in normal weight population.

For hypothesis 3, we used two different approaches to explore the role of BMI and WC as a mediator in the association of Mediterranean diet and DASH style diet with markers for insulin resistance and inflammation.

The traditional approach for mediation analysis included the following steps. First, linear regression analyses were applied to examine associations of Mediterranean diet and DASH style diet (the highest tertile vs. the first tertile, respectively) with BMI and WC, after adjusting for age, gender, race/ethnicity, educational attainment, living with spouse, income, smoking status, level of physical activity, family history of CHD, parental history of DM, and total energy intake, as well as alcohol consumption for the association of DASH style diet with BMI and WC (the estimate a in the Figure 3.1). Second, linear regression analyses were applied to examine the association of BMI and WC with markers for insulin resistance or inflammation, using the same covariates in addition to adjusting for MDS or DASH (the estimate b in the Figure 3.1). In this step, we added covariates to the model according to the characteristics of outcome variables (mean arterial pressure for markers for insulin resistance; multivitamin use for homocysteine). Third, the simple total effect of Mediterranean diet and DASH style diet was estimated by regressing the markers for insulin resistance or inflammation on MDS and DASH index while adjusting for the covariates used in the first step, but without adjusting for BMI or WC (the estimate c in the Figure 3.1). Fourth, BMI or WC was additionally

controlled in the model to estimate the direct effect of Mediterranean diet and DASH style diet on the markers for insulin resistance or inflammation (the estimate c in the Figure 3.1). Finally, the indirect effect by BMI or WC was estimated by calculating the product of the beta coefficient of the first regression model and the beta coefficient of the second regression model (the estimate a×b in the Figure 3.1) (Baron and Kenny 1986, Preacher and Hayes 2004). This approach also can be presented using the two models: $E\,[\,M \mid A = a, C = c\,] = \beta_0 + \beta_1 a + \beta_2{}' c$; $E\,[\,Y \mid A = a, M = m, C = c\,] = \theta_0 + \theta_1 a + \theta_2 m + \theta_4{}' c$ (a represents exposure, m represents mediator, and c represents confounders) in which the direct effect is evaluated by estimating θ_1; the indirect effect is evaluated by estimating $\beta_1\theta_2$ (Valeri and Vanderweele 2013). Then, we divided the indirect effect by its standard error and performed a z test under the null hypothesis that the indirect effect was equal to zero (Sobel 1982). In addition, proportion of mediation was calculated using the indirect effect as a numerator and the total effect as a denominator.

BMI or WC was considered as a mediator if the estimates a and b were significant (Baron and Kenny 1986). Another criterion for determining a mediator would be whether or not the estimate c was significant (Preacher and Hayes 2004). However, we did not include this criterion in the present study, because the effect of Mediterranean diet and DASH style diet on the markers for insulin resistance or inflammation may not be significant when direct and mediated effects have opposite signs (Rucker et al. 2011, Valeri and Vanderweele 2013).

In the causal mediation approach, we assessed the total, direct, and indirect effects of Mediterranean diet and DASH style diet on markers for insulin resistance or

inflammation with BMI or WC as a mediator, using the counterfactual framework (VanderWeele 2009, VanderWeele 2013). In this approach, total effect can be decomposed into direct effect (not mediated by BMI or WC) and indirect effect (mediated by BMI or WC). A causal directed acyclic graph (DAG) indicating these effects is presented in Figure 3.2. We applied the SAS macro developed by Valeri and VanderWeele to evaluate the natural direct effect, natural indirect effect, and marginal total effect of Mediterranean diet and DASH style diet on markers for insulin resistance or inflammation with BMI or WC as a mediator (Valeri and Vanderweele 2013).

Natural direct effect and natural indirect effects can be evaluated conceptually as follows. For instance, natural direct effect is the contrast between the counterfactual outcome if the subject were exposed at the highest tertile of Mediterranean diet, and the counterfactual outcome if the same subject were exposed at the lowest tertile of Mediterranean diet, with BMI assuming whatever value it would have taken at the reference value of the lowest tertile of Mediterranean diet. Natural indirect effect is the contrast, having set the Mediterranean diet at level of the highest tertile, between the counterfactual outcome if BMI assumed whatever value it would have taken at a value of the highest tertile of Mediterranean diet, and the counterfactual outcome if the mediator assumed whatever value it would have taken at a reference value of the lowest tertile of Mediterranean diet. Based on these individual approaches, the average natural indirect and direct effects at the population level were estimated (Robins and Greenland 1992, Richiardi, Bellocco, and Zugna 2013). Survey procedures were not applied in the causal mediation analysis because a statistical tool was not developed yet for this procedure.

For this causal mediation analysis, we made four assumptions. First, there is no unmeasured confounding in the relation of Mediterranean diet and DASH style diet with the markers for insulin resistance or inflammation given confounders; second, there is no unmeasured confounding in the relation of BMI or WC with the markers for insulin resistance or inflammation; third, there is no unmeasured confounding in the relation of Mediterranean diet and DASH style diet with BMI and WC; fourth, there is no effect of Mediterranean diet and DASH style diet affecting confounders of the relationship of BMI and WC with the markers for insulin resistance or inflammation (VanderWeele 2009). Under these assumptions, natural direct effect, natural indirect effect, marginal total effect can be estimated using two separate linear regressions: the first regression of markers for insulin resistance or inflammation on the exposure (MDS or DASH index), the mediator (BMI or WC) and the confounding variables; and a second regression of BMI or WC on the MDS or DASH index and the confounding variables. Then, two regressions were combined to estimate natural direct and indirect effects. In this causal mediation analysis, we evaluated whether there is an interaction of MDS or DASH index with BMI or WC in the regression of the marker for insulin resistance and inflammation. When there is a significant interaction, controlled direct effect, natural direct effect, and natural indirect effect were estimated using the following models for change in MDS or DASH index from level a* to level a: controlled direct effect =$(\theta_1 + \theta_3 m)(a - a*)$; natural direct effect = $(\theta_2\beta_1 + \theta_3\beta_1 a)(a - a*)$; natural indirect effect = $(\theta_1 + \theta_3 (\beta_0 + \beta_1 a* + \beta_2' c))(a - a*)$. When there is no significant interaction (θ_3 = 0), the controlled direct effect is equal to natural direct effect (Valeri and Vanderweele 2013). It is possible that the interaction may not be statistically significant due to low power. In addition, the

exposure-mediator interaction may be important in understanding the effects of mediation even when the interaction terms are not significant (VanderWeele 2015). Thus, we further evaluated whether there would be any substantial change in the magnitude of the natural indirect effect or the power to detect the natural indirect effect, with or without including the interaction terms of MDS or DASH index with BMI or WC in the model.

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Table 3.1 Food items of food frequency questionnaires in NHANES III for assessing the Mediterranean diet

Classification for Mediterranean diet	Food item
Grains	Cereals: all-bran, etc
	Cereals: total, etc
	Cooked, hot cereals
	Dark breads and rolls
	Corn bread, muffins, tortillas
Legume	Beans, lentils, chickpeas
	Peanuts, peanut butter, etc
Fruits	Citrus fruits
	Melons
	Peaches, nectarines, etc
	Any other fruits
Vegetables	Carrots
	Broccoli
	Brussel sprouts/cauliflower
	Tomatoes
	Spinach, greens, etc
	Tossed salad
	Cabbage, cole slaw, sauerkraut
	Hot red chili peppers
	Other peppers
	Any other vegetables
Fish	Shrimp, clams, etc
	Fish
Olive oil	Ratio of total saturated fatty acids (gm) to total monounsaturated fatty acids (gm)
Alcohol	Beer and lite beer
	Wine, etc
	Hard liquor
Milk and dairy products	Chocolate milk and hot cocoa
	Milk to drink or on cereal (Whole/regular; 2% /low fat milk)
	Yogurt and frozen yogurt
	Ice cream, ice milk, milkshakes
	Cheese, all types
	Cheese dishes
	Butter
Red meat and products	Bacon/sausage/processed meats
	Liver and other organ meats
	Beef
	Pork and ham
Poultry	Chicken and turkey

Table 3.2 Nutrient items of 24-h dietary recall in NHANES III for assessing DASH style diet index

DASH style diet index	Nutrient
Mellen's index	Protein (gm)
	Total fats (gm)
	Total saturated fatty acids (gm)
	Cholesterol (mg)
	Dietary fiber (gm)
	Calcium (mg)
	Magnesium (mg)
	Sodium (mg)
	Potassium (mg)

Table 3.3 Assessment of the Mediterranean Diet Score

	Frequency of consumption (times/week or otherwise stated)					
Score	0	1	2	3	4	5
Non-refined cereals (whole grain)	Never	1-6	7-12	13-18	19-31	≥32
Legumes	Never	<1	1-2	3-4	5-6	≥7
Fruits	Never	1-4	5-8	9-15	16-21	≥22
Vegetables	Never	1-6	7-12	13-20	21-32	≥33
Fish	Never	<1	1-2	3-4	5-6	≥7
MUFA:SFA	1st hexile	2nd hexile	3rd hexile	4th hexile	5th hexile	6th hexile
Score	5	4	3	2	1	0
Red meat and products	≤1	2-3	4-5	6-7	8-10	≥11
Poultry	≤3	4-5	5-6	7-8	9-10	≥11
Dairy products	≤10	11-15	16-20	21-28	29-30	≥31
Alcoholic beverages (g/d)	<28g (men), <14g (women)	Even intervals between two cutoffs				0g or >70g (men), >28g (women)

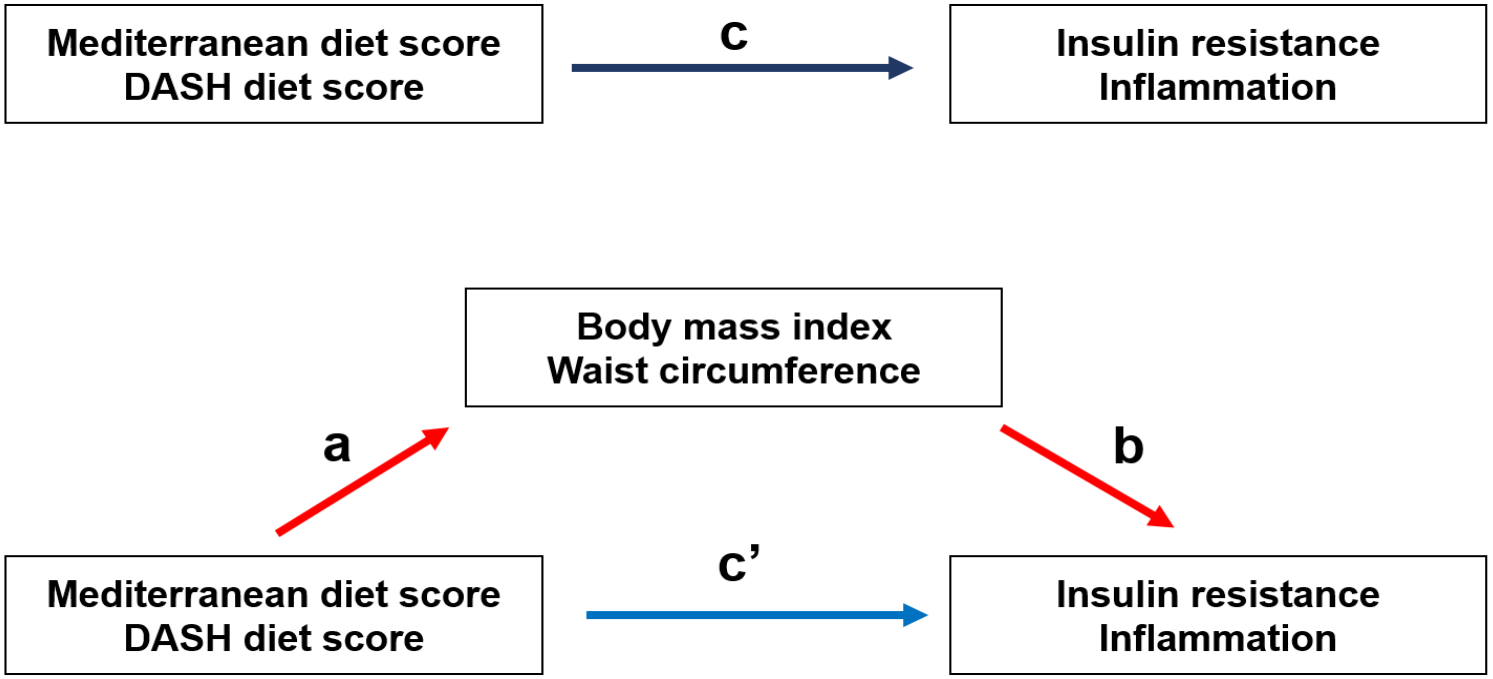

Figure 3.1 Traditional mediation model

This model is based on the association of Mediterranean diet score and DASH style diet score with the markers for insulin resistance and inflammation as body mass index and waist circumference as a mediator. Path a represents the regression coefficient of the association of The Mediterranean diet score (MDS) and DASH diet score with BMI and WC. Path b represents the regression coefficient of the association of BMI and WC with the markers for insulin resistance and inflammation. The product of regression coefficients of path a and path b represents the mediated effect of BMI or WC (path a × path b). Path c' represents the direct effect of MDS and DASH diet score with the markers for insulin resistance and inflammation, after the adjustment for BMI or WC. Path c represents the simple total effect of MDS and DASH diet score with the markers for insulin resistance and inflammation, without the adjustment for BMI or WC.

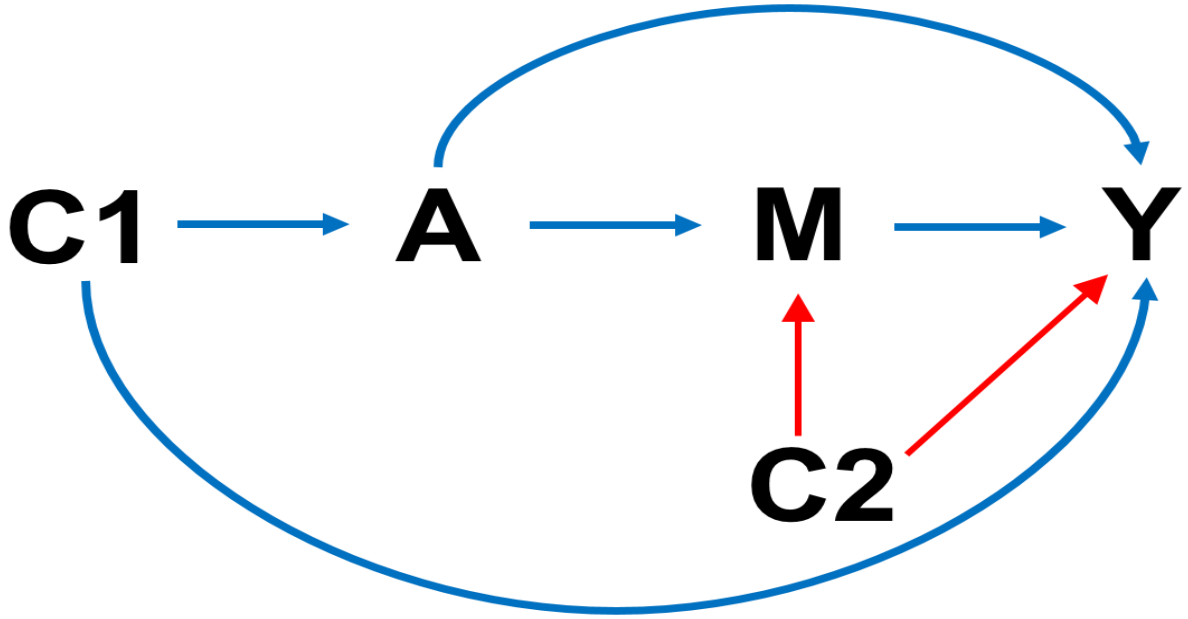

Figure 3.2 Causal directed acyclic graph (DAG) describing relations of Mediterranean diet and DASH style diet (A) with the markers for insulin resistance and inflammation (Y) as BMI and waist circumference as a mediator (M)

C1 and C2 include age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, and total calories (additionally adjusted for alcohol consumption in the model of DASH index).

CHAPTER 4

MEDITERRANEAN DIET, DIETARY APPROACHES TO STOP HYPERTENSION (DASH) STYLE DIET, AND METABOLIC HEALTH IN U.S. ADULTS [1]

[1] Yong-Moon Mark Park, Susan E. Steck, Teresa T. Fung, Jiajia Zhang, Linda J. Hazlett, Kyungdo Han, and Anwar M. Merchant. To be submitted to *Journal of Nutrition.*

Abstract

Background: There is little evidence on the relationship between Mediterranean diet, Dietary Approaches to Stop Hypertension (DASH) style diet, and metabolic health, especially in normal weight and obese population.

Objective: To investigate the association of Mediterranean diet and DASH style diet with metabolically healthy obese (MHO) and metabolically obese normal weight (MONW) phenotypes in a representative U.S. population.

Methods: Data from 2,767 adults aged 20-90 years without any prior diagnosis of cancer or cardiovascular disease were analyzed from the National Health and Nutrition Examination Survey III, 1988–1994. Mediterranean diet scores (MDS) and DASH index were calculated using food frequency questionnaires and 24-hr dietary recall data. MHO and MONW individuals were identified using criteria including high fasting glucose, insulin resistance, blood pressure, triglycerides, C-reactive protein, and low high-density lipoprotein-cholesterol. Logistic regression adjusting for potential confounders was used to examine associations for all participants and stratified by age groups (younger age group, <45 years for men or premenopausal women; older age group ≥45 for men or postmenopausal for women).

Results: With increasing MDS, there was a higher prevalence of MHO phenotype, especially in the younger age group (P for trend = 0.01). Higher MDS was associated with higher odds of MHO phenotype (tertile 3 vs 1; odds ratio, 2.57 [95% confidence interval, 1.04-6.35]; P trend = 0.04), whereas higher DASH index was associated with lower odds of MONW phenotype (tertile 3 vs 1; 0.59 [0.38-0.93]; P trend = 0.03) only in

the younger age group. No significant association of MDS and DASH index with MHO and MONW phenotypes was observed in the older age group.

Conclusions: Adherence to Mediterranean diet or DASH style diet was associated with MHO and MONW phenotypes only in the younger age group, suggesting that potential dietary intervention to prevent cardiometabolic disease differ by age group.

Keywords: Mediterranean diet, Dietary Approaches to Stop Hypertension style diet, metabolically healthy obese, metabolically obese normal weight, National Health and Nutrition Examination Survey III

Introduction

Obesity is associated with an increased risk of type 2 diabetes and cardiovascular disease (CVD) (1), but not all obese individuals are equally susceptible to cardiometabolic risk. Within the same category of body mass index (BMI), a subgroup of obese individuals who have normal metabolic characteristics has been identified as metabolically healthy obese (MHO) phenotype, compared with metabolically unhealthy obese (MUO) phenotype (2-4). In addition, a subgroup of normal weight individuals who are susceptible to metabolic abnormalities has been referred to as metabolically obese normal weight (MONW) phenotype (5-8).

It is well known that the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diets are associated with lower risk of cardiometabolic disease (9-12). Underlying mechanisms for cardiovascular health benefits of Mediterranean diet and DASH style diet are complex, but can be explained by the improvement of cardiometabolic profiles including insulin resistance, lipid profiles, blood pressure, and inflammatory markers (13). Therefore, it is plausible that adherence

to Mediterranean diet and DASH style diets might be positively associated with a metabolically healthy phenotype.

Several studies have explored the association between dietary factors and metabolic phenotype. However, these reports have been limited to MHO phenotype (14), especially without considering dietary pattern (15), or with specific age groups (16). A few studies have reported associations in MONW phenotype in non-US population (17), but with limited generalizability (18). Several studies demonstrated that dietary factors are related to metabolic phenotype in both obese and normal weight population (19), but only focused on women (20), or single foods such as olive oil (21). However, limited data exist on the relationship of Mediterranean diet and DASH diet with metabolic phenotypes, especially for a potential differential association by age groups with substantially different cardiovascular risk.

It has been suggested that the risk of developing coronary heart disease significantly increases after 45 years in men (22) and postmenopausal women (23). In addition, it is reported that the association between Mediterranean diet and atherothrombotic biomarkers is largely different between men <45 years and men ≥ 45 years as well as premenopausal and postmenopausal women (24). Another study showed that the Healthy Eating Index score was associated with MHO phenotype only in younger age group (14).

Therefore, we aimed to identify the association of Mediterranean diet and DASH style diet with MHO and MONW phenotypes in a nationally representative U.S. population, with a priori hypothesis that this association should be different according to

the two age groups exhibiting a substantial difference in cardiovascular risk; less than or greater than 45 years in men; before or after menopause in women.

Methods

Study population

We used data from the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994 for this analysis. NHANES III was carried out using a complex multi-stage stratified clustered probability sample design to achieve a nationally representative sample of the civilian, non-institutionalized US population. The survey included personal interviews, physical examinations, and laboratory measurements. We included 3,858 normal weight ($18.5 \leq BMI < 25$ kg/m^2) and obese ($BMI \geq 30$ kg/m^2) adults aged 20-90 years with complete data on FFQ and 24-hour dietary recall, as well as the variables for defining metabolic health including fasting glucose, insulin, blood pressure (BP), triglycerides, high-density lipoprotein cholesterol (HDL-C), and high-sensitivity C-reactive protein (hs-CRP) with at least 10 hours' fasting. We applied the strict fasting criteria to minimize misclassification of metabolic health status. Because dietary habits might change due to chronic disease, we excluded those who reported a history of myocardial infarction, stroke, congestive heart failure, or any prior diagnosis of cancer (n=463). To minimize reverse causation, we also excluded the participants who reported changing their dietary patterns due to any medical reason during the previous 12 months (n=489). Furthermore, we excluded those who reported implausible extreme energy intakes (<1st and >99th percentiles of energy intake/d in adults), those with hs-CRP >10mg/L, BMI > 60 kg/m^2, or pregnant or lactating women (n=139). A total of 2,767 individuals were analyzed in the present study where the younger age group

comprised men < 45 years and premenopausal women; and the older age group consisted of men ≥ 45 years and postmenopausal women. Women were designated as premenopausal if they reported having had a menstrual period during the previous 12 months; and postmenopausal if they did not or if they had a history of surgery with both ovaries removed (25).

Assessment of Mediterranean diet

An 81-item food frequency questionnaire (FFQ) and 24-hr dietary recall data, validated by the Nutrition Methodology Working Group (26), were used to assess dietary intake. Adherence to the Mediterranean diet was evaluated using the scoring methodology developed by Panagiotakos et al. (27, 28). In brief, scores 0 to 5 were assigned for the weekly consumption of food items assumed to be contributing to a Mediterranean dietary pattern, whereas scores on the inverse ordinal scale were assigned for the consumption of food items assumed to be against the Mediterranean dietary pattern. For instance, the scores assigned to the weekly consumption frequencies of legumes were as follows: no servings, less than 1 serving, 1–2 servings, 3–4 servings, 5–6 servings, and >6 servings were assigned scores of 0, 1, 2, 3, 4 and 5, respectively. Similar score assignments were used for the food items of whole grains, potatoes, fruits, vegetables, fish, and olive oil. Reverse scores were assigned for the components of red meat and products, poultry and full-fat dairy products. For the alcohol consumption, a score of 5 was assigned for consumption of less than 300 ml (36g) of alcohol per day, 0 for no consumption or for consumption of >700 ml (84g) per day. It has been shown that Mediterranean Diet Scores (MDS) are highly associated with prevalent cardio-metabolic

diseases, 10-year CVD risk, and inflammation and coagulation markers, in addition to capturing inherent characteristics of Mediterranean dietary pattern (24, 27, 28).

The NHANES III FFQ applied a 1-month reference period without recording portion sizes. Thus, we calculated the MDS, assuming that the number of servings per week were equivalent to the number of times that a food item was consumed per week. Potatoes were excluded in our MDS assessment, because the way potatoes are prepared in U.S. is quite different from European countries (29). The amount of alcohol consumed daily was estimated using the following assumption: 12.8g for 12-oz beer, 11g for 4-oz glass of wine, and 14g for an ounce of liquor based on the questionnaire provided. Additionally, gender-specific cut-offs were applied: a score of 5 was assigned for consumption of less than 28g and 14 g of alcohol per day, score 0 for no consumption or for consumption of greater than 70g and 28g per day in men and women, respectively, and the cutoffs for subcategories between 0 and 5 were reassigned with even intervals (30). Olive oil consumption was not measured in the NHANES III FFQ. Thus, we approximated olive oil consumption by calculating the ratio of total monounsaturated fatty acid to total saturated fatty acids using the 24-h dietary recall data, then dividing it into the six even intervals. Finally, the possible MDS score ranged from 0 to 50, with higher values of this MDS score indicating greater adherence to the Mediterranean diet.

Assessment of DASH style diet

We used 24-hr dietary recall data to calculate Mellen's index which is one of the established DASH index scores (31), because the NHANES FFQ did not provide information on servings. Mellen's index, a nutrient-based index with 9 components including saturated fat, total fat, protein, cholesterol, fiber, magnesium, calcium,

potassium, and sodium using target nutrient values used in clinical trials (32), applied absolute targets on the basis of a 2100-kcal diet for both men and women. Individuals fulfilling the goal for each component received 1 point, those who met an intermediate goal, defined as the midpoint between the DASH diet goal and the nutrient content of the DASH control diet (33) received one-half of a point, and those who met neither goal received 0 points. The possible DASH score ranged from 0 to 9, with higher values of this DASH score indicating greater adherence to the DASH style diet.

Assessment of Metabolic health

We determined metabolic health, using the metabolic parameters that were measured under quality control standards of Centers for Disease Control and Prevention. BMI was calculated as kg/m^2; height was measured to the nearest 0.1cm and weight to the nearest 0.01 kg. Waist circumference was measured at the level of the right iliac crest. BP was averaged over five separate measurements. Fasting glucose was measured in serum, using a modified hexokinase enzymatic method. Serum insulin was measured using a radioimmnuoassay (Pharmacia Diagnostics). HDL-C and triglycerides were measured using a Hitachi 704 analyzer (Boehringer-Mannheim Diagnostics). Serum hs-CRP concentrations were measured by latex-enhanced nephlometry (Department of Laboratory Medicine, Immunology Division, University of Washington, Seattle). Metabolic health was defined when the individual had fewer than two cardiometabolic abnormalities [systolic/diastolic BP ≥ 130/85 mm Hg or antihypertensive medication use, triglycerides ≥ 150 mg/dL or on cholesterol-lowering medication, fasting glucose ≥ 100 mg/dL or antidiabetic medication use, homoeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose (mg/dl) x fasting insulin (lU/mL)/405) > the 90th

percentile, hs-CRP > the 90th percentile, and HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on cholesterol-lowering medication] (34). Thus, obese individuals who had 0 or 1 component were defined as MHO phenotype, whereas normal weight individuals who had 2 or more components were defined as MONW phenotype.

Assessment of Covariates

Participants' sociodemographic characteristics, medical history, and lifestyle related characteristics were measured during the personal interview. Sociodemographic variables included age, gender, race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or others), educational attainment (<12 years, 12 years, or >12 years of education), living with spouse, and level of income measured based on poverty income ratio (PIR) which is the ratio of household income to the appropriate poverty threshold (low (PIR ≤ 1.3), middle (1.3<PIR ≤ 3.5), and high (PIR>3.5)). Family history of coronary heart disease (CHD) and parental history of DM were self-reported. Smoking status was categorized as never, former, and current. The amount of alcohol consumed daily was estimated using the above mentioned assumption. Moderate alcohol use was defined as consumption up to 28g and 14 g of alcohol per day in men and women (30). Physical activity was categorized based on the recommended levels of physical activity (35). Recommended physical activity was designated as a self-reported leisure time moderate activity (3 ≤ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs ≥ 6) three or more times per week; physical inactivity as no reported leisure time physical activity; and insufficient physical activity as not meeting the criteria for recommended levels of physical activity but not inactive.

Statistical Analysis

Descriptive statistics were computed for the sample across the presence or absence of MHO phenotype and MONW phenotype in each age group. Continuous variables were presented by mean (SE: standard error) and compared using linear regression analyses. Categorical variables were expressed by percentage with SE and were compared using Rao-Scott $\chi 2$ tests. We used the appropriate survey procedures to account for the complex sampling design by using weights used in NHANES III. For the subgroup analysis, domain option was applied in survey procedure to preserve appropriate subsample in the complex sampling design, and it utilized the entire samples to estimate the variance of subpopulations.

Multiple linear regression analyses were performed to test whether there is any difference in the consumption of Mediterranean diet, DASH style diet, and their components between metabolic phenotypes (Metabolically Healthy Normal Weight (MHNW) vs. MONW and MHO vs. MUO) after adjusting for educational attainment, income, living with spouse, smoking status, alcohol consumption, level of physical activity, family history of CHD, parental history of DM, total energy intake, and BMI. The consumption of dietary factors were also compared between MHO and MHNW phenotype. Considering multiple comparisons of Mediterranean diet and DASH style diet between metabolic phenotypes, a Bonferroni-corrected significance threshold (alpha = 0.0167) was applied (36).

Logistic regression analyses were conducted to generate odds ratios (ORs) and 95% confidence intervals (CIs) to quantify the associations of the tertiles of MDS and DASH score with the MHO and MONW phenotype. Since it was hypothesized that healthy

dietary patterns would be positively associated with MHO phenotype and inversely associated with MONW phenotype, the main outcomes of interest in the present study were MHO phenotype in the obese individuals and MONW phenotype in the normal weight individuals. For analyses in the obese population, the MUO phenotype was considered the referent group. For analyses in the normal weight population, the MHNW phenotype was designated as the referent group. Multivariable-adjusted model included age and total energy intake as continuous covariates; gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of CHD, parental history of DM as categorical covariates. We evaluated age-, gender-, and race/ethnicity- adjusted ORs in model 1; further adjusted for the educational attainment, living with spouse, income, smoking status, level of physical activity, family history of CHD, parental history of DM, and total energy intake in model 2. Alcohol consumption as a categorical variable was added in the model in which the association between DASH index and metabolic health was assessed. Obesity parameters such as BMI or waist circumference were not included in the model, assuming that these may be on the causal pathway between healthy dietary pattern and metabolic health.

All the statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), using the P value of less than .05 was considered statistically significant.

Results

MHO and MONW phenotypes were observed in 40.5% and 10.2 % in the younger age group; 19.0% and 40.5% in the older age group, respectively. Table 4.1 shows general and clinical characteristics according to the presence or absence of MHO

and MONW phenotypes in both age groups. MHO individuals were less likely to be non-Hispanic white; had more favorable metabolic parameters in both age groups, but were less likely to have CHD family history and DM parental history in younger age group; were less likely to be smokers and more likely to be physically active in older age group, compared to MUO individuals. MONW individuals were older, and had less favorable metabolic parameters compared to MHNW in both age groups as expected given that the metabolic parameters were used in categorizing metabolic health status. MONW individuals in the younger age group were more likely to be men and smokers and have a parental history of DM than their MHNW counterparts. MONW individuals in the older age group had less education and were less likely to be physically active, compared to their MHNW individuals.

Table 4.2 shows the distribution of weekly consumption frequencies of each component of Mediterranean diet according to the presence or absence of MHO and MONW phenotypes in both age groups. Overall, MDS was not very different between MHO and MUO; or between MONW and MHNW in either age group. MHO individuals consumed more grains and legumes than MUO individuals in the younger age group. MONW individuals consumed less fish in older age group. In addition, MONW individuals were less likely to consume fruits and poultry in younger age group; fruits and red meats in older age group, compared to MHNW individuals, all of which were marginally significant. When we compared MHO individuals with MHNW individuals in each age group, older MHO individuals consumed fewer vegetables. In addition, younger MHO individuals consumed fewer vegetables, whereas younger MHO individuals had

lower MDS and consumed more red meats; older MHO individuals consumed less dairy products, all of which were marginally statistically significantly different.

Table 4.3 shows the distribution of each components of DASH style diet according to the presence or absence of MHO and MONW phenotypes in both age groups. Overall, no difference of DASH score was observed between metabolic phenotypes in either age group, although MONW individuals had a lower average DASH score compared to MHNW individuals in the younger age group which was marginally significant. MHO individuals consumed less cholesterol than MUO individuals in older age group. MONW individuals consumed less fiber and magnesium in younger age group, and less protein in older age group, compared to MHNW individuals. When we compared MHO individuals with MHNW individuals, MHO individuals had a lower average DASH score; consumed more total fat and saturated fat, and less fiber.

Distribution of metabolic risk factors according to the tertiles of Mediterranean diet scores and DASH index by age group are shown in Table 4.4 for obese individuals and Table 4.5 for normal weight individuals, after adjusting for age, gender, and race/ethnicity. Overall, triglycerides tended to decrease with increasing MDS tertile in all subgroups; HDL-C was likely to increase with increasing MDS tertile, especially in older obese and younger normal weigh individuals. In contrast, triglycerides tended to increase with increasing DASH tertile although significant trend was observed only in young obese individuals. DBP tended to decrease with increasing DASH tertile except younger obese group. However, SBP was likely to increase with increasing MDS tertile in obese individuals; DBP increased in old obese individuals. BMI and waist circumference tended to decrease in younger obese individuals with increasing tertiles of MDS and

DASH index. Conversely, BMI tended to increase with increasing tertile of MDS in younger normal weight individuals. Decrease of hs-CRP was only observed in obese individuals. Total energy intake was decreased with increasing tertile of DASH index in all subgroups.

Figures 4.1a through 1d show the prevalence of MHO and MONW according to tertiles of MDS and DASH scores in younger and older age groups. With increasing MDS, there was a higher prevalence of MHO phenotype, especially in young age group (P for trend = 0.01). No significant trend was observed in other results.

Table 4.6 shows the association of MDS and DASH scores with MHO and MONW phenotypes in younger and older age groups. Among younger adults, those in the third vs. first tertile of MDS had higher odds of MHO phenotype (OR, 2.57 [95% CI, 1.04-6.35], P trend = 0.04), whereas those in the third vs. first tertile of DASH index had lower odds of MONW phenotype (OR, 0.59 [95% CI, 0.38-0.93], P trend = 0.03), after multivariable adjustment. Although those in the third vs. first tertile of MDS among older adults had marginally significantly higher odds of MHO phenotype in age-, gender-, and race/ethnicity- adjusted model (OR, 1.84 [95% CI, 0.99-3.43], P trend = 0.06), this association was no longer significant after multivariable adjustment. Otherwise, no significant association of MDS and DASH index with MHO and MONW phenotypes was observed.

Table 4.7 reports results of the logistic regression analyses examining whether level of physical activity was associated with MHO and MONW phenotypes. Individuals in the younger age group who reported getting recommended levels of physical activity had lower odds of MONW phenotype, whereas individuals in the older age group who

reported getting recommended levels of physical activity had both higher odds of MHO and lower odds of MONW phenotype.

Discussion

In this nationally representative sample of U.S. adults, the Mediterranean diet and DASH style diet were associated with metabolic phenotypes in the younger age group that included men < 45 years and premenopausal women. More specifically, MDS was positively associated with MHO phenotype, whereas the DASH index was inversely associated with the MONW phenotype, independent of a wide range of potential confounders. MDS and DASH index were not associated with MHO and MONW phenotypes in the older age group that included men ≥ 45 years and postmenopausal women, suggesting that healthy dietary patterns may be more effective in reducing the cardiometabolic risk among younger adults.

Several epidemiological studies have explored the association of healthy dietary patterns with MHO and MONW phenotypes. Phillips et al. showed that higher compliance with food pyramid recommendations and DASH diet score were likely to be associated with metabolic health in obese and non-obese, respectively, Irish population aged 45-74 years. However, the results were not significant after multivariable adjustment (19). The age distribution of this study was similar to the older age group in our study in which no significant association of high dietary quality with metabolic health existed. Camhi et al. demonstrated that Healthy Eating Index 2005 scores were higher in MHO women aged 19-44 years compared with MUO women, but no difference was found in adults 45-85 years based on NHANES 2007-2010 data (14). Suliga et al. evaluated a normal weight Polish population aged 37-66 using the prudent dietary pattern,

characterized by a high consumption of fish and whole grains, and a low consumption of refined grains, sugar, sweets and cold cured meat, and found that higher scores of the prudent dietary pattern were associated with lower odds of MONW phenotype (18).

Our study is unique in terms of assessing both Mediterranean diet and DASH style diet, measured by FFQ and 24 dietary recall, respectively, in the association with MHO and MONW phenotypes, according to age groups known to have distinct differential cardiovascular risk. Consistent with the findings from the previous research (14, 16, 19), the association between high diet quality and metabolic health was not apparent in older age group, although MDS was marginally significant with being more likely to be MHO phenotype in age-, gender-, and race/ethnicity- adjusted model. These findings suggest that other factors such as physical activity might be more important than diet in relation to metabolic abnormalities among older adults, which is supported by the fact that age-related changes in body composition, especially increase in visceral fat, may elevate the risk of cardiometabolic disease (37). In our study, individuals in the older age group who reported getting recommended levels of physical activity had higher odds of MHO and lower odds of MONW phenotype (Table 4.7).

Interestingly, the significant associations of MDS and DASH index with metabolic health in the younger age group differed according to MHO and MONW phenotypes; MDS was positively associated the MHO phenotype, whereas the DASH index was inversely associated with the MONW phenotype. The Mediterranean diet is rich in dietary fiber, antioxidant capacity, polyphenolic compounds, and magnesium (38), all of which are closely related to decreased insulin resistance, a key mechanism of metabolic abnormalities in obese individuals (39). We found that HOMA-IR, a surrogate

measure of insulin resistance, was significantly decreased with increasing MDS tertile, especially in younger obese individuals (Table 4.4). In addition, high intake of monounsaturated fatty acids (MUFAs), a key characteristic of the Mediterranean diet usually represented by olive oil consumption, contributes to lowering triglycerides and increasing HDL-C, two main components for determining metabolic health (40). Although intake of a single food cannot account for the underlying mechanism, higher consumption of legumes, another food source rich in MUFAs, in younger MHO individuals shown in Table 4.2 might contribute much to the association between higher MDS and MHO phenotype in the present population. Furthermore, the decreasing level of triglycerides with increasing tertile of MDS might support this association. In contrast, the increasing levels of triglycerides were observed with increasing tertiles of DASH index, indicating that a low fat diet may have an adverse effect on lipid profiles (Table 4.4) (41, 42).

In younger normal weight individuals, higher DASH index was associated with lower odds of MONW phenotype. MDS also showed the same direction with DASH index in the association with MONW, although it was not statistically significant. The DASH index used in the present study was developed based on the nutrient targets in the DASH trial (33). Among the components of DASH index, the consumption of fiber and magnesium was significantly lower in young MONW individuals, compared with their MHNW counterparts. Magnesium has been known to be associated with lower risk of metabolic syndrome (43) and type 2 DM (44). It is unclear whether dietary fiber and magnesium act synergistically or independently on decreasing the risk of metabolic disease such as type 2 DM (45). However, dietary fiber and magnesium are known to be

highly correlated (46), which may support our results that two factors were significantly different between MONW and MHNW phenotypes altogether.

It has been argued that the MHO phenotype might not be a favorable condition, compared with MHNW phenotype (47). Few studies have compared dietary consumption between MHNW and MHO phenotypes. A study conducted among white and black women aged 45-98 years demonstrated that food intake was not different between two phenotypes after multivariable adjustment (20). The present study showed that MHO individuals consumed less vegetables and fiber, higher saturated fat and total fat, and had a lower DASH score, especially in younger age group, suggesting that MHO individuals are less likely to have healthy dietary habit compared with MHNW individuals.

As expected, the prevalence and characteristics of MHO and MONW phenotypes were considerably different between younger and older age groups that were pre-defined by differential cardiovascular risk. The prevalence of MHO was relatively higher in younger age group whereas the prevalence of MONW was relatively higher in older age group, which also has been reported from other studies (34, 48). In addition, both MHO and MONW phenotypes were associated with DM parental history in younger age group; physical activity in older ager group altogether, suggesting that genetic factors in younger age group and lifestyle related factor in older age group might be predominantly related to metabolic health independent of obesity status.

Our study has several strengths. First, we used data from a valid and reliable nationally representative study, based on extensive laboratory and physical examinations using standardized protocols to minimize the influence of measurement errors. Second, we assessed the association between healthy dietary patterns and metabolic health in a

comprehensive way consisting of normal weight and obese populations; younger and older age groups with distinct differential cardiovascular risk, using MDS and DASH score. Finally, the present study adjusted for a wide range of potential confounders in multivariable analyses. There are also several limitations. First, this cross-sectional study cannot imply a causal relationship between healthy dietary pattern and metabolic health, although we tried to minimize reverse causality using strict exclusion criteria. Second, since the information on "servings per week" was not available at NHANES III FFQ, we used "times per week" in assessing the consumption frequency for MDS calculation. This approach might cause exposure misclassification, but the direction would be non-differential. In addition, we used nutrient data from the 24-hr dietary recall data in calculating DASH score, which might not be comparable to DASH score based on FFQ in other studies. However, we were able to compare the association between metabolic health and healthy dietary pattern in terms of food based approach and nutrient based approach. Third, there might be residual confounding due to not measuring the covariates in an objective way, such as self-reported physical activity and smoking status.

In conclusion, the prevalence of MHO and MONW phenotypes and their related characteristics were distinctly different in younger and older age groups. Adherence to Mediterranean diet or DASH style diet is likely to be beneficially associated with MHO and MONW phenotypes in younger adults, suggesting that potential interventions to prevent cardiometabolic disease may need to be tailored by age group. Prospective studies evaluating relations between healthy dietary patterns and the risk of metabolic health are warranted.

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Table 4.1 Comparison of general and clinical characteristics between the metabolic phenotypes in younger age group and older age group

Characteristics	Younger age group (men < 45 years and premenopausal women)									
	MHNW (n=1,096)		MONW (n=164)		P	MHO (n=284)		MUO (n=411)		P
Age, years	31.1	± 0.3	35.6	± 0.5	<0.001	35.1	± 0.6	36.1	± 0.7	0.27
Men, %	42.5	± 1.8	61.0	± 6.3	0.005	36.5	± 4.6	53.5	± 5.2	0.03
Race/ ethnicity, %										
Non-Hispanic white	74.6	± 3.0	70.7	± 6.5	0.83	61.9	± 5.1	66.1	± 4.0	<0.001
Non-Hispanic black	10.0	± 1.2	9.8	± 1.9		27.0	± 3.9	13.5	± 2.4	
Mexican-American	4.8	± 0.8	5.9	± 1.4		5.2	± 1.1	11.0	± 1.9	
Other	10.6	± 2.1	13.6	± 6.3		5.9	± 1.6	9.4	± 2.9	
Educational attainment, %										
<12 years	15.7	± 1.7	18.3	± 4.5	0.80	19.2	± 3.2	22.8	± 4.5	0.75
12 years	33.5	± 2.4	29.6	± 6.8		44.7	± 4.8	40.7	± 5.3	
>=13 years	50.8	± 2.4	52.2	± 6.6		36.1	± 4.4	36.5	± 5.6	
Income, %										
PIR=<1.3	19.5	± 2.3	15.7	± 3.6	0.14	23.2	± 4.4	17.8	± 2.3	0.41
PIR=<3.5	40.2	± 2.7	55.1	± 8.6		42.6	± 5.2	51.2	± 5.0	
PIR>3.5	40.3	± 2.6	29.3	± 7.8		34.2	± 5.1	31.0	± 4.5	
Living with spouse, %	55.6	± 2.4	64.2	± 6.4	0.24	69.9	± 4.2	67.4	± 4.7	0.64
Smoking status, %										
Never	50.5	± 2.4	34.6	± 4.4	0.02	51.8	± 6.4	44.5	± 4.7	0.08
Former	18.4	± 1.7	23.7	± 4.7		15.8	± 4.0	29.9	± 5.0	
Current	31.1	± 2.5	41.7	± 6.1		32.4	± 5.4	25.6	± 2.9	
Drinking alcohol, %										
Never	33.7	± 2.2	40.1	± 6.6	0.28	39.1	± 5.0	46.6	± 4.9	0.47
Moderate	63.8	± 2.2	59.3	± 6.6		59.9	± 5.0	52.6	± 4.9	
Heavy	2.5	± 0.5	0.6	± 0.5		1.1	± 0.6	0.7	± 0.5	
Physical activity, %										
Inactive	10.4	± 1.7	13.0	± 3.2	0.59	13.9	± 2.2	12.1	± 2.4	0.73

Insufficient activity	54.7	±	2.6	57.0	±	6.0		64.4	±	5.1	61.1	±	4.7	
Recommended Activity	35.0	±	2.7	30.0	±	4.9		21.7	±	4.7	26.8	±	5.1	
CHD family history, %	16.0	±	1.8	17.8	±	4.0	0.68	12.4	±	3.2	23.9	±	3.6	0.05
DM parental history, %	12.6	±	1.6	21.4	±	5.2	0.04	21.7	±	4.5	35.8	±	5.9	0.03
DM, %	0.03	±	0.03	2.3	±	1.6	<0.001	0.00	±	0.00	7.3	±	2.1	NA
Hypertension, %	3.2	±	0.6	19.3	±	4.9	<0.001	19.4	±	5.0	35.5	±	4.0	0.03
BMI, kg/m^2	22.0	±	0.1	22.8	±	0.1	<0.001	33.6	±	0.2	35.4	±	0.4	<0.001
Waist circumference, cm	77.8	±	0.2	84.8	±	0.8	<0.001	104.1	±	0.9	109.5	±	1.0	<0.001
Fasting glucose, mg/dl	89	±	0.3	98	±	1.0	<0.001	92	±	0.5	103	±	1.4	<0.001
HOMA-IR	1.48	±	0.03	2.32	±	0.09	<0.001	2.62	±	0.05	4.86	±	0.31	<0.001
SBP, mmHg	111	±	0.4	122	±	2.1	<0.001	118	±	1.0	125	±	0.7	<0.001
DBP, mmHg	69	±	0.5	77	±	1.2	<0.001	74	±	0.8	81	±	0.8	<0.001
hs-CRP, mg/dl	0.25	±	0.01	0.40	±	0.03	<0.001	0.43	±	0.02	0.55	±	0.04	0.003
Triglycerides, mg/dl	79	±	1.5	161	±	6.0	<0.001	98	±	3.6	181	±	7.2	<0.001
HDL-C, mg/dl	56	±	0.7	43	±	1.4	<0.001	50	±	0.9	38	±	0.8	<0.001

Table 4.1 (Continued)

Characteristics	Older age group (men ≥ 45 years and postmenopausal women)													
	MHNW (n=244)			MONW (n=223)			P	MHO (n=69)			MUO (n=276)			P
Age, years	59.0	±	0.7	64.4	±	0.8	<0.001	60.3	±	0.6	58.4	±	0.6	0.05
Men, %	57.2	±	4.0	54.7	±	4.6	0.64	28.5	±	6.7	50.2	±	4.4	0.01
Race/ ethnicity, %														
Non-Hispanic white	81.6	±	3.0	73.2	±	3.9	0.09	74.0	±	6.2	80.5	±	3.6	<0.001
Non-Hispanic black	7.4	±	1.8	10.8	±	1.9		21.5	±	5.4	9.2	±	1.4	
Mexican-American	2.0	±	0.5	3.7	±	1.0		4.0	±	1.4	4.1	±	0.8	
Other	9.0	±	2.2	12.3	±	3.2		0.5	±	0.5	6.1	±	3.4	
Educational attainment, %														
<12 years	24.2	±	3.4	39.1	±	4.3	0.01	19.2	±	3.2	22.8	±	4.5	0.75

12 years	28.2	±	4.7	30.8	±	4.5		44.7	±	4.8	40.7	±	5.3	
>=13 years	47.6	±	4.3	30.2	±	4.0		36.1	±	4.4	36.5	±	5.6	
Income, %														
PIR=<1.3	13.7	±	3.0	22.4	±	3.7	0.07	12.7	±	6.6	18.4	±	4.0	0.63
PIR=<3.5	38.9	±	4.1	42.3	±	3.9		43.0	±	8.7	44.9	±	3.8	
PIR>3.5	47.4	±	4.5	35.3	±	5.2		44.3	±	8.8	36.7	±	4.3	
Living with spouse, %	68.4	±	4.0	64.2	±	4.1	0.39	75.5	±	5.8	73.3	±	3.5	0.74
Smoking status, %														
Never	45.8	±	3.8	49.1	±	4.5	0.79	61.4	±	6.9	38.2	±	3.6	0.002
Former	25.0	±	3.5	22.3	±	4.1		32.8	±	7.1	38.4	±	4.0	
Current	29.1	±	3.6	28.5	±	3.5		5.8	±	3.1	23.4	±	3.1	
Drinking alcohol, %														
Never	44.8	±	4.9	48.7	±	5.8	0.65	63.4	±	8.5	58.0	±	4.2	0.43
Moderate	50.2	±	5.0	45.3	±	5.8		32.2	±	7.8	40.6	±	4.5	
Heavy	5.0	±	1.9	6.0	±	2.4		4.4	±	4.3	1.4	±	0.8	
Physical activity, %														
Inactive	10.3	±	1.9	21.0	±	3.8	0.02	16.8	±	4.2	22.6	±	3.0	0.02
Insufficient activity	48.3	±	4.4	37.4	±	4.1		36.9	±	9.9	51.8	±	3.8	
Recommended Activity	41.4	±	4.0	41.6	±	4.4		46.3	±	9.0	25.7	±	3.2	
CHD family history, %	9.9	±	2.6	8.7	±	2.7	0.72	10.1	±	4.5	20.1	±	3.4	0.11
DM parental history, %	17.6	±	4.5	13.8	±	2.6	0.38	14.4	±	5.2	29.3	±	4.6	0.09
DM, %	4.2	±	2.0	15.4	±	3.2	0.006	6.4	±	2.8	29.9	±	4.4	0.001
Hypertension, %	24.9	±	4.0	60.8	±	4.8	<0.001	36.2	±	7.7	61.3	±	5.1	0.02
BMI, kg/m^2	22.4	±	0.1	22.9	±	0.1	<0.001	32.8	±	0.2	34.2	±	0.3	<0.001
Waist circumference, cm	84.3	±	0.5	87.5	±	0.6	<0.001	104.8	±	0.6	111.6	±	0.7	<0.001
Fasting glucose, mg/dl	93	±	0.8	106	±	1.6	<0.001	95	±	0.6	121	±	3.2	<0.001
HOMA-IR	1.50	±	0.03	2.29	±	0.08	<0.001	2.59	±	0.07	5.69	±	0.41	<0.001
SBP, mmHg	123	±	1.0	140	±	1.5	<0.001	129	±	1.3	136	±	1.1	0.000
DBP, mmHg	73	±	0.5	78	±	0.9	<0.001	76	±	1.0	79	±	0.5	0.01
hs-CRP, mg/dl	0.26	±	0.01	0.36	±	0.02	<0.001	0.42	±	0.03	0.60	±	0.03	<0.001
Triglycerides, mg/dl	96	±	3.1	173	±	12.8	<0.001	108	±	4.4	202	±	7.6	<0.001

HDL-C, mg/dl	60	±	1.5	49	±	1.3	<0.001	58	±	1.1	44	±	1.2	<0.001

Data are presented as means ± standard error or proportion (%) ± standard error.
P values for continuous variables represent P for trend.
Abbreviations: MHNW, metabolically healthy normal weight; MONW, metabolically obese normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; PIR, poverty income ratio; CHD, coronary heart disease; DM, diabetes mellitus, BMI, body mass index; HOMA-IR, homoeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol.

Table 4.2 Comparison for each component of Mediterranean diet scores between the metabolic phenotypes in younger age group and older age group

	Younger age group (men < 45 years and premenopausal women)						
	MHNW (n=1,096)	MONW (n=164)	P	MHO (n=284)	MUO (n=411)	P	P (MHNW vs MHO)
Score range	(12 - 41)	(12 - 37)		(13 - 38)	(12 - 39)		
MDS	26.1 ± 0.2	26.0 ± 0.5	0.35	26.0 ± 0.4	25.2 ± 0.3	0.14	0.03
Grains (times/wk)	3.9 ± 0.2	4.1 ± 0.5	0.87	4.1 ± 0.3	5.0 ± 0.7	0.01	0.79
Legumes (times/wk)	2.7 ± 0.1	2.6 ± 0.2	0.33	2.9 ± 0.2	2.6 ± 0.2	**0.005**	0.92
Fruit (times/wk)	5.4 ± 0.3	4.8 ± 0.3	0.04	4.7 ± 0.6	4.7 ± 0.4	0.72	0.12
Vegetable (times/wk)	13.6 ± 0.5	14.2 ± 1.1	0.59	12.4 ± 0.5	12.1 ± 0.7	0.90	0.15
Fish (times/wk)	1.5 ± 0.1	1.5 ± 0.2	0.99	1.6 ± 0.2	1.2 ± 0.2	0.18	0.61
MUFA:SFA	1.20 ± 0.02	1.19 ± 0.03	0.59	1.18 ± 0.01	1.18 ± 0.02	0.38	0.41
Red meats (times/wk)	5.3 ± 0.1	5.9 ± 0.4	0.18	5.6 ± 0.6	5.6 ± 0.4	0.28	0.02
Poultry (times/wk)	2.2 ± 0.1	1.8 ± 0.1	0.03	2.1 ± 0.2	2.1 ± 0.1	0.36	0.59
Dairy products (times/wk)	12.7 ± 0.4	11.0 ± 0.8	0.08	10.5 ± 0.9	11.8 ± 0.7	0.15	0.43
Alcohol (g/d)	3.6 ± 0.3	4.0 ± 0.6	0.97	2.6 ± 0.3	2.4 ± 0.4	0.11	0.36
Total energy intake, kcal	2316 ± 46	2152 ± 89	**<0.001**	2132 ± 82	2478 ± 110	0.12	0.07

Table 4.2 (Continued)

	Older age group (men ≥ 45 years and postmenopausal women)						
	MHNW (n=244)	MONW (n=223)	P	MHO (n=69)	MUO (n=276)	P	P (MHNW vs MHO)
Score range	(14 - 42)	(16 - 40)		(15 - 36)	(15 - 40)		
MDS	27.5 ± 0.4	26.8 ± 0.4	0.64	26.2 ± 0.4	25.3 ± 0.4	0.47	0.34

Grains (times/wk)	5.4	±	0.3	6.1	±	0.5	0.98	7.1	±	1.0	4.9	±	0.4	0.22	0.61
Legumes (times/wk)	3.1	±	0.2	3.2	±	0.3	0.16	2.3	±	0.2	2.4	±	0.2	0.78	0.41
Fruit (times/wk)	8.5	±	0.5	6.8	±	0.6	0.03	7.2	±	0.4	7.2	±	0.4	0.16	0.18
Vegetable (times/wk)	17.1	±	0.7	16.0	±	0.7	0.04	14.5	±	0.8	13.5	±	0.5	0.60	**0.002**
Fish (times/wk)	1.7	±	0.1	1.2	±	0.1	**0.003**	1.4	±	0.1	1.2	±	0.1	0.10	0.73
MUFA:SFA	1.22	±	0.03	1.20	±	0.03	0.57	1.23	±	0.03	1.20	±	0.03	0.34	0.36
Red meats (times/wk)	5.0	±	0.3	4.4	±	0.3	0.02	4.4	±	0.2	5.3	±	0.3	0.20	0.16
Poultry (times/wk)	2.1	±	0.1	2.0	±	0.1	0.18	2.0	±	0.2	2.0	±	0.2	0.93	0.31
Dairy products (times/wk)	11.3	±	0.4	10.7	±	0.7	0.73	9.1	±	0.7	11.6	±	0.9	0.22	0.03
Alcohol (g/d)	6.0	±	1.1	5.0	±	0.9	0.56	2.2	±	0.3	2.7	±	0.8	0.99	0.30
Total energy intake, kcal	2051	±	53	1914	±	70	0.88	1690	±	68	1955	±	56	0.96	0.09

Data are presented as means ± standard error.

P values were obtained after adjusting for educational attainment, income, living with spouse, smoking status, alcohol consumption, level of physical activity, family history of CHD, parental history of DM, total energy intake, and body mass index. Alcohol consumption was excluded in the model for MDS and alcohol. Bold numbers indicate statistically significant differences between metabolic phenotypes, considering a Bonferroni-corrected significance threshold (alpha = 0.0167).

Abbreviations: MDS, Mediterranean diet scores; MHNW, metabolically healthy normal weight; MONW, metabolically obese normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

Table 4.3 Comparison for each component of DASH index between the metabolic phenotypes in younger age group and older age group

	Younger age group (men < 45 years and premenopausal women)								
	MHNW (n=1,096)	MONW (n=164)	P	MHO (n=284)	MUO (n=411)	P	P (MHNW vs MHO)
Score range	(0 - 7)	(0 - 6.5)		(0 - 6.5)	(0 - 8)		
DASH score	2.1 ± 0.1	2.0 ± 0.1	0.03	1.8 ± 0.1	1.8 ± 0.1	0.31	**0.008**
Saturated fat, % energy	11.3 ± 0.2	11.0 ± 0.3	0.84	11.5 ± 0.4	12.1 ± 0.4	0.98	**0.009**
Total fat, % energy	33.9 ± 0.5	33.0 ± 0.7	0.77	35.4 ± 0.8	36.7 ± 0.7	0.76	**0.008**
Protein, % energy	15.1 ± 0.2	14.7 ± 0.5	0.05	15.4 ± 0.5	15.1 ± 0.3	0.35	0.16
Cholesterol, mg	129 ± 4	125 ± 8	0.24	132 ± 7	133 ± 6	0.48	0.75
Fiber, mg	7.4 ± 0.1	6.7 ± 0.3	**<0.001**	6.7 ± 0.2	7.3 ± 0.3	0.18	**0.008**
Magnesium, mg	135 ± 2	127 ± 3	**<0.001**	126 ± 4	128 ± 3	0.76	0.03
Calcium, mg	372 ± 12	347 ± 18	0.48	336 ± 17	371 ± 19	0.91	0.60
Potassium, mg	1265 ± 16	1236 ± 31	0.08	1221 ± 38	1266 ± 23	0.58	0.99
Sodium, mg	1621 ± 20	1581 ± 49	0.52	1711 ± 42	1709 ± 65	0.85	0.03

Table 4.3 (Continued)

	Older age group (men ≥ 45 years and postmenopausal women)						
	MHNW (n=244)	MONW (n=223)	P	MHO (n=69)	MUO (n=276)	P	P (MHNW vs MHO)
Score range	(0 - 8.5)	(0 - 8)		(0 - 7.5)	(0 - 7.5)		
DASH score	2.8 ± 0.1	2.9 ± 0.2	0.93	2.5 ± 0.2	2.5 ± 0.2	0.56	0.12
Saturated fat, % energy	10.2 ± 0.3	10.0 ± 0.4	0.66	11.2 ± 0.5	11.2 ± 0.3	0.56	0.33

Total fat, % energy	31.7	±	0.6	30.5	±	0.8	0.27	34.1	±	0.8	33.9	±	0.7	0.53	0.74
Protein, % energy	16.1	±	0.4	15.0	±	0.4	**0.01**	16.1	±	0.4	15.6	±	0.3	0.46	0.23
Cholesterol, mg	130	±	11	129	±	14	0.45	128	±	6	136	±	6	**0.01**	0.39
Fiber, mg	9.5	±	0.4	9.2	±	0.5	0.34	8.5	±	0.4	8.4	±	0.3	0.89	0.08
Magnesium, mg	162	±	4	156	±	5	0.13	149	±	5	151	±	4	0.71	0.18
Calcium, mg	393	±	13	410	±	21	0.69	380	±	20	412	±	16	0.10	1.00
Potassium, mg	1561	±	36	1547	±	45	0.64	1485	±	50	1510	±	28	0.90	0.10
Sodium, mg	1639	±	38	1675	±	140	0.58	1773	±	65	1679	±	35	0.35	0.58

Data are presented as means ± standard error.
P values were obtained after adjusting for educational attainment, income, living with spouse, smoking status, alcohol consumption, level of physical activity, family history of CHD, parental history of DM, total energy intake, and body mass index.
Bold numbers indicate statistically significant differences between metabolic phenotypes, considering a Bonferroni-corrected significance threshold (alpha = 0.0167).
Abbreviations: MHNW, metabolically healthy normal weight; MONW, metabolically obese normal weight; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; DASH, Dietary Approaches to Stop Hypertension. The amount of each DASH dietary components are based on a 1,000 kcal diet.

Table 4.4 Comparison for metabolic risk factors according to the tertiles of Mediterranean diet scores and DASH index by age group in obese participants

Characteristics	Younger age group (men < 45 years and premenopausal women)					
	MDS			DASH index		
	Tertile 1 (n=385)	Tertile 3 (n=408)	P trend	Tertile 1 (n=399)	Tertile 3 (n=381)	P trend
Score range	(12 - 23)	(29 - 39)		(0 - 1)	(3 - 8)	
Index scores	20.6 ± 0.2	31.2 ± 0.1	<0.001	0.6 ± 0.0	4.0 ± 0.1	<0.001
Age, years	35.4 ± 0.8	36.0 ± 1.0	0.85	35.7 ± 0.7	37.0 ± 0.7	0.23
BMI, kg/m2	36.3 ± 0.5	34.1 ± 0.5	<0.001	35.0 ± 0.3	34.4 ± 0.5	0.10
Waist circumference, cm	112 ± 1.2	105 ± 1.2	<0.001	109 ± 1.0	105 ± 1.3	0.03
Fasting glucose, mg/dl	98 ± 1.4	100 ± 2.0	0.93	98 ± 0.7	100 ± 1.8	0.77
HOMA-IR	4.36 ± 0.29	3.50 ± 0.19	0.002	3.74 ± 0.25	4.12 ± 0.36	0.54
hs-CRP, mg/dl	0.52 ± 0.03	0.43 ± 0.05	0.02	0.53 ± 0.04	0.42 ± 0.04	0.001
SBP, mmHg	123 ± 0.9	126 ± 2.0	0.002	122 ± 0.9	120 ± 1.4	0.57
DBP, mmHg	79 ± 0.8	80 ± 1.1	0.49	78 ± 0.9	77 ± 1.5	0.78
Triglycerides, mg/dl	170 ± 9.2	141 ± 10.5	0.02	129 ± 5.7	150 ± 9.6	0.001
HDL-C, mg/dl	41 ± 0.9	44 ± 1.3	0.38	43 ± 1.4	45 ± 1.7	0.94
Total energy intake, kcal	2520 ± 109	2279 ± 154	0.05	2599 ± 100	2028 ± 62	<0.001

Table 4.4 (Continued)

Characteristics	Older age group (men ≥ 45 years and postmenopausal women)					
	MDS			DASH index		
	Tertile 1 (n=125)	Tertile 3 (n=174)	P trend	Tertile 1 (n=102)	Tertile 3 (n=221)	P trend
Score range	(15 - 23)	(29 - 40)		(0 - 1)	(3 - 7.5)	
Index scores	20.3 ± 0.3	31.2 ± 0.3	<0.001	0.5 ± 0.0	4.2 ± 0.2	<0.001
Age, years	58.6 ± 0.6	59.3 ± 0.7	0.07	59.9 ± 0.9	58.3 ± 0.7	0.25
BMI, kg/m2	33.8 ± 0.3	34.0 ± 0.6	0.20	33.1 ± 0.3	33.3 ± 0.3	0.92
Waist circumference, cm	111 ± 0.9	112 ± 1.3	0.66	108 ± 0.7	109 ± 1.0	0.87
Fasting glucose, mg/dl	119 ± 5.6	114 ± 1.6	0.24	107 ± 2.2	123 ± 5.9	0.01
HOMA-IR	5.60 ± 0.58	4.48 ± 0.29	0.21	3.99 ± 0.28	6.09 ± 0.66	0.12
hs-CRP, mg/dl	0.55 ± 0.04	0.55 ± 0.03	0.04	0.56 ± 0.05	0.53 ± 0.06	0.53
SBP, mmHg	131 ± 1.0	137 ± 2.5	0.02	135 ± 1.3	132 ± 1.5	0.14
DBP, mmHg	77 ± 0.7	80 ± 1.2	0.009	79 ± 0.7	76 ± 0.7	<0.001
Triglycerides, mg/dl	193 ± 10.2	176 ± 15.6	0.11	166 ± 8.6	193 ± 9.1	0.28
HDL-C, mg/dl	44 ± 1.4	50 ± 2.8	0.007	47 ± 1.3	46 ± 2.1	0.58
Total energy intake, kcal	1883 ± 64	1908 ± 125	0.68	2179 ± 87	1813 ± 94	<0.001

Data are presented as means ± standard error.
P values were obtained after adjusting for age, gender, and race/ethnicity. P values for HOMA-IR, hs-CRP, and triglycerides were calculated after log transformation of each observation due to their skewed distribution.
Abbreviations: BMI, body mass index; HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol.

Table 4.5 Comparison for metabolic risk factors according to the tertiles of Mediterranean diet scores and DASH index by age group in normal weight participants

Characteristics	Younger age group (men < 45 years and premenopausal women)					
	MDS			DASH index		
	Tertile 1 (n=385)	Tertile 3 (n=408)	P trend	Tertile 1 (n=399)	Tertile 3 (n=381)	P trend
Score range	(12 - 23)	(29 - 41)		(0 - 1)	(3 - 7)	
Index scores	20.4 ± 0.2	31.7 ± 0.2	<0.001	0.7 ± 0.0	3.9 ± 0.0	<0.001
Age, years	29.7 ± 0.5	33.6 ± 0.6	<0.001	29.9 ± 0.4	33.0 ± 0.6	<0.001
BMI, kg/m2	21.8 ± 0.1	22.2 ± 0.1	0.007	21.9 ± 0.1	22.2 ± 0.1	0.21
Waist circumference, cm	78 ± 0.6	78 ± 0.5	0.42	78 ± 0.4	78 ± 0.6	0.64
Fasting glucose, mg/dl	90 ± 0.6	90 ± 0.6	0.40	90 ± 0.7	91 ± 0.6	0.96
HOMA-IR	1.64 ± 0.05	1.51 ± 0.03	0.02	1.60 ± 0.06	1.52 ± 0.04	0.18
hs-CRP, mg/dl	0.28 ± 0.01	0.27 ± 0.01	0.46	0.27 ± 0.01	0.25 ± 0.01	0.21
SBP, mmHg	111 ± 0.8	112 ± 0.8	0.80	113 ± 1.0	112 ± 0.8	0.10
DBP, mmHg	69 ± 0.6	70 ± 0.6	0.72	71 ± 0.6	70 ± 0.6	0.02
Triglycerides, mg/dl	89 ± 3.0	87 ± 3.1	0.01	86 ± 2.4	88 ± 2.1	0.63
HDL-C, mg/dl	53 ± 1.0	57 ± 1.1	0.006	54 ± 0.9	56 ± 1.3	0.44
Total energy intake, kcal	2283 ± 75	2226 ± 78	0.23	2590 ± 75	2010 ± 64	<0.001

Table 4.5 (Continued)

Characteristics	Older age group (men ≥ 45 years and postmenopausal women)					
	MDS			DASH index		
	Tertile 1 (n=125)	Tertile 3 (n=174)	P trend	Tertile 1 (n=102)	Tertile 3 (n=221)	P trend
Score range	(14 - 23)	(29 - 42)		(0 - 1)	(3 - 8.5)	
Index scores	20.5 ± 0.2	31.7 ± 0.3	<0.001	0.6 ± 0.0	4.5 ± 0.1	<0.001
Age, years	61.6 ± 0.9	61.7 ± 1.0	0.92	59.6 ± 1.2	64.3 ± 0.9	<0.001
BMI, kg/m2	22.4 ± 0.2	22.6 ± 0.1	0.18	22.7 ± 0.1	22.5 ± 0.1	0.95
Waist circumference, cm	87 ± 0.8	86 ± 0.5	0.40	87 ± 0.9	85 ± 0.4	0.23
Fasting glucose, mg/dl	97 ± 1.5	98 ± 1.2	0.85	98 ± 0.9	99 ± 1.6	0.67
HOMA-IR	1.81 ± 0.10	1.80 ± 0.07	0.58	1.85 ± 0.07	1.78 ± 0.08	0.14
hs-CRP, mg/dl	0.31 ± 0.03	0.31 ± 0.02	0.91	0.32 ± 0.02	0.30 ± 0.01	0.29
SBP, mmHg	130 ± 1.5	130 ± 1.7	0.78	130 ± 1.7	132 ± 1.5	0.36
DBP, mmHg	75 ± 0.8	75 ± 0.6	0.49	76 ± 0.9	74 ± 0.5	0.06
Triglycerides, mg/dl	141 ± 17.0	112 ± 4.0	0.001	123 ± 7.2	138 ± 10.9	0.07
HDL-C, mg/dl	53 ± 2.0	57 ± 1.3	0.10	54 ± 1.5	55 ± 1.1	0.88
Total energy intake, kcal	1938 ± 71	1986 ± 59	0.71	2226 ± 88	1737 ± 63	<0.001

Data are presented as means ± standard error.
P values were obtained after adjusting for age, gender, and race/ethnicity. P values for HOMA-IR, hs-CRP, and triglycerides were calculated after log transformation of each observation due to their skewed distribution.
Abbreviations: BMI, body mass index; HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol.

Table 4.6 Adjusted odds ratios (ORs) of Metabolically Healthy Obese (MHO) and Metabolically Obese Normal Weight (MONW) phenotype according to the tertile categories in the Mediterranean diet score and DASH index

		Younger age group				Older age group			
MHO phenotype		Tertile 1	Tertile 2	Tertile 3	P trend	Tertile 1	Tertile 2	Tertile 3	P trend
	Mediterranean diet score, n (scores range)	207 (12 - 23)	300 (24 - 28)	188 (29 - 39)		103 (15 - 23)	124 (24 - 28)	118 (29 - 40)	
	Age, gender, and race/ethnicity adjusted OR	1.00 (ref.)	1.60 (0.82-3.10)	2.65 (1.19-5.90)	0.02	1.00 (ref.)	1.12 (0.63-1.98)	1.84 (0.99-3.43)	0.06
	Multivariable adjusted OR*	1.00 (ref.)	1.57 (0.83-2.95)	2.57 (1.04-6.35)	0.04	1.00 (ref.)	0.78 (0.43-1.41)	1.07 (0.56-2.06)	0.82
	DASH index, n (scores range)	249 (0 - 1)	268 (1.5 - 2.5)	178 (3 - 8)		75 (0 - 1)	131 (1.5 - 2.5)	139 (3 – 7.5)	
	Age, gender, and race/ethnicity adjusted OR	1.00 (ref.)	1.13 (0.63-2.02)	1.26 (0.70-2.27)	0.43	1.00 (ref.)	0.81 (0.53-1.24)	1.08 (0.60-1.93)	0.67
	Multivariable adjusted OR*	1.00 (ref.)	0.95 (0.59-1.54)	1.10 (0.72-1.67)	0.75	1.00 (ref.)	0.95 (0.53-1.72)	1.18 (0.55-2.51)	0.63
MONW phenotype		Tertile 1	Tertile 2	Tertile 3	P trend	Tertile 1	Tertile 2	Tertile 3	P trend
	Mediterranean diet score, n (scores range)	385 (12 - 23)	467 (24 - 28)	408 (29 - 41)		125 (14 - 23)	168 (24 - 28)	174 (29 - 42)	
	Age, gender, and race/ethnicity adjusted OR	1.00 (ref.)	0.86 (0.52-1.40)	0.69 (0.41-1.18)	0.17	1.00 (ref.)	1.06 (0.59-1.92)	0.75 (0.45-1.24)	0.17
	Multivariable adjusted OR*	1.00 (ref.)	0.95 (0.54-1.67)	0.73 (0.44-1.23)	0.23	1.00 (ref.)	1.14 (0.62-2.12)	0.87 (0.50-1.53)	0.53
	DASH index, n (scores range)	399 (0 - 1)	480 (1.5 - 2.5)	381 (3 - 7)		102 (0 - 1)	144 (1.5 - 2.5)	221 (3 – 8.5)	
	Age, gender, and race/ethnicity adjusted OR	1.00 (ref.)	0.79 (0.43-1.43)	0.67 (0.42-1.09)	0.11	1.00 (ref.)	0.85 (0.42-1.70)	0.90 (0.50-1.64)	0.81
	Multivariable adjusted OR*	1.00 (ref.)	0.72 (0.40-1.32)	0.59 (0.38-0.93)	0.03	1.00 (ref.)	0.84 (0.39-1.79)	1.04 (0.51-2.13)	0.86

Data are presented as odds ratio (95% confidence interval).
Abbreviations: OR, odds ratio; DASH, Dietary Approaches to Stop Hypertension; MHO, metabolically healthy obese; MONW, metabolically obese normal weight.

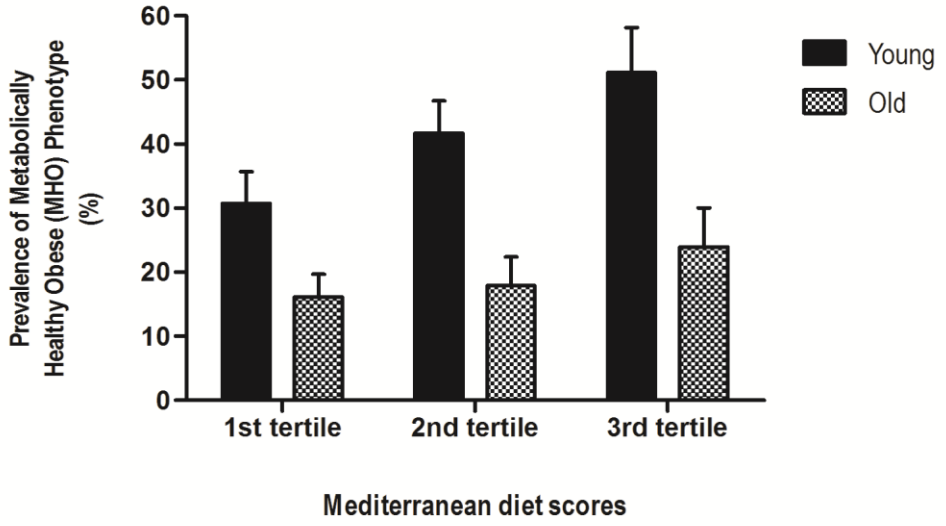
* adjusted for educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, and total calories (additionally adjusted for alcohol consumption in the model of DASH index).

Table 4.7 Multivariable adjusted odds ratios (ORs) of Metabolically Healthy Obese (MHO) and Metabolically Obese Normal Weight (MONW) phenotype according to the levels of physical activity

		Younger age group			Older age group		
		Being inactive	Insufficient activity	Recommended Activity	Being inactive	Insufficient activity	Recommended Activity
MHO phenotype	Multivariable adjusted OR*	1.00 (ref.)	0.74 (0.43-1.28)	0.57 (0.23-1.40)	1.00 (ref.)	1.01 (0.53-1.91)	2.82 (1.36-5.85)
	Multivariable adjusted OR †	1.00 (ref.)	0.67 (0.34-1.33)	0.56 (0.21-1.52)	1.00 (ref.)	1.02 (0.55-1.89)	2.81 (1.35-5.83)
MONW phenotype	Multivariable adjusted OR*	1.00 (ref.)	0.73 (0.38-1.39)	0.53 (0.33-0.86)	1.00 (ref.)	0.44 (0.23-0.86)	0.49 (0.28-0.86)
	Multivariable adjusted OR†	1.00 (ref.)	0.72 (0.38-1.36)	0.53 (0.33-0.86)	1.00 (ref.)	0.45 (0.22-0.91)	0.49 (0.26-0.89)

Data are presented as odds ratio (95% confidence interval).
Abbreviations: OR, odds ratio; MHO, metabolically healthy obese; MONW, metabolically obese normal weight.
All models were adjusted for educational attainment, income, living with spouse, smoking status, family history of coronary heart disease, parental history of diabetes mellitus, and total calories.
* Additionally adjusted for Mediterranean diet score.
† Additionally adjusted for Dietary Approaches to Stop Hypertension (DASH) diet index and alcohol consumption.

A



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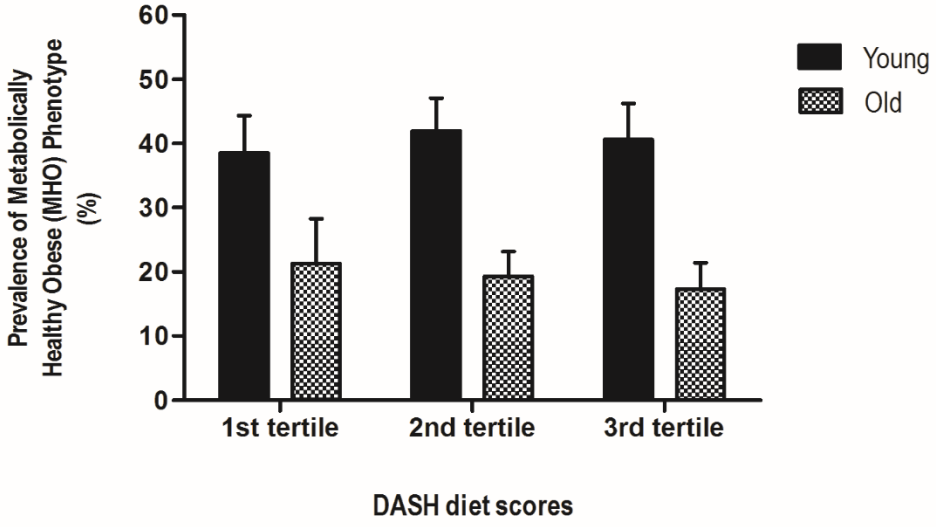

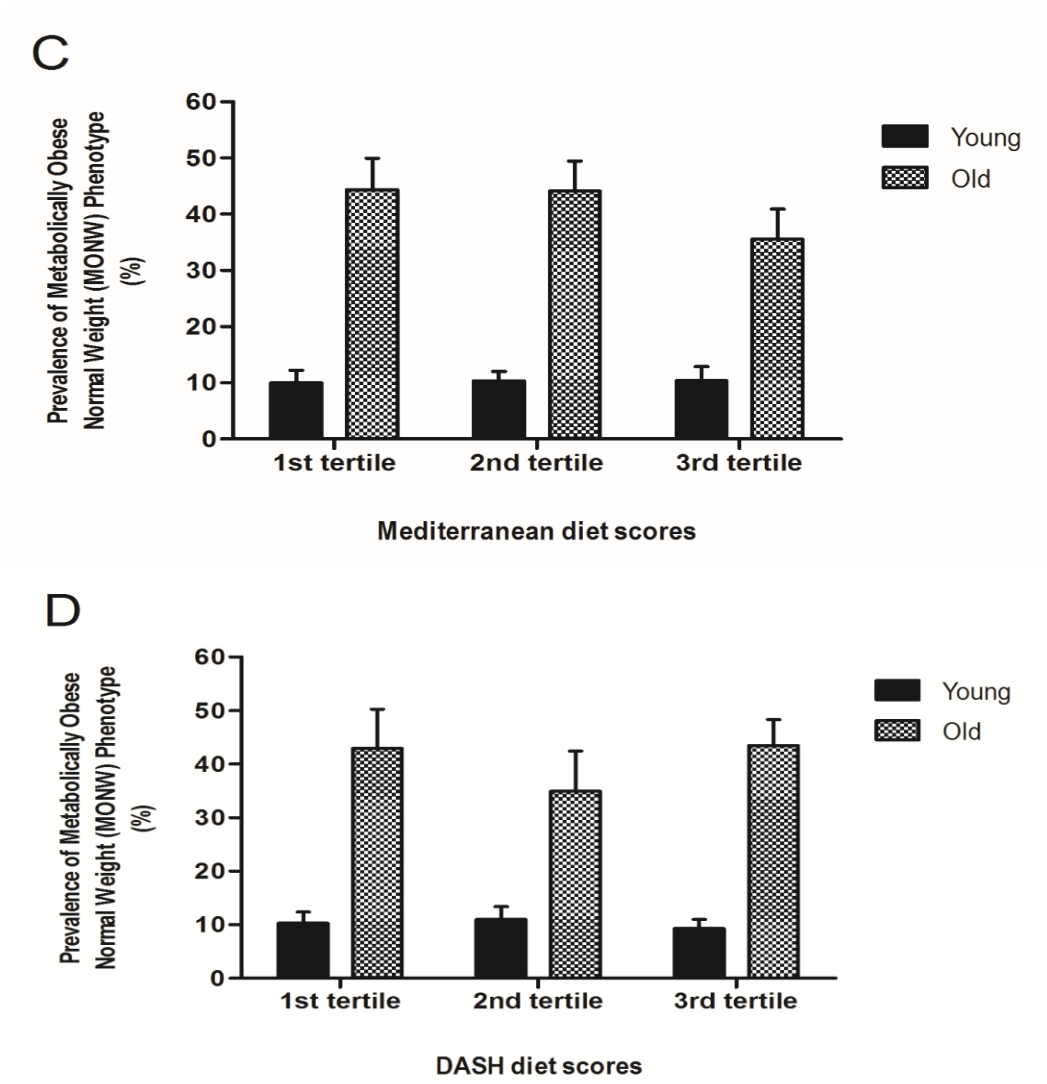

Figure 4.1 Prevalence of Metabolically Healthy Obese (MHO) and Metabolically Obese Normal Weight (MONW) phenotypes according to the tertiles of the Mediterranean diet scores (MDs) and the Dietary Approaches to Stop Hypertension (DASH) style diet index in younger and older age group

((A) prevalence of MHO phenotype according to the MDS tertile, (B) prevalence of MHO phenotype according to the tertile of DASH index, (C) prevalence of MONW phenotype according to the MDS tertile, and (D) prevalence of MONW phenotype according to the tertile of DASH index). The error bars represent standard errors. Younger age group includes men < 45 years and premenopausal women; older age group includes men ≥ 45 years and postmenopausal women. In Figure 4.1a, the prevalence of MHO phenotype increases with increasing MDS in younger age group (P for trend = 0.01).

CHAPTER 5

MEDITERRANEAN DIET AND MORTALITY RISK IN METABOLICALLY HEALTHY OBESE AND METABOLICALLY UNHEALTHY OBESE PHENOTYPES[1]

[1] Yong-Moon Mark Park, Susan E. Steck, Teresa T. Fung, Jiajia Zhang, Linda J. Hazlett, Kyungdo Han, and Anwar M. Merchant. To be submitted to *JAMA Internal Medicine.*

Abstract

IMPORTANCE: The Mediterranean diet has been consistently associated with reduced mortality risk. Few prospective studies have examined whether the benefits from a Mediterranean diet are equally shared by obese individuals with varying metabolic health.

OBJECTIVE: To investigate the association between Mediterranean diet, metabolic phenotypes, and mortality risk in a representative obese U.S. population.

DESIGN, SETTING, AND PARTICIPANTS: Data from 1,739 adults aged 20-88 years were analyzed from participants of the National Health and Nutrition Examination Survey III, 1988–1994 followed up for deaths until December 31, 2011 in a prospective cohort analysis. Mediterranean Diet Scores (MDS) were created to assess the adherence to Mediterranean diet using food frequency questionnaires, supplemented by 24-hr dietary recall data. Participants were classified as metabolically healthy obese (MHO) phenotype (0 or 1 metabolic abnormality) or metabolically unhealthy obese (MUO) phenotype (two or more metabolic abnormalities), based on high glucose, insulin resistance, blood pressure, triglycerides, C-reactive protein, and low high-density lipoprotein-cholesterol. Multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of mortality with increasing three MDS categories and 1-SD increments of MDS.

MAIN OUTCOMES AND MEASURES: All-cause, cardiovascular disease (CVD), and cancer mortality.

RESULTS: During a median follow-up of 18.5 years, there were 77 and 309 deaths in 598 MHO and 1,141 MUO individuals, respectively. In MHO individuals, the

multivariable-adjusted HR of all-cause mortality in the highest tertile compared to the first tertile of MDS was 0.44 (95% CI, 0.26-0.75; P for trend <0.001), after adjustment for potential confounders. The corresponding HR for cancer mortality was 0.23 (95% CI, 0.02-2.10; P trend = 0.03). A 1 SD increment in the adherence to MDS was associated with a 41% reduction in the risk of all-cause mortality (HR, 0.59; 95% CI, 0.37-0.94). Similar findings were obtained when we restricted our analyses to those with or without prevalent diabetes mellitus and hypertension. However, there was no mortality risk reduction in individuals with MUO phenotype.

CONCLUSIONS AND RELEVANCE: Adherence to a Mediterranean dietary pattern appears to improve longevity in the MHO phenotype, but not among the MUO phenotype in an obese population.

KEYWORDS: Mediterranean diet, metabolic health, obesity, mortality, National Health and Nutrition Examination Survey

Introduction

The Mediterranean diet, representing the traditional dietary pattern of the populations living around the Mediterranean Sea, is characterized by high intake of olive oil, legumes, grains, fish, fruits, and vegetables; moderate intake of milk, dairy products, and alcohol; and low intake of meat and meat products. [1] Main components of the Mediterranean diet have been operationalized through predefined indexes or scores to evaluate the adherence to the Mediterranean diet. Multiple epidemiological studies have demonstrated that the adherence to the Mediterranean diet is associated with lower risk of all-cause, cardiovascular disease (CVD), and cancer mortality, in addition to risk reduction of cardiometabolic disease. [2-5]

The risk of developing obesity-related metabolic complications corresponds to the degree of obesity. [6] However, the existence of these obesity-related metabolic abnormalities varies extensively among obese individuals. [7] Despite being outwardly obese, a subset of obese individuals appears to be protected or more resistant to the development of cardiometabolic abnormalities associated with obesity. These individuals with a metabolically healthy obese (MHO) phenotype, namely benign or uncomplicated obesity, demonstrate favorable metabolic characteristics such as high insulin sensitivity, relatively low visceral fat, lower liver enzyme profiles, no sign of dyslipidemia or hypertension [8-10] and lower risk of CVD, [11,12] compared to their metabolically unhealthy obese (MUO) counterparts. Thus, differentiating MHO and MUO individuals would be beneficial to make personalized risk assessment for prevention of obesity-related disease.[13]

Since healthy dietary behaviors are important to reduce obesity-related morbidity and mortality, [14] it would be important to understand how the beneficial effects of established healthy dietary patterns such as Mediterranean diet differ according to MHO and MUO phenotypes. A few studies examined the potential differential association of dietary factors between MHO and MUO phenotypes; however, many of these studies were cross-sectional, [15-18] without consideration of dietary pattern, [17] had small sample size, [19,20] or with a short follow-up. [17] [19,20] [21] In addition, it is unclear how dietary pattern may influence the natural history of these phenotypes differentially.

Therefore, in this analysis we tested hypotheses that Mediterranean diet would have differential health benefit of mortality risk reduction in MHO individuals and MUO individuals, in a nationally representative obese US population.

Methods

Study population

Data from 1,739 adults aged 20-88 years were analyzed from participants of the National Health and Nutrition Examination Survey III, 1988–1994, followed up for deaths until December 31, 2011 in a prospective cohort analysis. In NHANES III, complex multi-stage stratified clustered probability sampling scheme was applied to achieve a nationally representative sample of the civilian, non-institutionalized US population. The survey included personal interviews, physical examinations, and laboratory measurements.

We included 2,535 obese (a body mass index (BMI) $\geq$ 30 kg/m^2) adults aged 20 and older who were eligible for mortality follow-up and had complete data on food frequency questionnaire (FFQ) and 24-hour dietary recall, those with complete data on cardiometabolic parameters including fasting glucose, insulin, blood pressure (BP), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and high-sensitivity C-reactive protein (hs-CRP). We excluded those who reported a history of myocardial infarction, stroke, congestive heart failure, or cancer (other than skin cancer) (n=299). To minimize reverse causation, we excluded the participants who reported changing their dietary patterns during the previous 12 months because of pre-existing obesity, high blood pressure, high blood cholesterol, diabetes mellitus, or general health (n=415). In addition, we excluded those who reported implausible extreme energy intakes (<1st and >99th percentiles of energy intake/d in adults), those with hs-CRP >10mg/L, BMI > 60 kg/m^2, or pregnant or lactating women (n=82). Finally, a total of 1,739 individuals were analyzed.

Assessment of Mediterranean diet

Dietary intake was assessed using the FFQ and the 24-hr dietary recall data that were validated by the Nutrition Methodology Working Group. [22] Adherence to the Mediterranean diet was assessed using the scoring methodology developed by Panagiotakos et al. [23,24] In this methodology, scores 0 to 5 were assigned for the weekly consumption of food items assumed to be contributing to Mediterranean dietary pattern, whereas scores on the inverse ordinal scale were assigned for the consumption of food items assumed to be against the Mediterranean dietary pattern. For instance, the scores assigned to the weekly consumption frequencies of vegetables were as follows: no servings, 1–6 servings, 7–12 servings, 13–20 servings, 21–32 servings and >33 servings were assigned scores of 0, 1, 2, 3, 4 and 5, respectively. Similar score assignments were used for the food items of non-refined cereals, potatoes, fruits, legumes, fish, and olive oil. Reverse scores were assigned for the components of red meat and meat products, poultry and full-fat dairy products. For alcohol consumption, a score of 5 was assigned for consumption of less than 300 ml (36g) of alcohol per day, and 0 for no consumption or for consumption of >700 ml (84g) per day. This Mediterranean Diet Score (MDS) has been shown to be highly associated with prevalent cardiometabolic diseases, 10-year CVD risk, and inflammation and coagulation markers, in addition to capturing inherent characteristics of Mediterranean dietary pattern. [23-25]

The NHANES III FFQ applied a 1-month reference period without recording portion sizes. Thus, we calculated the MDS, assuming that the number of servings per week were equivalent to the number of times that a food item was consumed per week. Potatoes were excluded in our MDS assessment, because preparation methods for

potatoes in US are quite different from European countries. [26] In addition, assessment of alcohol consumption was modified in the present study as follows. The amount of alcohol consumed daily was estimated using the following assumption: 12.8g for 12-oz beer, 11g for 4-oz glass of wine, and 14g for an ounce of liquor based on the questionnaire provided. Then, gender-specific cut-offs were applied: score 5 was assigned for consumption of less than 28g and 14 g of alcohol per day, score 0 for no consumption or for consumption of greater than 70g and 28g per day in men and women, respectively, and the cutoffs for subcategories between 0 and 5 were reassigned with even intervals. [27] Olive oil consumption was not measured in the NHANES III FFQ. Thus, we approximated olive oil consumption by calculating the ratio of total monounsaturated fatty acid (MUFA) to total saturated fatty acids (SFA) using the 24-h dietary recall data, then dividing it into the six even intervals. The possible overall MDS score ranged from 0 to 50, with higher values of this MDS score indicating greater adherence to the Mediterranean diet.

Assessment of Metabolic health

Metabolic health was assessed using the metabolic parameters that were measured under quality control standards of Centers for Disease Control and Prevention. BMI was calculated as kg/m^2; height was measured to the nearest 0.1cm and weight to the nearest 0.01 kg. BP was averaged over five separate measurements. Glucose was measured in serum, using a modified hexokinase enzymatic method. Serum insulin was measured using radioimmnuoassay (Pharmacia Diagnostics). HDL-C and TG were measured using a Hitachi 704 analyzer (Boehringer-Mannheim Diagnostics). Serum CRP concentrations were measured by latex-enhanced nephlometry (Department of Laboratory Medicine,

Immunology Division, University of Washington, Seattle). Since NHANES III participants did not comply with fasting instruction strictly, six hour fasting data were used to increase the sample size. [28]

Metabolic health was defined when the individual had fewer than two cardiometabolic abnormalities [systolic/diastolic BP ≥ 130/85 mm Hg or antihypertensive medication use, TG ≥ 150 mg/L or on cholesterol-lowering medication, fasting glucose ≥ 100 mg/dL or antidiabetic medication use, homoeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose (mg/dl) x fasting insulin (lU/mL)/405) > the 90th percentile, hs-CRP > the 90th percentile, and HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on cholesterol-lowering medication]. [29]

Assessment of Mortality

To identify mortality and cause of death, the National Center for Health Statistics linked all participants aged 20 years and older to the National Death Index to 31 December 2011. Therefore, for each participant follow-up extended from the date of the examination to the date of death or 31 December 2011. The underlying cause listed on the death certificate was applied to determine cause of death that was identified using the underlying Cause of Death-113 groups (international classification of disease (ICD), 10th revision). Total mortality was defined as deaths with any underlying cause of death; CVD and cancer mortality were defined as deaths with underlying cause of death codes for ICD-10 I00-I69 and C00-C97, respectively. [30]

Assessment of Covariates

Demographic variables included age, gender, race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or others), educational attainment (<12

years, 12 years, or >12 years of education), living with spouse, level of income based on poverty income ratio (PIR) which is the ratio of household income to the appropriate poverty threshold (low (PIR ≤ 1.3), middle (1.3<PIR ≤ 3.5), and high (PIR>3.5)). The potential risk factors for CVD comprised smoking status (never, former, and current) and the presence of family history of CVD. Physical activity was classified based on the recommended levels of physical activity. [31] A group with recommended physical activity was defined as those who had self-reported leisure time moderate activity (3 ≤ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs ≥ 6) three or more times per week; physically inactive group as those with no reported leisure time physical activity; a group with insufficient physical activity as those who did not meet the criteria for recommended levels of physical activity but not inactive.

Statistical Analysis

The statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), using the appropriate survey procedures to account for the complex sampling design and weights. For the subgroup analysis, domain option was applied in survey procedure to preserve appropriate subsample in the complex sampling design, and it utilized the entire samples to estimate the variance of subpopulations. Continuous variables were presented by mean (SE: standard error) and compared using linear regression analyses. Categorical variables were expressed by percentage with SE and were compared using Rao-Scott $\chi 2$ tests. P value of less than .05 was considered statistically significant.

We used Cox proportional hazards regression to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause, CVD, and cancer mortality. The

proportional hazards assumption of the Cox models was evaluated with log of negative log survival curves based on Kaplan-Meier estimates for MDS tertile group as well as categorical age, gender, and race/ethnicity. In addition to crude HRs, we estimated age-, gender-, and race/ethnicity–adjusted HRs as well as the multivariable-adjusted HRs including age, BMI, and total energy intake as continuous covariates; gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of CHD as categorical covariates. Missing values in the covariates were added as a dummy variable in the multivariable models. To assess potential differential association of MDS by age (<65 vs. ≥65 years), sex, race/ethnicity, smoking (ever vs. never smoked), conformity to recommended physical activity (yes vs. no), BMI (<35 vs ≥35), and the presence or absence of chronic disease including diabetes mellitus and hypertension, we also stratified our analysis and performed interaction tests using all-cause mortality as the outcome by including the cross-product interaction terms in the Cox models based on F test. In addition, we performed sensitivity analyses with an alternative common definition of metabolic health as metabolic syndrome, [32] and after exclusion of subjects who died during the first five years of follow-up.

Results

The MHO phenotype (n=647) was observed in 34.8 % (SE, 1.7 %) of the obese sample from the present study. Table 5.1 shows general characteristics according to the MDS tertiles between MHO and MUO individuals. MHO individuals with the highest MDS tertile were more likely to have a higher income, lower insulin resistance, and higher diastolic BP than MHO individuals in other tertiles. MUO individuals with the highest MDS tertile tended to be older and not non-Hispanic white; were more likely to

have a higher education, higher income, lower proportion of current smokers and recommended physical activity, lower BMI and waist circumference, lower insulin resistance, higher systolic BP, lower triglycerides, and higher HDL-C than MUO individuals with other tertiles. Comparing MHO and MUO individuals, MHO individuals tended to be younger, female, not non-Hispanic white, non-smokers, and have a more favorable metabolic status.

The distribution of consumption frequency in each Mediterranean diet component according to the MDS tertile, MHO, and MUO phenotypes is shown in Table 5.2. In both phenotypes, consumption of grains, legumes, fruit, vegetable, fish, ratio of MUFA to SFA tended to increase with increasing tertile of MDS. In addition, consumption of red meats and dairy products tended to decrease with increasing tertile of MDS. However, consumption of poultry and alcohol consumption increased only in MUO individuals. Overall, MHO individuals consumed more MUFA (P=0.03), and less red meats and dairy products (P = 0.001 and 0.02, respectively), resulting in higher MDS scores for those components, compared to MUO individuals.

During a median follow-up of 18.5 years, there were 77 and 309 deaths in 598 MHO and 1,141 MUO individuals, respectively. Overall, higher mortality risk was observed in MUO than MHO after adjusting for potential confounders [HRs were 1.50 (95% CI, 1.12-2.00) for all-cause mortality, 2.50 (95% CI, 1.20-5.21 for CVD mortality, and 1.27 (95% CI, 0.81-1.98) for cancer mortality].

Table 5.3 shows adjusted HRs of all-cause, cardiovascular, and cancer mortality according to the tertile categories and a five-point increment in the MDS in MHO and MUO individuals. In MHO individuals, compared with the lowest tertile, HRs in the

second and the highest tertiles, respectively, were 0.35 (95% CI, 0.19-0.64) and 0.44 (0.26-0.75) (P for trend <0.001) for all-cause mortality; and 0.13 (0.06-0.26) and 0.23 (0.02-2.10) (P for trend =0.03) for cancer mortality, after multivariable adjustment. A five-point increment (1 SD of MDS) in the adherence to MDS was inversely associated with risk of all-cause mortality (HR, 0.59; 0.37-0.94). No mortality risk reduction was observed in MUO phenotypes with increasing MDS.

In stratified analyses for HRs of all-cause mortality with a five-point increment of MDS, stronger inverse associations were observed for individuals who were aged 65 years or older, non-Hispanic white, and with lower BMI < 35 kg/m^2, especially in men and those with recommended physical activity (P for interaction = 0.002 and 0.008, respectively) (Table 5.4). In addition, the inverse association between MDS and all-cause mortality in MHO individuals was consistent among individuals with and without prevalent chronic disease including diabetes mellitus and hypertension. There was a positive and inverse association between MDS and all-cause mortality in MUO individuals with and without the recommended physical activity, respectively (P for interaction = 0.003).

When data were analyzed after excluding subjects who died in the first five years of follow-up, the overall results were not different from those of the original dataset (Table 5.5). In addition, using an alternative definition of metabolic health as metabolic syndrome shows similar results in terms of the reduction of all-cause mortality only in MHO individuals (Table 5.6).

Discussion

In this nationally representative sample of obese U.S. adults, higher adherence to

Mediterranean diet was associated with a lower risk of all-cause mortality in the MHO phenotype, after adjustment for potential confounders. We observed a 41% reduction in all-cause mortality with each 1 SD increment in the MDS among MHO individuals. This association persisted when we restricted our analyses to those with or without prevalent chronic disease including diabetes mellitus and hypertension. However, the inverse association between the MDS and mortality was not observed in MUO phenotype. To our knowledge, the present study is the first to evaluate a differential beneficial effect of Mediterranean diet on the mortality risk reduction in MHO and MUO phenotype.

Several epidemiologic studies have explored the association between Mediterranean diet and the risk of mortality in the obese population in subgroup analyses, showing inconsistent results. Trichopoulou et al. [1] showed inverse association of MDS with all-cause mortality in Greek adults who had a BMI ≥ 28.06. In contrast, George et al. [33] showed that there was a weak association of MDS with all-cause mortality and no association with CVD and cancer mortality in US postmenopausal women with BMI ≥ 30. Mitrou et al. [34] also showed that the associations with all-cause mortality were only observed in ever-smoker in middle to older aged US adults with BMI ≥ 30. Based on the findings from these previous studies, the Mediterranean diet might not be a useful indicator in reducing the risk of mortality in obese population due to the possibility of underreporting of food intake [35] and physiological impact of obesity itself on mortality risk. [36]

However, these studies assumed that all obese individuals were at the same metabolic health and same risk of mortality. Previous studies have demonstrated that MHO individuals are not at increased risk of mortality and CVD compared with their

MUO counterparts. [37,38] The mechanisms explaining favorable cardiometabolic, hormonal, and inflammatory profiles in MHO individuals remain largely unknown. However, several potential pathophysiologic mechanisms include differences in adipose cell size, [39] degree of favorable gene markers' expression reflecting adipose cell differentiation, [40] and the role of key genes in numerous insulin-signaling pathways [41] and amino acid homeostasis [42] between MHO and MUO individuals.

Our results show that inverse associations of Mediterranean diet on risk of mortality were observed only in MHO individuals. These associations persisted in sensitivity analyses in which we analyzed the data using an alternative definition of metabolic health as metabolic syndrome and when we excluded the deaths at the first five year follow-ups. Underlying mechanisms for health benefits of Mediterranean diet are complex, but can be explained by the improvement of cardiometabolic profiles including insulin sensitivity, lipid profiles, blood pressure, endothelial dysfunction, reactive oxidation, and inflammatory markers. [43] [44] It has been reported that individuals with MHO phenotype are at increased risk of unfavorable long-term outcomes compared with metabolically healthy normal weight individuals, even with few metabolic abnormalities. [45] In addition, one third of MHO phenotype can be converted to MUO phenotype within a decade due to unhealthy life style. [46] Thus, our results suggest that there would be a synergistic physiological mechanism to prevent adverse health outcomes, along with improving the favorable cardiometabolic profiles linking MHO phenotype and the adherence to Mediterranean diet. Furthermore, those who reported to follow the recommended physical activity [moderate activity ($3 \leq METs<6$) of five or more times per week or vigorous activity ($METs \geq 6$) of three or more times per week] [31] had more

benefits in mortality reduction from Mediterranean diet, representing the possible synergistic effects of healthy life styles. Men also had more significant mortality reduction compared to women, which also has been shown in a randomized clinical trial study. [2] However, more evidence is needed to confirm gender difference in mortality risk reduction with Mediterranean diet in individuals with MHO phenotype.

Based on our findings, MUO individuals might not be as responsive to diet because they are already metabolically overburdened. [13] Cardiometabolic co-morbidities might explain the lack of association between Mediterranean diet and the risk of mortality in MUO individuals. However, a stratified analysis with and without the prevalent chronic disease including diabetes mellitus and hypertension showed similar results. Interestingly, the risk of mortality in MUO individuals showed U shaped association with increasing MDS tertile in which those with the 1st and 3rd tertile of MDS tended to have higher mortality than those with the 2nd tertile, even though MUO individuals in the 3rd tertile had favorable metabolic profiles compared to those in other tertiles at baseline. In addition, MUO individuals who met the recommended physical activity tended to have a higher risk of all-cause mortality, whereas MUO individuals who did not meet the recommended physical activity tended to have a lower risk of all-cause mortality in a subgroup analysis. Although we cannot completely rule out the possibility of reverse causality in this association, these results suggest that healthy life style alone might not be adequate to reverse the deleterious prognosis of MUO individuals. Thus, the MUO individuals may need to be prioritized for intensive weight loss program along with healthy dietary habit to reduce obesity related comorbidities. Furthermore, appropriate therapeutic approaches may be warranted to reduce the

mortality risk of MUO individuals. [47]

Several intervention studies have explored the impact of dietary interventions on cardiometabolic risk factors and whether MHO and MUO individuals had the same benefits. [20,21,48-51] However, the findings of these studies were inconsistent and their hypotheses have been focused on the short-term effect of energy-restricted diet along with exercise on change of cardiometabolic parameters in MHO and MUO phenotypes. Thus, the interpretation of these results might not be applicable to the present long-term prospective cohort study using a Mediterranean dietary pattern as an exposure. Therefore, more evidence would be necessary based on long-term follow-up studies evaluating the effects of healthy dietary pattern on mortality reduction, considering MHO and MUO phenotypes. [47]

Strengths of our study include its prospective study design with nearly 18 year follow-up for mortality based on the representative US population. In addition, data were collected based on extensive laboratory and physical examinations using standardized protocols to minimize the influence of measurement errors. Furthermore, we were able to replicate the findings using sensitivity analyses. However, our study has limitations. First, there was no information on "servings per week" that was applied in MDS calculation by Panagiotakos et al. [24] Because NHANES III FFQ did not have portion size, we used "times per week" in assessing the consumption frequency. This approach may cause exposure misclassification, but the direction would be non-differential. Second, due to a single measure of diet collected at baseline, we could not account for any changes in dietary intake over time. Third, self-assessment of food consumption may produce non-differential measurement error, although energy adjustment would mitigate this error to

some degree. [52] Finally, there may be residual confounding due to not measuring the covariates in an objective way, such as self-reported physical activity and smoking status.

Conclusion

Our results suggest that higher adherence to Mediterranean diet was associated with a lower risk of all-cause, CVD, and cancer mortality exclusively in the MHO phenotype, based on a nationally representative U.S. adult population. The lack of a beneficial association between adherence to Mediterranean diet with the mortality reduction in MUO individuals may warrant the need of an aggressive prevention approach to reduce mortality risk in MUO individuals.

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Table 5.1 Comparison of general characteristics according to the tertile categories of Mediterranean diet score between Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes at baseline

Characteristics	Metabolically Healthy Obese (MHO) phenotype									P
	Overall (n=598)			Tertile 1 (n=181)			Tertile 2 (n=240)			Tertile 3 (n=177)			
Age, years	40.7	±	0.6	40.8	±	0.9	40.5	±	0.8	40.8	±	1.3	0.76
Men, %	33.2	±	3.4	36.5	±	5.4	23.8	±	4.9	41.7	±	7.0	0.07
Race/ ethnicity, %													
Non-Hispanic white	68.7	±	3.4	68.7	±	5.6	68.8	±	5.4	68.5	±	3.8	0.43
Non-Hispanic black	19.5	±	2.4	21.5	±	3.8	21.6	±	3.8	14.9	±	2.5	
Mexican-American	4.5	±	0.7	2.3	±	0.7	4.7	±	1.0	6.4	±	1.1	
Other	7.3	±	1.7	7.5	±	4.2	4.9	±	2.8	10.3	±	2.7	
Educational attainment, %													
<12 years	24.3	±	2.2	29.1	±	4.9	24.8	±	4.3	19.0	±	4.5	0.39
12 years	40.0	±	3.0	39.8	±	5.4	43.6	±	5.7	35.6	±	7.6	
>=13 years	35.8	±	3.3	31.1	±	6.0	31.7	±	5.1	45.4	±	5.3	
Income, %													
PIR=<1.3	21.2	±	2.9	21.1	±	4.5	25.5	±	5.3	16.0	±	3.7	0.001
PIR=<3.5	42.2	±	3.2	60.7	±	6.0	37.0	±	5.6	30.5	±	5.7	
PIR>3.5	36.5	±	3.5	18.2	±	5.7	37.5	±	6.6	53.5	±	6.1	
Living with spouse, %	69.0		2.9	68.8		5.6	67.8		4.5	70.7		5.5	0.92
Smoking status, %													
Never	55.1	±	3.4	50.6	±	5.2	60.5	±	5.9	52.8	±	6.1	0.58
Former	21.8	±	2.9	27.7	±	5.0	16.7	±	3.7	22.6	±	5.1	
Current	23.1	±	3.2	21.8	±	4.7	22.8	±	5.5	24.6	±	6.4	
Physical activity, %													
Inactive	14.7	±	2.1	23.5	±	5.2	12.0	±	2.6	9.6	±	2.9	0.07

Insufficient activity	59.5 ± 3.4	54.1 ± 4.5	63.5 ± 6.0	59.8 ± 5.0	
Recommended activity	25.8 ± 3.4	22.4 ± 4.7	24.4 ± 5.3	30.6 ± 5.3	
CHD family history, %	16.9 ± 2.9	18.5 ± 4.5	15.4 ± 5.3	17.2 ± 4.8	0.89
Diabetes mellitus, %	3.8 ± 1.1	4.5 ± 1.7	4.4 ± 1.6	2.5 ± 2.2	0.73
Hypertension, %	23.6 ± 3.6	19.5 ± 3.2	26.0 ± 6.2	24.5 ± 5.5	0.58
BMI, kg/m^2	33.4 ± 0.1	34.0 ± 0.2	33.0 ± 0.2	33.2 ± 0.3	0.07
Waist circumference, cm	104.0 ± 0.7	106.3 ± 0.8	101.8 ± 1.1	104.4 ± 1.2	0.16
Fasting glucose, mg/dl	91 ± 0.4	91.0 ± 0.6	90.7 ± 0.8	90.9 ± 0.7	0.61
HOMA-IR	2.4 ± 0.0	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	<0.001
SBP, mmHg	120 ± 0.6	120 ± 0.8	119 ± 0.9	122 ± 1.6	0.24
DBP, mmHg	74 ± 0.5	73 ± 0.7	74 ± 0.7	77 ± 1.0	0.002
hs-CRP, mg/dl	0.4 ± 0.02	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03	0.56
Triglycerides, mg/dl	107 ± 5.0	111 ± 5.3	96 ± 3.4	117 ± 14.1	0.62
HDL-C, mg/dl	52 ± 0.8	49 ± 0.9	54 ± 1.3	51 ± 1.6	0.06

Table 5.1 (Continued)

Characteristics	Metabolically Unhealthy Obese (MUO) phenotype					
	Overall (n=1,141)	Tertile 1 (n=340)	Tertile 2 (n=465)	Tertile 3 (n=336)	P	P (MHO vs. MUO)
Age, years	47.2 ± 0.7	45.9 ± 1.1	46.8 ± 1.0	49.7 ± 0.8	0.05	<0.001
Men, %	49.9 ± 2.7	45.6 ± 3.9	53.5 ± 4.3	49.5 ± 3.9	0.29	<0.001
Race/ ethnicity, %						
Non-Hispanic white	75.0 ± 2.6	81.9 ± 2.4	73.9 ± 3.5	67.6 ± 4.0	<0.001	<0.001
Non-Hispanic black	11.2 ± 1.4	10.0 ± 1.7	11.9 ± 1.9	11.5 ± 1.7		
Mexican-American	7.1 ± 1.2	3.8 ± 0.9	6.3 ± 1.1	13.0 ± 2.5		
Other	6.7 ± 1.6	4.4 ± 1.7	7.9 ± 2.3	7.9 ± 3.0		

Educational attainment, %																
<12 years	27.1	±	2.4	30.6	±	3.8	24.3	±	2.8	27.3	±	4.3	0.02	0.55
12 years	37.0	±	2.5	42.0	±	3.2	37.6	±	4.1	29.4	±	3.1		
>=13 years	35.9	±	3.3	27.5	±	3.9	38.1	±	4.5	43.3	±	5.1		
Income, %																
PIR=<1.3	17.4	±	1.9	25.4	±	3.6	13.5	±	2.4	13.3	±	2.2	<0.001	0.28
PIR=<3.5	48.0	±	2.5	52.8	±	3.7	49.4	±	4.0	38.6	±	5.0		
PIR>3.5	34.6	±	3.1	21.8	±	3.0	37.2	±	4.9	48.1	±	4.9		
Living with spouse, %	30.5		1.8	65.6		3.5	72.2		3.4	70.1		3.8	0.41	0.88
Smoking status, %																
Never	42.4	±	2.1	40.9	±	3.1	44.0	±	3.2	41.7	±	5.5	0.004	0.003
Former	33.7	±	2.1	25.0	±	3.7	35.7	±	2.7	41.9	±	5.5		
Current	23.9	±	1.7	34.1	±	3.7	20.3	±	3.2	16.3	±	2.6		
Physical activity, %																
Inactive	16.2	±	1.7	22.6	±	2.9	13.3	±	2.3	12.9	±	2.6	0.04	0.60
Insufficient activity	55.8	±	2.3	56.2	±	4.5	56.1	±	3.6	54.6	±	4.2		
Recommended activity	28.0	±	2.1	21.2	±	4.1	30.6	±	3.3	32.5	±	3.8		
CHD family history, %	21.6	±	2.2	27.8	±	3.8	18.8	±	3.4	18.3	±	3.7	0.13	0.28
Diabetes mellitus, %	18.2	±	1.5	16.9	±	2.9	16.3	±	2.4	23.5	±	3.0	0.19	<0.001
Hypertension, %	49.4	±	2.6	46.9	±	3.9	47.7	±	3.9	56.0	±	4.6	0.27	<0.001
BMI, kg/m^2	34.8	±	0.2	35.8	±	0.3	34.3	±	0.2	34.3	±	0.4	0.001	<0.001
Waist circumference, cm	110.9	±	0.4	113.3	±	0.7	110.2	±	0.7	108.9	±	0.8	<0.001	<0.001
Fasting glucose, mg/dl	107	±	0.9	107.5	±	2.5	107.5	±	1.5	106.8	±	1.6	0.31	<0.001
HOMA-IR	5.0	±	0.2	5.3	±	0.2	5.1	±	0.3	4.4	±	0.2	<0.001	<0.001
SBP, mmHg	131	±	0.6	129	±	0.8	130	±	0.8	134	±	1.1	<0.001	<0.001
DBP, mmHg	80	±	0.5	80	±	0.4	80	±	0.5	81	±	1.2	0.21	<0.001
hs-CRP, mg/dl	0.6	±	0.02	0.6	±	0.02	0.6	±	0.03	0.6	±	0.04	0.19	<0.001
Triglycerides, mg/dl	195	±	4.0	208	±	6.0	187	±	5.8	190	±	9.0	0.02	<0.001
HDL-C, mg/dl	42	±	0.4	40	±	0.7	41	±	0.8	45	±	1.3	0.003	<0.001

Data are presented as mean ± SE or proportion (%) ± SE. P values for continuous variables represent P for trend.
Abbreviations: PIR, poverty income ratio; CHD, coronary heart disease; BMI, body mass index; HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol.

Table 5.2 Comparison of consumption frequency for each component of the Mediterranean diet in Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes at baseline

	Metabolically Healthy Obese (MHO) phenotype				
	Overall (n=598)	Tertile 1 (n=181)	Tertile 2 (n=240)	Tertile 3 (n=177)	P for trend
Score range	(13 - 39)	(13 - 23)	(24 - 28)	(29 - 39)	
MDS score	26.0 ± 0.2	20.8 ± 0.2	25.9 ± 0.1	31.1 ± 0.2	<0.001
Grains (times/wk)	4.7 ± 0.4	3.8 ± 0.7	4.2 ± 0.4	6.3 ± 0.6	<0.001
Legumes (times/wk)	2.6 ± 0.1	1.5 ± 0.1	3.0 ± 0.2	3.2 ± 0.3	<0.001
Fruit (times/wk)	5.9 ± 0.4	4.9 ± 0.4	4.6 ± 0.3	8.4 ± 1.0	0.002
Vegetable (times/wk)	13.1 ± 0.3	11.2 ± 0.6	12.3 ± 0.5	16.0 ± 0.9	<0.001
Fish (times/wk)	1.4 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	2.3 ± 0.3	<0.001
MUFA:SFA	1.23 ± 0.03	1.02 ± 0.02	1.26 ± 0.02	1.41 ± 0.10	<0.001
Red meats (times/wk)	5.2 ± 0.3	6.2 ± 0.3	5.3 ± 0.5	4.0 ± 0.2	<0.001
Poultry (times/wk)	2.3 ± 0.1	2.1 ± 0.2	2.3 ± 0.2	2.3 ± 0.3	0.47
Dairy products (times/wk)	10.3 ± 0.4	12.3 ± 0.6	10.2 ± 0.6	8.3 ± 0.5	<0.001
Alcohol (g/d)	2.7 ± 0.3	2.4 ± 1.0	2.3 ± 0.3	3.5 ± 0.4	0.15
Total energy intake, kcal	2032 ± 47	2121 ± 72	2016 ± 61	1966 ± 128	0.66

Table 5.2 (Continued)

	Metabolically Unhealthy Obese (MUO) phenotype					
	Overall (n=1,141)	Tertile 1 (n=340)	Tertile 2 (n=465)	Tertile 3 (n=336)	P for trend	P (MHO VS MUO)
Score range	(12 - 40)	(12 - 23)	(24 - 28)	(29 - 40)		

MDS score	25.5	±	0.2	20.5	±	0.1	26.0	±	0.1	31.2	±	0.1	<0.001	0.04
Grains (times/wk)	5.0	±	0.3	3.2	±	0.3	5.3	±	0.5	6.6	±	0.4	<0.001	0.49
Legumes (times/wk)	2.4	±	0.1	1.6	±	0.1	2.7	±	0.2	3.2	±	0.2	<0.001	0.14
Fruit (times/wk)	6.1	±	0.3	4.0	±	0.2	5.5	±	0.3	9.9	±	0.5	<0.001	0.59
Vegetable (times/wk)	13.5	±	0.4	10.1	±	0.4	13.8	±	0.5	17.7	±	0.7	<0.001	0.53
Fish (times/wk)	1.4	±	0.1	1.2	±	0.3	1.2	±	0.1	2.0	±	0.1	0.005	0.50
MUFA:SFA	1.19	±	0.02	1.06	±	0.02	1.20	±	0.02	1.35	±	0.05	<0.001	0.03
Red meats (times/wk)	5.6	±	0.2	6.7	±	0.3	5.5	±	0.3	4.1	±	0.2	<0.001	0.01
Poultry (times/wk)	2.1	±	0.1	2.0	±	0.1	2.1	±	0.1	2.2	±	0.1	0.010	0.12
Dairy products (times/wk)	11.6	±	0.4	14.2	±	0.8	10.9	±	0.5	9.2	±	0.3	<0.001	0.03
Alcohol (g/d)	2.7	±	0.2	2.2	±	0.7	2.3	±	0.3	4.0	±	0.3	0.01	0.96
Total energy intake, kcal	2222	±	47	2221	±	65	2235	±	69	2200	±	69	0.89	<0.001

Data are presented as mean ± SE.

Abbreviations: MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; MDS, Mediterranean Diet Score.

Table 5.3 Adjusted hazard ratio (HR) and 95% CI of all-cause, cardiovascular, and cancer mortality according to the tertile categories and a five-point increment in the Mediterranean diet score in Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes

	Metabolically Healthy Obese (MHO) phenotype					Metabolically Unhealthy Obese (MUO) phenotype				
	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS
No. of participants	181	240	177			340	465	336		
No. of person years	3191	4394	3284			5639	8006	5625		
Deaths from all causes, n	35	26	16			105	116	88		
Model 1	1.00 (ref.)	0.47 (0.26-0.84)	0.36 (0.23-0.57)	<0.001	0.67 (0.56-0.81)	1.00 (ref.)	0.75 (0.56-1.01)	0.98 (0.56-1.74)	0.84	1.01 (0.83-1.22)
Model 2	1.00 (ref.)	0.40 (0.24-0.66)	0.35 (0.20-0.59)	<0.001	0.59 (0.48-0.73)	1.00 (ref.)	0.69 (0.54-0.88)	0.77 (0.45-1.31)	0.28	0.91 (0.75-1.10)
Model 3	1.00 (ref.)	0.35 (0.19-0.64)	0.44 (0.26-0.75)	<0.001	0.59 (0.37-0.94)	1.00 (ref.)	0.74 (0.58-0.95)	0.92 (0.48-1.76)	0.66	0.96 (0.78-1.17)
CVD Deaths, n	6	5	5			26	33	27		
Model 1	1.00 (ref.)	0.64 (0.18-2.29)	1.15 (0.12-11.0)	0.89	0.92 (0.38-2.24)	1.00 (ref.)	1.57 (0.84-2.93)	1.76 (0.94-3.29)	0.07	1.08 (0.89-1.31)
Model 2	1.00 (ref.)	0.43 (0.12-1.49)	1.15 (0.12-10.7)	0.88	0.87 (0.28-2.67)	1.00 (ref.)	1.46 (0.75-2.83)	1.41 (0.79-2.50)	0.22	1.12 (0.93-1.35)
Model 3	1.00 (ref.)	0.18 (0.05-0.58)	1.25 (0.41-3.83)	0.65	1.01 (0.29-3.51)	1.00 (ref.)	1.28 (0.61-2.67)	1.61 (0.56-4.62)	0.34	1.15 (0.87-1.54)
* Cancer Deaths, n	11	6	3			25	25	24		
Model 1	1.00	0.12	0.19	0.03	0.45	1.00	0.58	1.07	0.99	1.13

	(ref.)	(0.07-0.22)	(0.04-0.95)		(0.21-0.97)	(ref.)	(0.19-1.72)	(0.41-2.80)		(0.73-1.77)
Model 2	1.00 (ref.)	0.11 (0.06-0.20)	0.16 (0.04-0.76)	0.01	0.36 (0.15-0.86)	1.00 (ref.)	0.54 (0.20-1.50)	0.90 (0.34-2.40)	0.78	1.07 (0.67-1.71)
Model 3	1.00 (ref.)	0.13 (0.06-0.26)	0.23 (0.02-2.10)	0.03	0.28 (0.04-1.79)	1.00 (ref.)	0.61 (0.34-1.09)	1.17 (0.41-3.33)	0.90	1.27 (0.77-2.10)

Data are presented as hazard ratio (95% confidence interval).

Abbreviations: MDS, Mediterranean diet score; CVD, cardiovascular disease.

Model 1 represents a crude model. Model 2 adjusted for age, gender, and race/ethnicity. Model 3 further adjusted for educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, body mass index, and total calorie intakes.

* those who had a history of skin cancer were also excluded.

Due to small sample sizes, cautious interpretation of finding is needed for CVD and cancer deaths in MHO individuals.

Table 5.4 Subgroup analyses of the association between a five-point increment in the Mediterranean diet score and the risk of all-cause mortality in Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes

	Metabolically Healthy Obese (MHO) phenotype			Metabolically Unhealthy Obese (MUO) phenotype		
	No. of participants (deaths)	HR for a 1 SD increase in MDS	P interaction	No. of participants (deaths)	HR for a 1 SD increase in MDS	P interaction
Age, y			0.55			0.79
< 65	535 (43)	0.59 (0.32-1.06)		908 (152)	0.97 (0.68-1.37)	
≥ 65	63 (34)	0.41 (0.23-0.75)		233 (157)	1.13 (0.95-1.34)	
Gender			0.002			0.68
Men	163 (22)	0.02 (0.01-0.67)		495 (146)	0.96 (0.76-1.21)	
Women	435 (55)	0.83 (0.50-1.38)		646 (163)	0.98 (0.70-1.38)	
Race/ ethnicity			0.29			0.52
Non-Hispanic white	175 (31)	0.39 (0.17-0.87)		432 (158)	1.02 (0.82-1.27)	
Others	423 (46)	0.76 (0.44-1.31)		709 (151)	0.84 (0.58-1.21)	
Body mass index, kg/m^2			0.46			0.36
< 35	429 (55)	0.43 (0.26-0.71)		720 (208)	1.01 (0.80-1.29)	
≥ 35	162 (22)	0.64 (0.31-1.36)		421 (101)	0.95 (0.70-1.30)	
Smoking status			0.07			0.96
Non-smoker	378 (40)	0.54 (0.25-1.18)		589 (136)	1.08 (0.76-1.52)	
Ever-smoker	220 (37)	0.20 (0.10-0.43)		552 (173)	0.96 (0.75-1.22)	
Recommended physical activity			0.008			0.003
Yes	148 (24)	0.30 (0.11-0.80)		278 (85)	1.29 (0.89-1.87)	
No	450 (53)	0.67 (0.45-1.00)		863 (224)	0.95 (0.75-1.20)	

Chronic disease			0.13			0.57
Absence	457 (35)	0.56 (0.29-0.95)		480 (61)	1.09 (0.60-1.95)	
Presence	141 (42)	0.50 (0.29-0.86)		661 (248)	0.92 (0.71-1.18)	

Models are adjusted as model 3 in Table 5.3, except for the stratifying factor.
A group with recommended physical activity was defined as those who had self-reported leisure time moderate activity (3 ≤ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs ≥ 6) three or more times per week.
Presence of chronic disease indicates having diabetes mellitus and hypertension.

Table 5.5 Multivariable adjusted hazard ratio (HR) * and 95% CI of all-cause mortality according to the tertile categories and a five-point increment in the Mediterranean diet score in Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes excluding the deaths at the first five year follow-up

	Metabolically Healthy Obese (MHO) phenotype					Metabolically Unhealthy Obese (MUO) phenotype				
	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS
No. of participants	189	256	184			378	502	355		
No. of person years	3371	4663	3408			6435	8630	6017		
Deaths from all causes, n	36	31	18			118	143	98		
	1.00 (ref.)	0.42 (0.42-0.78)	0.54 (0.28-0.95)	0.006	0.57 (0.44-0.72)	1.00 (ref.)	0.84 (0.68-1.03)	1.12 (0.68-1.83)	0.73	1.07 (0.89-1.28)
CVD deaths, n	8	8	5			37	43	29		
	1.00 (ref.)	1.41 (0.13-1.25)	1.26 (0.17-9.48)	0.88	0.46 (0.26-0.81)	1.00 (ref.)	1.22 (0.60-2.47)	1.27 (0.62-2.59)	0.47	1.07 (0.87-1.33)
* Cancer deaths, n	12	7	4			27	28	23		
	1.00 (ref.)	0.14 (0.07-0.27)	0.23 (0.05-0.99)	0.02	0.30 (0.09-1.02)	1.00 (ref.)	0.67 (0.39-1.17)	1.28 (0.52-3.14)	0.69	1.35 (0.86-2.13)

Data are presented as hazard ratio (95% confidence interval).

Abbreviations: MDS, Mediterranean diet score; CVD, cardiovascular disease

* : Adjusted for age, gender, and race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, body mass index, and total calories.

* those who had a history of skin cancer were also excluded.

Due to small sample sizes, cautious interpretation of finding is needed for CVD and cancer deaths in MHO individuals.

Table 5.6 Multivariable adjusted hazard ratio (HR) * and 95% CI of all-cause, cardiovascular, and cancer mortality according to the tertile categories and a five-point increment in the Mediterranean diet score in Metabolically Healthy Obese (MHO) and Metabolically Unhealthy Obese (MUO) phenotypes using metabolic syndrome† as a definition of metabolic health

	Metabolically Healthy Obese (MHO) phenotype					Metabolically Unhealthy Obese (MUO) phenotype				
	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS	Tertile 1	Tertile 2	Tertile 3	P trend	HR for a 1 SD increase in MDS
No. of participants	248	325	219			263	364	283		
No. of person years	4319	5948	4002			4354	6184	4701		
Deaths from all causes, n	51	41	25			83	98	78		
	1.00 (ref.)	0.51 (0.31-0.85)	0.65 (0.40-1.06)	0.02	0.74 (0.60-0.90)	1.00 (ref.)	0.73 (0.56-0.94)	1.02 (0.50-2.08)	0.92	0.95 (0.73-1.24)
CVD deaths, n	7	8	9			24	30	23		
	1.00 (ref.)	1.35 (0.03-25.7)	3.98 (0.17-62.4)	0.66	1.39 (0.10-22.2)	1.00 (ref.)	1.15 (0.54-2.46)	1.34 (0.45-4.00)	0.56	1.04 (0.72-1.52)
Cancer deaths, n	16	11	5			20	19	21		
	1.00 (ref.)	0.21 (0.10-0.45)	0.63 (0.11-3.47)	0.24	0.63 (0.27-1.47)	1.00 (ref.)	0.58 (0.33-1.03)	1.07 (0.34-3.40)	0.99	1.12 (0.63-1.99)

Data are presented as hazard ratio (95% confidence interval).

Abbreviations: MDS, Mediterranean diet score; CVD, cardiovascular disease.

Those who had a history of skin cancer were excluded in calculating HRs in cancer deaths.

* : Adjusted for age, gender, and race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, body mass index, and total calories.

†: Metabolic syndrome was defined when the individual had fewer than three cardiometabolic abnormalities (systolic/diastolic blood pressure ≥ 130/85 mm Hg or antihypertensive medication use, triglycerides ≥ 150 mg/L, fasting plasma glucose ≥ 100 mg/dL or antidiabetic medication use, high density lipoprotein-cholesterol (HDL-C) < 40 mg/dL in men or < 50 mg/dL in women, and waist circumference ≥ 102 cm in men or ≥ 88 cm in women.

CHAPTER 6

DIET QUALITY AND MORTALITY RISK IN METABOLICALLY OBESE NORMAL WEIGHT ADULTS[1]

[1] Yong-Moon Mark Park, Teresa T. Fung, Susan E. Steck, Jiajia Zhang, Linda J. Hazlett, Kyungdo Han, and Anwar M. Merchant. To be submitted to *Diabetes Care*.

Abstract

Objective: To examine the association between the Dietary Approaches to Stop Hypertension (DASH) style diet, Healthy Eating Index (HEI), metabolic health, and mortality risk in a representative normal weight U.S. population.

Research Design and Methods: Data were from normal weight ($18.5 \leq$ body mass index < 25 kg/m^2) adults ≥ 40 years in the National Health and Nutrition Examination Survey III, 1988–1994 followed up for deaths until December 31, 2011. A total of 1,333 participants without known cardiovascular disease (CVD) and cancer at baseline were included in this prospective cohort study. Metabolic health was defined based on high glucose, blood pressure, triglycerides, C-reactive protein, insulin resistance, and low high-density lipoprotein-cholesterol.

Results: During a median follow-up of 17.9 years, there were 313 and 250 deaths in the metabolically obese normal weight (MONW) phenotype and metabolically healthy normal weight (MHNW) phenotype, respectively. In MONW individuals, one SD increment in the adherence to DASH style diet and HEI (2 points and 14 points, respectively) was significantly associated with 23% and 27% reductions in the risk of all-cause mortality (Hazard ratio (HR), 0.77 [95% confidence interval (CI), 0.66-0.90]; HR, 0.73 [95% CI, 0.63-0.85], respectively), after adjustment for potential confounders. The corresponding HRs for CVD mortality were 0.70 (95% CI, 0.53-0.93) and 0.74 (95% CI, 0.61-0.91), respectively. Similar findings for all-cause mortality were observed regardless of diabetes or hypertension status. However, no association was observed in MHNW phenotype**.**

Conclusions: Higher diet quality scores were associated with a lower risk of

mortality in MONW adults.

Keywords: Dietary Approaches to Stop Hypertension (DASH) style diet, Health Eating Index, metabolic obesity, mortality, National Health and Nutrition Examination Survey

Introduction

Despite being apparently non-obese, a subset of normal weight individuals appear to be more susceptible to insulin resistance, type 2 diabetes mellitus (DM) and cardiovascular disease (CVD) (1). These individuals display a metabolically obese normal weight (MONW) phenotype characterized by lower energy expenditure, higher visceral adiposity, impaired insulin sensitivity, and a more atherogenic lipid profile (1). These unfavorable characteristics of MONW phenotype contribute to an increased risk of cardiometabolic disease (2-4), compared to their metabolically healthy normal weight (MHNW) counterparts. It has been reported that the prevalence of MONW phenotype ranges from 7.1% to 30.1% depending on the criteria used in U.S. population (5; 6). Thus, identification of modifiable risk factors in MONW individuals who are apparently healthy, but at high risk of cardiometabolic disease would be beneficial to prevent the development of cardiometabolic morbidity and mortality (7).

Healthy dietary pattern is associated with a reduced risk of CVD and cancer (8). In the United States, the Dietary Approaches to Stop Hypertension (DASH) score and the Healthy Eating Index (HEI) are diet quality indexes of substantial public health importance (9). The DASH style diet was developed to prevent hypertension (10), and it has been reported that the adherence to the DASH style diet is related to a reduced risk of CVD (11) and type 2 DM (12). HEI was developed by US Department of Agriculture

researchers to measure the overall dietary quality in the US population, has been associated with lower inflammation and risk of chronic disease (13; 14).

To date, the intervention studies on the effect of healthy dietary patterns on the reduced risk of chronic disease have been limited to an obese population (15-17). Even in observational studies, there is little evidence on the association between healthy dietary patterns and risk of chronic disease in normal weight population (11; 18). As the MONW phenotype also has risk of developing cardiometabolic disease, it would be important to examine the potential of high diet quality on reducing the morbidity and mortality in these individuals. Furthermore, the role of the healthy dietary pattern in the natural history of MONW phenotype is unknown.

Therefore, we addressed the question whether high quality diet measured by DASH style diet and HEI have health benefits in relation to mortality risk reduction in MONW individuals in a nationally representative normal weight US population.

Methods

Study population

We used data from the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994, followed up for deaths until December 31, 2011 in this prospective cohort analysis. NHANES III was carried out using a complex multi-stage stratified clustered probability sample design to achieve a nationally representative sample of the civilian, non-institutionalized US population. The survey included personal interviews, physical examinations, and laboratory measurements.

We included 1,786 normal weight ($18.5 \leq$ body mass index (BMI) < 25 kg/m^2) adults aged 40-90 years who were eligible for mortality follow-up and had complete data

on food frequency questionnaire (FFQ) and 24-hour dietary recall, and cardiometabolic parameters including fasting glucose, insulin, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), blood pressure (BP), and high-sensitivity C-reactive protein (hs-CRP). We excluded those who reported a history of myocardial infarction, stroke, congestive heart failure, or cancer (other than skin cancer) (n=304). To minimize reverse causation, we excluded the participants who reported changing their dietary patterns during the previous 12 months because of pre-existing obesity, high blood pressure, high blood cholesterol, DM, or general health (n=129). We also excluded those who reported implausible extreme energy intakes (<1st and >99th percentiles of energy intake/d in adults), pregnant or lactating women, and those with hs-CRP >10mg/L (n=20). Finally, a total of 1,333 individuals were included in thess analyses and followed-up for mortality outcomes. Since there is a large difference in mean age between MONW and MHNW phenotypes (5; 19), we limited our study to subjects aged 40 and older to minimize the age effect in comparing the association between high quality diet and the mortality risk in both MONW and MHNW phenotype.

Assessment of DASH style diet

The DASH style diet is characterized by high intake of fruits, vegetables, nuts and legumes, low fat dairy products, and whole grains; and low intake of fat (total/saturated), sodium, sweets, and red meats (20). NHANES FFQ did not provide information on servings, thus, we did not use FFQ data for these analyses. Dietary intake was assessed using the 24-hr dietary recall data, validated by the Nutrition Methodology Working Group (21). We calculated Mellen's index which is one of the established DASH index scores from the 24-hr dietary recall data (22). Mellen's index, a nutrient-based index with

9 components including target nutrient values used in clinical trials (23), applied absolute targets on the basis of a 2100-kcal diet for both men and women. Individuals satisfying the goal for each component received 1 point, those who meet an intermediate goal, defined as the midpoint between the DASH diet goal and the nutrient content of the DASH control diet received one-half of a point, and those who meet neither goal received 0 points (10). The possible DASH score ranged from 0 to 9, with higher values of this DASH score indicating greater adherence to the DASH style diet.

Assessment of HEI scores

In 2000, the Centers for Disease Control and Prevention proposed the HEI as a measure for diet quality (24). Ten main factors used to calculate HEI include grains, fruits, vegetables, dairy, meats, total fat, saturated fat, cholesterol, sodium, and dietary variety (25). HEI scores range from 0 to 100, with a score of each component ranging from 0 to 10. Among the HEI components, grains, fruits, vegetables, meats and dairy products were based on the Food Guide Pyramid's recommended number of servings in which the maximum score was given to the recommended servings. The remaining components complied with the Dietary Guidelines for Americans considering the recommended intakes of total fat, saturated fat, cholesterol and sodium (25). In addition, dietary variety was assessed by adding up the number of different foods eaten by an individual in amounts sufficient to contribute at least one-half of a serving in a food group (26). The data for HEI and its component scores were determined from dietary information collected by a single 24-h recall using an automated, interactive interview (27).

Assessment of Metabolic health

Metabolic health was assessed using the metabolic parameters that were measured under quality control standards of Centers for Disease Control and Prevention. BMI was calculated as kg/m^2; height was measured to the nearest 0.1cm and weight to the nearest 0.01 kg. Waist circumference was measured at the level of the right iliac crest. BP was averaged over five separate measurements. Glucose was measured in serum, using a modified hexokinase enzymatic method. Serum insulin was measured using a radioimmnuoassay (Pharmacia Diagnostics). HDL-C and TG were measured using a Hitachi 704 analyzer (Boehringer-Mannheim Diagnostics). Serum CRP concentrations were measured by latex-enhanced nephlometry (Department of Laboratory Medicine, Immunology Division, University of Washington, Seattle). Since NHANES III participants did not comply with fasting instruction strictly, six hour fasting data were used to increase the sample size (28).

Metabolic health was defined when the individual had fewer than two cardiometabolic abnormalities [systolic/diastolic BP ≥ 130/85 mm Hg or antihypertensive medication use, triglycerides ≥ 150 mg/L on cholesterol-lowering medication, fasting glucose ≥ 100 mg/dL or antidiabetic medication use, homoeostasis model assessment of insulin resistance (HOMA-IR = fasting glucose (mg/dl) x fasting insulin (lU/mL)/405) > the 90th percentile, hs- CRP > the 90th percentile, and HDL-C < 40 mg/dL in men or < 50 mg/dL in women on cholesterol-lowering medication] (5).

Assessment of Mortality

To determine the vital status and cause of death, the National Center for Health Statistics linked all participants aged 20 years and older to the National Death Index

through 31 December 2011. Therefore, for each participant follow-up extended from the date of the examination to the date of death or 31 December 2011. The underlying cause listed on the death certificate was applied to determine cause of death that was identified using the underlying Cause of Death-113 groups (international classification of disease (ICD), 10th revision). Total mortality was defined as deaths with any underlying cause of death; CVD and cancer mortality were defined as deaths with underlying cause of death codes for ICD-10 I00-I69 and C00-C97, respectively (29).

Assessment of Covariates

Demographic variables included age, gender, race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or others), educational attainment (<12 years, 12 years, or >12 years of education), living with spouse, and level of income measured based on poverty income ratio (PIR) which is the ratio of household income to the appropriate poverty threshold (low (PIR $\leq$ 1.3), middle (1.3<PIR $\leq$ 3.5), and high (PIR>3.5). The potential risk factors for CVD comprised smoking status (never, former, and current), and the presence of family history of coronary heart disease (CHD). The amount of alcohol consumed daily was estimated using the following assumption: 12.8g for 12-oz beer, 11g for 4-oz glass of wine, and 14g for an ounce of liquor based on the questionnaire. Then, moderate alcohol use was defined as up to 28g and 14 g of alcohol per day in men and women (30). Physical activity was classified based on the recommended levels of physical activity (31). A group with recommended physical activity was defined as those who had self-reported leisure time moderate activity (3 $\leq$ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs $\geq$ 6) three or more times per week; physically inactive group as

those with no reported leisure time physical activity; a group with insufficient physical activity as those who did not meet the criteria for recommended levels of physical activity but not inactive.

Statistical Analysis

We used the appropriate survey procedures to account for the complex sampling design and weights. For the subgroup analysis, domain analysis was applied to preserve the complex sampling design in which the entire samples were used for estimating the variance of subpopulations. Continuous variables were presented by mean (SE: standard error) and compared using linear regression analyses. Categorical variables were expressed by percentage with SE and were compared using Rao-Scott $\chi 2$ tests.

Cox proportional hazards regression model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for all-cause, CVD, and cancer mortality. The proportional hazards assumption of the Cox models was evaluated with log of negative log survival curves based on Kaplan-Meier estimates for DASH or HEI tertile group as well as age, gender, and race/ethnicity. Multivariable-adjusted HRs included the following covariates: age, gender, race/ethnicity, educational attainment, living with spouse, income, smoking status, alcohol consumption, level of physical activity, family history of CHD, waist circumference, and total energy intake. Missing values in the covariates were added as a dummy variable in the multivariable models.

To evaluate the potential effect modification of DASH index or HEI according to the variables with public health importance, we stratified our analysis and tested for interactions of DASH index and HEI with the following variables using all-cause mortality as the outcome by including the cross-product interaction terms in the Cox

models based on F test: age (<65 vs. ≥65 years), sex, race/ethnicity, smoking (current/former smoker vs. never smoked), physical activity (physically inactive vs. physically active), alcohol consumption (moderate drinker vs. non- or heavy drinker), and the presence or absence of chronic disease including DM and hypertension. Furthermore, we performed sensitivity analyses after exclusion of subjects who died during the first five year follow-up. All the statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), and a P value of less than .05 was considered statistically significant.

Results

The MONW phenotype (n=525) was observed in 31.2 (SE, 1.7) % of the normal weight sample from the present study. Table 6.1 shows general characteristics according to the tertiles for DASH index and HEI in MONW and MHNW individuals. Overall, MONW individuals tended to be older and male; had less education and income; were less likely to be non-Hispanic whites and moderate drinkers; were more likely to be physically active; and had more DM, hypertension, and less favorable metabolic parameters, compared with MHNW individuals. In MONW individuals, those with the higher tertile in both DASH index and HEI were more likely to be older and female. In MHNW individuals, those with the higher tertile in DASH index and HEI were more likely to be older and physically active; had more income; were less likely to be a current smoker; and had a lower diastolic BP.

The distribution of components of DASH style diet and HEI according to the each tertile in MONW and MHNW phenotypes is shown in Table 6.2. Overall, no difference of total DASH and HEI scores were found between MONW and MHNW individuals. For

the DASH style diet, trends of all nutrients in DASH style diet were significant with increasing tertile of DASH scores except sodium in MHNW individuals. Percentage of energy in saturated fat and total fat was lower in MONW individuals (P=0.01 and 0.001, respectively). For the HEI scores, trends of all components in HEI were significant with increasing tertile of HEI scores except HEI score for meats in both MONW and MHNW individuals. Total energy intake was not different between each tertile of MONW and MHNW individuals. HEI score for fats and dietary variety were higher and lower in MONW individuals (P=0.01 and 0.04, respectively).

During a median follow-up of 17.9 years, there were 313 and 248 deaths in MONW and MHNW phenotypes, respectively. Overall, higher mortality risk was observed in MONW phenotype compared to MHNW phenotype for all-cause mortality (HR, 1.36 [95% CI, 1.11-1.67]), CVD mortality (HR, 2.24 [95% CI, 1.55-3.24]), and cancer mortality (HR, 1.47 [95% CI, 1.02-2.11]) after adjusting for potential confounders. Table 6.3 shows adjusted HR of all-cause, cardiovascular, and cancer mortality according to the tertile categories and a 1 SD increment in the DASH and HEI scores in MONW and MHNW individuals. Significant inverse associations of increasing DASH index and HEI with all-cause and CVD mortality were observed in MONW individuals after multivariable adjustment. For all-cause mortality, compared with the lowest tertile, HRs were 0.61 (0.44-0.86) (P for trend = 0.006) for DASH index and 0.50 (0.35-0.73) (P for trend < 0.001) for HEI in the highest tertile. For CVD mortality, HRs were 0.40 (0.24-0.65) (P for trend < 0.001) for DASH index and 0.49 (0.26-0.93) (P for trend = 0.007) for HEI in the highest tertile, compared with the lowest tertile. A 1 SD increment (2 points for DASH index and 14 points for HEI) in the adherence to DASH style diet and HEI

was inversely associated with a reduction in the risk of all-cause mortality (HR, 0.77 [0.66-0.90]; HR, 0.73 [0.63-0.85], respectively), and CVD mortality (HR, 0.70 [0.53-0.93]; HR, 0.74 [0.61-0.91], respectively). However, no association was observed in MHNW phenotypes with increasing DASH score and HEI.

In stratified analyses for HRs of all-cause mortality with a SD increment of DASH index and HEI, stronger inverse associations were observed for MONW individuals who were aged 65 years or older, non- or former smokers (for DASH index), or physically active, although interaction term was not significant. MONW individuals with or without DM or hypertension had mortality reduction in higher DASH index and HEI, altogether. In MHNW individuals, the inverse association was stronger among those who were race/ethnicity other than non-Hispanic white (P interaction <0.01 in both indexes) and physically inactive (P for interaction=0.002 for DASH index and 0.06 for HEI).

When we analyzed the data after excluding subjects who died at follow-up during the first five years, the overall results are not materially different from the main analysis. Furthermore, the strength of association tended to increase and the significant inverse association was observed between the increasing HEI score and cancer mortality in MONW individuals (Table 6.5).

Discussion

Our findings suggest that high diet quality such as DASH style diet and HEI is associated with a lower risk of all-cause, and CVD mortality in the MONW individuals, independent of potential confounders, based on the nationally representative sample of normal weight U.S. adults. We observed a 23% and 27% reduction in all-cause mortality

with each 1 SD increment in the DASH score and HEI, respectively, among MONW individuals. This association persisted when we confined our analyses to those with or without prevalent DM and hypertension. We also observed greater risk reductions in CVD mortality for both DASH index and HEI. However, these beneficial effects of high quality diet on the reduction of mortality were not observed in MHNW phenotype. To our knowledge, this is the first study demonstrating that high diet quality such as DASH style and HEI may reduce the mortality risk in MONW individuals.

The inverse associations of DASH and HEI with all-cause and CVD mortality were consistent with previous studies showing that higher-quality diets are associated with reduction in the risk of all-cause mortality (32-35) and CVD mortality (32; 33; 35; 36) in DASH; all-cause mortality (35; 37) and CVD mortality (35; 37; 38) in HEI. Among these previous studies, only one study demonstrated that the inverse association between the DASH diet score and all-cause mortality was obvious in non-obese subjects (BMI <30) in their subgroup analysis (34).

Underlying mechanisms for the beneficial effect of the adherence to the DASH-style diet and HEI on reducing the risk of mortality in MONW individuals might be complex. Those with MONW phenotype are at high risk of developing diseases in which proinflammatory markers such as CRP, interleukins, and tumor necrosis factor alpha play a major role (39; 40). In addition, it is known that the DASH-style diet reduces plasma CRP and fibrinogen levels (41), and that HEI score is inversely associated with serum CRP concentrations (14), indicating that both DASH-style diet and HEI have anti-inflammatory effects. Thus, our results suggest that high quality dietary patterns such as DASH and HEI may antagonize the unfavorable cardiometabolic profiles in those with

MONW phenotype, resulting in lowering the risk of mortality.

In our subgroup analysis in MONW individuals, both DASH and HEI indexes were associated with reduced mortality among older age individuals (≥ 65 years), although interaction terms were not significant. This finding might be explained by the potential cumulative exposure to the healthy dietary pattern with increasing age or that the diet measure more accurately reflected eating patterns before the event (42). An alternative explanation would be a reduced statistical power due to the smaller number of deaths in the younger age group. Although the interaction terms were not significant, the observation that the physically active MONW subjects had a risk reduction in mortality on both DASH and HEI scores reflect the possible synergistic effects of healthy life styles.

The prevalence of hypertension and DM at baseline tended to increase with increasing DASH tertile in both MONW and MHNW phenotype, although these were not statistically significant. This finding indicates that there might be a reverse causation between DASH score, hypertension, and DM, because those with diagnosed hypertension and DM were more likely to follow healthy dietary habits. However, when stratified to those with or without hypertension and DM, there was a consistent inverse association of DASH and HEI score with all-cause mortality, indicating that high quality diets appear to have a long-term benefit on mortality reduction in MONW phenotype independent of prevalent hypertension and DM.

Interestingly, high quality dietary patterns were not associated with mortality reduction in MHNW individuals. This finding might suggest that high quality diet does not operate independently in reducing the risk of mortality for those at lower risk of

dying. Instead, high quality diet may contribute to decreasing the mortality risk in MHNW individuals who had unfavorable lifestyle characteristics. In a stratified analysis, the large reduction of mortality risk was observed only in physically inactive individuals in MHNW phenotype, in both DASH and HEI scores (P for interaction=0.002 for DASH index and 0.06 for HEI). Similar findings were found in current smokers although the interaction terms were not significant.

Our study has several strengths. First, a prospective study design with nearly 18 year follow-up for mortality based on the representative US population allowed us to evaluate a long-term effect of high diet quality on the reduction of mortality risk. Second, data were collected based on extensive laboratory and physical examinations using standardized protocols to minimize the influence of measurement errors. Finally, we were able to assess the potential effect modifications and replicate the findings using a sensitivity analysis. There are also several limitations. First, because NHANES III FFQ did not have portion size, we used only nutrient data from the 24-hr dietary recall data in calculating DASH and HEI scores, which might not be comparable to scores based on FFQ in other studies. Second, due to a single measure of diet collected at baseline, we could not account for any changes in dietary intake over time. Third, self-assessment of food consumption may produce non-differential measurement error, although energy adjustment would reduce this error to some degree (43). Finally, there may be residual confounding due to not measuring the covariates in an objective way, such as self-reported physical activity and smoking status.

To conclude, adherence to high quality diet such as DASH style diet and HEI is associated with a lower risk of all-cause, and CVD mortality in the MONW phenotype,

based on a nationally representative U.S. adult population. High quality diets may be particularly beneficial for normal weight individuals with metabolic abnormalities.

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Table 6.1 Comparison of general characteristics between the lowest and highest tertile for DASH index and Healthy Eating Index in Metabolically Obese Normal Weight (MONW) and Metabolically Healthy Normal Weight (MHNW) phenotypes at baseline

Characteristics	Metabolically Obese Normal Weight (MONW) phenotype				
		DASH		HEI	
	Overall (n=525)	Tertile 1 (n=199)	Tertile 3 (n=216)	Tertile 1 (n=232)	Tertile 3 (n=232)
Age, years	61.4 ± 0.6	58.7 ± 1.3	64.2 ± 1.1*	57.5 ± 1.1	64.6 ± 1.3*
Men, %	50.2 ± 3.4	58.6 ± 4.5	39.2 ± 4.8†	51.4 ± 5.0	39.9 ± 6.0‡
Race/ ethnicity, %					
Non-Hispanic white	78.7 ± 3.0	80.5 ± 5.3	76.8 ± 4.2	70.7 ± 5.5	86.3 ± 3.4‡
Non-Hispanic black	7.4 ± 1.2	9.3 ± 2.3	5.1 ± 1.1	13.5 ± 2.7	2.8 ± 0.7
Mexican-American	3.0 ± 0.6	2.8 ± 1.0	2.6 ± 0.7	3.6 ± 1.2	2.3 ± 0.6
Other	11.0 ± 2.5	7.4 ± 5.0	15.5 ± 4.0	12.2 ± 4.4	8.6 ± 3.1
Educational attainment, %					
<12 years	33.9 ± 3.5	31.6 ± 5.0	32.9 ± 5.0	41.7 ± 5.7	30.9 ± 6.1
12 years	32.4 ± 3.6	36.6 ± 6.7	31.6 ± 3.9	36.7 ± 5.4	30.1 ± 5.1
>=13 years	33.7 ± 4.0	31.8 ± 6.3	35.5 ± 5.3	21.6 ± 5.7	39.0 ± 6.8
Income, %					
PIR=<1.3	16.9 ± 2.1	18.2 ± 3.8	18.3 ± 3.6	23.5 ± 3.2	13.3 ± 3.3
1.3 <PIR=<3.5	41.2 ± 3.4	38.1 ± 6.1	39.7 ± 6.2	43.4 ± 5.2	41.0 ± 6.3
PIR>3.5	41.9 ± 3.9	43.7 ± 6.3	42.0 ± 6.6	33.1 ± 6.1	45.8 ± 6.6
Living with spouse, %	68.5 ± 2.5	72.0 ± 3.9	63.6 ± 3.7	68.9 ± 4.8	69.2 ± 3.8
Smoking status, %					
Never	42.3 ± 3.3	36.4 ± 5.2	48.0 ± 6.0	33.4 ± 5.5	54.0 ± 5.8
Former	28.1 ± 2.6	27.0 ± 5.8	31.4 ± 4.7	27.0 ± 4.5	31.5 ± 4.5
Current	29.7 ± 2.9	36.5 ± 6.2	20.6 ± 4.8	39.6 ± 5.4	14.5 ± 3.7
Drinking alcohol, %					
Never	51.9 ± 4.4	44.4 ± 7.4	64.1 ± 5.8	47.5 ± 6.3	62.2 ± 6.3*
Moderate	41.7 ± 4.0	48.4 ± 7.3	29.2 ± 5.5	48.9 ± 6.6	36.9 ± 6.2

Heavy	6.4	±	2.1	7.3	±	4.5	6.6	±	2.9	3.6	±	2.1	0.9	±	0.6
Physical activity, %															
Inactive	20.1	±	2.8	22.2	±	5.7	18.7	±	4.1‡	21.8	±	4.0	15.8	±	3.1
Insufficient activity	41.0	±	2.5	49.2	±	6.6	28.5	±	3.7	48.4	±	6.2	39.1	±	4.8
Recommended activity	38.8	±	2.6	28.7	±	5.7	52.8	±	5.3	29.7	±	5.5	45.1	±	5.5
CHD family history, %	12.0	±	2.1	15.6	±	6.0	10.9	±	3.3	15.6	±	4.2	7.0	±	2.3
Diabetes mellitus, %	14.5	±	2.1	10.2	±	2.4	17.5	±	3.5	16.5	±	3.7	12.8	±	2.8
Hypertension, %	54.6	±	3.3	52.5	±	6.5	63.2	±	3.7	52.7	±	5.0	54.7	±	5.8
BMI, kg/m^2	22.9	±	0.1	23.0	±	0.1	22.6	±	0.1†	22.8	±	0.1	22.9	±	0.1
Waist circumference, cm	87.3	±	0.3	89.3	±	0.8	85.3	±	0.7*	87.2	±	0.6	86.4	±	0.5
Fasting glucose, mg/dl	104	±	1.5	99	±	1.0	109	±	4.5‡	101	±	1.6	103	±	3.0
HOMA-IR	2.5	±	0.2	2.1	±	0.1	3.1	±	0.7	2.2	±	0.1	3.0	±	0.7‡
SBP, mmHg	137	±	1.5	133	±	1.4	140	±	1.5†	136	±	2.0	136	±	2.3
DBP, mmHg	77	±	1.5	78	±	1.0	77	±	0.8	79	±	1.1	75	±	0.7
hs-CRP, mg/dl	0.43	±	0.03	0.42	±	0.04	0.43	±	0.03	0.49	±	0.05	0.43	±	0.04
Triglycerides, mg/dl	172	±	3.8	162	±	3.4	185	±	14.4	180	±	9.7	174	±	6.4
HDL-C, mg/dl	48	±	0.6	47	±	1.3	50	±	1.1	47	±	1.4	49	±	1.2

Table 6.1 (Continued)

Characteristics	Metabolically Healthy Normal Weight (MHNW) phenotype												P (MONW vs. MHNW)
				DASH				HEI					
	Overall (n=808)			Tertile 1 (n=408)			Tertile 3 (n=417)			Tertile 1 (n=408)			Tertile 3 (n=474)			
Age, years	52.4	±	0.5	50.4	±	0.7	55.8	±	0.8*	48.6	±	0.6	54.4	±	1.0*	<0.001
Men, %	34.8	±	2.4	35.1	±	4.5	30.1	±	3.7	38.9	±	4.0	31.3	±	3.6	<0.001
Race/ ethnicity, %																
Non-Hispanic white	84.7	±	2.2	84.9	±	3.0	85.8	±	3.0	79.9	±	3.6	86.5	±	2.1†	0.03
Non-Hispanic black	6.3	±	1.0	7.9	±	1.7	4.5	±	1.1	9.2	±	1.7	4.1	±	0.8	
Mexican-American	1.6	±	0.3	1.5	±	0.3	1.5	±	0.3	1.6	±	0.4	1.2	±	0.3	

Other	7.4	±	1.8	5.7	±	2.0	8.2	±	2.8	9.2	±	3.1	8.2	±	1.6			
Educational attainment, %																		
<12 years	15.9	±	2.2	17.2	±	2.9	16.5	±	3.5	19.8	±	3.1	11.1	±	2.8*	<0.001		
12 years	28.6	±	2.7	32.0	±	4.1	22.7	±	4.0	32.1	±	3.9	23.3	±	3.4			
>=13 years	55.5	±	3.6	50.8	±	5.6	60.9	±	5.3	48.1	±	5.4	65.6	±	3.6			
Income, %																		
PIR=<1.3	10.0	±	1.8	13.7	±	2.5	6.2	±	1.8‡	15.2	±	3.2	5.7	±	1.8†	0.002		
1.3<PIR=<3.5	35.3	±	3.3	37.7	±	4.6	34.1	±	4.9	37.1	±	4.4	33.8	±	4.6			
PIR>3.5	54.7	±	3.4	48.6	±	4.8	59.6	±	5.0	47.7	±	4.8	60.5	±	4.5			
Living with spouse, %	68.4	±	2.7	70.2	±	4.2	70.2	±	3.9	71.7	±	3.6	66.7	±	4.8	0.98		
Smoking status, %																		
Never	45.7	±	2.8	35.8	±	3.4	57.9	±	3.9*	34.1	±	4.9	56.7	±	4.6*	0.32		
Former	29.7	±	2.2	32.5	±	3.4	30.7	±	4.1	27.2	±	4.4	30.3	±	4.0			
Current	24.5	±	2.2	31.7	±	4.5	11.4	±	2.1	38.7	±	5.1	13.0	±	2.6			
Drinking alcohol, %																		
Never	39.4	±	3.7	36.6	±	4.8	48.7	±	5.9†	37.2	±	5.1	40.3	±	5.4	0.002		
Moderate	56.1	±	3.7	61.2	±	4.9	47.5	±	6.2	58.7	±	4.9	54.8	±	5.8			
Heavy	4.5	±	0.9	2.2	±	0.9	3.9	±	1.5	4.1	±	2.0	4.9	±	1.8			
Physical activity, %																		
Inactive	9.8	±	1.4	10.5	±	1.8	9.5	±	2.0†	15.5	±	2.8	4.6	±	1.7*	<0.001		
Insufficient activity	51.5	±	2.0	59.6	±	3.1	43.2	±	3.8	58.2	±	4.1	48.0	±	3.0			
Recommended activity	38.7	±	2.3	29.9	±	2.9	47.3	±	4.1	26.3	±	4.0	47.5	±	2.8			
CHD family history, %	13.3	±	1.6	16.5	±	3.3	8.3	±	2.1	15.6	±	3.8	11.6	±	2.7	0.59		
Diabetes mellitus, %	4.6	±	0.9	3.5	±	1.5	4.7	±	1.5	2.1		1.3	6.3	±	1.8	<0.001		
Hypertension, %	16.0	±	1.9	13.2	±	2.1	17.7	±	2.4	12.8	±	2.7	17.7	±	2.5	<0.001		
BMI, kg/m^2	22.4	±	0.1	22.4	±	0.1	22.4	±	0.1	22.3	±	0.1	22.4	±	0.1	<0.001		
Waist circumference, cm	81.8	±	0.3	82.1	±	0.6	81.4	±	0.5	81.7	±	0.6	81.4	±	0.5	<0.001		
Fasting glucose, mg/dl	91	±	0.3	91	±	0.5	92	±	0.6	91	±	0.5	92	±	0.5‡	<0.001		
HOMA-IR	1.4	±	0.0	1.4	±	0.0	1.4	±	0.0	1.3	±	0.0	1.4	±	0.0	<0.001		
SBP, mmHg	119	±	0.3	118	±	1.0	120	±	1.0	118	±	0.9	120	±	1.2	<0.001		
DBP, mmHg	73	±	0.3	72	±	0.6	71	±	0.4†	73	±	0.5	73	±	0.5†	<0.001		
hs-CRP, mg/dl	0.27	±	0.01	0.29	±	0.01	0.26	±	0.01	0.28	±	0.01	0.25	±	0.01	<0.001		

Triglycerides, mg/dl	88	±	1.5	85	±	2.5	93	±	2.3†	87	±	2.7	91	±	2.1	<0.001
HDL-C, mg/dl	60	±	0.7	59	±	1.2	60	±	0.6	58	±	1.4	60	±	1.1	<0.001

Data are presented as means ± standard error or proportion (%) ± standard error.
* P <0.001, † P <0.01, and ‡ P <0.05 for comparison across each tertile of DASH index and HEI. P values for continuous variables represent P for trend.
Abbreviations: DASH, Dietary Approaches to Stop Hypertension; HEI, Healthy Eating Index; PIR, poverty income ratio; CHD, coronary heart disease; BMI, waist circumference; HOMA-IR, homoeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol.

Table 6.2 Comparison for each component of DASH-style diet and Healthy Eating Index in Metabolically Obese Normal Weight (MONW) and Metabolically Healthy Normal Weight (MHNW) phenotypes at baseline

	Metabolically Obese Normal Weight (MONW) phenotype				
DASH	Overall (n=525)	Tertile1 (n=178)	Tertile2 (n=156)	Tertile3 (n=191)	P for trend
Score range	(0 - 8)	(0 - 1.5)	(2 - 3)	(3.5 - 8)	
DASH score	2.8 ± 0.1	1.0 ± 0.1	2.5 ± 0.0	4.7 ± 0.1	<0.001
Saturated fat, % energy	10.3 ± 0.2	13.3 ± 0.3	10.4 ± 0.5	7.4 ± 0.3	<0.001
Total fat, % energy	31.0 ± 0.5	38.9 ± 0.4	31.3 ± 1.3	23.6 ± 0.5	<0.001
Protein, % energy	15.2 ± 0.2	13.9 ± 0.3	15.3 ± 0.5	16.4 ± 0.4	<0.001
Cholesterol, mg	122.4 ± 6.5	143.4 ± 8.1	129.8 ± 9.8	97.7 ± 14.6	<0.001
Fiber, mg	9.0 ± 0.2	6.3 ± 0.1	7.8 ± 0.4	12.3 ± 0.5	<0.001
Magnesium, mg	156.7 ± 3.0	121.8 ± 2.3	145.0 ± 6.8	197.4 ± 5.9	<0.001
Calcium, mg	416.5 ± 11.3	356.2 ± 12.5	386.9 ± 20.0	493.7 ± 26.4	<0.001
Potassium, mg	1520 ± 23	1230 ± 23	1407 ± 53	1870 ± 50	<0.001
Sodium, mg	1639 ± 28	1741 ± 42	1612 ± 51	1563 ± 50	0.003
Total energy intake, kcal	1977 ± 53	2268 ± 110	2014 ± 103	1683 ± 75	<0.001
HEI	Overall (n=525)	Tertile1 (n=272)	Tertile2 (n=263)	Tertile3 (n=273)	P for trend
Score range	(21.3 - 97.7)	(21.3 - 59.2)	(59.3 - 72.8)	(72.9 - 97.7)	
HEI score	67.2 ± 0.7	50.7 ± 0.5	66.4 ± 0.5	81.3 ± 0.4	<0.001
Grains, no. of servings	6.6 ± 0.2	5.7 ± 0.3	6.6 ± 0.3	7.3 ± 0.6	0.02
Fruits, no. of servings	1.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.1	3.3 ± 0.2	<0.001
Vegetables, no. of servings	3.3 ± 0.1	2.5 ± 0.2	3.1 ± 0.2	4.3 ± 0.3	<0.001
Dairy, no. of servings	1.9 ± 0.1	1.8 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	0.62
Meats, no. of servings	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.8 ± 0.2	0.47

Legumes, no. of servings	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.16
Grains, HEI score	6.7 ± 0.2	6.0 ± 0.3	6.6 ± 0.2	7.4 ± 0.2	<0.001
Fruits, HEI score	4.7 ± 0.2	2.0 ± 0.2	3.2 ± 0.3	8.2 ± 0.2	<0.001
Vegetables, HEI score	6.3 ± 0.2	4.8 ± 0.3	6.2 ± 0.3	7.6 ± 0.3	<0.001
Dairy, HEI score	6.2 ± 0.2	5.3 ± 0.4	6.5 ± 0.3	6.7 ± 0.4	0.01
Meats, HEI score	6.8 ± 0.2	6.5 ± 0.4	6.7 ± 0.3	7.0 ± 0.2	0.12
Fats, HEI score	7.1 ± 0.2	4.7 ± 0.5	7.1 ± 0.3	9.2 ± 0.1	<0.001
Saturated fat, HEI score	7.0 ± 0.2	4.2 ± 0.4	7.2 ± 0.3	9.0 ± 0.2	<0.001
Cholesterol, HEI score	8.3 ± 0.2	6.0 ± 0.4	8.8 ± 0.2	9.7 ± 0.1	<0.001
Sodium, HEI score	6.4 ± 0.3	5.9 ± 0.4	6.2 ± 0.4	7.0 ± 0.3	0.001
Dietary variety, HEI score	7.7 ± 0.2	5.4 ± 0.4	7.8 ± 0.3	9.5 ± 0.1	<0.001
Total energy intake, kcal	1977 ± 53	1917 ± 88	2023 ± 116	1985 ± 93	0.51

Table 6.2 (Continued)

DASH	Metabolically Healthy Normal Weight (MHNW) phenotype					
	Overall (n=808)	Tertile1 (n=293)	Tertile2 (n=255)	Tertile3 (n=260)	P for trend	P (MONW vs MHNW)
Score range	(0 - 8.5)	(0 - 1.5)	(2 - 3)	(3.5 - 8.5)		
DASH score	2.7 ± 0.1	1.0 ± 0.0	2.5 ± 0.0	4.7 ± 0.1	<0.001	0.12
Saturated fat, % energy	10.7 ± 0.1	13.0 ± 0.2	10.9 ± 0.2	8.0 ± 0.2	<0.001	0.01
Total fat, % energy	32.9 ± 0.4	39.4 ± 0.4	33.7 ± 0.5	25.0 ± 0.4	<0.001	0.001
Protein, % energy	15.6 ± 0.2	14.6 ± 0.2	15.2 ± 0.3	17.0 ± 0.4	<0.001	0.12
Cholesterol, mg	124.1 ± 3.9	156.6 ± 6.4	113.9 ± 5.8	97.9 ± 6.3	<0.001	0.78
Fiber, mg	9.0 ± 0.2	6.3 ± 0.2	8.3 ± 0.2	12.7 ± 0.4	<0.001	0.83
Magnesium, mg	157.4 ± 2.2	121.5 ± 1.5	147.0 ± 2.0	207.4 ± 3.6	<0.001	0.85
Calcium, mg	390.2 ± 9.2	323.9 ± 9.0	364.0 ± 15.9	489.6 ± 17.0	<0.001	0.10

Potassium, mg	1514	±	24	1210	±	27	1432	±	25	1931	±	36	<0.001	0.84
Sodium, mg	1639	±	23	1642	±	31	1613	±	50	1661	±	39	0.62	0.99
Total energy intake, kcal	1997	±	29	2187	±	66	2123	±	56	1662	±	38	<0.001	0.70
HEI	Overall (n=808)			Tertile1 (n=272)			Tertile2 (n=263)			Tertile3 (n=273)			P for trend	P (MONW vs MHNW)
Score range	(23.5 - 96.7)			(23.5 - 59.2)			(59.3 - 72.8)			(72.9 - 96.7)				
HEI score	66.8	±	0.5	50.5	±	0.5	66.2	±	0.3	80.8	±	0.2	<0.001	0.60
Grains, no. of servings	6.4	±	0.1	5.4	±	0.2	6.5	±	0.2	7.2	±	0.3	<0.001	0.34
Fruits, no. of servings	1.9	±	0.1	0.6	±	0.1	1.5	±	0.1	3.3	±	0.2	<0.001	0.56
Vegetables, no. of servings	3.3	±	0.1	2.6	±	0.1	3.2	±	0.1	3.9	±	0.2	<0.001	0.92
Dairy, no. of servings	2.0	±	0.1	1.9	±	0.2	1.8	±	0.2	2.2	±	0.2	0.26	0.63
Meats, no. of servings	2.0	±	0.0	2.4	±	0.1	1.9	±	0.1	1.8	±	0.1	0.001	0.02
Legumes, no. of servings	0.1	±	0.1	0.1	±	0.1	0.1	±	0.0	0.1	±	0.0	0.69	0.62
Grains, HEI score	6.5	±	0.2	5.5	±	0.2	6.4	±	0.1	7.3	±	0.2	<0.001	0.08
Fruits, HEI score	4.7	±	0.1	1.8	±	0.1	4.2	±	0.3	7.6	±	0.3	<0.001	0.83
Vegetables, HEI score	6.4	±	0.2	4.8	±	0.2	6.6	±	0.2	7.7	±	0.2	<0.001	0.53
Dairy, HEI score	6.4	±	0.2	5.7	±	0.4	5.6	±	0.3	7.6	±	0.2	<0.001	0.43
Meats, HEI score	6.9	±	0.2	7.0	±	0.2	6.8	±	0.2	6.9	±	0.3	0.72	0.45
Fats, HEI score	6.6	±	0.1	4.1	±	0.3	6.6	±	0.2	8.7	±	0.2	<0.001	0.01
Saturated fat, HEI score	6.8	±	0.2	4.0	±	0.3	6.8	±	0.3	9.1	±	0.1	<0.001	0.31
Cholesterol, HEI score	8.0	±	0.2	5.7	±	0.4	8.7	±	0.2	9.4	±	0.1	<0.001	0.10
Sodium, HEI score	6.4	±	0.1	5.9	±	0.3	6.2	±	0.3	6.9	±	0.3	0.002	0.89
Dietary variety, HEI score	8.1	±	0.1	6.1	±	0.3	8.2	±	0.1	9.7	±	0.1	<0.001	0.03
Total energy intake, kcal	1997	±	29	2052	±	65	1932	±	49	1998	±	52	0.52	0.70

Data are presented as means ± standard error.
Abbreviations: DASH, Dietary Approaches to Stop Hypertension; HEI, Healthy Eating Index;
The amount of each DASH dietary components are based on a 1,000 kcal diet.

Table 6.3 Adjusted hazard ratio (HR)* of all-cause, cardiovascular, and cancer mortality according to the tertile categories and a linear increment in the DASH diet score and Healthy Eating Index in Metabolically Obese Normal Weight (MONW) and Metabolically Healthy Normal Weight (MHNW) phenotypes during a median follow-up of 17.9 years

	n	No. of person years	Deaths from all causes, n	All-cause mortality, HR (95% CI)	CVD deaths, n	CVD mortality, HR (95% CI)	Cancer deaths, n	Cancer mortality, HR† (95% CI)
Metabolically Obese Normal Weight (MONW) phenotype								
DASH								
Tertile 1	178	2392	103	1.00 (ref.)	34	1.00 (ref.)	23	1.00 (ref.)
Tertile 2	156	2012	95	0.84 (0.59-1.19)	35	0.86 (0.54-1.38)	16	0.94 (0.40-2.21)
Tertile 3	191	2617	115	0.61 (0.44-0.86)	37	0.40 (0.24-0.65)	19	0.92 (0.56-1.49)
P trend				0.006		<0.001		0.77
HR for a 1 SD increase in DASH				0.77 (0.66-0.90)		0.70 (0.53-0.93)		0.79 (0.62-1.01)
HEI								
Tertile 1	172	2268	98	1.00 (ref.)	32	1.00 (ref.)	20	1.00 (ref.)
Tertile 2	181	2327	111	0.79 (0.56-1.12)	40	1.21 (0.78-1.89)	21	0.43 (0.19-0.97)
Tertile 3	172	2426	104	0.50 (0.35-0.73)	34	0.49 (0.26-0.93)	17	0.54 (0.22-1.34)
P trend				<0.001		0.007		0.21
HR for a 1 SD increase in HEI				0.73 (0.63-0.85)		0.74 (0.61-0.91)		0.66 (0.46-0.95)

Metabolically Healthy Normal Weight (MHNW) phenotype								
DASH								
Tertile 1	293	4845	91	1.00 (ref.)	21	1.00 (ref.)	29	1.00 (ref.)
Tertile 2	255	4289	69	0.77 (0.54-1.10)	19	0.74 (0.35-1.58)	18	0.42 (0.17-1.08)
Tertile 3	260	4195	90	0.96 (0.68-1.35)	25	0.99 (0.41-2.37)	19	0.69 (0.39-1.24)
P trend				0.77		0.98		0.31
HR for a 1 SD increase in DASH				1.03 (0.84-1.24)		1.06 (0.73-1.53)		0.96 (0.64-1.43)
HEI								
Tertile 1	272	4523	83	1.00 (ref.)	19	1.00 (ref.)	26	1.00 (ref.)
Tertile 2	263	4226	85	0.71 (0.44-1.15)	24	0.72 (0.38-1.33)	22	0.71 (0.27-1.89)
Tertile 3	273	4580	82	0.66 (0.39-1.13)	22	0.70 (0.40-1.22)	18	0.51 (0.19-1.37)
P trend				0.14		0.22		0.18
HR for a 1 SD increase in HEI				0.85 (0.68-1.05)		0.92 (0.70-1.21)		0.90 (0.62-1.30)

Data are presented as hazard ratio (95% confidence interval).

Abbreviations: DASH, Mediterranean diet score; HEI, Healthy Eating Index; CVD, cardiovascular disease

* Adjusted for age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, alcohol consumption, level of physical activity, family history of coronary heart disease, waist circumference, and total calorie intakes.

† those who had a history of skin cancer were also excluded.

Table 6.4 Subgroup analyses of the association of one SD increment in the DASH and HEI score with the risk of all-cause mortality in Metabolically Obese Normal Weight (MONW) and Metabolically Healthy Normal Weight (MHNW) phenotypes

	Metabolically Obese Normal Weight (MONW) phenotype				
	No. of participants (deaths)	HR for a 1 SD increase in DASH	P interaction	HR for a 1 SD increase in HEI	P interaction
Age, y			0.28		0.09
< 65	229 (70)	0.90 (0.63-1.28)		0.72 (0.50-1.03)	
≥ 65	273 (226)	0.69 (0.59-0.80)		0.71 (0.61-0.82)	
Gender			0.99		0.27
Men	273 (174)	0.85 (0.64-1.11)		0.77 (0.60-0.98)	
Women	229 (122)	0.63 (0.51-0.79)		0.69 (0.55-0.86)	
Race/ ethnicity			0.41		0.16
Non-Hispanic white	279 (182)	0.74 (0.57-0.95)		0.77 (0.64-0.93)	
Others	223 (114)	0.64 (0.48-0.85)		0.68 (0.50-0.93)	
Smoking status			0.06		0.48
Current smoker	153 (90)	0.80 (0.45-1.41)		0.60 (0.41-0.86)	
Non- or former smoker	349 (206)	0.75 (0.63-0.89)		0.76 (0.64-0.91)	
Drinking alcohol			0.25		0.84
Moderate drinker	190 (105)	0.79 (0.53-1.20)		0.79 (0.59-1.06)	
Non- or heavy drinker	312 (191)	0.82 (0.67-1.02)		0.67 (0.55-0.81)	
Physical activity			0.95		0.82
Physically inactive	126 (78)	1.33 (0.70-2.54)		0.81 (0.64-1.04)	
Physically active	376 (218)	0.75 (0.64-0.87)		0.72 (0.62-0.85)	
Chronic disease			0.06		0.20
Absence	162 (68)	0.65 (0.48-0.88)		0.55 (0.38-0.79)	
Presence	340 (228)	0.83 (0.69-0.99)		0.79 (0.64-0.97)	

Table 6.4 (Continued)

	Metabolically Healthy Normal Weight (MHNW) phenotype				
	No. of participants (deaths)	HR for a 1 SD increase in DASH	P interaction	HR for a 1 SD increase in HEI	P interaction
Age, y			0.61		0.29
< 65	568 (77)	0.85 (0.56-1.27)		0.73 (0.53-1.01)	
≥ 65	218 (159)	1.12 (0.96-1.31)		0.90 (0.75-1.07)	
Gender			0.73		0.45
Men	301 (107)	1.00 (0.74-1.34)		0.94 (0.74-1.19)	
Women	412 (97)	1.14 (0.89-1.47)		0.71 (0.48-1.05)	
Race/ ethnicity			0.001		0.005
Non-Hispanic white	476 (157)	1.06 (0.87-1.28)		0.87 (0.72-1.05)	
Others	310 (79)	0.72 (0.44-1.16)		0.67 (0.45-0.99)	
Smoking status			0.62		0.26
Current smoker	223 (74)	0.82 (0.56-1.22)		0.67 (0.48-0.93)	
Non- or former smoker	563 (162)	1.06 (0.85-1.33)		0.88 (0.66-1.18)	
Drinking alcohol			0.22		0.14
Moderate drinker	387 (87)	0.82 (0.56-1.22)		0.93 (0.74-1.16)	
Non- or heavy drinker	399 (149)	1.06 (0.85-1.33)		0.78 (0.63-0.97)	
Physical activity			0.002		0.06
Physically inactive	130 (53)	0.37 (0.23-0.59)		0.63 (0.46-0.85)	
Physically active	656 (183)	1.08 (0.86-1.34)		0.87 (0.66-1.13)	
Chronic disease			0.94		0.14
Absence	582 (129)	1.00 (0.70-1.42)		0.73 (0.55-0.98)	
Presence	204 (107)	0.96 (0.77-1.18)		0.97 (0.73-1.30)	

Data are presented as hazard ratio (95% confidence interval).
Abbreviations: SD, standard deviation; DASH, Mediterranean diet score; HEI, Healthy Eating Index; CVD, cardiovascular disease
Models are adjusted as Table 6.3, except for the stratifying factor. Being physically inactive indicates no reported leisure time physical activity; Presence of chronic disease indicates having diabetes mellitus or hypertension.

Table 6.5 Multivariable adjusted hazard ratio (HR) of all-cause mortality according to the tertile categories and a SD increment in the DASH diet and HEI scores in Metabolically Obese Normal Weight (MONW) and Metabolically Healthy Normal Weight (MHNW) phenotypes excluding the deaths at the first five year follow-up

	n	No. of person years	Deaths from all causes, n	All-cause mortality, HR (95% CI)	CVD deaths, n	CVD mortality, HR (95% CI)	Cancer deaths, n	Cancer mortality, HR† (95% CI)
Metabolically Obese Normal Weight (MONW) phenotype								
DASH								
Tertile 1	152	2315	77	1.00 (ref.)	26	1.00 (ref.)	18	1.00 (ref.)
Tertile 2	123	1927	62	0.72 (0.47-1.11)	20	0.59 (0.33-1.05)	10	0.71 (0.23-2.14)
Tertile 3	164	2550	88	0.57 (0.42-0.77)	24	0.35 (0.22-0.56)	14	0.76 (0.34-1.71)
P trend				<0.001		<0.001		0.37
HR for a 1 SD increase in DASH				0.75 (0.64-0.88)		0.69 (0.49-0.96)		0.70 (0.48-1.02)
HEI								
Tertile 1	140	2178	66	1.00 (ref.)	20	1.00 (ref.)	16	1.00 (ref.)
Tertile 2	147	2241	77	0.66 (0.47-0.93)	27	1.25 (0.76-2.05)	12	0.19 (0.07-0.48)
Tertile 3	152	2373	84	0.43 (0.31-0.60)	23	0.49 (0.26-0.93)	14	0.30 (0.12-0.79)
P trend				<0.001		<0.001		0.04
HR for a 1 SD increase in HEI				0.72 (0.62-0.83)		0.72 (0.59-0.88)		0.55 (0.38-0.79)
Metabolically Healthy								

Normal Weight (MHNW) phenotype								
DASH								
Tertile 1	275	4795	73	1.00 (ref.)	14	1.00 (ref.)	24	1.00 (ref.)
Tertile 2	242	4257	56	0.73 (0.48-1.11)	11	0.54 (0.19-1.54)	16	0.54 (0.19-1.52)
Tertile 3	239	4133	70	0.99 (0.66-1.49)	14	0.83 (0.32-2.16)	16	0.83 (0.43-1.56)
P trend				0.93		0.68		0.77
HR for a 1 SD increase in DASH				1.06 (0.84-1.33)		0.93 (0.57-1.52)		1.07 (0.69-1.65)
HEI								
Tertile 1	256	4479	67	1.00 (ref.)	14	1.00 (ref.)	22	1.00 (ref.)
Tertile 2	245	4168	68	0.62 (0.36-1.06)	14	0.39 (0.20-0.74)	19	0.69 (0.24-1.97)
Tertile 3	255	4531	64	0.54 (0.39-0.99)	11	0.33 (0.15-0.73)	15	0.50 (0.18-1.41)
P trend				0.05		0.01		0.19
HR for a 1 SD increase in HEI				0.80 (0.63-1.01)		0.69 (0.50-0.96)		0.92 (0.62-1.34)

Data are presented as hazard ratio (95% confidence interval).
Abbreviations: DASH, Mediterranean diet score; CVD, cardiovascular disease
* Adjusted for age, gender, and race/ethnicity, educational attainment, income, smoking status, level of physical activity, family history of coronary heart disease, a past history of coronary heart disease or stroke, a past history of any cancer, waist circumference, and total calories.
† those who had a history of skin cancer were also excluded.

CHAPTER 7

OBESITY AS A MEDIATOR IN THE ASSOCIATION BETWEEN HEALTHY DIETARY PATTERNS AND INSULIN RESISTANCE AND INFLAMMATION: COMPARISON BETWEEN TRADITIONAL APPROACH AND CAUSAL MEDIATION APPROACH[1]

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Abstract

Background: The association between healthy dietary patterns and efficient glucose metabolism or anti-inflammatory effects is well known. However, the extent to which obesity may act as a confounder or mediator in this association is unclear.

Objective: We investigated whether the associations of the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diet with markers for insulin resistance and inflammation are mediated by body mass index (BMI) or waist circumference (WC) in a representative U.S. population.

Design: Data from 4,700 adults aged 20-90 years without any prior diagnosis of cancer, cardiovascular disease, diabetes, or hypertension were analyzed from the National Health and Nutrition Examination Survey III, 1988–1994. Mediterranean diet scores (MDS) and DASH index were calculated using food frequency questionnaires and the 24-hr dietary recall data. Markers for insulin resistance included fasting glucose, insulin, homoeostasis model assessment of insulin resistance (HOMA-IR), post-load glucose, hemoglobin A1c (HbA1c); inflammatory markers included high-sensitivity C-reactive protein (hs-CRP), white blood cell (WBC), fibrinogen, homocysteine, and lipoprotein (a). Traditional and causal mediation analyses were applied using multiple linear regression models.

Results: Higher MDS was inversely associated with BMI, WC, log insulin, log HOMA-IR, fasting glucose, HbA1c, WBC, and fibrinogen after adjusting for potential confounders. BMI mediated the association between MDS and log insulin, log HOMA-IR, fasting glucose, and HbA1c, whereas WC mediated the association between MDS with log insulin, log HOMA-IR, fasting glucose, post-load glucose, HbA1c, log hs-CRP,

WBC, and fibrinogen. In addition, the mediated effects of WC were greater than those of BMI consistently in all markers in both traditional and causal mediation analysis. Furthermore, the association between MDS and fasting glucose was fully mediated by adiposity, especially by WC in younger individuals including men < 45 years and premenopausal women. However, no mediation effect by adiposity was observed in the association of DASH index with the markers for insulin resistance and inflammation.

Conclusions: WC mediates the association of the Mediterranean diet with insulin resistance and inflammation more so than BMI, suggesting that lowering abdominal obesity may be one of the pathways through which the Mediterranean diet reduces insulin resistance and inflammation.

Keywords: waist circumference, body mass index, Mediterranean diet, insulin resistance, inflammation, mediation

Introduction

Much evidence shows that the Mediterranean diet and the Dietary Approaches to Stop Hypertension (DASH) style diets are associated with lower risk of cardiometabolic disease (1-3). Underlying mechanisms for cardiovascular health benefits of Mediterranean diet and DASH style diet are complex, but can be explained by the combined effect of improving metabolic profiles including lipid profiles, blood pressure, insulin resistance, and inflammatory markers (4, 5).

The Mediterranean diet and DASH style diets appear to be independently associated with cardiometabolic risk after adjusting for adiposity (6). However, it is unclear how much the adjustment for adiposity, such as general obesity measured by body mass index (BMI) or abdominal obesity measured by waist circumference (WC),

modifies or attenuates the association of the Mediterranean diet and DASH style diet with cardiometabolic risk. Using mediation analysis, the role of adiposity underlying the relationship of the Mediterranean diet and DASH style diet with cardiometabolic risk could be clarified (7). Furthermore, the degree of mediation may be different between general obesity and abdominal obesity, because abdominal obesity affects metabolic disturbance to a greater extent than general obesity (8).

Mediation analysis is a statistical procedure that can be used to explain the process underlying the relationship between the exposure and the outcome, and the extent to which this relationship can be mediated by a third variable (9). The approach to mediation analysis proposed by Baron and Kenny (9) focused on comparing two regression models in which one model was conditioned on the mediator and the other one was not. The exposure coeffients in the regression models would be interpreted as a direct effect in the model adjusted for the mediator, and as a total effect in the model unadjusted for the mediator (7). This traditional approach to evaluate mediation tends to produce a bias by not considering mediator-outcome confounding and interaction between exposure and mediator. Using the counterfactual framework in causal mediation analysis, unbiased valid estimates of direct effect and indirect effect can be obtained (7, 10).

Limited data exist on the mediator role of adiposity on the relationship of the Mediterranean diet and DASH style diet with cardiometabolic risk, especially in a causal mediation analytic approach. Therefore, we aimed to examine the association of Mediterranean diet and DASH style diet with markers for insulin resistance and inflammation. In addition, we investigated whether this association is mediated by BMI

and WC, using both approaches in traditional mediation analysis and causal mediation analysis.

Methods

Study population

We used data from the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994. NHANES III was conducted using a complex multi-stage stratified clustered probability sample design to attain a nationally representative sample of the civilian, non-institutionalized US population. The survey included personal interviews, physical examinations, and laboratory measurements. We included 7,871 adults with BMI $\geq$ 18.5 kg/m^2 aged 20-90 years who had complete data on FFQ, 24-hour dietary recall, BMI, and WC with at least 10 hours' fasting. Because dietary habits might change due to chronic illness, we excluded those who reported a history of myocardial infarction, stroke, congestive heart failure, or any prior diagnosis of cancer (n=988). In addition, we excluded those who were diagnosed or treated for diabetes mellitus (DM) and hypertension, or taking cholesterol-lowering medication (n=1,485). To minimize reverse causation, we additionally excluded the participants who reported changing their dietary patterns due to any medical reason during the previous 12 months (n=547). Because one of the main outcomes was an inflammatory marker, we also excluded those with rheumatoid arthritis or hs-CRP > 10mg/L. Furthermore, we excluded pregnant or lactating women, those who reported implausible extreme energy intakes (<1st and >99th percentiles of energy intake/d in adults) or those with BMI > 60 kg/m^2 (n=151). Finally, a total of 4,700 individuals were analyzed in the present study.

Assessment of Mediterranean diet

We used 81-item food frequency questionnaires (FFQ) and the 24-hr dietary recall data, validated by the Nutrition Methodology Working Group (11), to evaluate dietary intake. Adherence to the Mediterranean diet was assessed using the scoring methodology developed by Panagiotakos et al. (12, 13). In brief, scores 0 to 5 were assigned for the weekly consumption of food items assumed to be contributing towards Mediterranean dietary pattern, whereas scores on the inverse ordinal scale were assigned for the consumption of food items assumed to be against the Mediterranean dietary pattern. For instance, the scores assigned to the weekly consumption frequencies of legumes were as follows: no servings, less than 1 serving, 1–2 servings, 3–4 servings, 5–6 servings, and >6 servings were assigned scores of 0, 1, 2, 3, 4 and 5, respectively. Similar score assignments were used for the food items of non-refined cereals, potatoes, fruits, vegetables, fish, and olive oil. Reverse scores were assigned for the components of red meat and products, poultry and full-fat dairy products. For the alcohol consumption, a score of 5 was assigned for consumption of less than 300 ml (36g) of alcohol per day, and 0 for no consumption or for consumption of >700 ml (84g) per day. It has been shown that Mediterranean Diet Score (MDS) is highly associated with prevalent cardiometabolic diseases, 10-year CVD risk, and inflammation and coagulation markers, in addition to capturing inherent characteristics of Mediterranean dietary pattern (12-14).

The NHANES III FFQ applied a 1-month reference period without recording portion sizes. Thus, we calculated the MDS, assuming that the number of servings per week were equivalent to the number of times that a food item was consumed per week. We excluded potatoes for MDS assessment, because the way potatoes are prepared in US

is different from European countries (15). Daily alcohol consumption was estimated using the following assumption: 12.8g for 12-oz beer, 11g for 4-oz glass of wine, and 14g for an ounce of liquor based on the questionnaire provided. Then, gender-specific cut-offs were applied: a score of 5 was assigned for consumption of less than 28g and 14 g of alcohol per day, 0 for no consumption or for consumption of greater than 70g and 28g per day in men and women, respectively, and the cutoffs for subcategories between 0 and 5 were reassigned with even intervals (16). Since olive oil consumption was not measured in the NHANES III FFQ, we calculated the ratio of total monounsaturated fatty acid to total saturated fatty acids with six even intervals using the 24-h dietary recall data as a proxy variable of olive oil consumption. Finally, the possible MDS score ranged from 0 to 50, with higher values of this MDS score indicate greater adherence to the Mediterranean diet.

Assessment of DASH style diet

One day 24-hr dietary recall data was used to calculate Mellen's index which is one of established DASH index scores (17), because the serving data was not available in NHANES FFQ. Mellen's index, a nutrient-based index with 9 components including saturated fat, total fat, protein, cholesterol, fiber, magnesium, calcium, potassium, and sodium using target nutrient values used in clinical trials (18), applied absolute targets on the basis of a 2100-kcal diet for both men and women. One point was assigned to the individuals who achieved the goal for each component; one-half of a point was assigned to those who met an intermediate goal, defined as the midpoint between the DASH diet goal and the nutrient content of the DASH control diet (19); and 0 point was assigned to those who met neither goal.

Assessment of the markers for insulin resistance and inflammation

Metabolic parameters were measured under quality control standards of Centers for Disease Control and Prevention. BMI was calculated as kg/m^2; height was measured to the nearest 0.1cm and weight to the nearest 0.01 kg. Waist circumference (WC) was measured at the level of the right iliac crest. Blood pressure (BP) was averaged over five separate measurements. Mean arterial pressure was calculated using the formula of [(2 × diastolic BP + systolic BP) / 3]. Glucose was measured in serum, using a modified hexokinase enzymatic method. Serum insulin was measured using a radioimmnuoassay (Pharmacia Diagnostics). Homoeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the formula [fasting glucose (mg/dl) × fasting insulin (lU/mL)/405]. Hemoglobin A1c (HbA1c) was measured on whole blood with an ion-exchange high-performance liquid chromatography (HPLC) method (Bio-Rad Diamat HPLC; interassay coefficient of variation 2.0). Post-load glucose was measured using the 2 hour oral glucose tolerance testing for the participants aged 40-74 years. Thus, analyses for post-load glucose were limited to this age group. Serum high sensitivity C reactive protein (hs-CRP) concentrations were measured by latex-enhanced nephlometry (Department of Laboratory Medicine, Immunology Division, University of Washington, Seattle). The Coagamate XC Plus automated coagulation analyzer was used to measure fibrinogen levels (Organon Technika, Durham, NC). The Coulter Counter Model S-PLUS JR with Coulter histogram differential, a quantitative, automated hematology analyzer, was used to determine white blood cell (WBC) ($\times 10^3$ cells). Serum homocysteine was measured by reverse phase, high-performance liquid chromatography and fluorescence detection (US Department of Agriculture Human Nutrition Research Center on Aging at Tufts

University, Boston, MA). Lipoprotein (a) was measured by enzyme-linked immunosorbent assay (ELISA) (Strategic Diagnostics, Newark, DE).

Assessment of Covariates

Participants' sociodemographic characteristics, medical history, and lifestyle related characteristics were measured during the personal interview. Sociodemographic variables included age, gender, race or ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or others), educational attainment (<12 years, 12 years, or >12 years of education), living with spouse, and level of income measured based on poverty income ratio (PIR) which is the ratio of household income to the appropriate poverty threshold (low (PIR $\leq$ 1.3), middle (1.3<PIR $\leq$ 3.5), and high (PIR>3.5)). Family history of coronary heart disease (CHD) and parental history of DM were self-reported. Smoking status was categorized as never, former, and current. Consumption up to 28g and 14 g of alcohol per day in men and women was considered as moderate alcohol use (16). Physical activity was categorized based on the recommended levels of physical activity (20). A recommended physical activity was designated as a self-reported leisure time moderate activity (3 $\leq$ metabolic equivalents (METs) <6) of five or more times per week or leisure time vigorous activity (METs $\geq$ 6) three or more times per week; a physical inactivity as no reported leisure time physical activity; an insufficient physical activity as not meeting the criteria for recommended levels of physical activity but not inactive.

Statistical Analysis

All the statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA), and a P-value of less than .05 was considered statistically significant. Descriptive statistics were computed for the sample across tertiles of

Mediterranean diet and DASH style diet. Continuous variables were presented by mean (SE: standard error) and compared using linear regression analyses. Skewed variables such as fasting insulin, HOMA-IR, hs-CRP, homocysteine, and lipoprotein (a) were log-transformed to improve their distribution towards normal. Categorical variables were expressed by percentages with SEs and were compared using Rao-Scott $\chi 2$ tests. For the traditional mediation analysis, we used the appropriate survey procedures to account for the complex sampling design and weights used in NHANES III. For the subgroup analyses, domain analysis was applied to preserve the complex sampling design in which the entire samples were used for estimating the variance of subpopulations. For the outcome variables in our mediation analyses, fasting insulin, HOMA-IR, fasting glucose, post-load glucose, and HbA1c were selected as markers for insulin resistance; hs-CRP, WBC, fibrinogen, homocysteine, and lipoprotein (a) for inflammatory markers.

The traditional approach for mediation analysis included the following steps. First, linear regression analyses were applied to examine associations of Mediterranean diet and DASH style diet (the highest tertile vs. the first tertile, respectively) with BMI and WC, after adjusting for age, gender, race/ethnicity, educational attainment, living with spouse, income, smoking status, level of physical activity, family history of CHD, parental history of DM, and total energy intake, as well as alcohol consumption for the association of DASH style diet with BMI and WC (the estimate a in the Figure 7.1). Second, linear regression analyses were applied to examine the association of BMI and WC with markers for insulin resistance or inflammation, using the same covariates in addition to adjusting for MDS or DASH (the estimate b in the Figure 7.1). In this step, we added covariates to the model according to the characteristics of outcome variables (mean

arterial pressure for markers for insulin resistance; multivitamin use for homocysteine). Third, the simple total effect of Mediterranean diet and DASH style diet was estimated by regressing the markers for insulin resistance or inflammation on MDS and DASH index while adjusting for the covariates used in the first step, but without adjusting for BMI or WC (the estimate c in the Figure 7.1). Fourth, BMI or WC was additionally controlled in the model to estimate the direct effect of Mediterranean diet and DASH style diet on the markers for insulin resistance or inflammation (the estimate c in the Figure 7.1). Finally, the indirect effect by BMI or WC is estimated by calculating the product of the beta coefficient of the first regression model and the beta coefficient of the second regression model (the estimate a×b in the Figure 7.1) (9, 21). This approach also can be presented using the two models: $E\,[\,M \mid A = a, C = c\,] = \beta_0 + \beta_1 a + \beta_2' c$; $E\,[\,Y \mid A = a, M = m, C = c\,] = \theta_0 + \theta_1 a + \theta_2 m + \theta_4' c$ (a represents exposure, m represents mediator, and c represents confounders) in which the direct effect is evaluated by estimating θ_1; the indirect effect is evaluated by estimating $\beta_1\theta_2$ (22). Then, we divided the indirect effect by its standard error and performed a z test under the null hypothesis that the indirect effect is equal to zero (23). In addition, proportion of mediation was calculated using the indirect effect as a numerator and the total effect as a denominator.

BMI or WC was considered as a mediator if the estimates a and b were significant (9). Another criterion for determining a mediator would be whether or not the estimate c was significant (21). However, we did not include this criterion in the present study, because the effect of Mediterranean diet and DASH style diet on the markers for insulin

resistance or inflammation may not be significant when direct and mediated effects have opposite signs (22, 24).

In the causal mediation approach, we assessed the total, direct, and indirect effects of Mediterranean diet and DASH style diet on markers for insulin resistance or inflammation with BMI or WC as a mediator, using the counterfactual framework (25, 26). In this approach, total effect can be decomposed into direct effect (not mediated by BMI or WC) and indirect effect (mediated by BMI or WC). A causal directed acyclic graph (DAG) indicating these effects is presented in Figure 7.2. We applied the SAS macro developed by Valeri and VanderWeele to evaluate the natural direct effect, natural indirect effect, and marginal total effect of Mediterranean diet and DASH style diet on markers for insulin resistance or inflammation with BMI or WC as a mediator (22).

Natural direct effect and natural indirect effects can be evaluated conceptually as follows. For instance, natural direct effect is the contrast between the counterfactual outcome if the subject were exposed at the highest tertile of Mediterranean diet, and the counterfactual outcome if the same subject were exposed at the lowest tertile of Mediterranean diet, with BMI assuming whatever value it would have taken at the reference value of the lowest tertile of Mediterranean diet. Natural indirect effect is the contrast, having set the Mediterranean diet at level of the highest tertile, between the counterfactual outcome if BMI assumed whatever value it would have taken at a value of the highest tertile of Mediterranean diet, and the counterfactual outcome if BMI assumed whatever value it would have taken at a reference value of the lowest tertile of Mediterranean diet. Based on these individual approaches, the average natural indirect

and direct effects at the population level were estimated (7, 10). Survey procedures were not applied in the causal mediation analysis.

For this causal mediation analysis, we made four assumptions. First, there is no unmeasured confounding in the relation of Mediterranean diet and DASH style diet with the markers for insulin resistance or inflammation given confounders; second, there is no unmeasured confounding in the relation of BMI or WC with the markers for insulin resistance or inflammation; third, there is no unmeasured confounding in the relation of Mediterranean diet and DASH style diet with BMI and WC; fourth, there is no effect of Mediterranean diet and DASH style diet affecting confounders of the relationship of BMI and WC with the markers for insulin resistance or inflammation (25). Under these assumptions, natural direct effect, natural indirect effect, marginal total effect can be estimated using two separate linear regressions: the first regression of markers for insulin resistance or inflammation on the exposure (MDS or DASH index), the mediator (BMI or WC) and the confounding variables; and a second regression of BMI or WC on the MDS or DASH index and the confounding variables. Then, two regressions were combined to estimate natural direct and indirect effects. In this causal mediation analysis, we evaluated whether there is an interaction of MDS or DASH index with BMI or WC in the regression of the marker for insulin resistance and inflammation. When there is a significant interaction, controlled direct effect, natural direct effect, and natural indirect effect were estimated using the following models for change in MDS or DASH index from level a* to level a: controlled direct effect =$(\theta_1 + \theta_3 m)(a - a*)$; natural direct effect = $(\theta_2\beta_1 + \theta_3\beta_1 a)(a - a*)$; natural indirect effect = $(\theta_1 + \theta_3 (\beta_0 + \beta_1 a* + \beta_2' c))(a - a*)$. When there is no significant interaction (θ_3 = 0), the controlled direct

effect is equal to natural direct effect (22). It is possible that the interaction may not be statistically significant due to low power. In addition, the exposure-mediator interaction may be important in understanding the effects of mediation even when the interaction terms are not significant (27). Thus, we further evaluated whether there would be any substantial change in the magnitude of the natural indirect effect or the power to detect the natural indirect effect, with or without including the interaction terms of MDS or DASH index with BMI or WC in the model.

For the fair comparison between traditional and causal mediation approach, we additionally analyzed the data using the traditional approach without considering complex survey design of NAHANES III.

In addition, we assessed whether the association of MDS or DASH index with markers of insulin resistance and inflammation was modified by age group (before and after 45 years for men; before and after menopause for women) or gender by including the interaction terms in the model. Only age group showed the significant interaction in the association between MDS and fasting glucose (P=0.03). Thus, an additional subgroup analysis was performed by age group for the association between MDS and fasting glucose.

Results

Table 7.1 shows general and clinical characteristics with increasing tertiles of MDS and DASH index. As both MDS and DASH index increased, there was an increased age; a lower proportion of non-Hispanic black and higher proportion of Mexican-American; a higher proportion of high income and recommended physical activity; a lower proportion of current smoker; decreased BMI, WC, and WBC. With

increasing MDS tertile only, there was a higher proportion of high income, moderate alcohol consumption, and hypertension; decreased HbA1c, fasting insulin, HOMA-IR, and fibrinogen. With increasing tertile of DASH index only, there was a lower proportion of men and a decreased homocysteine.

Table 7.2 shows the distribution in weekly consumption of Mediterranean diet components and nutrient consumption of DASH style diet with increasing tertiles of MDS and DASH index. Overall, the consumption in each component of Mediterranean diet and DASH style diet tended to increase with increasing tertiles of MDS and DASH index. Total energy intake was not different between MDS tertiles, but increased with increasing tertiles of DASH index.

Compared with the lowest tertile of MDS, highest tertile of MDS was associated with 0.77 kg/m^2 lower BMI and 2.67 cm lower WC (P=0.004 and <0.001, respectively) in multivariable adjusted model. However, no significant association of DASH (highest tertile vs. lowest tertile) was observed with BMI and WC. In addition, when we examined the association of MDS and DASH index with the markers for insulin resistance or inflammation in multivariable model without adjusting for BMI or WC, a significant association representing a total effect was observed in log insulin, log HOMA, fasting glucose, HbA1c, WBC, and fibrinogen for MDS; only log homocysteine for DASH index (Table 7.3).

With increasing BMI and WC, there was a significant increase in log insulin, log HOMA, fasting glucose, post-load glucose, HbA1c, log hs-CRP, WBC, and fibrinogen, based on the multivariable model with adjusting for MDS or DASH index. However, no significant association was observed in log homocysteine and log lipoprotein (a) with

BMI and WC (Table 7.4). Based on the results from Table 7.3 and Table 7.4, with MDS as an exposure; log insulin, log HOMA, fasting glucose, post-load glucose, HbA1c, log hs-CRP, WBC, and fibrinogen as the outcomes were included in the mediation analysis.

Table 7.5 shows the total effect, direct effect, indirect effect, and Sobel statistics for testing indirect effect. Overall, the mediated effects of BMI and WC were significant on the association of MDS with log insulin, log HOMA, fasting glucose, HbA1c, log hs-CRP, WBC, and fibrinogen, except post-load glucose in which BMI was a mediator. In addition, the mediated effects of WC were greater than those of BMI consistently in all markers. Although the estimates of direct effects were not significant in either marker, the direction of direct effect and indirect effect was opposite in log hs-CRP in which proportion of mediation may not be interpretable. Otherwise, the mediated effects by BMI were higher in log HOMA showed the highest mediated effects by adiposity. Among the inflammatory markers, log hs-CRP showed the highest mediated effects by adiposity.

In causal mediation analysis, the overall proportion of mediated effect was decreased compared with traditional mediation analysis. In addition, the significance of mediated effect of BMI disappeared in post-load glucose, log hs-CRP, WBC and fibrinogen; became nearly marginal in the markers for insulin resistance including log insulin, log HOMA, fasting glucose, and HbA1c. However, the significance of mediated effect by WC remained (Table 7.6). In both traditional approach and causal mediation approach, the association between MDS and fasting glucose was fully mediated by BMI or WC. In addition, the association between MDS, post-load glucose and hs-CRP was fully mediated by WC (Table 7.5 and 6).

There was no statistically significant interaction of MDS with BMI and WC. When we also assessed the change in the magnitude of the natural indirect effect with or without including the interaction terms of MDS with BMI or WC in the model, the ratios of estimates in natural indirect effect ranged between 0.82 and 1.32, meaning that there was no remarkable change in the estimates of natural indirect effect. Furthermore, there was no substantial increase in the power to detect the indirect effect (Table 7.7). Taken together, we could not detect the significant interaction of MDS with BMI and WC. Thus, the controlled direct effect was equivalent to the natural indirect effect in our data.

When we analyzed the data using the traditional approach without considering complex survey design of NHANES III, the proportion of mediation in the traditional approach approximated that of the causal mediation approach, especially in fasting glucose and post-load glucose with WC as a mediator (Table 7.8).

When stratified by the age group in the association between MDS and fasting glucose, mediation effect by adiposity was prominent in younger age group, especially in WC (Table 7.9 & Figure 7.3).

Discussion

The present study is, to the best of our knowledge, the first to evaluate the role of adiposity in the relationship of Mediterranean diet with markers of insulin resistance and inflammation in a nationally representative sample of U.S. adults, using mediation analyses in both traditional and causal mediation approaches. We observed an inverse association of MDS with markers of insulin resistance including fasting insulin, HOMA-IR, fasting glucose, and HbA1c; and with inflammatory markers such as WBC and fibrinogen. WC mediated the association of MDS with log insulin, log HOMA-IR,

fasting glucose, post-load glucose, HbA1c, log hs-CRP, WBC, and fibrinogen. Furthermore, the mediation effect in this association was greater in WC representing abdominal obesity than BMI representing general obesity, in both traditional and causal mediation approaches. However, no mediation effect by adiposity was observed in the association of DASH index with the markers for insulin resistance and inflammation.

Consistent evidence shows that the Mediterranean diet has a beneficial effect on a reduced risk of type 2 DM (28). Several biological mechanisms may explain the inverse association between the Mediterranean diet and insulin resistance which is a key pathophysiological trait of type 2 DM. It has been shown that adherence to the Mediterranean diet is related to increased antioxidant capacity (29). This antioxidant effect of Mediterranean diet may protect oxidative stress accumulation which has been proposed to develop insulin resistance and beta-cell dysfunction (30). In addition, magnesium, abundant in Mediterranean diet, especially in vegetables, legumes, and nuts may promote insulin-mediated glucose uptake (31). Another potential mechanism can be explained by a high intake of dietary fiber through the Mediterranean diet, which contributes to lowering plasma glucose and insulin levels (32). Moderate alcohol consumption is also associated with improving insulin sensitivity and decreased basal insulin secretion (33). In addition, monounsaturated fats, rich in the Mediterranean diet, may have beneficial effects on insulin sensitivity and glucose metabolism (34).

Furthermore, the inverse relationship between the Mediterranean diet and insulin resistance can be explained by the role of the Mediterranean diet in obesity prevention (35). Although the epidemiological evidence on the relationship between the Mediterranean diet and obesity prevention has not been consistent, likely due to

methodological differences in previous observational studies, a potential physiological role of the Mediterranean diet in preventing obesity has been hypothesized (36). In addition, a recent meta-analysis showed that the Mediterranean diet was effective in reducing body weight based on clinical trials (37). It has been reported that WC representing abdominal obesity is a better predictor for type 2 DM than BMI representing general obesity (8). Under the condition of abdominal obesity, plenty of free fatty acids are released from expanded adipose tissue, which inhibits insulin-stimulated glucose metabolism in skeletal muscle and stimulates gluconeogenesis in the liver (35); increased flow of plasma free fatty acids aggravates altered lipid metabolism in the liver (38), all of which contributes to insulin resistance (39). The above mentioned beneficial health effects of the Mediterranean diet may play a role in protecting against obesity-induced insulin resistance, especially from abdominal fat. This underlying mechanism is supported by our findings in which higher MDS was inversely associated with fasting insulin and HOMA-IR, fasting glucose, and HbA1c; the mediation effect in this association was greater in WC than BMI in both traditional approach and causal mediation approach.

In the present study, the association between MDS and fasting glucose was fully mediated by adiposity, because there was no significant association between the two after adjusting for BMI or WC (40). Despite a difference in the results from traditional approach and causal mediation approach, the mediation effect by WC was consistently observed, especially in younger individuals including men < 45 years and premenopausal women (Table 7.9 & Figure 7.3), suggesting that the effect of the Mediterranean diet on

reducing glucose level might be greater through lowering abdominal obesity than general obesity in men < 45 years and premenopausal women.

It has been reported that adherence to the Mediterranean diet is associated with decreased inflammation which is a potential risk factor for atherosclerosis and CVD (41). Low-grade chronic inflammation is crucial in the initial phase of developing atherosclerosis, the main cause of coronary heart disease (42). In addition, inflammatory markers such as CRP and interleukin-6 are associated with the risk of type 2 DM (43). Obesity is considered to be a sub-clinical inflammatory condition to enhance the production of pro-inflammatory factors that contribute to insulin resistance (44), and abdominal obesity may play a key role in this process (45). Thus, the underlying mechanism on the beneficial effect of the Mediterranean diet on prevention of inflammation may be supported by our findings in which higher MDS was inversely associated with WBC and fibrinogen; the mediation effect in this association was greater in WC than BMI in both traditional approach and causal mediation approach.

In the present study, the Mediterranean diet was not associated with hs-CRP with or without adjusting for BMI or WC. Instead, the indirect effect of hs-CRP was consistently highly significant in both traditional approach and causal mediation approach, especially in WC as a mediator, suggesting that the effect of Mediterranean diet might be fully mediated by adiposity, especially abdominal obesity. This finding may be supported by a recent study in which combining weight loss with Mediterranean diet had no impact on plasma CRP, but a group with substantial decrease of WC showed reduction of plasma CRP (46).

The DASH style diet was not considered for mediation analyses in the present study, because there was no significant association with obesity, after adjusting for potential confounders. Moreover, no association between DASH index and the markers for insulin resistance was found. These findings were consistent with the previous research in which the association of the DASH style diet with insulin sensitivity and weight control was reported to be unclear (47, 48).

Our study has several strengths. First, we used data from a valid and reliable nationally representative study, based on extensive laboratory and physical examinations using standardized protocols to minimize the influence of measurement errors. Second, we assessed the mediation effect of general obesity and abdominal obesity in the association of healthy dietary patterns with markers for insulin resistance and inflammation in a comprehensive way using a traditional approach and causal mediation analysis as well as exploring the possible interaction effect. Finally, the present study adjusted for a wide range of potential confounders in multivariable analyses. There are also several limitations. First, this cross-sectional study cannot imply a causal and temporal relationship between healthy dietary pattern, adiposity, and the markers for insulin resistance and inflammation, although we tried to minimize reverse causality using strict exclusion criteria. Second, since the information on "servings per week" was not available on the NHANES III FFQ, we used "times per week" in assessing the consumption frequency for MDS calculation. This approach might cause exposure misclassification, but the direction would be non-differential. In addition, we used nutrient data from the 24-hr dietary recall data in calculating DASH score, which might not be comparable to DASH score based on FFQ in other studies. Third, there might be

residual confounding due to not measuring the covariates in an objective way.

In conclusion, the association between Mediterranean diet, obesity, insulin resistance and inflammation suggest that obesity, especially abdominal obesity, may play a crucial role in the relationship of the Mediterranean diet with decreased insulin resistance and inflammation. Further prospective studies are warranted to identify the role of adiposity in the prevention effect of the Mediterranean diet on reducing the risk of type 2 DM and CVD.

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Table 7.1 Distribution of general and clinical characteristics according to tertiles of MDS and DASH index

Characteristics	MDS				P
	Overall (n=4,700)	Tertile 1 (n=1,729)	Tertile 2 (n=1,501)	Tertile 3 (n=1,470)	
Age, years	38.5 ± 0.5	37.3 ± 0.5	38.1 ± 0.5	40.6 ± 0.8	<0.001
Men, %	54.1 ± 0.9	54.5 ± 2.2	57.0 ± 2.2	50.6 ± 2.1	0.22
Race/ ethnicity, %					
Non-Hispanic white	73.9 ± 1.6	77.6 ± 2.0	71.7 ± 2.4	71.1 ± 2.3	<0.001
Non-Hispanic black	11.3 ± 0.7	12.0 ± 0.9	12.3 ± 1.1	9.3 ± 1.0	
Mexican-American	6.5 ± 0.6	3.9 ± 0.4	6.5 ± 0.6	10.0 ± 1.1	
Other	8.3 ± 1.2	6.5 ± 1.5	9.5 ± 1.9	9.6 ± 1.8	
Educational attainment, %					
<12 years	20.1 ± 1.2	23.6 ± 1.6	19.8 ± 1.8	15.6 ± 1.5	<0.001
12 years	35.3 ± 1.1	41.2 ± 1.7	32.9 ± 1.8	29.5 ± 1.9	
>=13 years	44.7 ± 1.4	35.2 ± 2.0	47.3 ± 2.4	54.9 ± 2.1	
Income, %					
PIR=<1.3	17.9 ± 1.2	20.3 ± 2.2	17.2 ± 1.5	15.2 ± 1.7	<0.001
PIR=<3.5	45.5 ± 2.0	51.3 ± 3.0	44.6 ± 2.3	38.2 ± 2.5	
PIR>3.5	36.7 ± 2.1	28.4 ± 3.3	38.3 ± 2.3	46.6 ± 2.3	
Smoking status, %					
Never	47.2 ± 1.2	43.4 ± 1.9	50.5 ± 1.8	49.0 ± 2.4	<0.001
Former	21.4 ± 0.8	19.4 ± 1.3	19.3 ± 1.6	26.3 ± 1.8	
Current	31.4 ± 1.3	37.3 ± 1.7	30.2 ± 1.8	24.7 ± 2.2	
Drinking alcohol, %					
Never	38.5 ± 1.4	65.7 ± 1.9	26.4 ± 1.7	14.0 ± 1.3	<0.001
Moderate	59.1 ± 1.4	31.5 ± 2.0	71.1 ± 1.8	84.2 ± 1.3	
Heavy	2.4 ± 0.2	2.8 ± 0.7	2.5 ± 0.6	1.9 ± 0.6	
Physical activity, %					
Inactive	12.0 ± 0.9	15.3 ± 1.5	10.3 ± 1.2	9.2 ± 1.2	<0.001
Insufficient activity	55.1 ± 1.2	55.3 ± 1.8	58.4 ± 1.8	51.2 ± 2.2	

Recommended Activity	32.9	±	1.3	29.4	±	1.9	31.3	±	1.8	39.6	±	2.3			
CHD family history, %	15.7	±	0.8	15.9	±	1.5	17.2	±	1.4	13.7	±	1.7	0.34		
DM parental history, %	16.9	±	0.9	15.6	±	1.6	18.5	±	1.4	17.2	±	1.7	0.42		
DM, %	2.7	±	0.3	2.5	±	0.4	3.1	±	0.7	2.5	±	0.6	0.63		
Hypertension, %	9.4	±	0.8	8.1	±	0.9	8.2	±	1.3	12.3	±	1.6	0.02		
BMI, kg/m^2	25.8	±	0.1	26.0	±	0.2	26.0	±	0.2	25.3	±	0.2	0.01		
WC, cm	89.1	±	0.4	89.9	±	0.5	90.0	±	0.6	87.2	±	0.5	<0.001		
Percentage body fat, %	29.0	±	0.3	29.3	±	0.5	29.2	±	0.3	28.5	±	0.3	0.32		
Fasting glucose, mg/dl	95	±	0.3	95	±	0.4	95	±	0.4	94	±	0.5	0.66		
Post load glucose, mg/dl	116	±	1.5	116	±	2.4	119	±	3.5	114	±	2.1	0.34		
HbA1c, %	5.2	±	0.0	5.2	±	0.0	5.2	±	0.0	5.1	±	0.0	0.03		
Fasting insulin, mg/dl	9.3	±	0.2	9.8	±	0.2	9.4	±	0.3	8.3	±	0.2	<0.001		
HOMA-IR	2.21	±	0.05	2.34	±	0.06	2.27	±	0.08	1.98	±	0.05	<0.001		
hs-CRP, mg/dl	0.33	±	0.01	0.35	±	0.01	0.32	±	0.01	0.33	±	0.02	0.39		
WBC ($\times 10^3$ cells)	6.8	±	0.1	7.1	±	0.1	6.8	±	0.1	6.5	±	0.1	<0.001		
Homocysteine, umol/L	9.4	±	0.2	9.5	±	0.3	9.4	±	0.3	9.3	±	0.4	0.94		
Fibrinogen, mg/dl	291	±	3.2	303	±	4.8	287	±	5.4	282	±	4.4	<0.001		
Lipoprotein (a), mg/dl	22	±	1.2	24	±	2.0	20	±	1.4	23	±	1.6	0.06		

Table 7.1 (Continued)

Characteristics	DASH index									P
	Tertile 1 (n=1,480)			Tertile 2 (n=1,770)			Tertile 3 (n=1,450)			
Age, years	36.7	±	0.5	37.7	±	0.5	41.3	±	0.8	<0.001
Men, %	56.7	±	2.3	56.6	±	1.5	48.1	±	1.9	0.006
Race/ ethnicity, %										
Non-Hispanic white	73.9	±	1.9	73.7	±	2.2	74.2	±	1.9	<0.001
Non-Hispanic black	15.4	±	1.3	10.3	±	0.7	8.1	±	0.7	
Mexican-American	5.1	±	0.5	6.2	±	0.6	8.3	±	1.0	

Other	5.6	±	1.1	9.8	±	1.9	9.3	±	1.5	
Educational attainment, %										
<12 years	19.0	±	1.8	19.4	±	1.6	22.1	±	1.9	0.004
12 years	39.6	±	2.2	37.2	±	1.5	28.0	±	2.5	
>=13 years	41.4	±	2.4	43.4	±	2.0	49.9	±	2.8	
Income, %										
PIR=<1.3	16.7	±	2.0	18.2	±	1.5	18.7	±	1.7	0.05
PIR=<3.5	48.6	±	3.2	47.1	±	2.2	39.8	±	2.5	
PIR>3.5	34.7	±	3.2	34.7	±	2.1	41.5	±	3.2	
Smoking status, %										
Never	44.0	±	1.9	44.7	±	2.3	53.9	±	2.2	<0.001
Former	19.2	±	1.5	22.7	±	1.5	22.0	±	1.7	
Current	36.7	±	2.1	32.6	±	2.0	24.1	±	1.9	
Drinking alcohol, %										
Never	37.9	±	2.4	37.1	±	2.0	41.1	±	2.5	0.58
Moderate	59.7	±	2.4	60.8	±	2.0	56.0	±	2.6	
Heavy	2.4	±	0.5	2.1	±	0.4	2.8	±	0.8	
Physical activity, %										
Inactive	11.4	±	1.1	12.4	±	1.0	12.0	±	1.7	<0.001
Insufficient activity	61.4	±	2.0	54.9	±	2.2	48.6	±	2.2	
Recommended Activity	27.2	±	1.8	32.7	±	2.2	39.4	±	2.3	
CHD family history, %	16.9	±	1.3	16.6	±	1.5	13.2	±	1.4	0.14
DM parental history, %	17.9	±	1.6	16.1	±	1.6	17.0	±	1.4	0.68
DM, %	2.5	±	0.5	2.3	±	0.5	3.3	±	0.9	0.55
Hypertension, %	8.4	±	1.2	9.7	±	1.1	10.0	±	1.3	0.58
BMI, kg/m^2	25.8	±	0.2	26.1	±	0.2	25.4	±	0.2	0.005
WC, cm	89.1	±	0.5	90.2	±	0.6	87.8	±	0.6	0.002
Percentage body fat, %	29.1	±	0.4	28.9	±	0.3	29.2	±	0.5	0.80
Fasting glucose, mg/dl	94	±	0.4	95	±	0.4	95	±	0.6	0.66
Post load glucose, mg/dl	115	±	2.4	112	±	2.2	121	±	3.1	0.07
HbA1c, %	5.2	±	0.0	5.2	±	0.0	5.2	±	0.0	0.75

Fasting insulin, mg/dl	9.3	±	0.2	9.6	±	0.3	8.8	±	0.2	0.05
HOMA-IR	2.21	±	0.06	2.29	±	0.09	2.11	±	0.07	0.15
hs-CRP, mg/dl	0.32	±	0.01	0.35	±	0.02	0.32	±	0.01	0.47
WBC ($\times 10^3$ cells)	7.0	±	0.1	6.9	±	0.1	6.6	±	0.1	0.001
Homocysteine, umol/L	10.1	±	0.4	9.4	±	0.3	8.7	±	0.2	0.009
Fibrinogen, mg/dl	290	±	5.3	290	±	4.8	293	±	5.1	0.85
Lipoprotein (a), mg/dl	21	±	1.7	23	±	1.3	23	±	1.7	0.20

Data are presented as means ± standard error or proportion (%) ± standard error.
P values for continuous variables represent P for trend.
Abbreviations: MDS, Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension; PIR, poverty income ratio; CHD, coronary heart disease; DM, diabetes mellitus; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.

Table 7.2 Comparison of each component of Mediterranean diet scores and DASH index by the tertiles

	MDS			
	Overall (n=4,700)	Tertile 1 (n=1,729)	Tertile 2 (n=1,501)	Tertile 3 (n=1,470)	P for trend
Score range	(8 - 42)	(8 - 24)	(25 - 28)	(29 - 42)	
MDS score	25.8 ± 0.2	21.0 ± 0.1	26.5 ± 0.0	31.5 ± 0.1	<0.001
Grains (times/wk)	4.4 ± 0.1	3.2 ± 0.1	4.5 ± 0.2	6.2 ± 0.3	<0.001
Legumes (times/wk)	2.6 ± 0.1	1.8 ± 0.1	2.8 ± 0.1	3.6 ± 0.2	<0.001
Fruit (times/wk)	5.7 ± 0.2	3.9 ± 0.1	5.3 ± 0.2	8.8 ± 0.3	<0.001
Vegetable (times/wk)	13.3 ± 0.3	10.0 ± 0.3	12.6 ± 0.3	18.4 ± 0.5	<0.001
Fish (times/wk)	1.4 ± 0.1	1.0 ± 0.0	1.3 ± 0.1	2.2 ± 0.1	<0.001
MUFA:SFA	1.18 ± 0.01	1.06 ± 0.01	1.16 ± 0.01	1.36 ± 0.02	<0.001
Red meats (times/wk)	5.7 ± 0.1	7.2 ± 0.2	5.4 ± 0.1	4.0 ± 0.1	<0.001
Poultry (times/wk)	2.0 ± 0.0	1.8 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	0.001
Dairy products (times/wk)	12.3 ± 0.3	14.5 ± 0.4	11.5 ± 0.3	10.2 ± 0.3	<0.001
Alcohol (g/d)	3.9 ± 0.2	3.0 ± 0.4	4.3 ± 0.3	4.7 ± 0.2	0.001
Total energy intake, kcal	2313 ± 28	2322 ± 51	2399 ± 47	2208 ± 52	0.18
	DASH index			
	Overall (n=4,700)	Tertile 1 (n=1,480)	Tertile 2 (n=1,770)	Tertile 3 (n=1,450)	P for trend
Score range	(0 - 8)	(0 - 1)	(1.5 - 2.5)	(3 - 8)	
DASH score	2.1 ± 0.0	0.6 ± 0.0	1.9 ± 0.0	4.0 ± 0.0	<0.001
Saturated fat, % energy	11.5 ± 0.1	13.6 ± 0.1	11.7 ± 0.2	8.8 ± 0.1	<0.001
Total fat, % energy	34.2 ± 0.3	40.2 ± 0.3	35.0 ± 0.3	26.6 ± 0.4	<0.001
Protein, % energy	15.1 ± 0.1	14.2 ± 0.2	15.0 ± 0.2	16.2 ± 0.2	<0.001
Cholesterol, mg	129 ± 2	157 ± 3	126 ± 3	103 ± 4	<0.001

Fiber, mg	7.5	±	0.1	5.5	±	0.1	6.9	±	0.1	10.5	±	0.2	<0.001
Magnesium, mg	137	±	2	107	±	1	128	±	2	180	±	3	<0.001
Calcium, mg	371	±	6	313	±	5	362.1	±	8.1	444.8	±	12.2	<0.001
Potassium, mg	1310	±	14	1054	±	10	1231	±	16	1694	±	30	<0.001
Sodium, mg	1631	±	14	1715	±	19	1658	±	32	1504	±	27	<0.001
Total energy intake, kcal	2313	±	28	2587	±	54	2333	±	32	1990	±	41	<0.001

Data are presented as means ± standard error.

Abbreviations: MDS, Mediterranean Diet Score; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; DASH, Dietary Approaches to Stop Hypertension.

The amount of each DASH dietary component is based on a 1,000-kcal diet.

Table 7.3 Beta-coefficients (95% CI) of the association of MDS and DASH index with body mass index, waist circumference, and markers for insulin resistance and inflammation in conventional analyses

	MDS					DASH index				
Mediator	Estimate (a)	95% CI			P	Estimate (a)	95% CI			P
BMI	-0.770	(-1.284	-0.256	)	0.004	-0.285	(-0.742	0.171	)	0.22
WC	-2.670	(-3.994	-1.347	)	<0.001	-0.988	(-2.373	0.397	)	0.16
Outcome	Estimate (c)	95% CI			P	Estimate (c)	95% CI			P
Log insulin	-0.153	(-0.195	-0.110	)	<0.001	-0.056	(-0.112	0.000	)	0.05
Log HOMA	-0.163	(-0.210	-0.116	)	<0.001	-0.059	(-0.121	0.003	)	0.06
Fasting glucose	-1.416	(-2.578	-0.253	)	0.02	-0.337	(-1.636	0.963	)	0.61
Post-load glucose	-1.720	(-6.866	3.427	)	0.51	3.505	(-3.37	10.38	)	0.31
HbA1c	-0.099	(-0.149	-0.049	)	<0.001	-0.002	(-0.061	0.057	)	0.94
Log hs-CRP	-0.015	(-0.076	0.045	)	0.61	-0.023	(-0.078	0.032	)	0.41
WBC	-0.323	(-0.547	-0.098	)	0.006	-0.267	(-0.503	-0.031	)	0.03
Fibrinogen	-17.44	(-26.99	-7.88	)	0.001	1.066	(-14.09	16.22	)	0.89
Log homocysteine	0.018	(-0.036	0.072	)	0.50	-0.073	(-0.143	-0.004	)	0.04
Log lipoprotein (a)	-0.006	(-0.251	0.239	)	0.96	0.191	(-0.052	0.433	)	0.12

Abbreviations: CI, confidence interval; MDS, Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA-, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates were adjusted for age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, mean arterial pressure, and total calories (additionally adjusted for alcohol consumption in the model including DASH index; mean arterial pressure in the model for markers of insulin resistance; multivitamin use in the model for homocysteine).
Paths a and c are explained in the Figure 7.1.

Table 7.4 Beta-coefficients (95% CI) of the association of body mass index and waist circumference on the markers for insulin resistance and inflammation in conventional analyses

		MDS					DASH				
Mediator	Outcomes	Estimate (b)	95% CI			P	Estimate (b)	95% CI			P
BMI	Log insulin	0.055	(0.050	0.060	)	<0.001	0.055	(0.050	0.060	)	<0.001
	Log HOMA	0.060	(0.055	0.066	)	<0.001	0.060	(0.055	0.066	)	<0.001
	Fasting glucose	0.503	(0.359	0.646	)	<0.001	0.505	(0.367	0.643	)	<0.001
	Post-load glucose	0.814	(0.112	1.517	)	0.02	0.876	(0.188	1.564	)	0.01
	HbA1c	0.015	(0.010	0.019	)	<0.001	0.015	(0.011	0.019	)	<0.001
	Log hs-CRP	0.031	(0.025	0.037	)	<0.001	0.031	(0.024	0.037	)	<0.001
	WBC	0.055	(0.036	0.074	)	<0.001	0.055	(0.036	0.075	)	<0.001
	Fibrinogen	1.732	(0.677	2.786	)	0.002	1.778	(0.762	2.795	)	0.001
	Log homocysteine	0.001	(-0.004	0.007	)	0.65	0.001	(-0.004	0.007	)	0.62
	Log lipoprotein (a)	0.002	(-0.016	0.020	)	0.81	0.001	(-0.019	0.021	)	0.94
WC	Log insulin	0.023	(0.021	0.024	)	<.0001	0.023	(0.021	0.025	)	<0.001
	Log HOMA	0.025	(0.023	0.027	)	<.0001	0.025	(0.023	0.027	)	<0.001
	Fasting glucose	0.229	(0.168	0.289	)	<.0001	0.230	(0.171	0.288	)	<0.001
	Post-load glucose	0.477	(0.203	0.751	)	0.001	0.494	(0.228	0.759	)	0.001
	HbA1c	0.006	(0.004	0.007	)	<.0001	0.006	(0.004	0.008	)	<0.001
	Log hs-CRP	0.012	(0.010	0.014	)	<.0001	0.012	(0.010	0.014	)	<0.001
	WBC	0.020	(0.013	0.028	)	<.0001	0.021	(0.013	0.028	)	<0.001
	Fibrinogen	0.625	(0.194	1.057	)	0.005	0.657	(0.249	1.065	)	0.002
	Log homocysteine	0.001	(-0.002	0.003	)	0.53	0.001	(-0.002	0.003	)	0.51
	Log lipoprotein (a)	-0.001	(-0.007	0.006	)	0.89	-0.001	(-0.009	0.006	)	0.76

Abbreviations: CI, confidence interval; MDS, Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates were adjusted for the same covariates used in Table 7.3 in addition to MDS or DASH index.
Path b is explained in the Figure 7.1.

Table 7.5 Direct effect and indirect effects of the Mediterranean diet on the markers for insulin resistance and inflammation with BMI and waist circumference as a mediator using traditional mediation analysis

		Direct effect (c')				Indirect effect (a*b)					Proportion of mediation
Mediator	Outcomes	Estimate	95% CI		P	Estimate	95% CI		Sobel test statistic	P	
BMI	Log insulin	-0.104	(-0.143	-0.065)	<0.001	-0.042	(-0.070	-0.014)	-2.981	0.003	27.7%
	Log HOMA	-0.110	(-0.154	-0.065)	<0.001	-0.046	(-0.077	-0.016)	-2.982	0.003	28.4%
	Fasting glucose	-0.982	(-2.191	0.227)	0.11	-0.387	(-0.661	-0.113)	-2.766	0.006	27.3%
	Post-load glucose	-0.895	(-6.183	4.393)	0.74	-0.627	(-1.294	0.040)	-1.842	0.07	36.5%
	HbA1c	-0.086	(-0.136	-0.037)	0.001	-0.011	(-0.019	-0.003)	-2.731	0.006	11.5%
	Log hs-CRP	0.007	(-0.054	0.067)	0.82	-0.024	(-0.040	-0.008)	-2.880	0.004	NA
	WBC	-0.282	(-0.501	-0.062)	0.01	-0.042	(-0.073	-0.011)	-2.672	0.008	13.1%
	Fibrinogen	-15.98	(-25.93	-6.04)	0.002	-1.33	(-2.51	-0.16)	-2.22	0.03	7.6%
WC	Log insulin	-0.085	(-0.128	-0.043)	<0.001	-0.061	(-0.090	-0.031)	-4.004	<0.001	39.8%
	Log HOMA	-0.090	(-0.138	-0.042)	0.001	-0.067	(-0.099	-0.034)	-4.010	<0.001	41.0%
	Fasting glucose	-0.757	(-1.983	0.469)	0.22	-0.611	(-0.945	-0.276)	-3.578	<0.001	43.1%
	Post-load glucose	0.258	(-5.288	5.804)	0.93	-1.273	(-2.215	-0.331)	-2.649	0.008	74.0%
	HbA1c	-0.083	(-0.132	-0.034)	0.001	-0.015	(-0.024	-0.006)	-3.408	0.001	15.3%
	Log hs-CRP	0.015	(-0.044	0.074)	0.60	-0.032	(-0.049	-0.016)	-3.809	<0.001	NA
	WBC	-0.270	(-0.490	-0.049)	0.02	-0.054	(-0.088	-0.021)	-3.224	0.001	16.9%
	Fibrinogen	-15.21	(-25.21	-5.21)	0.004	-1.67	(-3.05	-0.29)	-2.36	0.02	9.6%

Abbreviations: CI, confidence interval; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates for direct effect were adjusted for the same covariates used in Table 7.3.
Paths a, b, and c' are explained in the Figure 7.1.

Table 7.6 Marginal total effect, natural direct effect, and natural indirect effect on the Mediterranean diet with markers for insulin resistance and inflammation as BMI and waist circumference as a mediator using causal mediation analysis

		Marginal total effect					Natural direct effect				
Mediator	Outcomes	Estimate	95% CI			P	Estimate	95% CI			P
BMI	Log insulin	-0.082	(	-0.118	-0.045	) <0.001	-0.061	(	0.000	-0.093	) 0.02
	Log HOMA	-0.092	(	-0.132	-0.051	) <0.001	-0.070	(	-0.105	-0.035	) <0.001
	Fasting glucose	-1.571	(	-3.002	-0.139	) 0.03	-1.346	(	-2.763	0.072	) 0.06
	Post-load glucose	-5.508	(	-12.46	1.45	) 0.12	-5.156	(	-12.11	1.80	) 0.15
	HbA1c	-0.078	(	-0.129	-0.028	) 0.003	-0.071	(	-0.122	-0.021	) 0.006
	Log hs-CRP	-0.043	(	-0.088	0.002	) 0.06	-0.031	(	-0.074	0.012	) 0.16
	WBC	-0.178	(	-0.324	-0.032	) 0.02	-0.161	(	-0.307	-0.016	) 0.03
	Fibrinogen	-9.61	(	-18.95	-0.27	) 0.04	-8.66	(	-17.96	0.64	) 0.07
WC	Log insulin	-0.082	(	-0.118	-0.046	) <0.001	-0.050	(	-0.081	-0.019	) 0.001
	Log HOMA	-0.093	(	-0.133	-0.052	) <0.001	-0.057	(	-0.092	-0.023	) 0.001
	Fasting glucose	-1.573	(	-3.005	-0.142	) 0.03	-1.187	(	-2.601	0.227	) 0.10
	Post-load glucose	-5.138	(	-12.08	1.81	) 0.15	-4.327	(	-11.27	2.62	) 0.22
	HbA1c	-0.079	(	-0.130	-0.028	) 0.002	-0.067	(	-0.117	-0.017	) 0.009
	Log hs-CRP	-0.043	(	-0.088	0.002	) 0.06	-0.025	(	-0.068	0.018	) 0.26
	WBC	-0.179	(	-0.325	-0.032	) 0.02	-0.153	(	-0.298	-0.008	) 0.04
	Fibrinogen	-9.42	(	-18.77	-0.07	) 0.05	-8.03	(	-17.36	1.30	) 0.09

Table 7.6 (Continued)

Mediator	Outcomes	Natural indirect effect					Proportion of mediation
		Estimate	95% CI			P	
BMI	Log insulin	-0.020	(	-0.039	-0.002	) 0.03	24.7%
	Log HOMA	-0.022	(	-0.043	-0.002	) 0.03	24.2%
	Fasting glucose	-0.225	(	-0.437	-0.013	) 0.04	14.3%
	Post-load glucose	-0.352	(	-0.745	0.041	) 0.08	6.4%
	HbA1c	-0.007	(	-0.014	0.000	) 0.04	9.4%
	Log hs-CRP	-0.012	(	-0.024	0.000	) 0.05	28.2%
	WBC	-0.017	(	-0.034	0.000	) 0.06	9.3%
	Fibrinogen	-0.944	(	-1.927	0.038	) 0.06	9.8%
WC	Log insulin	-0.032	(	-0.051	-0.013	) 0.001	39.0%
	Log HOMA	-0.035	(	-0.057	-0.014	) 0.001	38.3%
	Fasting glucose	-0.386	(	-0.630	-0.143	) 0.002	24.5%
	Post-load glucose	-0.811	(	-1.413	-0.209	) 0.008	15.8%
	HbA1c	-0.012	(	-0.019	-0.004	) 0.002	14.8%
	Log hs-CRP	-0.018	(	-0.030	-0.006	) 0.003	42.3%
	WBC	-0.026	(	-0.044	-0.008	) 0.005	14.4%
	Fibrinogen	-1.391	(	-2.392	-0.390	) 0.006	14.8%

Abbreviations: CI, confidence interval; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates were adjusted for the same covariates used in Table 7.3.

Table 7.7 Comparison of the estimates in indirect (mediated) effects with or without the interaction terms of MDS with BMI and waist circumference in causal mediation analysis

		With interaction			Without interaction			
Mediator	Outcomes	NIE 1	P	PM	NIE 2	P	PM	NIE1/NIE2
BMI	Log insulin	-0.021	0.03	25.5%	-0.020	0.03	24.7%	1.03
	Log HOMA	-0.023	0.03	25.2%	-0.022	0.03	24.2%	1.04
	Fasting glucose	-0.243	0.05	15.5%	-0.225	0.04	14.3%	1.08
	Post-load glucose	-0.465	0.11	7.9%	-0.352	0.08	6.4%	1.32
	HbA1c	-0.008	0.05	10.5%	-0.007	0.04	9.4%	1.11
	Log hs-CRP	-0.010	0.06	23.8%	-0.012	0.05	28.2%	0.85
	WBC	-0.014	0.09	7.6%	-0.017	0.06	9.3%	0.82
	Fibrinogen	-0.943	0.08	9.8%	-0.944	0.06	9.8%	1.00
WC	Log insulin	-0.033	0.001	40.0%	-0.032	0.001	39.0%	1.03
	Log HOMA	-0.036	0.001	39.2%	-0.035	0.001	38.3%	1.03
	Fasting glucose	-0.381	0.004	24.2%	-0.386	0.002	24.5%	0.99
	Post-load glucose	-0.989	0.02	16.6%	-0.811	0.008	15.8%	1.22
	HbA1c	-0.012	0.006	14.9%	-0.012	0.002	14.8%	1.00
	Log hs-CRP	-0.015	0.004	36.3%	-0.018	0.003	42.3%	0.85
	WBC	-0.022	0.02	12.1%	-0.026	0.005	14.4%	0.84
	Fibrinogen	-1.199	0.03	14.1%	-1.391	0.006	14.8%	0.86

Abbreviations: NIE, natural indirect effect; PM, Proportion of mediation; CI, confidence interval; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates were adjusted for the same covariates used in Table 7.3.

Table 7.8 Indirect (mediated) effect of the Mediterranean diet on the markers for insulin resistance and inflammation as BMI and waist circumference as a mediator using traditional mediation analysis without considering complex survey design of NHANES III

		Indirect effect (a*b)						Proportion of mediation
Mediator	Outcomes	Estimate	95% CI			Sobel test statistic	P	
BMI	Log insulin	-0.010	(-0.020	0.000	)	-2.021	0.043	24.1%
	Log HOMA	-0.011	(-0.022	0.000	)	-1.988	0.047	23.4%
	Fasting glucose	-0.108	(-0.219	0.003	)	-1.914	0.056	13.9%
	Post-load glucose	-0.308	(-0.653	0.037	)	-1.751	0.080	10.9%
	HbA1c	-0.004	(-0.007	0.000	)	-1.911	0.056	9.2%
	Log hs-CRP	-0.006	(-0.012	0.000	)	-1.826	0.068	26.8%
	WBC	-0.008	(-0.016	0.001	)	-1.750	0.080	8.7%
	Fibrinogen	-0.738	(-1.494	0.017	)	-1.916	0.055	14.6%
WC	Log insulin	-0.016	(-0.026	-0.006	)	-3.025	0.002	37.7%
	Log HOMA	-0.017	(-0.029	-0.006	)	-2.963	0.003	36.7%
	Fasting glucose	-0.186	(-0.313	-0.058	)	-2.859	0.004	23.8%
	Post-load glucose	-0.740	(-1.265	-0.215	)	-2.762	0.006	26.2%
	HbA1c	-0.006	(-0.009	-0.002	)	-2.810	0.005	14.4%
	Log hs-CRP	-0.006	(-0.012	0.000	)	-1.826	0.068	26.8%
	WBC	-0.012	(-0.021	-0.003	)	-2.613	0.009	13.5%
	Fibrinogen	-1.059	(-1.815	-0.303	)	-2.745	0.006	20.9%

Abbreviations: CI, confidence interval; BMI, body mass index; WC, waist circumference; HbA1c, glycated hemoglobin; HOMA, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; WBC, white blood cell.
All estimates were adjusted for the same covariates used in Table 7.3.

Table 7.9 Marginal total effect, natural direct effect, and natural indirect effect on the association of Mediterranean diet score with fasting glucose as body mass index and waist circumference as a mediator in younger age group and older age group

		Marginal total effect					Natural direct effect				
Age group	Outcomes	Estimate	95% CI			P	Estimate	95% CI			P
Younger	BMI	-1.835	(	-3.366	-0.304	) 0.02	-1.559	(	-3.076	-0.041	) 0.04
	WC	-1.832	(	-3.363	-0.301	) 0.02	-1.379	(	-2.894	0.137	) 0.07
Older	BMI	-1.530	(	-4.827	1.768	) 0.36	-1.108	(	-4.372	2.156	) 0.51
	WC	-1.543	(	-4.840	1.754	) 0.36	-1.046	(	-4.297	2.206	) 0.53

Table 7.9 (Continued)

		Natural indirect effect					Proportion of mediation
Age group	Outcomes	Estimate	95% CI			P	
Younger	BMI	-0.276	(	-0.501	-0.052	) 0.02	15.1%
	WC	-0.453	(	-0.711	-0.194	) 0.001	24.7%
Older	BMI	-0.422	(	-0.938	0.095	) 0.11	27.6%
	WC	-0.497	(	-1.090	0.096	) 0.10	32.2%

Abbreviations: CI, confidence interval; BMI, body mass index; WC, waist circumference. All estimates were adjusted for age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, mean arterial pressure, and total calorie intakes. All estimates were adjusted for the same covariates used in Table 7.3.

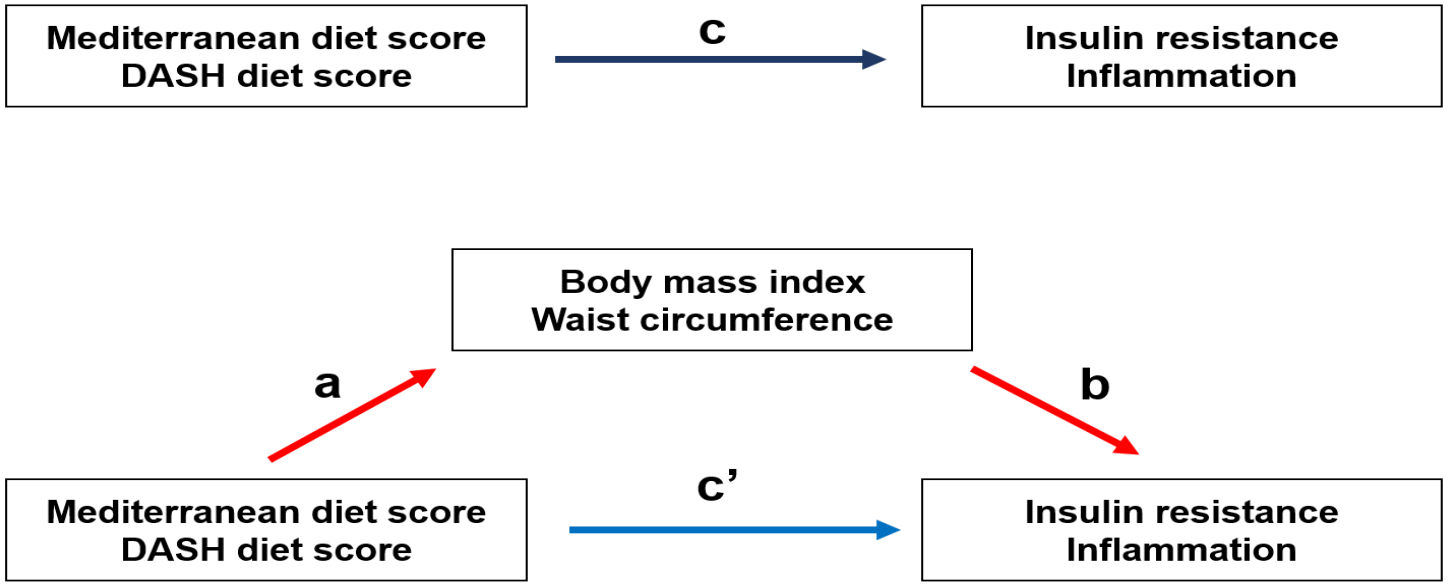

Figure 7.1 Traditional mediation model for the association of Mediterranean diet score and DASH style diet score with the markers for insulin resistance and inflammation as body mass index and waist circumference as a mediator

Path a represents the regression coefficient of the association of the Mediterranean diet score (MDS) and DASH diet score with BMI and WC. Path b represents the regression coefficient of the association of BMI and WC with the markers for insulin resistance and inflammation. The product of regression coefficients of path a and path b represents the mediated effect of BMI or WC (path a × path b). Path c' represents the direct effect of MDS and DASH diet score with the markers for insulin resistance and inflammation, after the adjustment for BMI or WC. Path c represents the simple total effect of MDS and DASH diet score with the markers for insulin resistance and inflammation, without the adjustment for BMI or WC.

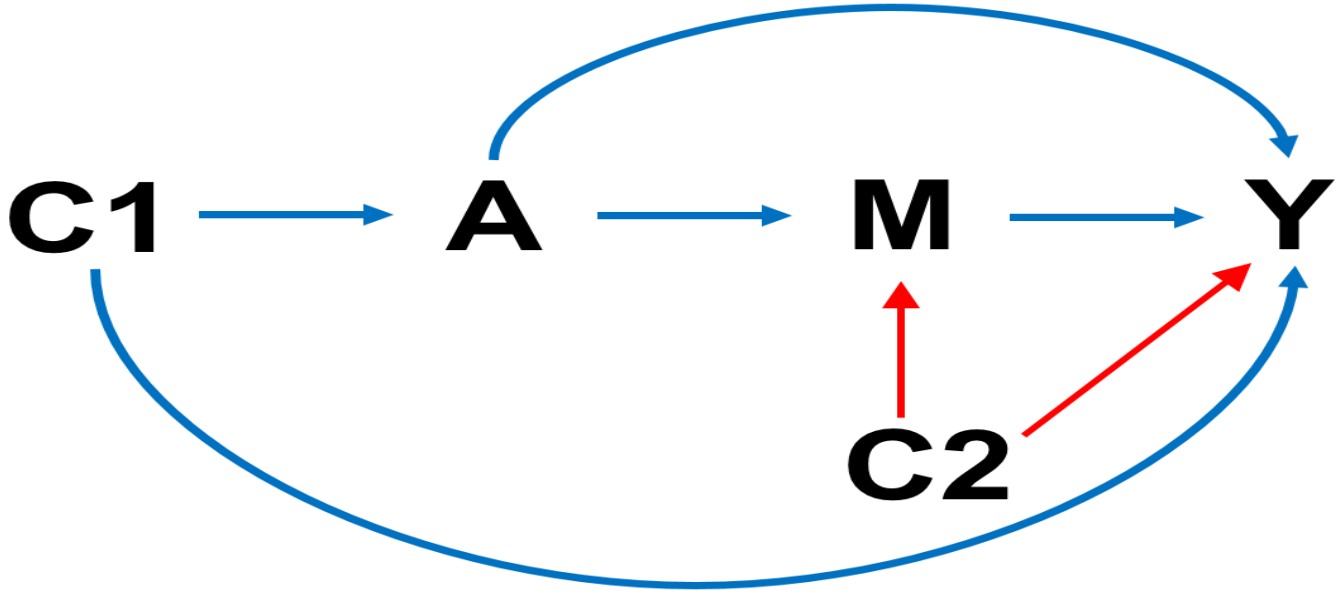

Figure 7.2 Causal directed acyclic graph (DAG) describing relations of Mediterranean diet and DASH style diet (A) with the markers for insulin resistance and inflammation (Y) as BMI and waist circumference as a mediator (M)

C1 and C2 include age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, and total calories (additionally adjusted for alcohol consumption in the model of DASH index).

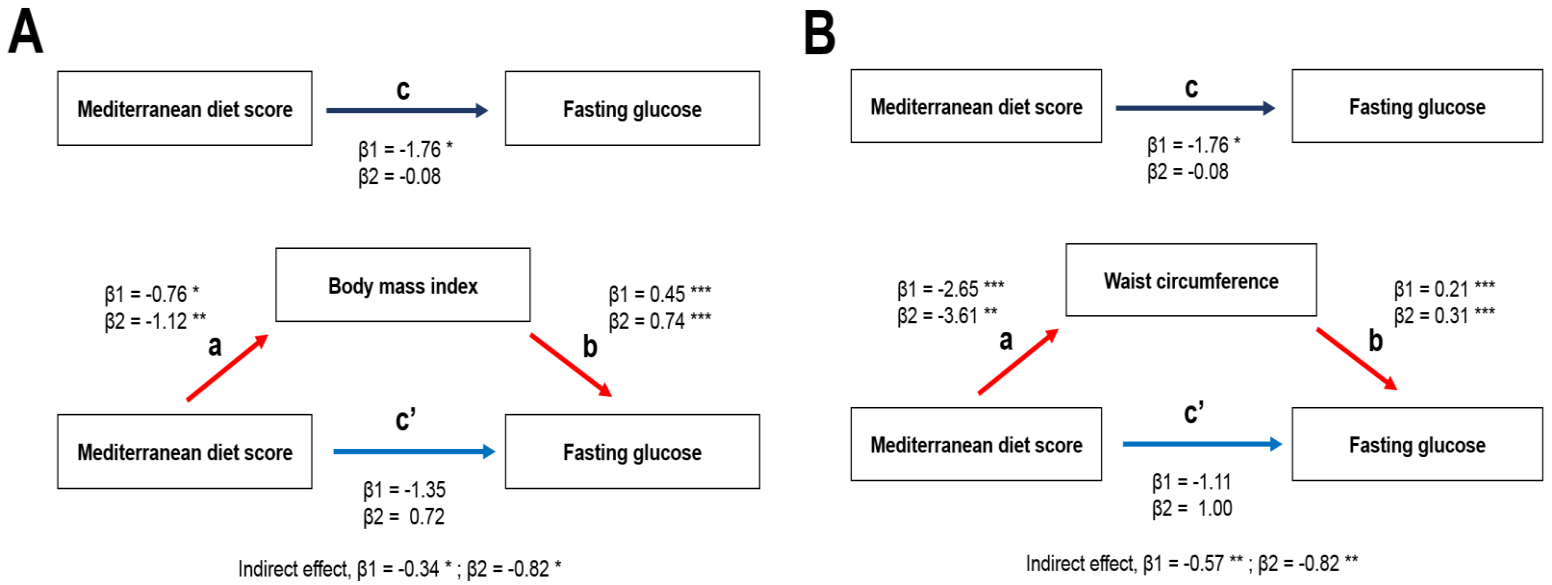

Figure 7.3 Mediation model for the association of Mediterranean diet score with fasting glucose as body mass index (A) and waist circumference (B) as a mediator in younger age group and older age group

a, b, c, and c' indicate the estimates in the same way as Figure 7.1. β1 represents the beta coefficients of younger age group including men < 45 years and premenopausal women; β2 represents the beta coefficients of older age group including men ≥ 45 years and postmenopausal women. All estimates were adjusted for age, gender, race/ethnicity, educational attainment, income, living with spouse, smoking status, level of physical activity, family history of coronary heart disease, parental history of diabetes mellitus, mean arterial pressure, and total calorie intakes. Proportion of mediation by body mass index and waist circumference is 19.3 % and 32.1% in the younger age group; 10.3 % and 14.1% in the older age group, respectively (*: P < 0.05, **: P < 0.01, and ***: P < 0.001)

CHAPTER 8

DISSERTATION SUMMARY

8.1 Summary of results

Our hypothesis for the present dissertation was as follows: 1) adherence to the Mediterranean diet or DASH style diet would be associated with metabolic health in both normal weight and obese populations; 2) adherence to the Mediterranean diet or DASH style diet would be associated with reduced all-cause and CVD mortality risk in individuals with unfavorable metabolic phenotype in both normal weight and obese populations; 3) adiposity would be a mediator of the influence of the Mediterranean diet or DASH style diet on cardiometabolic risk.

In the cross-sectional study based on specific aim #1, the Mediterranean diet and DASH style diets were associated with metabolic phenotypes in the younger age group that included men < 45 years and premenopausal women. More specifically, MDS was positively associated with MHO phenotype, whereas the DASH index was inversely associated with the MONW phenotype, after adjustment for a wide range of potential confounders. MDS and DASH index were not associated with MHO and MONW phenotypes in the older age group that included men ≥ 45 years and postmenopausal women, suggesting that healthy dietary patterns may be more effective in reducing the cardiometabolic risk among younger adults.

In the prospective study based on specific aim #2, higher adherence to

Mediterranean diet was associated with a lower risk of all-cause mortality in the MHO phenotype, after adjustment for potential confounders. We observed a 41% reduction in all-cause mortality with each 5-point increment in the MDS among MHO individuals. This association persisted when we restricted our analyses to those with or without prevalent chronic disease including diabetes mellitus and hypertension. However, the inverse association between the MDS and mortality was not observed among participants with the MUO phenotype.

In addition, DASH style diet was associated with a lower risk of all-cause and CVD mortality in the MONW individuals, after adjustment for potential confounders. We observed a 23% reduction in all-cause mortality with each 1 SD increment in the DASH score among MONW individuals. This association persisted when we confined our analyses to those with or without prevalent diabetes mellitus and hypertension. However, these health benefits on the reduction of mortality were not observed in individuals with the MHNW phenotype.

In the cross-sectional study based on specific aim #3, we observed an inverse association of MDS with the markers of insulin resistance including fasting insulin, HOMA-IR, fasting glucose, and HbA1c; and with inflammatory markers such as WBC and fibrinogen. WC mediated the association of MDS with log insulin, log HOMA-IR, fasting glucose, post-load glucose, HbA1c, log hs-CRP, WBC, and fibrinogen. Furthermore, the mediation effect of this association was greater in WC representing abdominal obesity than BMI representing general obesity, in both traditional and causal mediation approaches. However, no mediation effect by adiposity was observed in the association of DASH index with the markers for insulin resistance and inflammation.

8.2 Strengths and limitations

Our study has several strengths. We used data from a well-characterized and reliable nationally representative dataset, based on extensive laboratory and physical examinations using standardized protocols to minimize the influence of measurement errors. In addition, we assessed the associations between healthy dietary patterns and metabolic health in a comprehensive way comprised of normal weight and obese populations, using MDS and DASH index. In a mediation analyses, we assessed the mediation effect of general obesity and abdominal obesity in the association of healthy dietary patterns with the markers of insulin resistance and inflammation in a comprehensive way using a traditional approach and causal mediation analysis; in the latter analysis we also explored possible interaction between dietary patterns and the mediator (adiposity measures).

For the prospective study, we were able to evaluate a long-term health benefit of high diet quality on the reduction of mortality risk with nearly 18 years follow-up-. Furthermore, we were able to assess potential effect modifications and replicate the findings using a sensitivity analysis. Finally, the present studies adjusted for a wide range of potential confounders in multivariable analyses.

There are also several limitations. First, the cross-sectional study design in specific aims #1 and #3 cannot imply a causal and temporal relationship between healthy dietary pattern, metabolic health, and cardiometabolic risk factors, although we tried to minimize reverse causality using strict exclusion criteria. Second, since the information on “servings per week” was not available at NHANES III FFQ, we used “times per week” in assessing the consumption frequency for MDS calculation. This approach might cause

exposure misclassification, but the direction would be non-differential. In addition, we used nutrient data from the 24-hr dietary recall data in calculating DASH index, which might not be comparable to DASH score based on FFQ in other studies. However, we were able to compare the association between metabolic health and healthy dietary pattern in terms of food based approach and nutrient based approach. Third, self-assessment of food consumption may produce non-differential measurement error, although energy adjustment would reduce this error to some degree (Kipnis et al. 2003). Furthermore, there might be residual confounding due to not measuring the covariates in an objective way.

8.3 Public health implications

Adherence to the Mediterranean diet or DASH style diet is likely to be beneficially associated with MHO and MONW phenotypes in younger adults including men < 45 years and premenopausal women, suggesting that potential interventions to prevent cardiometabolic disease may need to be tailored by age group. Since individuals with unhealthy metabolic status are highly likely to develop CVD (Aung et al. 2014); adherence to the Mediterranean diet or DASH style diet may contribute to primary prevention of CVD in younger adults.

The lack of a beneficial association between adherence to Mediterranean diet and mortality reduction in MUO individuals, even in those who were physically active, may warrant the need of an aggressive prevention approach to reduce mortality risk in MUO individuals. In addition, the MUO individuals may need to be prioritized for intensive weight loss program along with improving dietary habits to reduce obesity-related comorbidities. Normal weight individuals with metabolic abnormalities may benefit from

healthy dietary patterns such as DASH style diet for CVD prevention and mortality risk reduction.

Obesity, especially abdominal obesity, may play a crucial role in the relationship of the Mediterranean diet with decreased insulin resistance and inflammation. In addition, a decrease in abdominal obesity might be considered an intermediate outcome for evaluating the effect of healthy dietary pattern on reducing cardiometabolic risk.

8.4 Suggestions for future research

Future prospective studies are warranted to establish the causal relationship between healthy dietary patterns and the risk of metabolic health, especially cohorts that may consider the change of dietary pattern of the participants. In addition to assessing the risk of incident unhealthy metabolic phenotype, it is necessary to evaluate whether healthy dietary patterns can contribute to the regression from unhealthy metabolic phenotype to healthy metabolic phenotype in normal weight and obese individuals.

More evidence would be necessary based on long-term follow-up studies with enough power to evaluate the effects of healthy dietary pattern on mortality reduction, especially for cause-specific mortalities including CVD and cancer deaths. Based on the fact that metabolic phenotype is a dynamic state instead of being sustained for years (Soriguer et al. 2013), change of metabolic phenotype with advancing years should be considered, especially for the transition between MHO and MUO phenotypes.

In addition, future prospective studies with multiple sequential measurements for dietary patterns, obesity parameters, and cardiovascular outcomes are warranted to more accurately clarify the degree to which adiposity mediates the beneficial association between the Mediterranean diet and markers of insulin resistance and inflammation.

Evaluating the temporal relationship between healthy dietary patterns and obesity together with the temporal relationship between obesity and cardiometabolic risk factors would confirm the causal mediated role of adiposity.

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