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A modified Dietary Approaches to Stop Hypertension pattern and its association with non-alcoholic hepatic steatosis and fibrosis

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Thesis

A *MODIFIED DIETARY APPROACHES TO STOP HYPERTENSION* PATTERN AND ITS ASSOCIATION WITH NON-ALCOHOLIC HEPATIC STEATOSIS AND FIBROSIS

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A *MODIFIED DIETARY APPROACHES TO STOP HYPERTENSION* PATTERN AND ITS ASSOCIATION WITH NON-ALCOHOLIC HEPATIC STEATOSIS AND FIBROSIS

KYLIE WARD

ABSTRACT

Background: The benefits of a Dietary Approaches to Stop Hypertension (DASH) dietary pattern in association with non-alcoholic fatty liver disease (NAFLD), specifically steatosis and fibrosis, and its association with odds of NAFLD have been studied by few.

Objectives: This study examined the prospective association between adherence to a newly modified DASH dietary pattern (mDASH) and NAFLD odds, specifically the relative odds of hepatic steatosis and fibrosis, assessed via vibration-controlled transient elastography (VCTE) measures of controlled attenuation parameter (CAP) and liver stiffness measure (LSM), among non-heavy drinking men and women in the Framingham Generation 3 (Gen III), Omni 2, and New Offspring Spouse (NOS) cohorts. In secondary analyses, we explored the modification of the association between mDASH adherence and steatosis by factors such as body mass index (BMI), waist circumference, waist-to-height ratio (WsHtR), physical activity, smoking, alcohol drinking, and cardiometabolic risk (CMR) traits (diabetes, hypertension, dyslipidemia, or an elevated TG: HDL ratio). Sensitivity analyses were also done by adding the group of heavy drinkers (>14 drinks/week females; > 21 drinks/week males) back into the study sample and repeating some primary analyses.

Methods: The mDASH score was derived from a semi-quantitative food frequency questionnaire collected at examination visit 2 in all three cohorts (Gen III, Omni 2, and NOS). Measures of hepatic steatosis and fibrosis were derived from a VCTE machine called FibroScan, at the third examination visit in all cohorts. Subjects included 2573 participants (1390 females and 1183 males), aged 24 and older, who were free of all cancers except non-melanoma skin, and were not heavy drinkers (>14 drinks/week females; >21 drinks/week males). The mDASH score was classified into tertiles and dichotomized (mDASH score 8.0-21.5 vs. 22.0-39.5) to evaluate the association between mDASH and measures of hepatic steatosis and fibrosis. Multivariable logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for each of four NAFLD stages (no hepatic steatosis or fibrosis, hepatic steatosis without fibrosis, hepatic fibrosis without steatosis, and hepatic steatosis with fibrosis) for all subjects and females and males separately. Multivariable linear regression models were used to assess the continuous association between mDASH and measures of CAP and log-transformed LSM as indicators of hepatic steatosis and fibrosis, respectively, for all subjects. These multivariable regression models were also used to explore whether the effects of mDASH on CAP (using linear models) or the occurrence of steatosis (using logistic models) were modified by other factors. All analyses were adjusted for confounding by age, sex, education, energy intake, cigarettes per day, and alcohol drinking status. Further adjustments for confounding by BMI, WsHtR, and CMR were also done. Factors found not to confound the effects of mDASH on NAFLD were excluded from the final models.

Results: Overall, 729 individuals met the VCTE criteria for hepatic steatosis (122 of whom had concurrent fibrosis and 607 without any fibrosis) and 96 met the VCTE criteria for fibrosis without steatosis. 1748 individuals had neither steatosis nor fibrosis. Individuals in the middle and highest categories of mDASH had 35% lower odds of any hepatic steatosis (95% CI_{T2}: 0.52-0.81; 95% CI_{T3}:0.51-0.83; p-trend: 0.0008) compared to individuals in the lowest category of mDASH. When stratifying by sex, males had a higher prevalence of steatosis than females. Females in tertile 3 (vs. tertile 1) had 40% lower odds (95% CI: 0.41-0.87) of steatosis versus 37% lower odds (95% CI: 0.45-0.88) for males in tertile 3. However, when further adjusting for BMI, WsHtR, or CMR, mDASH was more protective against steatosis in males than females. Individuals in tertile 2 and tertile 3 (vs. tertile 1) of mDASH had 34% and 29% lower odds (95% CI_{T2}: 0.52-0.84 and 95% CI_{T3}: 0.55-0.92; p-trend: 0.0144) of steatosis without fibrosis. Those in the highest category of mDASH had 56% lower odds of steatosis with fibrosis while those in the middle category of adherence to mDASH had 44% lower odds of steatosis with fibrosis when compared to those in the lowest category of mDASH (95% CI_{T3}: 0.26-0.75; 95% CI_{T2}: 0.35-0.88; p-trend: 0.0023). All odds ratios were attenuated when further adjusting for BMI, WsHtR, or CMR. No association was found between mDASH adherence and the odds of hepatic fibrosis without steatosis. We then examined the associations between mDASH and the continuous measures of steatosis (CAP) and fibrosis (LSM) using linear regression models. For every 5-point increase in mDASH, the CAP measurement was 4.95 dB/m (SE: 1.15; $p<0.0001$) lower while the log-transformed LSM measure was 0.03 kPa (SE: 0.01; $p=0.0007$) lower. The associations between

mDASH adherence and steatosis were modified by some other factors. Individuals with a low BMI or waist circumference and high adherence to mDASH had the greatest protective effects compared to those with higher BMI or waist circumference, respectively, and low adherence to mDASH ($OR_{LOW\ BMI+HIGH\ MDASH}$ 0.13, 95% CI: 0.10-0.17; $OR_{LOW\ WC+HIGH\ MDASH}$: 0.12, 95% CI: 0.09-0.16). There was no statistical interaction between mDASH and these anthropometric measures of body fat. However, overweight individuals (higher BMI) with higher mDASH scores had a 23% lower risk of steatosis than overweight individuals with lower mDASH scores. Thus, mDASH had an independent beneficial effect on risk of steatosis among overweight participants. The effects of mDASH were modified by physical activity. Individuals with higher physical activity levels and greater adherence to mDASH had 48% lower odds of steatosis (95% CI: 0.39-0.68) compared to those with lower mDASH adherence and lower physical activity levels. Even those with lower activity levels had a 27% lower risk of steatosis associated with adherence to the mDASH eating pattern. Higher mDASH adherence was also protective in individuals with-light or moderate alcohol consumption. Those with higher mDASH adherence and moderate alcohol consumption (>3 but ≤14 drinks/week for females; >3 but ≤21 drinks/week for males) had 44% lower odds of steatosis (95% CI: 0.41-0.76). High mDASH adherence was also protective in current, former, and nonsmokers, with the greatest protective association being seen in nonsmokers (OR: 0.62; 95% CI: 0.39-1.00). Beneficial effects of mDASH among current and former smokers were weaker and non-statistically significant. Finally, those participants with no cardiometabolic dysfunction were found to have lower relative odds of steatosis,

particularly with higher adherence to mDASH. However, even those with cardiometabolic dysfunction seemed to benefit from higher adherence to mDASH (vs. those with cardiometabolic dysfunction with lower adherence to mDASH) (OR: 0.75; 95% CI: 0.59-0.96). When no cardiometabolic traits were present, individuals who were highly adherent to mDASH, had 78% lower odds of steatosis (95% CI: 0.16-0.29).

Conclusions: In this large prospective cohort study, higher adherence to an mDASH dietary pattern was associated with lower odds of hepatic steatosis with or without fibrosis in females and males aged 24 years or older who were not heavy drinkers (>14 drinks/week in females; >21 drinks/week in males). These beneficial effects were apparent even among certain higher-risk individuals, including those with a higher BMI, less physical activity, and prevalent cardiometabolic dysfunction.

Keywords: hepatic steatosis and fibrosis, mDASH, NAFLD risk

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LIST OF ABBREVIATIONS

AASLD ..... American Association of the Study of Liver Diseases

AHEI ..... Alternative Healthy Eating Index

ATP III ..... Adult Treatment Panel III

β ..... Beta coefficient

BMI ..... Body Mass Index

CAP ..... Controlled Attenuation Parameter

CDC ..... Center for Disease Control

CI ..... Confidence Interval

cm ..... centimeters

cm/m² ..... centimeters per meter squared

CMR ..... cardiometabolic risk

CPD ..... cigarettes per day

CVD ..... cardiovascular disease

DASH ..... Dietary Approaches to Stop Hypertension

D-AII ..... Dietary Anti-inflammation Index

DBP ..... diastolic blood pressure

dB/m ..... decibels per meter

DII ..... Dietary Inflammatory Index

DNL ..... de novo lipogenesis

EASD ..... European Association for the Study of Diabetes

EASL ..... European Association for the Study of the Liver

EASO ..... European Association for the Study of Obesity

FDA..... Food and Drug Administration

FFA ..... free fatty acids

FFQ ..... food frequency questionnaire

FHS ..... Framingham Heart Study

GEN III ..... Generation 3

HCC ..... hepatocelluar carcinoma

HDL ..... high-density lipoprotein

HEI..... Healthy Eating Index

IFG ..... impaired fasting glucose

IL-6 ..... interleukin-6

IQR..... interquartile range

JNC-7 . Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

JNK ..... c-Jun N-terminal kinase

kcals/d ..... kilocalories per day

kg..... kilograms

kg/m² ..... kilograms per meter squared

kPa..... kilopascals

LDL..... low-density lipoprotein

LSM ..... liver stiffness measurement

mDASH..... modified Dietary Approaches to Stop Hypertension

MED..... Mediterranean Diet

MetS.....metabolic syndrome

METs..... metabolic equivalents

mg/dL..... milligram per deciliter

MV-PAI .....moderate-vigorous physical activity index

N.....sample size

NAFL .....nonalcoholic fatty liver

NAFLD ..... nonalcoholic fatty liver disease

NASH.....nonalcoholic steatohepatitis

NCEP ..... National Cholesterol Education Program

NHLBI .....National Heart, Lung, and Blood Institute

NOS.....New Offspring Spouse

OR..... odds ratio

PA .....physical activity

PAI..... physical activity index

PNPLA3..... patatin-like phospholipase

Q..... quintile

Ref..... reference

SBP .....systolic blood pressure

SD .....standard deviation

SE.....standard error

Sex-spec. ..... sex-specific

SSBs.....sugar-sweetened beverages

T.....tertile

TEE..... total energy expenditure

TG..... triglycerides

TG: HDL.....triglyceride to high-density lipoprotein ratio

TM6SF2..... Transmembrane 6 superfamily member 2

TNF-α.....tumor necrosis factor alpha

T2DM..... type 2 diabetes mellitus

UPR.....unfolded protein response

VCTE..... vibration controlled transient elastography

VLDL..... very low-density lipoproteins

WGO..... World Gastroenterology Organization

WsHtR.....waist-to-height ratio

XL..... extra large

INTRODUCTION

Non-Alcoholic Fatty Liver Disease – Disease Background & Pathogenesis

NAFLD is a disease of growing concern as it has become the most common liver disease globally[38] and a common reason for liver transplantation[8, 69, 90]. The World Gastroenterology Organization (WGO), [102] defines NAFLD as a condition of excessive fat accumulation in the liver, also called hepatic steatosis, without heavy alcohol consumption (defined as >3 drinks/day for males and >2 drinks/day for females) [38, 71]. NAFLD can be described as a spectrum of disease stages ranging from simple steatosis (≥5% of fat in hepatocytes) in non-alcoholic fatty liver (NAFL), liver steatosis with ballooning or lobular inflammation in non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis[97] (Figure 1). The pathogenesis of NAFLD has been described by a 'multiple hit hypothesis' [7,29,92,97]. The 'multiple hit hypothesis' suggests that multiple factors, including insulin resistance, elevated plasma-free fatty acids (FFA), oxidative stress, liver inflammation, adipose tissue secreted hormones, nutrition, gut microbiota, and genetic/epigenetic factors interact with each other and induce NAFLD[97].

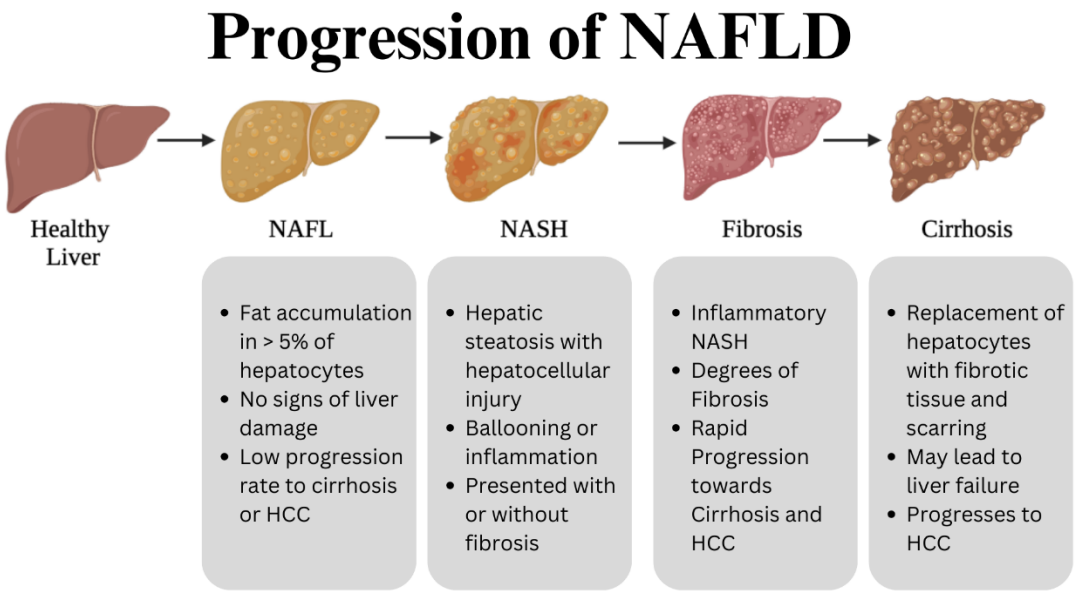

Figure 1: Stages of Nonalcoholic Fatty Liver Disease (NAFLD) and their pathophysiology. Abbreviations: HCC, Hepatocellular Carcinoma; NAFL, Nonalcoholic Fatty Liver; NASH, Nonalcoholic Steatohepatitis.

Simple steatosis is a reversible stage induced via four possible mechanisms: (1) increased fatty acid uptake to the liver; (2) reduced fat transport via very low-density lipoproteins (VLDL); (3) decreased fatty acid β-oxidation; and (4) increased de novo lipogenesis (DNL) [7, 97]. An individual's dietary habits, and environmental, and genetic factors can promote insulin resistance, obesity, and changes in the gut microbiome. Insulin resistance is a key driver in NAFLD development due to the cascade of events it can trigger to promote adipose tissue dysfunction and inflammation. Specifically, in an insulin-resistant state, DNL is upregulated, adipose tissue lipolysis is increased, and an increase in FFA occurs in the liver [97]. As FFAs build up in hepatocytes, they further affect insulin signaling pathways to promote insulin resistance and inhibit β-oxidation of FFAs to promote hepatic lipid accumulation [7]. Increased lipid accumulation suppresses

the autophagic function necessary to regulate lipid metabolism, only further promoting lipid accumulation at the NAFL stage. Increased levels of cholesterol, FFA, and other lipids lead to lipotoxicity and mitochondrial dysfunction. In the lipotoxic state, endoplasmic reticulum stress and unfolded protein responses (UPRs) activate two pro-inflammatory pathways, c-Jun terminal kinase (JNK) and SREBP-1c [97]. The variety of hits mentioned thus far all work together to promote an inflammatory state, which triggers the shift from the NAFL to NASH state.

The *European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), and European Association for the Study of Obesity (EASO)* [22] classify NAFL and NASH as two distinct conditions due to their different prognoses. NAFL is an early stage of the disease where liver steatosis occurs with no signs of damage, however as steatosis increases, it leads to hepatocellular injury. With the buildup of all hits mentioned earlier and the activation of pro-inflammatory pathways, JNK-AP-1 and IKK-NF-kB [7], inflammatory cytokines are produced that drive the changes seen in the livers of patients with NASH. This shift is important as NASH is associated with a greater risk for complications including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), due to damage to hepatocytes [69]. As the adipose tissue becomes more dysfunctional, there is an increased production of inflammatory cytokines, Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), as well as the hormone leptin, and decreased adiponectin[92] to promote continued steatosis and the development of fibrosis. The fibrotic stage is characterized by inflammation in the liver that can lead to

tissue scarring and altered liver function as fibrosis progresses. At the fibrotic state, the gut microbiome has enhanced small bowel permeability from increased circulating metabolites mentioned earlier which assist in the promotion and activation of inflammasomes [7], ER stress, and pro-inflammatory cytokines. Genetic and epigenetic factors, patatin-like phospholipase (PNPLA3), and transmembrane 6 superfamily member 2 (TM6SF2) [7,59,69,84,97] are associated with steatosis and fibrosis in humans [7]. As fibrosis reaches an advanced stage, the disease can progress to cirrhosis, which is defined by the replacement of hepatocytes with fibrotic tissue and scarring that leads to liver failure.

Disease progression occurs rather slowly with a majority of patients with NAFL not at risk for disease complications[71,101]. Disease progression takes a dramatic turn when patients develop NASH, as their fibrosis progression rate is estimated to be one stage of progression over 7 years versus one stage of progression over 14 years in those with NAFL[97]. Thus, individuals with NASH have a greater risk of disease complications than those with NAFL[71]. Furthermore, NAFLD has been described as the hepatic manifestation of metabolic syndrome[60] (MetS), due to its association with other metabolic risk factors such as insulin resistance, dyslipidemia, hypertension, type 2 diabetes (T2DM), and obesity [4]. It is of no surprise then that NAFLD is associated with T2DM[37] and cardiovascular disease (CVD) [35]. NAFLD is also associated with hepatic outcomes such as HCC[35] and extrahepatic outcomes such as extrahepatic cancers[35] and chronic kidney disease[39]. As NAFLD is estimated to affect 25% of the global population[27] and its

prevalence is growing, concern rests on its associations with other diseases and risk of mortality.

NAFLD Incidence

NAFLD incidence varies globally due to different environmental, socioeconomic, and lifestyle factors impacting the risk factors of NAFLD. Riazi et al[74] estimated NAFLD incidence globally to be 46.9 cases per 1,000 person-years and prevalence to be 32.4%, with men having higher incidence and prevalence than women. Studies have estimated the prevalence of NAFLD in North America to be between 35.3-47.8% [48,74,90]. Estes et al[27] developed a model to predict the burden of NAFLD and estimated that in the United States, NAFLD cases would increase by 21% from 83.1 million cases to 100.9 million cases by 2030. By 2040, global prevalence is estimated to reach 55.4%[90], which would increase the risk of further complications from NAFLD. Many papers have found that the prevalence of NAFLD is highest in Hispanics, followed by White individuals, and lowest in Black individuals[73,76,77]. Meta-analyses on NAFLD-related diseases have found that NAFLD increases overall mortality by 57% from both liver-related and cardiovascular-related causes [8], as well as a two-fold increased risk of T2DM[61] and chronic kidney disease [8].

NAFLD – Hepatic & Extrahepatic Outcomes

NAFLD & T2DM

NAFLD and T2DM have been so consistently correlated with one another that it is advised that the presence of either condition should lead to screening for the other. It has been estimated that more than 75% of individuals with T2DM have NAFLD [69] and that among those with prevalent NAFLD, 23% are estimated to have T2DM; in those with NASH, 44-47% have been found to have T2DM[27]. T2DM is a known risk factor for NAFLD, with multiple studies finding an increased risk for various stages of NAFLD in those with T2DM [8,39,69]. T2DM doubles the risk of developing hepatic steatosis, cirrhosis, and HCC[39]. Additionally, T2DM is the leading predictor of liver-related mortality [39], with those with T2DM having a 3 fold increased risk of dying from chronic liver diseases such as NAFLD [8]. After 13.7 years, 71% of NASH patients had T2DM and 46% of NAFL patients had T2DM[22], suggesting that the prevalence of T2DM occurs at both stages of NAFLD. Another study looked at the association between insulin resistance, obesity, and fatty liver on the risk of T2DM after 5-year follow-up[8]. They found that all three factors were independently associated with a two-fold increased risk of T2DM and a fourteen-fold increased risk when all three factors are present together[8]. Such findings suggest a relationship between obesity, insulin resistance, fatty liver, and T2DM. The direct relationship between NAFLD and T2DM is not easily understood, although it has been hypothesized that it is due to ectopic fat accumulation. This can lead to increased inflammatory hepatokine secretion, gluconeogenesis, and decreased glycogen synthesis [8], driving the inhibition of insulin signaling and therefore insulin resistance.

NAFLD & CVD

NAFLD is the hepatic manifestation of MetS, a known risk factor for CVD, and thus, these two disorders (NAFLD and MetS) are strongly correlated. A meta-analysis of the prevalence of various risk factors for CVD among individuals with NAFLD estimated 39% to have hypertension, 69% to have hyperlipidemia, and 51% to be obese[27]. Another meta-analysis of twenty-seven cross-sectional studies found that NAFLD was strongly associated with markers of atherosclerosis[66]. When assessing retrospective and large prospective studies, Byrne et al[8] found that NAFLD was associated with increased all-cause, CVD, and liver-related mortalities, as well as an increased risk of fatal and non-fatal CVD events. Both hepatic steatosis[26] and hepatic fibrosis[8,38,89] have been associated with risk of CVD. Hepatic fibrosis has been found to be the strongest determinant of the risk of extrahepatic outcomes such as CVD as well as all-cause, CVD-related, and liver-related mortality[8,89]. A study by Long et al[56] found hepatic fibrosis to be associated with increased odds of obesity, MetS, T2DM, and hypertension, once again suggesting that there is a link between these CVD risk factors and fibrosis. A sedentary lifestyle, poor dietary habits, and obesity have been a part of a proposed[94] link between NAFLD and CVD. Other review papers [8,35,38,89] have mentioned pro-inflammatory mediators, low-grade systemic inflammation, and pro-atherogenic lipid profiles as other possible links between the two.

NAFLD & HCC

NAFLD is the most common cause of HCC[38] and the fastest-growing indication for liver transplantation in the United States today[35]. Studies on NAFLD-related HCC have found that these cases are associated with worsened morbidity arising from the presence of more co-morbidities compared to non-NAFLD-related HCC [38], as well as poor survival outcomes, later diagnoses, and larger tumors with greater recurrence rates [38]. The majority of cases occur at the cirrhotic stage [35,38], however, this has been estimated to be roughly 50% of cases[70]. This suggests that both NAFL and NASH contribute some proportion of NAFLD-related HCC cases, with the fibrotic stage determining those who are at higher risk for HCC [35]. In terms of non-cirrhotic HCC cases, NAFLD contributes the highest proportion of cases compared with other diseases[87]. An overall increase in the occurrence of HCC has been observed over time as the rates of obesity and MetS have also increased globally, again suggesting a link between NAFLD and HCC [35]. NAFLD often presents with insulin resistance and obesity. In this state, chronic inflammation, altered lipid metabolism, and a pro-carcinogenic state [35] are believed to be the association between NAFLD and HCC but more research is needed in this area.

The relation between NAFLD, hepatic fibrosis, and the above diseases speaks to the importance of targeting NAFLD before or at the fibrotic state to lower disease risk. A prospective study by Sanyal et al [83] found that advanced stages of fibrosis were associated with the greatest increased risk of liver-related complications and death

compared to other stages. Hepatic fat accumulation is the greatest risk factor for NAFLD and drives its progression, while hepatic fibrosis has become the most important prognostic factor in NAFLD due to its relation to liver-related outcomes and mortality[97]. Aside from hepatic fat other risk factors include, age, sex, genetics, obesity, insulin resistance, T2DM, premature CVD, and family history of fatty liver[8,75]. As the rates of obesity and T2DM continue to increase, the prevalence of NAFLD is expected to do the same. As NAFLD cases increase and the population gets older, the risk of advanced fibrosis and cirrhosis shall increase and put greater risk for both liver-related and non-liver-related mortality[71]. Proper diagnostic protocols must be used to identify NAFLD and effective treatments to target the early reversible stages of the disease.

Treatment of NAFLD

The primary goal of NAFLD treatments is centered on targeting underlying risk factors such as insulin resistance, T2DM, dyslipidemia, and obesity[80]. NAFLD has been found in up to 80% of those with obesity [71]. At the forefront of treatment is weight reduction[28], which can be targeted through lifestyle modifications such as physical activity and diet, as recommended by both the EASL-EASD-EASO[22] and the American Association of the Study of Liver Diseases (AASLD) [79]. Both guidelines have recognized that small weight reductions of 3-5% can improve steatosis while greater weight reductions of 7-10% can provide improvement in NASH and fibrosis [28,79,80,84]. A prospective study by Vilar-Gomez et al[98] found a dose-response relationship in terms of weight loss percentage and histological changes. Specifically, weight reduction greater

than 10% resolved NASH or improved one stage of fibrosis, while weight reductions of 7-10% brought resolution to steatosis, inflammation, and ballooning[98]. The idea behind weight loss reduction is the fact that NAFLD itself is a disease of hepatic fat accumulation. Targeting hepatic fat accumulation through weight loss can also target other factors closely correlated with obesity. Hepatic fat accumulation comes from three primary sources, (1) an influx of FFA from fat depots; (2) excess dietary fat intake, and (3) DNL [4], which can be targeted by both physical activity and diet. The AASLD[79] also notes that in some patients with NAFLD where weight loss is not needed, dietary modifications and improved lifestyle changes can still improve overall health and comorbidities associated with NAFLD. Some lifestyle factors that have been studied regarding NAFLD include alcohol[2,3,19,37,40,47,100] and smoking[43]. Today, there are no FDA-approved drugs for the treatment of NAFLD[77]. Medications such as Vitamin E, thiazolidinediones[22,79], antioxidants, and lipid-lowering agents have shown improvements in NAFLD populations in clinical trials but still, no recommendations exist for the usage of any as a treatment today. For these reasons, physical activity and diet remain primary intervention practices in patients with NAFLD.

Role of Physical Activity and Diet in Treating NAFLD

Physical inactivity is a rising concern today as sedentary behavior leading to physical inactivity is increasing. Sedentary behavior has been found to be higher in people with MetS, obesity, and T2DM and increases the odds of all-cause mortality[80]. As sedentary behavior is associated with risk factors of NAFLD, it may be involved with

NAFLD risk and development. Cross-sectional studies have linked NAFLD patients with lower levels of physical activity than healthy subjects[80]. Physical activity has a wide range of known benefits on overall human health and metabolism. One benefit of increased physical activity is a reduced risk of T2DM and impaired insulin signaling. A systematic review of 24 exercise studies found that exercise, without weight loss, can produce a 20-30% reduction in intrahepatic lipids[80] and improve peripheral insulin sensitivity[84]. Aside from physical activity, diet is a promising intervention as dietary intake can be linked to liver fat accumulation and different diets may have different effects.

Diet is a modifiable factor that introduces nutrients to patients daily that may elicit a beneficial effect on health and be associated with disease. Diet has become a primary treatment option for NAFLD patients as it is related to weight reduction, lipid accumulation, and manifestations of MetS[80]. Nutritional assessments of NAFLD patients found that their diets tend to contain more low-nutrient quality foods, high-sodium foods, fewer full-fat dairy products[12], fiber[12], and decreased intakes of protein and fruits[4]. A variety of dietary components have been associated with increased NAFLD risk including ultra-processed foods[52], added sugars such as sucrose and fructose[41,68,80], red and processed meats[84], and alcohol[2,3,19,37,40,47,100]. Studies assessing diet quality have found that higher diet quality can prevent adiposity and metabolic consequences of NAFL[58] and lower the likelihood of having NAFLD[11,57].

A variety of dietary patterns have been well described concerning NAFLD and its extrahepatic outcomes, including, the Mediterranean Diet[11,16,43,46,57,67,75,80,91,94](MED), the Alternative Healthy Eating Index[16,57,58,91](AHEI), the Healthy Eating Index[36,58,67,91](HEI), DASH[13,16,30,31,32,58,60,67,72,75,91,94,103], and the Dietary Inflammatory Index[91](D-II). Of these diets, MED is the most well-documented diet with evidence of prevention and management of components of MetS and therefore is commonly recommended for NAFLD patients[75,84]. Specifically, the diet has been found to lower the risk of hepatic steatosis[43,46], as well as be protective against NAFLD-related diabetes and CVD[34,46]. As a dietary intervention, the MED diet is known to induce weight loss and improve metabolic factors. The MED diet is rich in fruit, vegetables, whole grains, nuts, fish, and olive oil and reduces intakes of processed foods & meats, refined grains, and sugar-sweetened beverages[84]. Prior research showed that higher adherence to HEI-2015 resulted in 40% lower odds of NAFLD, suggesting that healthy diets are associated with reduced risks of NAFLD[36]. D-II assesses the inflammatory potential of a diet. An anti-inflammatory index (D-AII) was modeled after D-II and was found to have an inverse association with NAFLD suggesting that higher anti-inflammatory diets are less associated with NAFLD compared to high inflammatory diets[95]. As NAFLD is a disease with inflammation, it is possible that decreasing inflammation in the diet may lower the risk of NAFLD. Multiple papers have looked to compare various diets and their effectiveness in NAFLD prevention and management[32,58,67,91]. Gao et al assessed the relationship between diet quality and hepatic fibrosis. The diets included in this study were the MED diet, AHEI, and DASH. Higher DASH scores were found to be cross-sectionally associated with

lower CAP and LSM measurements, suggesting that the diet may improve hepatic steatosis and fibrosis[32]. Higher diet quality scores for all three diets were found to be associated with lower odds of hepatic steatosis, hepatic fibrosis, and advanced hepatic fibrosis, with the overall probability of steatosis and fibrosis decreasing with higher diet quality scores. Similarly, Tian et al[91] assessed diet quality and NAFLD phenotypes with MED, DASH, DII, AHEI, and HEI diets. The DII diet showed a positive linear association with measures of liver steatosis (p-trend=0.009), suggesting pro-inflammatory diets increase liver fat. The other four diets were all inversely associated with NAFLD, with higher dietary scores being associated with less risk of both NAFLD and fibrosis (p-trend <0.05). Such findings suggest that improving diet quality can reduce the development and progression of NAFLD, and may significantly reduce disease risk. Although the MED diet is commonly recommended, there have been promising studies using the DASH diet.

The DASH Diet- Its Applicability & Limitations

The DASH diet originated as a dietary intervention to target hypertension and was successful in lowering blood pressure[1]. Since then it has been applied to a variety of chronic diseases, including hypertension[1], T2DM[16], coronary heart disease[30], colorectal cancer[31,60], and fatty liver [32,60,67,72,91,94,103]. The DASH diet promotes high consumption of fruits, vegetables, and low-fat dairy products, including whole grains, poultry, fish, nuts, and legumes, and reduces intakes of total fat, saturated fats, red meat, sweets sugar-sweetened beverages, and cholesterol. The diet composition consists of an increased

intake of potassium, magnesium, calcium, and fiber. Evidence at the time of the original trial showed that diets rich in fiber and minerals, such as potassium and magnesium, with lower fat content, could reduce blood pressure[1]. Dietary guidelines at this time promoted a reduction in total and saturated fat consumption with the belief that all dietary fats promoted weight gain and drove disease. Although the original trial did not determine which parts of the diet were effective in reducing blood pressure, the primary components were considered to be fruits, vegetables, and low-fat dairy. It was concluded that the DASH diet can lower blood pressure among those with pre-hypertension as well as those with hypertension. Since the original clinical trials evaluating the DASH eating pattern, additional data have been published showing, for example, that full-fat dairy has the same beneficial effects on cardiovascular outcomes as low-fat dairy[12,105]. Dietary guidelines at the time of the creation of DASH had believed that fat was a perpetrator of cardiovascular disease and low fat was the best option; however, the evidence since then has shown that this is not the case. A study examining the associations of saturated fats from dairy and nondairy sources on measurements of CMR found that saturated fats from dairy sources were beneficially associated with cardiometabolic markers (body fat, inflammatory biomarkers, cholesterol, and lipid particles)[105]. Another study found that a high-fat DASH diet lowered blood pressure to the same extent as the traditional DASH diet, while also decreasing triglycerides and VLDL levels, suggesting that the strict low-fat constraint of the DASH diet may be unnecessary[13]. Another study found that increased intakes of dairy and the protein that comes with that are inversely associated

with the risk of NAFLD[50]. As a result of evolving evidence, our research group felt that it was time to modify the DASH eating pattern to incorporate these changes.

mDASH as a Potential Dietary Intervention for NAFLD

The mDASH index was recently developed and tested for its validity in predicting hypertension and cardiovascular risk[104]. The mDASH diet promotes high intakes of fruits, vegetables, and dairy products, including whole grains, nuts, seeds and legumes, and fish, and lowers intakes of red and processed meats and sugar-sweetened beverages. The mDASH index incorporates up-to-date evidence on foods associated with lower risk of known DASH outcomes and provides greater food choices. Higher mDASH adherence (quintile 5 vs. quintile 1) lowered incident HTN and CVD by 31% and 29%[104]. It was also found that higher mDASH adherence (tertile 3 vs. tertile 1) was associated with a 29% lower NAFLD risk, and substantially smaller annualized increases in liver fat over 6 years[104]. Based on these findings, we hypothesized that greater mDASH adherence will be associated with lower odds of NAFLD at various stages of severity.

Purpose and Aims of This Study

The goal of this thesis was to evaluate the associations of an mDASH diet on non-alcoholic hepatic steatosis and fibrosis in participants from the Framingham NOS, Gen III, and Omni 2 cohorts. Specifically, this thesis examined the prospective association between adherence to the mDASH index and liver fat measurements of LSM and CAP. We further examined the prospective association between mDASH adherence and the

presence of hepatic steatosis and fibrosis. More specifically, we examined the following NAFLD stages as outcomes: (a) neither steatosis nor fibrosis; (b) hepatic steatosis without fibrosis; (c) hepatic fibrosis without steatosis; and (d) both hepatic steatosis and fibrosis. This work adds to the limited literature on the mDASH diet, and provides a greater understanding of the impact diet has on different stages of NAFLD. This research is important as the prevalence of NAFLD is increasing globally and it is known to increase overall mortality by 57% due to liver-related and cardiovascular-related causes, as well as a two-fold increased risk of T2DM[61] and chronic kidney disease. As mentioned earlier, hepatic fibrosis is the strongest predictor of all-cause and disease-specific mortalities, which emphasizes the importance of reducing the prevalence of fibrosis. The VCTE FibroScan data was used to define the stages of NAFLD by measuring hepatic fat accumulation and fibrosis. To date, few studies of mDASH have been completed, making the current study of particular importance in understanding the association of a DASH-like diet on non-alcoholic hepatic steatosis and fibrosis.

METHODS

Study Sample

The study sample included participants from the Framingham Heart Study's (FHS) Third Generation, Omni 2, and NOS cohorts. These three cohorts were selected due to the availability of relevant data across three consecutive exam periods that were used to look into dietary exposures, covariates, and outcomes in this study. The Gen III cohort began in 2002 with the recruitment of 4095 participants who were the offspring of

an adult in the Offspring Cohort of the FHS[5]. In addition, 103 individuals who were spouses of the original Offspring Study participants and had at least two children in the Gen III cohort were recruited in 2003 to be a part of NOS. In 1994, the FHS investigators enrolled a population of 507 men and women who were ethnically diverse and all residents of or near Framingham, as this population was underrepresented. This new cohort was called Omni 1. Omni 2 began in 2003 with the recruitment of 410 ethnically diverse participants, some of whom were relatives of Omni 1 individuals, from the Framingham area[5]. In this study, only Omni 2 participants were included because their data examination periods were at the same time as Gen III and NOS, thus providing comparable data for these analyses as only these three cohorts (Gen III, Omni 2, and NOS) underwent VCTE FibroScans to look at liver-related outcomes in this study. At exam 1, there were a total of 4608 participants in the VCTE cohorts. After exam one, there was a six-year period until exam 2, and then an eight-year interval between exam 2 and exam 3. A total of 3800 participants from all 3 cohorts attended the second exam visit that occurred from 2008-2011. Of those who attended, 3411 were from Gen III, 321 from Omni 2, and 68 from NOS.

At each examination visit, data collected from each participant included anthropometric and urinary measures, demographic data, laboratory measurements, medical history, and lifestyle habits, including diet, physical activity, alcohol intake, and smoking. The food frequency questionnaire (FFQ) at Exam 2 provided dietary intake data for the analysis, as it is a reflection of dietary habits before Exam 3. Of the 3800 who

attended exam 2, 3586 participants completed the FFQ at exam 2. Of those participants, 94 were excluded due to the following: (a) extremes of energy intake (<600 kcals/d or >4000 kcals/d for women; <600 kcals/d or >4200 kcals/day for men) or (b) missing > 12 FFQ food items, leaving a total of 3492 participants.

The outcomes for these analyses were derived from VCTE Fibro Scan data completed at exam 3. VCTE measurements included measures of hepatic steatosis (using the controlled attenuation parameter or CAP) and fibrosis (based on the liver stiffness measurement or LSM). Of the 3492 participants, only 3016 attended exam 3. Other exclusion criteria included those missing CAP and LSM data or those with unreliable VCTE data. Valid measures were defined as those in which the IQR/median was ≤ 30%. However, it has been shown that an IQR/median > 30% combined with an LSM < 7.1 kilopascals (kPa) also provides measures that are comparable to those with an IQR/median ≤ 30%; therefore, these scans will be kept and defined as 'reliable'[6]. Thus, unreliable VCTE data excluded for excess variability was defined as an IQR/median > 30% in combination with an LSM ≥ 7.1 kPa[6]. After excluding participants without reliable and complete VCTE data, 2862 participants remained in the study. Further exclusions included the following: (a) heavy alcohol consumption (i.e. greater than 14 drinks per week for women and greater than 21 drinks per week for men)[2,3,47]; (b) history of cancer except for non-melanoma skin cancer; and (c) those missing all blood lipids. After all exclusions, a total of 2573 participants remained eligible in this study (Figure 2).

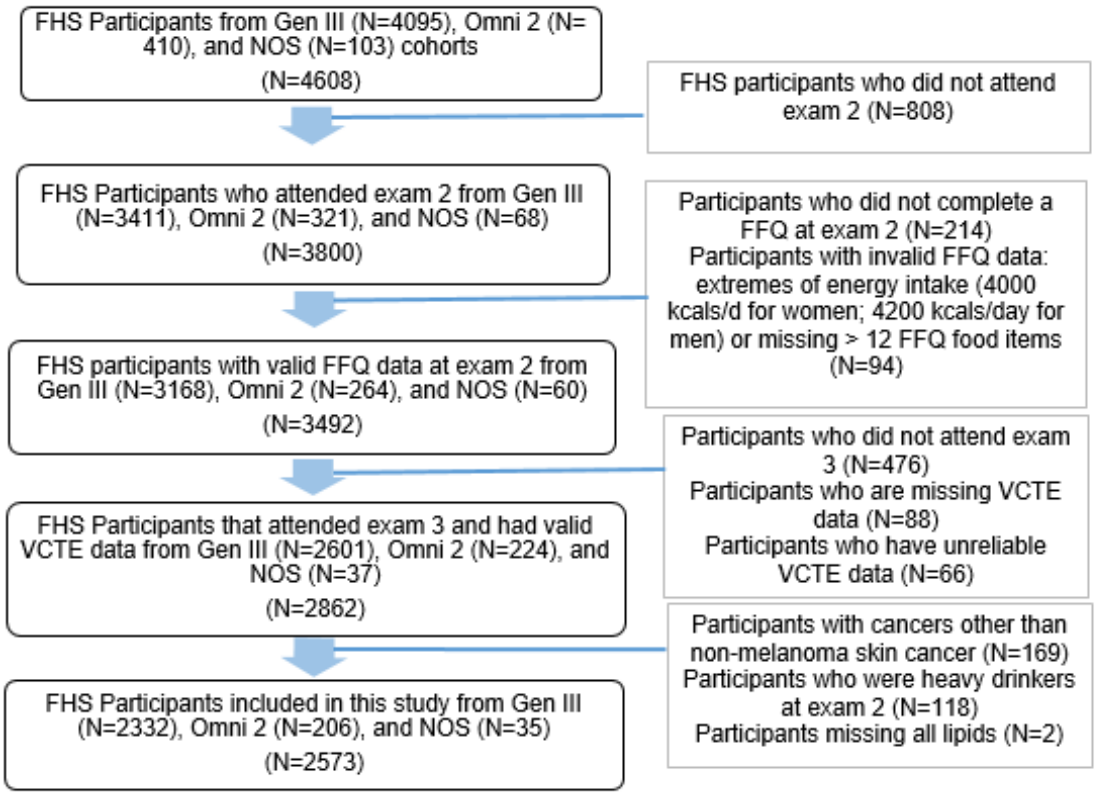

Figure 2: Flowchart of study participants from Framingham Heart Study cohort. Abbreviations: FHS, Framingham Heart Study; FFQ, food frequency questionnaire; Gen III, generation 3; N, sample size; NOS, New Offspring Spouse; VCTE, vibration-controlled transient elastography.

Assessment of adherence to a modified DASH index

Assessment of adherence to mDASH was based on self-reported dietary intakes from the Harvard semi-quantitative FFQ in which participants were asked about their typical intakes of foods and beverages over the last year as a way to understand their usual eating patterns. Specifically, each food included on the FFQ was listed with a

standardized serving size and a choice of intake frequencies ranging from “never or less than one serving per month” to “six or more servings per day”. Participants were also asked to report any additional food items that were regularly consumed that were not listed on the FFQ. Nutrient intakes were then calculated from the frequency of consumption for each item multiplied by the nutrient content of the specified portion of the food. Although FFQs can be limited in their ability to fully capture typical eating patterns due to reporter bias and error, the Harvard FFQ has been validated for intake of selected nutrients in previous literature[78,82].

The mDASH index as described above in the background section was developed in 2023[104] and is described here briefly. Adherence to the mDASH index was assessed for each participant by assigning a score of 1 to 5 to the sex-specific intakes of eight food-based components. The food components include the following: (1) total vegetables; (2) fruit & fruit juice; (3) total dairy products; (4) whole grains; (5) legumes, nuts & seeds; (6) fish; (7) red & processed meats; and (8) sugar-sweetened beverages (SSBs). Table 1 describes the FFQ-reported foods that made up each mDASH score component. As mentioned earlier, individual FFQ food frequency responses ranged from “never or <1 serving/month” to “≥6 servings/day”. FFQ items were then classified into their respective food groups of mDASH (Table 1). Each category of intake on the FFQ was then converted to mean servings per day in that category. Each food component was then classified into quintiles of intake based on the estimated servings per day reported on the FFQ. For the six components in which higher intakes are desired (vegetables, fruit &

fruit juice, total dairy, whole grains, legumes, nuts & seeds, and fish), the lowest quintile received a score of 1 with the highest quintile receiving a score of 5. In components where lower intake is desired (red & processed meats and SSBs), the lowest quintile of intake received a score of 5 with the highest quintile receiving a score of 1. The total sex-specific scores for the mDASH index had a possible range from 8-40 (Table 2).

Table 1: Food items classified in the final score components for the mDASH index.

Score components	FFQ items classified into score components
Total vegetable	FFQ listed items: Tomatoes, tomato juice, tomatoes sauce, broccoli, cabbage/Cole slaw, cauliflower, Brussels sprouts, carrot, mixed vegetables, winter squash, eggplant/zucchini/summer squash, spinach, kale/mustard/chard, iceberg lettuce, romaine lettuce, celery, beets, alfalfa sprouts, garlic, string beans, red chili sauce, corn, veggies from pizza, yams/sweet potatoes, potato/corn chips, French fried potatoes, peas/lima beans, red chili sauce, chowder.
Total fruit	FFQ listed items: Raisins/grapes, prunes, bananas, cantaloupe, watermelon, apples/pears, apple juice/cider, oranges, orange juice, grapefruit, grapefruit juice, strawberry, blueberries, other fruit juice, peaches/apricot/plums.
Total dairy	FFQ listed items: Skim/low-fat milk, whole milk, yogurt, cottage/ricotta cheese, other cheese (American or cheddar), cheese from pizza, ice milk, ice cream, and dairy from chowder.
Wholegrains	FFQ listed items: Cooked oatmeal, breakfast cereals with ≥25% whole-grain or bran content by weight, dark bread, brown rice, a mixture of bulgur, kasha, and couscous, popcorn, and whole grains from pancakes/waffles, muffins/biscuits, and cookies,
Legumes, nuts and seeds	FFQ listed items: Nuts, peanut butter, soybean/tofu, beans/lentils
Fish	FFQ listed items: canned tuna, dark fish including salmon, sardines, bluefish or swordfish, other fish, shrimp/shellfish/lobster
Red and processed meats	FFQ listed items: Processed meats, hot dogs, bacon, hamburger, beef/pork/lamb, and liver.
SSBs	FFQ listed items: Regular and decaffeinated, and all other carbonated beverages with sugar, and non-carbonated fruit drinks including lemonade, and Hawaiian punch.

Abbreviations: FFQ, food frequency questionnaire; mDASH, modified Dietary Approaches to Stop Hypertension; SSBs, sugar-sweetened beverages

Table 2: Standards for maximum scores on the mDASH index.

	mDASH index* Sex-spec. score
Score components for which higher intakes receive a higher score	
Total vegetable	Q5
Total fruit	Q5
Total dairy	Q5
Whole grains	Q5
Legumes, nuts, seeds	Q5
Fish	Q5
Score components for which lower intakes receive a higher score	
Red and processed meats	Q1
SSBs	Q1
Total score (points)	8–40

*Quintile intakes are expressed as servings as stated on the FFQ per day. For components whose higher intake is desired, participants receive a score from 1 (lowest quintile) to 5 (highest quintile); for components in which lower intake is desired, participants receive a score from 5 (lowest quintile) to 1 (highest quintile).
Abbreviations: FFQ, food frequency questionnaire; mDASH, modified Dietary Approaches to Stop Hypertension; Sex-spec., sex-specific; SSBs, sugar-sweetened beverages; Q, quintile.

Hepatic Steatosis and Fibrosis Outcomes

Hepatic steatosis and fibrosis were measured using the VCTE FibroScan (Echosens). FibroScan is a non-invasive protocol that uses ultrasound to estimate CAP, an indicator of steatosis, and LSM, an indicator of fibrosis. The FibroScan measured the velocity of a 50 Hz shear wave as it propagated through the skin and the liver, thereby providing the LSM. The frequency signals from the ultrasound propagated a 3.5 MHz wave through the body, detecting fat and provided the CAP score as an indicator of steatosis. Investigators asked participants about food and beverage intake prior to the VCTE scan but no participants were excluded based on fasting time. Each participant was positioned supine with the right leg crossed over the left ankle and the right arm above the head. The ultrasound probe was placed in the intercostal space over the right lobe of the liver. Probe selection of medium (M) or extra-large (XL) was based on the recommendations provided by the device. During the examination, 10 valid liver measurements were obtained to ensure reliable and accurate measures of average CAP and LSM. The device calculated the median CAP and LSM, as well as the interquartile range.

Based on previous studies[9,23,24,25,32,49,54,99] cutoffs were selected to define the following: (a) Hepatic Fibrosis: LSM $\geq$ 8.2 kPa; (b) Hepatic Steatosis: CAP $\geq$ 290 decibels per meter (dB/m). Based on these cutoff values, four stages of NAFLD were

defined based on the combined presence of hepatic steatosis and/or fibrosis as follows: (a) neither hepatic fibrosis nor steatosis: LSM < 8.2 kPa and CAP < 290 dB/m; (b) hepatic steatosis without fibrosis: LSM < 8.2 kPa and CAP ≥ 290 dB/m; (c) hepatic fibrosis without steatosis: LSM ≥ 8.2kPa and CAP < 290 dB/m; and (d) hepatic steatosis with fibrosis: LSM ≥ 8.2 kPa and CAP ≥ 290 dB/m.

Potential confounding and effect modifying variables

Potential confounders and effect modifiers were obtained from data from the clinical exam and the medical history questionnaire at exam 2. For any missing covariate information, data substitution was done using non-missing data from exam 1 and exam 3. Demographic and lifestyle data taken from exam 2 questionnaires include sex, age, race and ethnicity, highest level of education completed, income over the past year, medication use, alcohol intake, smoking, and a moderate-vigorous physical activity index (MV-PAI).

Height and weight were measured with the participant shoeless in a hospital gown using a stadiometer and a beam balance scale. From these measurements, BMI in kg/m^2 was calculated from the exam 2 weight (kg) divided by the average height of the participant (meters squared) at all three exams. Average height was used to address possible measurement errors or any height loss that may occur when aging. Waist circumference (cm) was measured in inches and converted to centimeters. The WsHtR (cm/m^2) was calculated from waist circumference and average height at all 3 exams. The

highest level of education was classified into two categories, those who completed a Bachelor's degree or further education or those who completed less than a Bachelor's degree. For those missing education at all three exams, the median education level for their income level was substituted.

Due to the direct effect of alcohol consumption on fatty liver, it was critical to analyze and include alcohol consumption in this study. The lifestyle questionnaire asked participants about their alcohol consumption with questions on monthly and weekly drinking patterns for each alcohol type, the age at which the participant started and stopped drinking alcohol, the maximum number of alcoholic beverages consumed over 24 hours, and drinking frequency. Any missing values from the questionnaire at Exam 2 were substituted for using values taken from the Exam 3 questionnaire. To assess the typical drinking amount for each participant, a total alcohol variable (drinks/week) was created by summing the weekly intakes of beer, wine, and liquor. If this value was greater than the number of days per week in which the participant drank alcohol, then this value was taken as total alcohol intake. If the days per week that participants drank were greater than the sum of alcoholic beverages per week, then days per week were reported as total drinks per week. Some participants reported their usual alcohol intake as 0 drinks per week for all types of alcohol and also reported usually drinking 0 days per week. Despite this, they also reported a maximum number of drinks they had consumed in 24 hours during the past month. Thus the reported drinking pattern for these individuals could reflect intermittent (social) drinking or binge drinking, dependent on the amount

consumed on a single occasion. In the case of social drinkers, their drink amount was assigned a low total alcohol amount based on the participant's sex. In the case of females: (a) 1 drink over 24 hours correlated to 0.25 drinks per week ; (b) 2 drinks over 24 hours correlated to 0.50 drinks per week ; (c) 3 drinks over 24 hours correlated to 0.75 drinks per week. In the case of males: (a) 1 or 2 drinks over 24 hours correlated to 0.25 drinks per week ; (b) 3 drinks over 24 hours correlated to 0.50 drinks per week ; (c) 4 drinks over 24 hours correlated to 0.75 drinks per week. The amounts were chosen as these participants had reported 0 intake for each type of alcoholic beverage and usually drinking on 0 days weekly but they do report drinking on occasion. Thus, they did not drink 1 alcoholic beverage per week but rather less than that.

Binge drinkers were classified based on guidelines from the *National Institute on Alcohol Abuse and Alcoholism*[21], which defines binge drinking as (a) females who consume 4 or more alcoholic beverages in a day or (b) males who consume 5 or more alcoholic beverages in a day. Our data set featured 344 (25%) binge drinkers among females and 501 (42%) binge drinkers among males. To differentiate between those near the binge drinker cut-off and those drinking far greater amounts, CDC guidelines[65] were used to classify heavy binge drinking as 8 or more drinks over 24 hours for females or 10 or more drinks over 24 hours for males. Finally, the alcohol data were used to classify drinking status as follows: Never drinkers (those who reported never drinking at Exam 2 and 3), Former Drinkers (those who reported at exam 2 or 3 an age at which they stopped drinking and who did not currently drink), Light Drinkers (< 3 drinks/week for men and

women), and Moderate Drinkers (>3 drinks/week but ≤14 for women or >3 drinks/week but ≤21 for men), while heavy drinkers were excluded. The cutoffs for light and moderate drinkers were based on the previous drinking definition[65].

The questionnaire asked participants about their smoking history, as well as the frequency and amount of current smoking. From this information, smoking status was defined as never smokers, former smokers, and current smokers. Current cigarette smoking was defined and analyzed as both a continuous (cigarettes smoked per day) and a categorical variable. In these cohorts, a majority of participants were current nonsmokers, with their cigarettes per day being zero. Few participants smoked more than a pack per day. Due to this, the cigarettes per day were classified as follows: (a) nonsmokers: less than 1 cigarette per day; (b) light smokers: 1 -< 10 cigarettes per day; and (c) heavy smokers: ≥10 cigarettes per day.

Participants were asked about their typical physical activity levels in a day, including how many hours they spent sleeping, being sedentary, doing slight activity, moderate activity, and vigorous activity. The Physical Activity Index was calculated by multiplying the self-reported hours for each level of activity by a weight derived from oxygen consumption required for that level of activity and then summing the products for all levels of activity. MV-PAI was also created which only included the sum of those two activity levels. Total energy expenditure (TEE) was calculated from the sex, age, weight, and physical activity of each participant and applied to the following equations[33]: (a) for

males: 864 – (9.72 x AGE) + PA x (14.2 x Weight + 503 x Height); (b) for females: 387 – (7.31 x AGE) + PA x (10.9 x Weight + 660.7 x Height). PA in the above equations is the physical activity coefficient for the physical activity level that the participant falls into. The physical activity index (PAI) cutoffs and PA coefficients were defined as (a) Sedentary – $0 \leq PAI < 32.3$ and PA=1.0; (b) Low activity- $32.3 \leq PAI < 35.7$ and PA=1.12; (c) Active – $35.7 \leq PAL < 41.6$ and PA=1.27; (d) Very Active- $41.6 < PAI$ and PA=1.45. Typically, physical activity level cutoffs are obtained via the doubly labeled water technique and come from the multiplication of the sum of activity time by the basal metabolic rate for each activity, however, data in FHS does not provide basal metabolic rate and therefore to define the PAL cutoffs in this study, the cutoffs of the quartiles of physical activity index at Exam 2 were used.

A variety of lab values were obtained on blood pressure and lipid levels. Blood pressures, including diastolic and systolic, were measured with a standard mercury sphygmomanometer (Baumanometer) at each examination visit. Two measurements were taken on the left arm with the participant seated and the mid-point of the cuff placed at the level of the heart. The participant rested for at least 30 seconds between measures. The average of the two measures was calculated. Hypertension was defined based on modified criteria from the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure* (JNC 7)[64] as follows: (a) a single visit with a mean systolic blood pressure ≥ 160 or a mean diastolic blood pressure ≥ 95; or (b) the first visit at which SBP was 140-159, or DBP was 90-94 mm Hg when

followed by a subsequent visit at which SBP was ≥ 140 or DBP was ≥ 90; or (3) the first visit at which the participant reported new use of anti-hypertensive medications. The date of hypertension diagnosis was the mid-point between the last known visit when the participant was not hypertensive and the first visit at which they met the criteria for being hypertensive.

Participants were asked to fast for 12 hours before all lab measurements; they were asked the last time they had eaten and total hours fasting were calculated. All fasting variables were those at which fasting time was ≥ 8 hours, with those fasting <8 hours excluded. Blood samples were taken from the antecubital vein and stored in aliquots at -80˚ centigrade. High-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and blood glucose were collected, and quality control was done to ensure accuracy. Due to the non-normal distribution of fasting triglycerides, a log transformation was done to normalize the values. The Triglyceride to HDL ratio (TG: HDL) was also calculated, as it is a known marker for individuals at risk for MetS and CVD, and possibly NAFLD [10,45,71,93]. The sex-specific cutoff value for the TG: HDL ratio that was considered to reflect a higher risk of metabolic dysfunction was based on the following definition from the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria: >3.8 for men and >3.0 for women[51].

Dyslipidemia was defined based on previously accepted definitions[22,56]. Specifically, dyslipidemia was defined as follows: (a) total cholesterol ≥ 240 or use of lipid-lowering medication; (b) if HDL was < 50 for women or <40 for men; (c) triglycerides levels were ≥ 150; or (d) if LDL ≥ 160[22]. Standard protocols were used to define individuals with T2DM at each examination visit starting at enrollment. A diagnosis of T2DM was defined as (a) non-fasting glucose ≥ 200 mg/dL; (b) 8+ hour fasting glucose ≥126 mg/dL; (c) use of glucose-lowering medications; (d) history of diagnosed T2DM with a non-fasting glucose of 126-200 mg/dL; or (e) a self-reported diagnosis of T2DM at one exam followed by a diagnosis of definite diabetes at the next examination visit. Impaired fasting glucose (IFG), also known as prediabetes, was defined when the above criteria were not met, but an 8-hour fasting glucose level was between 100-125 mg/dL or non-fasting glucose was ≥126 mg/dL with no history of a diabetes diagnosis. CMR was a categorical variable (yes/no) used to define the cardiometabolic risk of each individual. CMR was defined as a yes if an individual had any of the following present: (a) T2DM/IFG; (b) elevated TG: HDL ratio; (c) dyslipidemia; or (d) hypertension.

Statistical Analysis

Statistical analyses were done using SAS statistical software (version 9.4; SAS Institute, Cary, NC). The overall goal of this thesis was to examine the association between the mDASH index and non-alcoholic hepatic steatosis and fibrosis. The mDASH scores from exam visit 2 were normally distributed. We classified them into tertiles for

analysis to evaluate the cumulative incidence of each outcome at exam 3. Outcomes included stages of non-alcoholic fatty liver disease that were classified as follows: (1) no hepatic steatosis or fibrosis, (2) hepatic steatosis without fibrosis, (3) hepatic fibrosis without steatosis, and (4) both hepatic steatosis and fibrosis. Each outcome was first evaluated individually. A secondary analysis examined the risk of developing any hepatic steatosis (vs. none). To assess the unconfounded association between mDASH tertiles and the above stages of NAFLD, multivariable logistic regression models were used and odds ratios were obtained.

Linear regression models were used to assess the continuous association between mDASH and measures of CAP and log-transformed LSM as indicators of hepatic steatosis and fibrosis. LSM was log-transformed because it was non-normally distributed. All models were first adjusted for age and sex. Next, potential confounders were examined to determine the extent to which they altered the age and sex-adjusted-parameter estimates by approximately 10% or more and included examination of the following variables: (1) education (< Bachelor's Degree/≥ Bachelor's Degree); (2) Income (< $100,000 / ≥ $100,000); (3) Race and Ethnicity (Non-Hispanic White, Non-Hispanic Black/Hispanic); (4) physical activity (METs/hours, continuous); (5) moderate-vigorous activity (METs/hours, continuous); (6) total alcohol intake (drinks/week, continuous); (7) drinking status (nondrinkers, former drinkers, light drinkers, and moderate drinkers); (8) Binge Drinker (yes/no, categorical); (9) Heavy Binge Drinker (yes/no), categorical); (10) current smoker (yes/no, categorical); (11) Smoking Status

(neversmokers, former smokers and current smokers); (12) cigarettes per day (continuous); (13) cigarettes per day (nonsmokers, light smokers and heavy smokers); (14) energy intake (kcal/d, continuous); (15) total energy expenditure (kcal/d, continuous); (16) multivitamin use (yes/no, categorical); (17) sodium intake (mg, continuous); (18) body mass index (kg/m^2, continuous); (19) waist circumference (cm, continuous); (20) waist to height ratio (kg/m, continuous); (21) diabetes status at exam 2 (yes/no, dichotomous); (22) TG:HDL Ratio (normal/elevated, dichotomous); (23) TG:HDL ratio (continuous); (24) HDL (mg/dL, continuous); (25) 8-hour fasting LDL (mg/dL, continuous); (26) 8-hour fasting triglycerides (mg/dL, continuous); (27) 8-hour fasting blood glucose (mg/dL continuous); (28) dyslipidemia at exam 2 (yes/no, dichotomous); (29) hypertension at Exam 2 (yes/no, dichotomous); (30) systolic blood pressure (mmHg, continuous); (31) diastolic blood pressure (mmHg, continuous); CMR (yes/no, dichotomous). After testing all potential confounders, the confounders that remained in the models were age, sex, education status, energy intake, cigarettes per day (categorical), and drinking status.

Logistic regression models were also used to explore whether the effects of mDASH on NAFLD stages were modified by other factors. To optimize statistical power for the latter analyses, a sensitivity analysis was done to dichotomize the mDASH score. Based on these analyses, the mDASH score was classified as follows: lower mDASH score (T1:8-21.5) and higher mDASH score (T2+T3:22-39.5). Overall and sex-specific sensitivity analyses were done to determine appropriate cutoffs for potential effect

modifiers. Effect modifiers included BMI, waist circumference, MV-PAI, CMR, and total alcohol.

Sensitivity analyses were also done to determine the effect of adding heavy drinkers back into the study sample. Logistic regression models from the primary analysis were done again with heavy drinkers added to the study sample. For hepatic steatosis regardless of fibrosis status, sex stratification was done.

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RESULTS

The mean mDASH score of the three cohorts at exam 2 was 24.2 (±5.4) (range: 8.0-39.5) (Figure 3) out of the maximum possible score of 40. Table 3 shows the baseline characteristics of subjects across tertiles of mDASH scores. Compared with individuals in

the lowest tertile of mDASH score, those in the highest tertile were an average of 2.7 years older, had higher educational levels, higher income levels, lower BMI and WsHtRs, higher energy intakes, and higher MV-PAI. While self-reported energy intakes increased across categories of mDASH, the estimated TEE values did not, suggesting that energy intakes may be subject to some amount of bias in these analyses. Individuals in the highest tertile category of mDASH were also less likely to smoke, had lower rates of T2DM/IFG, and elevated TG: HDL ratios. After the exclusion of heavy drinkers, females in the three cohorts averaged 2.0 (IQR: 1.0-5.3) drinks of alcohol per week while males averaged 4.3 (IQR: 1.0-9.0) drinks of alcohol per week (Table 4). Female individuals were also more likely to be never drinkers and less likely to be moderate alcohol drinkers or binge drinkers compared with male individuals (Table 4).

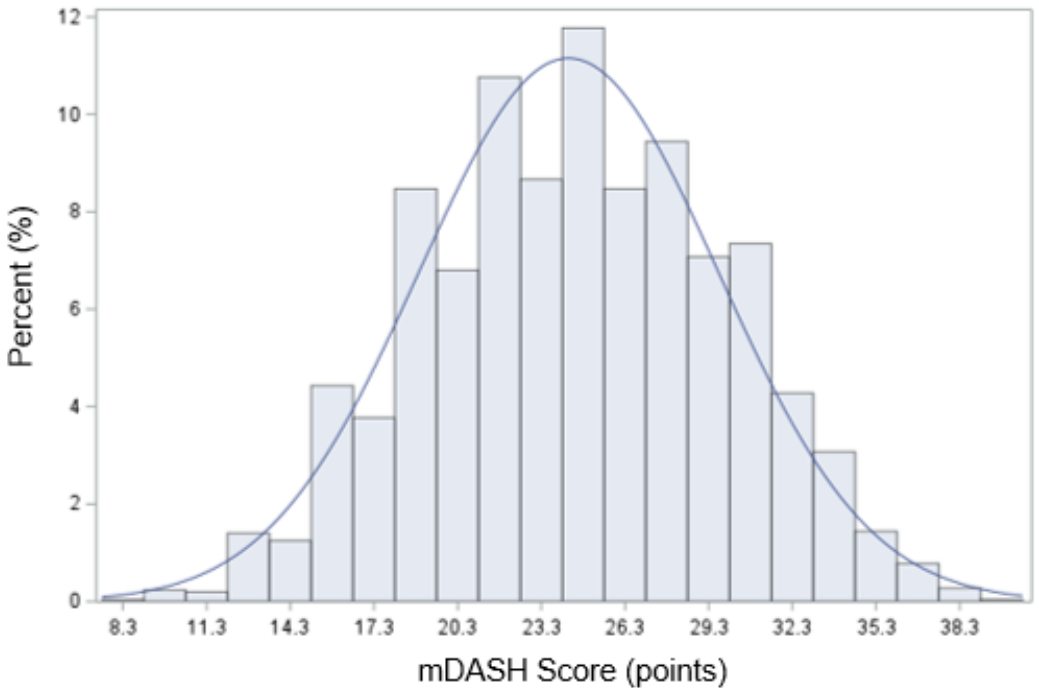


Figure 3: The distribution of the mDASH score in the 2nd examination in the Framingham VCTE cohorts (Gen III, Omni 2, and NOS) cohorts (N =2573). Mean (± standard deviation): 24.23 (± 5.4); median (min, max): 24.00 (8.00, 39.50).
Abbreviations: Gen III, generation 3; mDASH, modified Dietary Approaches to Stop Hypertension; min, minimum; max, maximum; NOS, new offspring spouse; VCTE, vibration-controlled transient elastography.

Table 3: Baseline characteristics by mDASH tertiles in the Framingham VCTE cohorts (Gen III, Omni 2, and NOS)

	Tertile 1 (8.0-21.5) N=836	**Tertile 2 (22.0-26.5) N=869**	**Tertile 3 (27.0-39.5) N=868**
Females, N (%)	457 (54.7)	455 (52.4)	478 (55.1)
Age, mean (±SD)	45.2 (8.7)	46.5 (8.9)	47.9 (9.4)
≥ Bachelor's degree, N (%)	412 (49.3)	526 (60.5)	592 (68.2)
Family income (≥ $100,000), N (%)	322 (38.5)	399 (45.9)	417 (48.0)
Non-Hispanic White, N (%)	790 (94.5)	829 (95.4)	828 (95.4)
BMI (kg/m^2), mean (±SD)	28.3 (5.8)	27.5 (5.4)	27.0 (5.1)
WsHtR (kg/m), median (IQR)	0.6 (0.5-0.6)	0.5 (0.5-0.6)	0.5 (0.5-0.6)
mDASH Score, mean (±SD)	18.2 (2.6)	24.1 (1.4)	30.2 (2.5)
TEE (kcal/d), mean (±SD)	2578 (560)	2584 (541)	2535 (507)
Total calories (kcal/d), mean (±SD)	1672 (564)	1955 (578)	2322 (607)
Moderate-vigorous index (METs/hour), median (IQR)	9.8 (7.2-17.0)	12.0 (7.4-17.0)	12.0 (7.4-17.0)
Current Smoker, N (%)	96 (11.5)	63 (7.3)	30 (3.5)
Cigarettes Per Day, mean (±SD)	1.6 (5.2)	0.6 (3.0)	0.3 (2.2)
T2DM/IFG, N (%)	233 (27.9)	202 (23.3)	213 (24.5)

Hypertension, N (%)	171 (20.5)	169 (19.5)	189 (21.8)
Dyslipidemia at Exam 2, N (%)	388 (46.4)	336 (38.7)	319 (36.8)
Elevated TG: HDL ratio, N (%)	160 (19.1)	112 (12.9)	112 (12.9)

Abbreviations: BMI, body mass index; Gen III, generation three; IQR, interquartile range; kcals/d, kilocalories per day; kg/m^2, kilograms per meter squared; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; NOS, new offspring spouse; SD, standard deviation; TEE, total energy expenditure; TG: HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 4: Baseline alcohol characteristics in participants by sex: the Framingham VCTE cohorts

Alcohol Characteristics	**Study sample including heavy drinkers**		**Study sample excluding heavy drinkers**	
	Females (N=1436)	Males (N=1255)	Females (N= 1390)	Males (N=1183)
Total Alcohol (drinks/week), median (IQR)	2.0 (1.0-5.3)	5.0 (1.0-10.0)	2.0 (1.0-5.3)	4.3 (1.0-9.0)
Alcohol Types (drinks/week), median (IQR)				
Beer	0.0 (0.0-0.8)	1.5 (0.0-5.0)	0.0 (0.0-0.8)	1.5 (0.0-5.0)
Wine	0.8 (0.0-3.0)	0.3 (0.0-2.0)	0.8 (0.0-3.0)	0.3 (0.0-2.0)
Liquor	0.0 (0.0-0.5)	0.0 (0.0-1.0)	0.0 (0.0-0.5)	0.0 (0.0-1.0)
Drink status, N (%)				
Never drinker	143 (10.0)	78 (6.2)	143 (10.3)	78 (6.5)
Former drinker	94 (6.6)	94 (7.5)	94 (6.8)	94 (8.0)
Light drinker	659 (45.9)	343 (27.3)	659 (47.4)	343 (29.0)
Moderate drinker	494 (34.4)	668 (53.2)	494 (35.5)	668 (56.5)
Heavy drinker	46 (3.2)	72 (5.7)	0 (0.0)	0 (0.0)
Drinking patterns, N (%)				
Binge	351 (24.5)	476 (37.9)	311 (22.4)	405 (34.2)
Heavy Binge	43 (3.0)	132 (10.5)	33 (2.4)	96 (8.1)

Total alcohol was the sum of total beer, wine, and liquor reported by participants. For drink status, never drinkers were those who reported never consuming alcohol throughout exams 1-3. Former drinkers were those who had reported drinking before exam 2 but no longer drinking at exam 2. Light drinkers were defined as ≤ 3 drinks/week for men and women. Moderate Drinkers were defined as: >3 drinks/week but ≤14 for women or >3 drinks/week but ≤21 for men. Heavy drinkers were defined as > 14 drinks/week for women and > 21 drinks/week for men. Binge Drinkers were defined as females who consume 4 or more alcoholic beverages in a day or males who consume 5 or more alcoholic beverages in a day. Heavy Binge Drinkers were defined as (a) 8 or more drinks over 24 hours for women; or (b) 10 or more drinks over 24 hours for men.
Abbreviations: IQR, interquartile range; N, sample size; VCTE, vibration-controlled transient elastography.

Odds of Hepatic Steatosis and Fibrosis Across mDASH Tertiles

Among individuals without heavy alcohol consumption, 729 (28.3%) had met the VCTE criteria of hepatic steatosis (122 with concurrent fibrosis and 607 without any fibrosis). There were also 96 (3.7%) individuals who had met the VCTE criteria of fibrosis without steatosis. Finally, 1748 (67.9%) had neither steatosis nor fibrosis. Table 5 shows adjusted odds ratios for the association between mDASH tertiles and hepatic steatosis, regardless of fibrosis status. It appears that the overall prevalence of hepatic steatosis decreased to around 25% in the middle and highest tertiles of mDASH compared with the lowest tertile (34%). When adjusting for age, sex, education, energy intake, cigarettes per day, and drinking status, individuals in the middle and highest categories of mDASH had 35% lower odds of steatosis compared with those in the lowest category of mDASH (95% $CI_{tertile2}$: 0.52-0.81; 95% $CI_{tertile3}$:0.51-0.83; p-trend:0.0008). When further adjusting for BMI, WsHtR, or CMR, the odds ratios were attenuated with individuals in tertile 2 of mDASH having a lower risk of steatosis than individuals in tertile 1 or 3. CMR factors such as T2DM, hypertension, dyslipidemia, and elevated TG: HDL ratio are often seen in conjunction with NAFLD. When adjusting for having any of the CMR factors mentioned above, individuals in tertiles 2 and 3 of mDASH had 29% (95% CI: 0.56-0.89) and 28% (95% CI: 0.56-0.92) reduced odds of hepatic steatosis compared with individuals in tertile 1 of mDASH with results being statistically significant (p-trend: 0.0112).

Of the 1390 females, 305 had hepatic steatosis at exam 3. The prevalence of steatosis decreased across the tertiles, with females in tertile 3 having 40% lower odds (95% CI: 0.41-0.87; p-trend: 0.0074) of steatosis compared with females in tertile 1 of mDASH. When further adjusting for BMI, WsHtR, or CMR, the odds ratios were attenuated. When adjusting for CMR, females in the highest tertile of mDASH had 28% lower odds (95% CI: 0.49-1.05; p-trend: 0.0896) of steatosis compared to females in the lowest category of mDASH. Of the 1183 males, 424 had steatosis at exam 3. Although males had a higher prevalence of steatosis than females, their relative odds of steatosis were stronger than that of females across all models. Males in tertile 2 had 44% reduced odds (95% CI: 0.41-0.76; p-trend: 0.0061) of steatosis at exam 3 compared to males in tertile 1 of mDASH. When further adjusting for BMI, WsHtR, or CMR, there was slight attenuation.

Table 5: Adjusted ORs for the association between mDASH tertiles and hepatic steatosis in FHS VCTE cohorts.

mDASH Score tertiles	Cases	Prevalence (%)	N	OR (95% CI)[1]	OR (95% CI)[2]	OR (95% CI)[3]	OR (95% CI)[4]
Overall							
T1 (8.0-21.5)	284	33.97	836	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22-26.5)	220	25.32	869	0.65 (0.52-0.81)	0.71 (0.55-0.90)	0.73 (0.57-0.93)	0.71 (0.56-0.89)
T3 (27-39.5)	225	25.92	868	0.65 (0.51-0.83)	0.78 (0.60-1.02)	0.81 (0.62-1.07)	0.72 (0.56-0.92)
P-trend				0.0008	0.0856	0.1555	0.0112
Females							
T1 (9–21.5)	114	24.95	457	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.5)	94	20.66	455	0.72 (0.52-1.00)	0.83 (0.57-1.20)	0.81 (0.56-1.17)	0.82 (0.59-1.16)
T3 (27–39.5)	97	20.29	478	0.60 (0.41-0.87)	0.82 (0.54-1.25)	0.84 (0.55-1.28)	0.72 (0.49-1.05)
P-trend				0.0074	0.3550	0.4072	0.0896
Males							
T1 (8–21.0)	170	44.85	379	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.0)	126	30.43	414	0.56 (0.41-0.76)	0.60 (0.44-0.84)	0.65 (0.47-0.90)	0.60 (0.44-0.81)
T3 (27–38.0)	128	32.82	390	0.63 (0.45-0.88)	0.70 (0.49-1.00)	0.74 (0.52-1.06)	0.67 (0.48-0.93)
P-trend				0.0061	0.0504	0.0987	0.0178

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day), and drink status (never drinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for
males).
Model 2: Model 1 + BMI
Model 3: Model 1 + WsHtR
Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels)
Abbreviations: BMI, body mass index; CI, confidence intervals; CMR, cardiometabolic risk; FHS, Framingham Heart Study; HTN, hypertension; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; OR, odds ratio; Ref., reference; T, tertile; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography; WsHtR, waist to height ratio.

Table 6: Adjusted ORs for the association between mDASH tertiles and NAFLD stages in FHS VCTE cohorts.

mDASH Score tertiles	Cases	Prevalence (%)	N	OR (95% CI)[1]	OR (95% CI)[2]	OR (95% CI)[3]	OR (95% CI)[4]
Hepatic Steatosis without Fibrosis							
T1 (8.0-21.5)	229	27.39	836	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22-26.5)	183	21.06	869	0.66 (0.52-0.84)	0.73 (0.56-0.95)	0.76 (0.58-0.98)	0.71 (0.56-0.91)
T3 (27-39.5)	195	22.47	868	0.71 (0.55-0.92)	0.87 (0.65-1.15)	0.90 (0.67-1.19)	0.77 (0.59-1.01)
P-trend				0.0144	0.3712	0.5066	0.0733
Hepatic Fibrosis without Steatosis							
T1 (9–21.5)	25	2.99	836	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.5)	28	3.22	869	0.93 (0.53-1.66)	0.98 (0.55-1.75)	1.00 (0.56-1.79)	0.93 (0.52-1.65)
T3 (27–39.5)	43	4.95	868	1.37 (0.76-2.47)	1.49 (0.83-2.69)	1.53 (0.84-2.77)	1.37 (0.76-2.46)
P-trend				0.2242	0.1407	0.1220	0.2311
Hepatic Steatosis and Fibrosis							
T1 (8–21.0)	55	6.58	836	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.0)	37	4.26	869	0.56 (0.35-0.88)	0.70 (0.42-1.16)	0.77 (0.46-1.29)	0.61 (0.38-0.97)
T3 (27–38.0)	30	3.46	868	0.44 (0.26-0.75)	0.63 (0.35-1.11)	0.70 (0.39-1.24)	0.49 (0.29-0.84)
P-trend				0.0023	0.1066	0.2182	0.0087

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day), and drink status (never drinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for males).
Model 2: Model 1 + BMI
Model 3: Model 1 + WsHtR
Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels)
Abbreviations: BMI, body mass index; CI, confidence intervals; CMR, cardiometabolic risk; FHS, Framingham Heart Study; HTN, hypertension; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; OR, odds ratio; Ref., reference; T, tertile; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography; WsHtR, waist to height ratio.

In Table 6, we examined the association between the tertiles of mDASH and individual stages of NAFLD. While the prevalence of hepatic steatosis without any fibrosis appeared to be reverse J shaped, with the middle category of mDASH showing the lowest prevalence of the disease, the width of the confidence intervals suggests that tertiles 2 and 3 may be quite similar. Individuals in tertile 2 of mDASH had 34% reduced odds (95% CI: 0.52-0.84; p-trend: 0.0144) of steatosis without fibrosis compared to those in tertile 1 of mDASH. When accounting for BMI or WsHtR, the OR estimates dropped to 27% and 24% lower odds of steatosis without fibrosis compared to those in tertile 1 of mDASH (95% CI_{BMI}: 0.56-0.95; 95% CI_{WsHtR}: 0.58-0.98). When accounting for CMR (T2DM, hypertension, dyslipidemia, or elevated TG: HDL ratios), individuals in the middle and highest categories of mDASH showed 29% and 23% lower odds (95% CI: $OR_{tertile2}$: 0.56-0.91; $OR_{tertile3}$:0.59-1.01) of steatosis without fibrosis compared to those in the lowest category of mDASH.

Unlike steatosis without fibrosis, there was an inverse linear trend between mDASH and steatosis with fibrosis (Table 6). Both the prevalence and odds ratios of steatosis with fibrosis decreased across the tertiles of mDASH, with those in the highest category of mDASH having the greatest protective effects from mDASH. Those in the highest category of mDASH had 56% lower odds versus 44% decreased odds in the middle category of mDASH when compared with the lowest category of mDASH (95% $CI_{tertile3}$:0.26-0.75; 95% $CI_{tertile2}$:0.35-0.88;p-trend: 0.0023). After adjusting for BMI,

WsHtR, and CMR, a strong inverse association between mDASH and the risk of hepatic steatosis with fibrosis remained.

Participants who developed hepatic fibrosis without steatosis appeared to be distinctly different from those with steatosis, suggesting that different pathways may be at play in steatosis vs. fibrosis. In the adjusted models, those in the highest tertile of mDASH had the highest odds of hepatic fibrosis in the absence of steatosis. It should be noted that the power of these analyses is limited, as there are only 96 cases of fibrosis without steatosis. None of the p-values for trend in this category of NAFLD outcomes reached statistical significance. Individuals in tertile 2 of mDASH had 7% reduced odds (95% CI: 0.53-1.66) compared to individuals in tertile 1 of mDASH. Those in tertile 3 had 37% increased odds (95% CI: 0.76-2.47) of fibrosis without steatosis compared to those in tertile 1 of mDASH. Although the confidence intervals are wide, the increased risk of fibrosis across the tertile of mDASH was observed across all models. When adjusting for BMI or WsHtR in addition to the factors in model 1, the odds ratios were strengthened, while adjusting for CMR did not affect the results.

Descriptive Tables of Steatosis and Fibrosis

In an attempt to better understand the differences in the results for various stages of NAFLD, tables 7-10 explore the characteristics of the individuals in various NAFLD stages across tertiles of mDASH, stratified by sex. First, in both females and males with hepatic steatosis regardless of fibrosis status, we saw that across increasing tertiles of

mDASH, individuals were more likely to be older, have more education and higher incomes, and be never drinkers and nonsmokers (Table 7). Females in tertile 3 of mDASH also had a slightly lower BMI. There were no consistent patterns of association between CMR associated with mDASH in either females or males.

Table 8 shows the subset of individuals with hepatic steatosis who do not also have fibrosis. The characteristics of those with steatosis without fibrosis are similar to those of Table 7 and this may be largely due to the overlap in the participants in the two tables. Overall, individuals with steatosis without fibrosis in the highest tertile of mDASH were older, had higher educational level or income status, were more often never-drinkers or nonsmokers, and had higher energy intake than individuals in the lowest tertile of mDASH. Of note, they did not have higher estimated TEE levels. In fact, those with the lowest mDASH scores had much higher TEE levels but lower self-reported energy intakes, again suggesting that energy intake in the lowest mDASH tertile may be under-estimated. Once again, the association between mDASH and CMR was inconsistent. These results suggest that aside from CMR, age, education, waist circumference, energy intake, total alcohol, and cigarettes smoked per day are all potential confounders or effect modifiers.

Table 7: Sex-specific baseline characteristics by mDASH tertiles in those with hepatic steatosis.

	Hepatic steatosis (N=729)					
	Females (N=305)			Males (N=424)		
	T1 (9.0-21.5) N=114	T2 (22.0-26.5) N=94	T3 (27.0-39.5) N=97	T1 (8.0-21.0) N=170	T2 (22.0-26.0) N=126	T3 (27.0-38.0) N=128
Age, mean (±SD)	47.6 (8.5)	49.3 (8.2)	49.2 (9.0)	45.8 (7.9)	47.8 (8.5)	48.8 (8.6)
≥ Bachelor's degree, N (%)	43 (37.7)	52 (55.3)	50 (51.6)	70 (41.2)	79 (62.7)	84 (65.6)
Family income (≥ $100,000), N (%)	30 (26.3)	34 (36.2)	32 (33.0)	63 (37.1)	55 (43.7)	71 (55.5)
BMI (kg/m²), mean (±SD)	31.6 (6.2)	32.4 (6.8)	31.0 (5.6)	31.3 (5.2)	31.0 (4.6)	31.1 (4.4)
Waist Circumference (cm), mean (±SD)	103.7 (14.3)	107.7 (16.5)	102.8 (13.4)	108.1 (12.6)	107.1 (12.2)	108.4 (10.7)
WsHtR, median (IQR)	0.6 (0.6-0.7)	0.7 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.6)	0.6 (0.6-0.6)
Moderate-vigorous index (METs/hour), median (IQR)	9.6 (4.8-16.8)	9.8 (7.2-14.6)	9.8 (7.2-14.6)	12.2 (7.4-19.6)	12.2 (7.4-22.2)	9.8 (7.3-14.6)
TEE (kcal/d), mean (±SD)	2318 (330)	2382 (356)	2283 (316)	3144 (482)	3125 (478)	3044 (390)
Total calories (kcal/d), mean (±SD)	1560 (537)	1926 (541)	2342 (539)	1829 (639)	1998 (591)	2360 (669)
Drink Status						
Never drinker	12 (10.5)	11 (11.7)	19 (19.6)	11 (6.5)	7 (5.6)	14 (10.9)
Former drinker	14 (12.3)	9 (9.6)	8 (8.3)	18 (10.6)	9 (7.1)	10 (7.8)

Light drinker	63 (55.3)	52 (55.3)	40 (41.2)	58 (34.1)	32 (25.4)	41 (32.0)
Moderate drinker	25 (21.9)	22 (23.4)	30 (30.9)	83 (48.8)	78 (61.9)	63 (49.2)
Total Alcohol (drinks/week), median (IQR)	1.0 (0.5-3.0)	1.0 (0.3-3.0)	1.0 (0.0-4.0)	3.0 (1.0-9.0)	5.9 (1.0-10.0)	3.0 (1.0-9.0)
Cigarettes Per Day, N (%)						
Nonsmoker	101 (88.6)	85 (90.4)	93 (95.8)	150 (88.2)	117 (92.9)	124 (96.9)
Moderate smoker	3 (2.6)	3 (3.2)	2 (2.1)	3 (1.8)	4 (3.2)	2 (1.6)
Heavy smoker	10 (8.8)	6 (6.4)	2 (2.1)	17 (10.0)	5 (4.0)	2 (1.6)
T2DM/IFG, N (%)	35 (30.7)	39 (41.5)	34 (35.1)	78 (45.9)	49 (38.9)	66 (51.6)
Hypertension, N (%)	36 (31.5)	27 (29.0)	36 (37.1)	48 (28.2)	50 (39.7)	47 (36.7)
Dyslipidemia at Exam 2, N (%)	63 (55.3)	49 (52.1)	49 (50.5)	114 (67.1)	77 (61.1)	79 (61.7)
Elevated TG: HDL Levels, N (%)	27 (23.7)	18 (19.2)	22 (22.7)	60 (35.3)	38 (30.2)	38 (29.7)

Abbreviations: BMI, Body Mass Index; cm, centimeters; IQR, interquartile range; kcals/d, kilocalories per day; kg/m^2, kilograms per meter squared; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; SD, standard deviation; T, tertile; TEE, total energy expenditure; TG: HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 8: Sex-specific baseline characteristics by mDASH tertiles in those with hepatic steatosis without fibrosis.

	Hepatic steatosis without fibrosis (N=607)					
	Females (N=263)			**Males (N=344)**		
	T1 (9.0-21.5) N=100	**T2 (22.0-26.5) N=76**	**T3 (27.0-39.5) N=87**	**T1 (8.0-21.0) N=129**	**T2 (22.0-26.0) N=107**	**T3 (27.0-38.0) N=108**
Age, mean (±SD)	46.8 (8.3)	49.0 (8.4)	48.8 (9.0)	45.6 (8.0)	47.7 (8.7)	48.6 (8.8)
≥ Bachelor's degree, N (%)	39 (39.0)	43 (56.6)	45 (51.7)	55 (42.6)	68 (63.6)	73 (67.6)
Family income (≥ $100,000), N (%)	26 (26.0)	27 (35.5)	29 (33.3)	46 (35.7)	44 (41.1)	59 (54.6)
BMI (kg/m²), mean (±SD)	31.2 (5.4)	31.6 (6.0)	30.7 (5.4)	30.6 (4.8)	31.0 (4.7)	30.8 (4.0)
Waist Circumference (cm), mean (±SD)	102.4 (13.2)	105.6 (16.1)	102.1 (12.7)	106.2 (11.5)	107.0 (12.6)	107.9 (10.3)
WsHtR, median (IQR)	0.6 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.7)	0.6 (0.6-0.6)	0.6 (0.6-0.6)	0.6 (0.6-0.6)
Moderate-vigorous index (METs/hour), median (IQR)	9.6 (4.8-16.8)	9.8 (7.2-14.6)	9.8 (7.2-14.6)	12.0 (7.4-19.4)	12.2 (7.4-21.6)	9.8 (7.4-14.6)
TEE (kcal/d), mean (±SD)	2297 (324)	2357 (353)	2266 (304)	3097 (461)	3116 (462)	3040 (400)
Total calories (kcal/d), mean (±SD)	1609 (547)	1916 (555)	2385 (583)	1832 (628)	1959 (592)	2347 (662)
Drink Status						
Never drinker	10 (10.0)	8 (10.5)	17 (19.5)	9 (7.0)	6 (5.6)	12 (11.1)
Former drinker	11 (11.0)	8 (10.5)	5 (5.8)	15 (11.6)	8 (7.5)	3 (3.7)
Light drinker	57 (57.0)	42 (55.3)	36 (41.4)	40 (31.0)	28 (26.2)	37 (34.3)
Moderate drinker	22 (22.0)	18 (23.7)	29 (33.3)	65 (50.4)	65 (60.8)	55 (50.9)
Total Alcohol (drinks/week), median (IQR)	1.0 (1.0-3.0)	1.0 (0.3-3.0)	1.0 (0.0-4.5)	3.3 (1.0-9.0)	5.3 (1.0-9.0)	3.6 (1.0-11.0)
Cigarettes Per Day, N (%)						
Nonsmoker	89 (89.0)	68 (89.5)	84 (96.6)	114 (88.4)	99 (92.5)	104 (96.4)
Moderate smoker	2 (2.0)	2 (2.6)	1 (1.2)	2 (1.6)	4 (3.7)	2 (1.8)
Heavy smoker	9 (9.0)	6 (7.9)	2 (2.3)	13 (10.1)	4 (3.7)	2 (1.8)

T2DM/IFG, N (%)	29 (29.0)	28 (36.8)	28 (32.2)	54 (41.9)	41 (38.3)	59 (54.6)
Hypertension, N (%)	29 (29.0)	17 (22.7)	30 (34.5)	36 (27.9)	41 (38.3)	42 (38.9)
Dyslipidemia at Exam 2, N (%)	54 (54.0)	37 (48.7)	42 (48.3)	86 (66.7)	68 (63.6)	66 (61.1)
Elevated TG: HDL Levels, N (%)	21 (21.0)	15 (19.7)	19 (21.8)	46 (35.7)	32 (29.9)	34 (31.5)

Abbreviations: BMI, body mass index; cm, centimeters; IQR, interquartile range; kcals/d, kilocalories per day; kg/m^2, kilograms per meter squared; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; SD, standard deviation; T, tertile; TEE, total energy expenditure; TG: HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 9 shows the sex-specific baseline characteristics according to tertiles of mDASH for individuals developing fibrosis without steatosis. The very small sample sizes in many of these categories may provide unreliable results. Overall, individuals with fibrosis without steatosis in tertile 3 of mDASH were more likely to have higher education levels, higher self-reported energy intakes (but not higher TEE levels), and less likely to be never-drinkers compared with those in tertile 1 of mDASH. Males with fibrosis without steatosis who were in the highest tertile of mDASH were more physically active and drank more alcohol intake per week compared with females who had fibrosis without steatosis.

Finally, Table 10 shows that individuals who developed both steatosis and fibrosis who were in the highest tertile of mDASH (vs. the lowest) were older, more frequently nonsmokers, and had a lower BMI and waist circumference. Males in the highest mDASH tertile also had higher incomes and were more cardiometabolically healthy than males in the lowest mDASH tertile. Females in this stage appeared to be older, less active, and less cardiometabolically healthy than males. When comparing the tables of each stage, there are a few differences that should be noted. Individuals with fibrosis without any steatosis had lower anthropometric measurements (especially females), higher intakes of alcohol weekly, and were cardiometabolically healthier compared to individuals with steatosis with or without fibrosis. As was shown in earlier tables, the greatest protective effects of mDASH were seen in those with both steatosis and fibrosis. These individuals generally had the highest anthropometric measurements and appeared

more cardiometabolically unhealthy than individuals with steatosis without fibrosis or fibrosis without steatosis. These results again suggest that anthropometric measures as well as lifestyle variables and CMR may be confounders or effect modifiers in these analyses.

Table 9: Sex-specific baseline characteristics by mDASH tertiles in those with hepatic fibrosis without steatosis.

	Hepatic fibrosis without steatosis (N=96)					
	Females (N=60)			Males (N=36)		
	T1 (9.0-21.5) N=16	T2 (22.0-26.5) N=14	T3 (27.0-39.5) N=30	T1 (8.0-21.0) N=9	T2 (22.0-26.0) N=14	T3 (27.0-38.0) N=13
Age, mean (±SD)	44.9 (8.0)	52.9 (9.9)	46.9 (9.2)	54.2 (8.5)	49.6 (10.5)	50.3 (10.2)
≥ Bachelor's degree, N (%)	9 (56.3)	7 (50.0)	23 (76.7)	5 (55.6)	9 (64.3)	10 (76.9)
Family income (≥ $100,000), N (%)	5 (31.3)	6 (42.9)	12 (40.0)	5 (55.6)	6 (42.9)	4 (30.8)
BMI (kg/m²), mean (±SD)	25.5 (3.3)	25.7 (3.1)	25.3 (5.2)	29.3 (4.4)	31.1 (8.6)	30.4 (8.1)
Waist Circumference (cm), mean (±SD)	90.6 (10.2)	91.8 (11.1)	87.7 (14.2)	103.0 (11.2)	107.1 (22.0)	103.9 (19.4)
WsHtR, median (IQR)	0.6 (0.5-0.6)	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.6 (0.6-0.6)	0.6 (0.5-0.7)	0.6 (0.5-0.7)
Moderate-vigorous index (METs/hour), median (IQR)	12.0 (6.0-13.5)	13.3 (9.6-17.0)	12.1 (7.2-14.8)	9.8 (7.4-19.6)	7.4 (4.8-12.2)	14.6 (7.4-24.4)
TEE (kcal/d), mean (±SD)	2226 (411)	2169 (351)	2191 (301)	2813 (437)	3041 (629)	3092 (464)
Total calories (kcal/d), mean (±SD)	1627 (372)	1898 (462)	2276 (541)	1299 (398)	1900 (539)	2484 (577)
Drink Status						
Never drinker	2 (12.5)	2 (14.3)	1 (3.3)	2(22.2)	1 (7.1)	0 (0.0)
Former drinker	0 (0.0)	1 (7.1)	2 (6.7)	1 (11.1)	1 (7.1)	1 (7.7)
Light drinker	9 (56.3)	5 (35.7)	16 (53.3)	0 (0.0)	2 (14.3)	3 (23.1)
Moderate drinker	5 (31.3)	6 (42.9)	11 (36.7)	6 (66.7)	10 (71.4)	9 (69.2)

Total Alcohol (drinks/week), median (IQR)	1.0 (1.0-6.8)	3.0 (1.0-6.3)	2.3 (1.0-5.0)	10.5 (0.0-12.0)	5.9 (1.0-8.8)	4.0 (3.0-10.0)
Cigarettes Per Day, N (%)						
Nonsmoker	14 (87.5)	14 (100.0)	29 (96.7)	8 (88.9)	14 (100.0)	11 (84.6)
Moderate smoker	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)
Heavy smoker	2 (12.5)	0 (0.0)	1 (3.3)	1 (11.1)	0 (0.0)	1 (7.7)
T2DM/IFG, N (%)	2 (12.5)	2 (14.3)	3 (10.0)	5 (55.6)	4 (28.6)	7 (53.9)
Hypertension, N (%)	1 (6.3)	2 (14.3)	6 (20.0)	6 (66.7)	4 (28.6)	3 (23.1)
Dyslipidemia, Exam 2, N (%)	10 (62.5)	4 (28.6)	6 (20.0)	5 (55.6)	5 (35.7)	6 (46.2)
Elevated TG: HDL Levels, N (%)	3 (18.8)	1 (7.1)	3 (10.0)	2 (22.2)	2 (14.3)	3 (23.1)

Abbreviations: BMI, Body Mass Index; cm, centimeters; IQR, interquartile range; kcals/d, kilocalories per day; kg/m^2, kilograms per meter squared; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; SD, standard deviation; T, tertile; TEE, total energy expenditure; TG: HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 10. Sex-specific baseline characteristics by mDASH tertiles in those with hepatic steatosis and fibrosis.

	Hepatic steatosis and fibrosis (N=122)					
	Females (N= 42)			Males (N=80)		
	T1 (9.0-21.5) N=14	T2 (22.0-26.5) N=18	T3 (27.0-39.5) N=10	T1 (8.0-21.0) N=41	T2 (22.0-26.0) N=19	T3 (27.0-38.0) N=20
Age, mean (±SD)	52.7 (8.2)	50.4 (7.3)	53.1 (8.0)	46.6 (33.6)	48.0 (7.0)	49.7 (7.7)
≥ Bachelor's degree, N (%)	4 (28.6)	9 (50.0)	5 (50.0)	15 (36.6)	11 (57.9)	11 (55.0)
Family income, (≥ $100,000), N (%)	4 (28.6)	7 (38.9)	3 (30.0)	17 (41.5)	11 (57.9)	12 (60.0)
BMI (kg/m²), mean (±SD)	34.6 (10.1)	35.7 (8.8)	33.1 (7.4)	33.6 (6.0)	31.3 (4.0)	32.8 (5.6)
Waist Circumference (cm), mean (±SD)	113.0 (18.5)	116.5 (15.8)	109.6 (17.3)	114.0 (14.1)	107.4 (10.6)	111.2 (12.8)
WsHtR, median (IQR)	0.6 (0.5-0.6)	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.6 (0.6-0.6)	0.7 (0.6-0.7)	0.6 (0.6-0.7)
Moderate-vigorous index (METs/hour), median (IQR)	10.9 (9.6-22.0)	9.8 (4.8-16.8)	9.7 (7.2-17.0)	12.2 (7.4-24.2)	12.2 (4.8-29.4)	12.2 (4.8-14.7)
TEE (kcal/d), mean (±SD)	2465 (342)	2487 (360)	2428 (390)	3291 (524)	3172 (570)	3067 (339)
Total calories (kcal/d), mean (±SD)	1503 (460)	1966 (491)	1967 (570)	1819 (680)	2217 (548)	2426 (717)
Drink Status						
Never drinker	2 (14.3)	3 (16.7)	2 (20.0)	2 (4.9)	1 (5.3)	2 (10.0)
Former drinker	3 (21.4)	1 (5.6)	3 (30.0)	3 (7.3)	1 (5.3)	6 (30.0)
Light drinker	6 (42.9)	10 (55.6)	4 (40.0)	18 (43.9)	4 (21.05)	4 (20.0)
Moderate drinker	3 (21.4)	4 (22.2)	1 (10.0)	18 (43.9)	13 (68.4)	8 (40.0)
Total Alcohol (drinks/week), median (IQR)	0.6 (0.0-3.0)	1.0 (0.5-3.0)	0.5 (0.0-1.0)	2.5 (1.0-9.0)	7.0 (1.0-13.3)	1.1 (0.0-5.0)
Cigarettes Per Day, N (%)						
Nonsmoker	12 (85.7)	17 (94.4)	9 (90.0)	36 (87.8)	18 (94.7)	20 (100.0)

Moderate smoker	1 (7.1)	1 (5.6)	1 (10.0)	1 (2.4)		
Heavy smoker	1 (7.1)			4 (9.8)	1 (5.3)	
T2DM/IFG, N (%)	6 (42.9)	11 (61.1)	6 (60.0)	24 (58.5)	8 (42.1)	7 (35.0)
Hypertension, N (%)	7 (50.0)	10 (55.6)	6 (60.0)	12 (29.3)	9 (47.4)	5 (25.0)
Dyslipidemia, Exam 2, N (%)	9 (64.3)	12 (66.7)	7 (70.0)	28 (68.3)	9 (47.4)	13 (65.0)
Elevated TG: HDL Levels, N (%)	6 (42.9)	3 (16.7)	3 (30.0)	14 (34.2)	6 (31.6)	4 (20.0)

Abbreviations: BMI, body mass index; cm, centimeters; IQR, interquartile range; kcals/d, kilocalories per day; kg/m^2, kilograms per meter squared; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; SD, standard deviation; T, tertile; TEE, total energy expenditure; TG: HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

The effect of mDASH tertiles on VCTE measurements of steatosis and fibrosis

To understand how mDASH scoring directly impacts degrees of steatosis and fibrosis, we examined the adjusted mean values of CAP, a measure of steatosis, and linear regression model results for CAP and LSM, as a measure of fibrosis. Table 11 looks at the adjusted mean CAP values for all individuals as well as stratified by sex in our models. Overall, when adjusting for age, sex, education, energy intake, cigarettes smoked per day, and drinking status, the mean CAP value decreased across the tertiles, with the highest tertile of mDASH lowering CAP by 12 dB/m ($Mean_{T3}$:261.45 dB/m, SE_{T3}:3.49; $Mean_{T1}$:273.56, SE_{T1}:3.31, p-trend <.0001) compared with the lowest tertile. When further adjusting for BMI, WsHtR, or CMR, the differences across tertiles of CAP were attenuated. When stratifying by sex, mDASH appeared to have a greater protective association with CAP in males than females, with males in the highest tertile having a CAP value that was 17.76 dB/m lower than that of males in the lowest tertile ($Mean_{T3}$: 268.70 dB/m, SE_{T3}:5.06; MeanT1: 286.46 dB/m, SE_{T1}:4.80; p-trend <.0001). It appeared that the mean CAP values were relatively similar across tertile 2 ad 3 for males. Although not as strong, there was still a protective association of mDASH with CAP, among females, with those in the highest tertile averaging a CAP of 253.90 dB/m (SE: 4.83) versus a CAP of 263.35 dB/m (SE: 4.57) in the lowest tertile of mDASH (p-trend <.0001). For every 5-point increase in mDASH, CAP decreased by nearly 5 dB/m (SE: 1.15; p-value <.0001), when adjusted for age, sex, education, energy intake, cigarettes per day, and drinking status (Table 12). LSM was highly skewed so its values were log-transformed for these analyses. For every 5-unit increase in mDASH score, log-

transformed LSM values decreased by 0.03 kPa (SE: 0.01; p-value=0.0007) when adjusted by demographic and lifestyle factors in model 1 (Table 12). These findings suggest that increasing adherence to mDASH may decrease liver steatosis and fibrosis.

Table 11: Adjusted mean values of CAP, a measure of liver fat, by mDASH tertiles

mDASH Score Tertiles	N	Mean (±SE)[1]	Mean (±SE)[2]	Mean (±SE)[3]	Mean (±SE)[4]
Overall					
T1 (8-21.5)	836	273.56 (±3.31)	271.51 (±2.91)	269.82 (±2.90)	270.90 (±3.20)
T2 (22–26.5)	869	263.32 (±3.32)	264.98 (±2.93)	264.09 (±2.92)	263.45 (±3.21)
T3 (27–39.5)	868	261.45 (±3.49)	265.61 (±3.07)	265.05 (±3.06)	262.01 (±3.37)
P-trend		<.0001	0.0323	0.0860	0.0026
Females					
T1 (9–21.5)	457	263.35 (±4.57)	259.20 (±3.97)	259.06 (±3.95)	260.62 (±4.38)
T2 (22–26.5)	455	257.75 (±4.56)	257.58 (±3.96)	257.39 (±3.93)	258.53 (±4.36)
T3 (27–39.5)	478	253.90 (±4.83)	257.84 (±4.20)	258.52 (±4.17)	255.90 (±4.62)
P-trend		<.0001	0.7051	0.8817	0.2271
Males					
T1 (8–21.0)	379	286.46 (±4.80)	278.65 (±3.06)	283.02 (±4.31)	284.07 (±4.70)
T2 (22–26.0)	414	269.72 (±4.89)	264.78 (±3.00)	272.18 (±4.38)	269.45 (±4.77)
T3 (27–38.0)	390	268.70 (±5.06)	264.45 (±3.13)	271.84 (±4.54)	268.32 (±4.94)
P-trend		<.0001	0.0012	0.0048	0.0002

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day), and drink status (never drinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for males).
Model 2: Model 1 + BMI
Model 3: Model 1 + WsHtR
Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels) Abbreviations:
BMI, body mass index; CAP, controlled attenuation parameter; CMR, cardiometabolic risk; HTN, hypertension; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; SE, standard error; T, tertile; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 12: Association between 5-unit increases in mDASH score and VCTE measurements

	β (±SE)[1]	β (±SE)[2]	β (±SE)[3]	β (±SE)[4]
CAP				
Sex-Specific mDASH Score	-4.95 (±1.15)	-2.63 (±1.02)	-2.04 (±1.01)	-3.42 (±1.12)
P-value	<.0001	0.0097	0.0443	0.0022
LSM				
Sex-Specific mDASH Score	-0.03 (±0.01)	-0.02 (±0.01)	-0.02 (±0.01)	-0.03 (±0.01)
P-value	0.0007	0.0141	0.0244	0.0035

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day), and drink status (never drinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for males).
Model 2: Model 1 + BMI
Model 3: Model 1 + WsHtR
Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels)
Abbreviations: BMI, body mass index; CAP, controlled attenuation parameter; CMR, cardiometabolic risk; HTN, hypertension; LSM, liver stiffness measure; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; OR, odds ratio; SE, standard error; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography; WsHtR, waist to height ratio.

Effect modifiers in the relationship between mDASH and hepatic steatosis

In Figure 4, we explored potential effect modification by anthropometric measures such as BMI and waist circumference, lifestyle factors such as physical activity, alcohol drinking, and smoking, and the presence of cardiometabolic dysfunction with the adherence to mDASH and risk of hepatic steatosis. Across many of these factors, there was evidence of effect modification on an additive scale for higher adherence to mDASH on the risk of hepatic steatosis. In terms of anthropometric measures, a healthy BMI (≤28 M; ≤ 25 F) or waist circumference (≤102 M; ≤88 F) alone was associated with 77% reduced odds of hepatic steatosis (95% CI_{BMI}: 0.16-0.32; 95% CI_{WC}: 0.16-0.32).

Individuals who had either a healthy BMI or a lower waist circumference combined with a higher adherence to mDASH had the strongest inverse association with the risk of steatosis (OR_{BMI}: 0.13, 95% CI: 0.10-0.17; OR_{WC}: 0.12, 95% CI: 0.09-0.16). High MV-PAI levels alone were not as protective as mDASH alone, with the two factors together resulting in 48% lower odds of steatosis (95% CI:0.39-0.68) compared to those with lower mDASH adherence and lower MV-PAI levels. Surprisingly, when assessing levels of weekly alcohol consumption, the greatest protective effect was observed in those with higher mDASH adherence and moderate alcohol consumption (>3 but ≤14 drinks/week for females; >3 but ≤21 drinks/week for males). These individuals had 44% reduced odds of steatosis (95% CI: 0.41-0.76). In those with lower mDASH adherence, there were non-statistically significant higher odds of steatosis associated with both light alcohol consumption and no alcohol consumption compared to those who moderately consumed alcohol and had a lower mDASH score. Among those with high adherence to mDASH, those who drank no alcohol had no reduction in the odds of steatosis. In regards to smoking, it appears that high mDASH adherence was protective in current, former, and nonsmokers, with the greatest protective effects being seen in nonsmokers (OR: 0.62; 95% CI: 0.39-1.00). Higher adherence to mDASH was associated with 25% lower odds of steatosis among those with at least one cardiometabolic outcome while a higher mDASH score resulted in 78% lower odds of steatosis among those with no presence of cardiometabolic dysfunction (95% CI: 0.16-0.29).

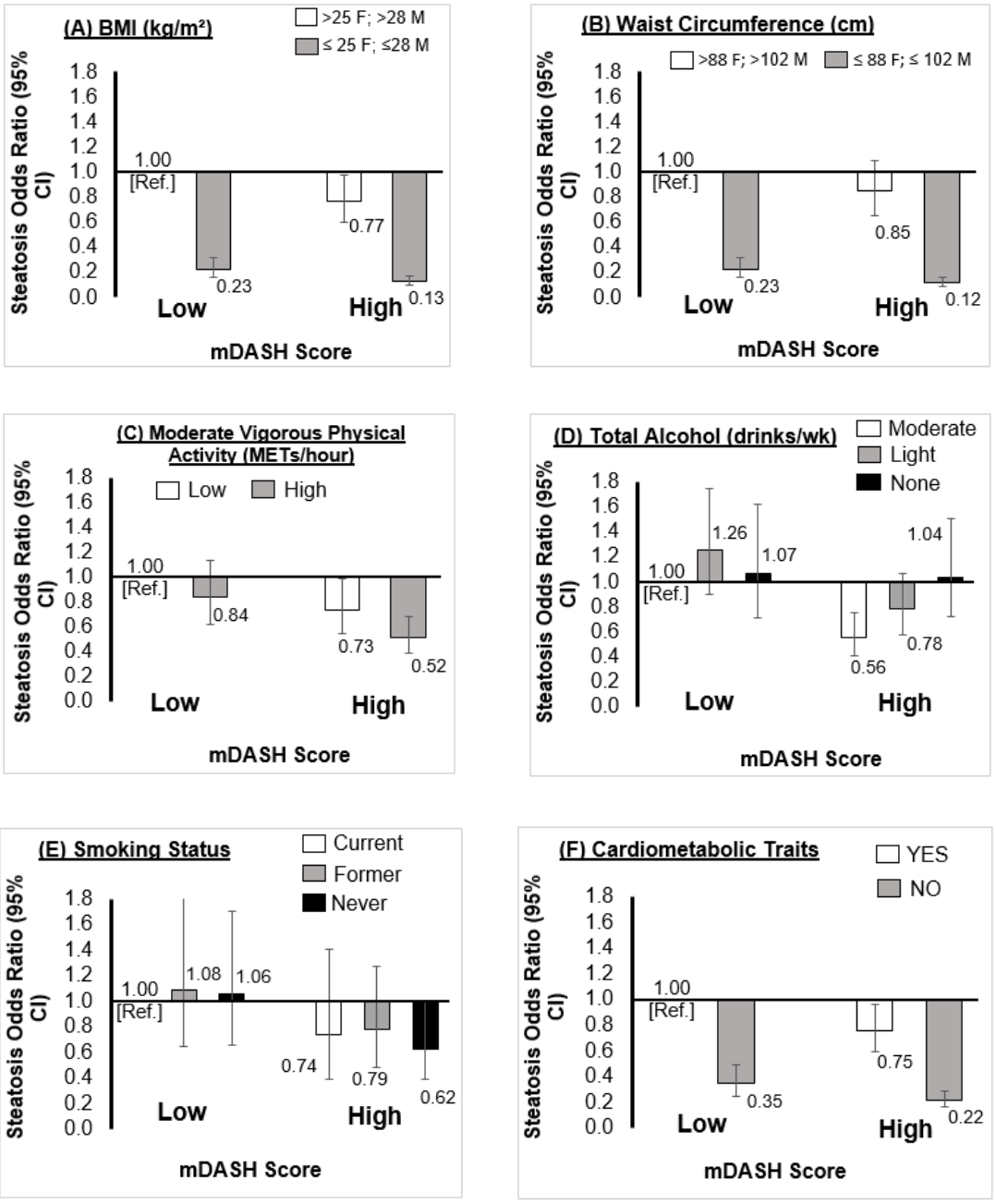


Figure 4: Modification of the association between adherence to mDASH and hepatic steatosis in Framingham VCTE cohorts. mDASH scores were classified as low adherence (Tertile 1: 8.0-21.5) and high adherence (Tertile 2 + Tertile 3: 22.0-39.5).
Analyses were adjusted for age, sex, education, energy intake, cigarettes per day (except analyses related to smoking status), and drinking status (except analyses related to alcohol status).
Abbreviations: BMI, body mass index; CI, confidence interval; cm, centimeters; F, females; kg/m², kilograms per meters squared; M, males; mDASH, modified Dietary Approaches to Stop

Hypertension; METs, metabolic equivalents; Ref, reference; VCTE, vibration controlled transient elastography.

Due to the strong protective effects seen with the lack of cardiometabolic dysfunction, figure 5 looks at the effect modification of mDASH adherence and steatosis by each trait individually. Across all cardiometabolic traits, evidence of effect modification was observed. In all traits, the effect of not having said cardiometabolic trait had a stronger protective association than mDASH alone. Similarly, in all traits, the combined effect of higher mDASH adherence and lack of cardiometabolic dysfunction resulted in the greatest protective effect. In all cases, mDASH still had protective effects even in the presence of adverse cardiometabolic traits. Such results suggest that various cardiometabolic traits such as T2DM, hypertension, dyslipidemia, and metabolic syndrome may have independent impacts on steatosis risk and therefore should be considered in prevention and treatment.

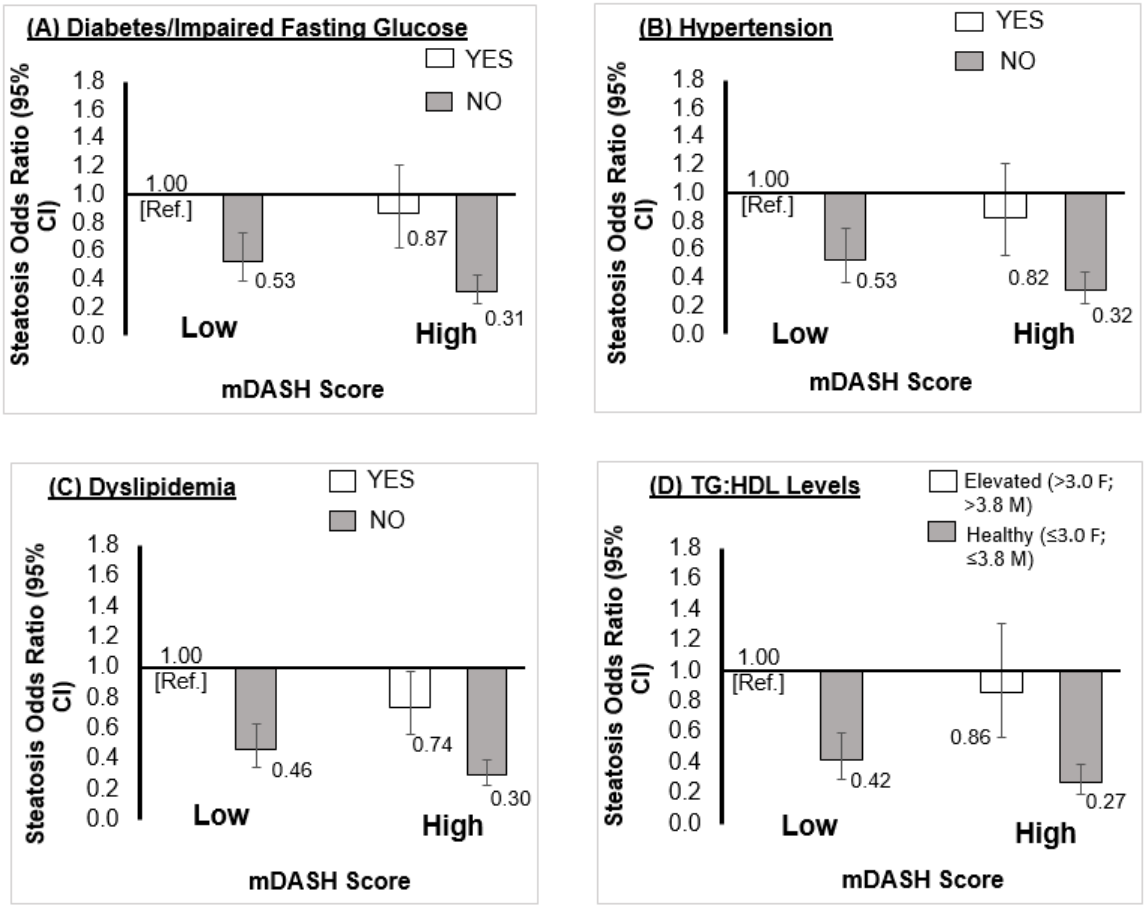
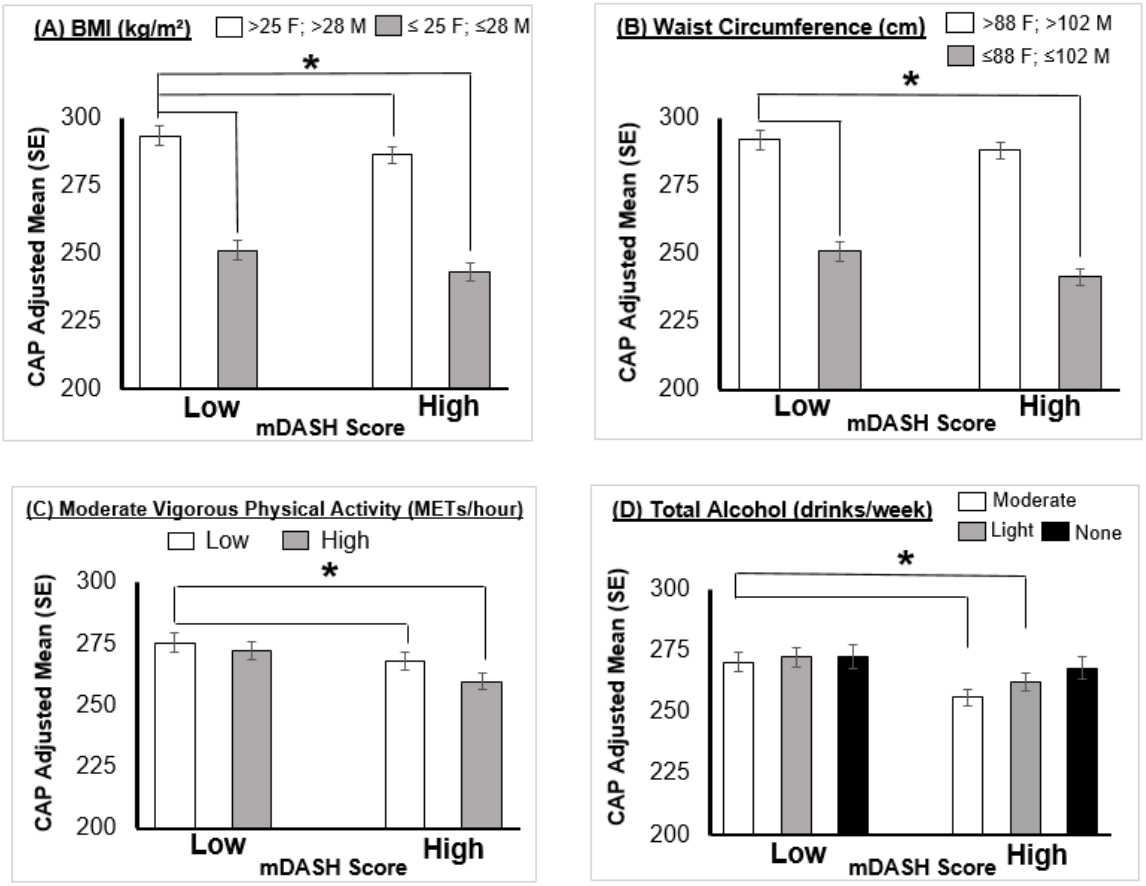


Figure 5: Cardiometabolic modification of the association between adherence to mDASH and hepatic steatosis in Framingham VCTE cohorts. mDASH scores were classified as low adherence (Tertile 1: 8.0-21.5) and high adherence (Tertile 2 + Tertile 3: 22.0-39.5).
Analyses were adjusted for age, sex, education, energy intake, cigarettes per day, and drinking status.
Abbreviations: CI, confidence interval; F, females; kg/m², M, males; mDASH, modified Dietary Approaches to Stop Hypertension; Ref, reference; TG: HDL, triglyceride to high-density lipoprotein ratio; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography.

Figure 6 addresses whether the effects of mDASH on mean CAP values were modified by other factors. In individuals who were overweight and had greater subcutaneous and visceral fat (higher BMIs/ or waist circumference, respectively), mDASH had minor protective effects on liver fat measures. With healthy anthropometric

values, the protective effects were relatively similar between lower and higher mDASH adherence with all findings being statistically significant. However, the strongest protective effect was seen with higher adherence ($CAP_{LOW\ BMI+LOW\ mDASH}$: 251.2, SE: 3.5; $CAP_{LOW\ BMI+HIGH\ mDASH}$: 243.2, SE: 3.1; $CAP_{LOW\ WC+LOW\ mDASH}$: 251.0, SE: 3.5; $CAP_{LOW\ WC+HIGH\ mDASH}$: 241.6, SE: 3.1). Protective effects were observed in those with higher mDASH adherence regardless of physical activity level, suggesting that diet may have greater protective effects than physical activity. However, high mDASH adherence with higher physical activity levels had lower odds of steatosis than those with lower physical activity levels. Those with lower mDASH adherence with or without alcohol consumption had no meaningful difference in CAP values while higher adherence to mDASH with or without alcohol consumption was associated with lower CAP. Moderate drinkers with high mDASH adherence saw the greatest protective association with CAP; their average CAP was 14.2 dB/m (SE: 3.2, p-value <.0001) lower than that of individuals with lower mDASH adherence and moderate consumption. Light drinkers with high mDASH adherence had a CAP that was lower by 7.9 dB/m (SE: 3.4, p-value: 0.0367) compared to those with low mDASH adherence and moderate alcohol consumption. Similar to alcohol consumption, mDASH was shown to be associated with lower CAP levels regardless of smoking status. Nonsmokers with higher mDASH adherence had the lowest CAP levels by 20.6 dB/m (SE: 1.9, p-value: 0.0005) compared to those with low mDASH adherence who were current smokers. The lack of any prevalent cardiometabolic dysfunction was associated with a dramatically lower CAP (by 34 dB/m, SE: 3.9, p-value <.0001) compared to those with cardiometabolic dysfunction

who had low mDASH adherence, while higher mDASH adherence had little effect on its own. For individual cardiometabolic characteristics, those without a particular trait had lower CAP values regardless of diet. Nonetheless, higher (vs. lower) mDASH adherence among those with some cardiometabolic dysfunction was associated with a non-statistically significant lowering of CAP values (figure 7).



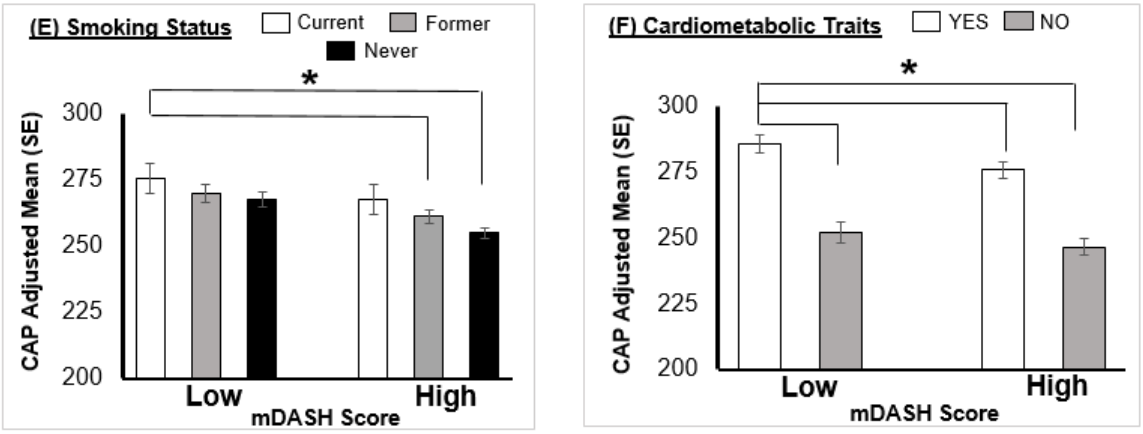


Figure 6: Modification of the association between adherence to mDASH and CAP in Framingham VCTE cohorts. mDASH scores were classified as low adherence (Tertile 1: 8.0-21.5) and high adherence (Tertile 2 + Tertile 3: 22.0-39.5). Analyses were adjusted for age, sex, education, energy intake, cigarettes per day (except analyses related to smoking status), and drinking status (except analyses related to alcohol status). Bar(s) with an asterisk above (*) represent statistically significant results (p-value < 0.05).
Abbreviations: BMI, body mass index; CAP, controlled attenuation parameter; cm, centimeters; F, females; kg/m², kilograms per meters squared; M, males; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; SE, standard error; VCTE, vibration controlled transient elastography.

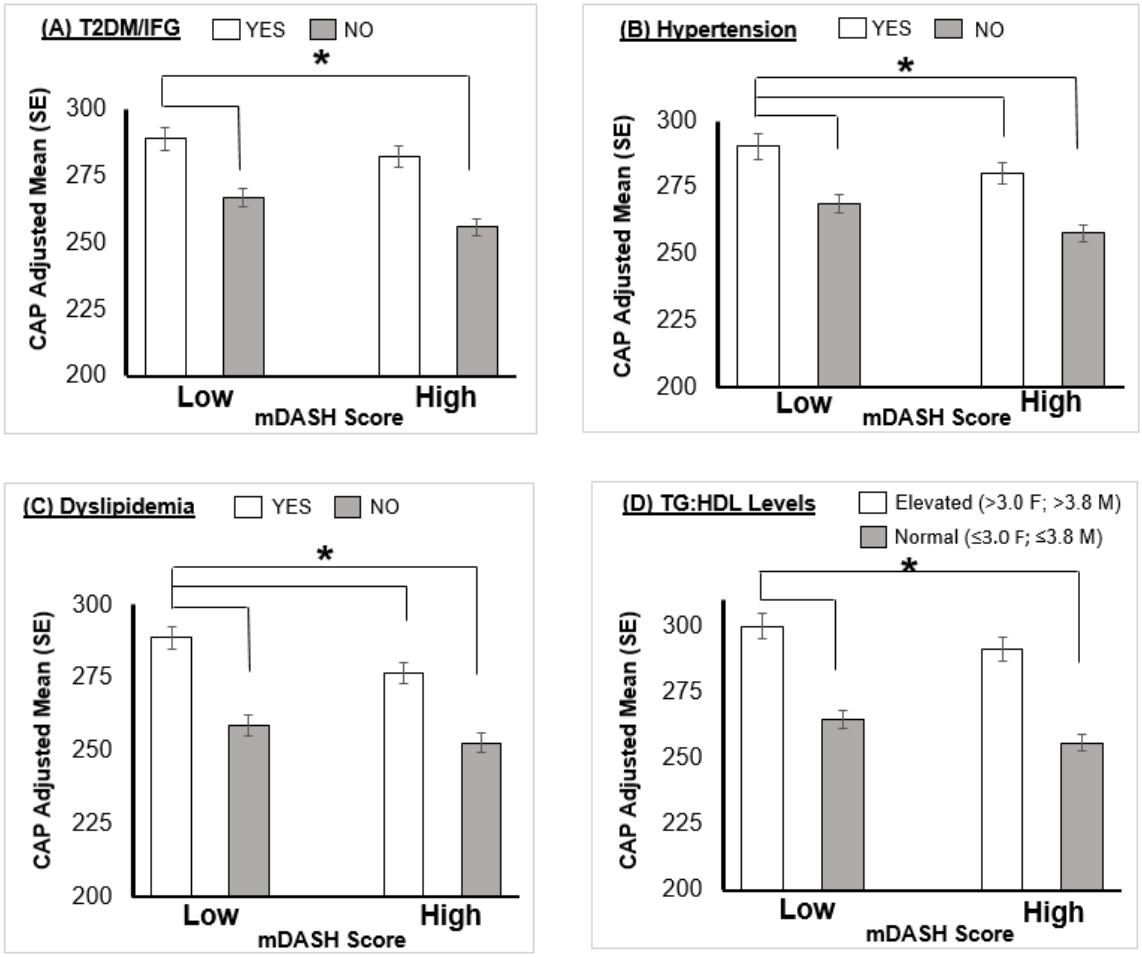


Figure 7: Cardiometabolic modification of the association between adherence to mDASH and CAP in Framingham VCTE cohorts. mDASH scores were classified as low adherence (Tertile 1: 8.0-21.5) and high adherence (Tertile 2 + Tertile 3: 22.0-39.5).
Analyses were adjusted for age, sex, education, energy intake, cigarettes per day, and drinking status. Bar(s) with an asterisk above (*) represent statistically significant results (p-value < 0.05).
Abbreviations: CAP, controlled attenuation parameter; cm, F, females; M, males; mDASH, modified Dietary Approaches to Stop Hypertension; SE, standard error; TG: HDL, triglyceride to high-density lipoprotein ratio; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography.

Sensitivity Analysis: Adding Heavy Drinkers

Tables 13-15 show results from sensitivity analyses done to determine the effect of adding heavy drinkers back into the analysis. Adding 118 heavy drinkers back into the sample did not change the baseline characteristics across the tertiles of mDASH. Individuals in the highest tertile of mDASH had higher educational levels, higher income levels, lower BMI and WsHtRs, higher energy intakes, and higher MV-PAI compared to those in the lowest tertile of mDASH. Individuals in the highest tertile category of mDASH were also less likely to smoke, had lower rates of T2DM/IFG, and elevated TG: HDL ratios than those in the lowest tertile of mDASH. Compared to the primary analysis, when running the logistic regression models to assess the association between mDASH and hepatic steatosis, little change occurred (Table 14). Individuals in tertile 2 or 3 of mDASH had 44% lower odds (95% $CI_{T2:}$ 0.53-0.82; 95% $CI_{T3:}$ 0.52-0.84) compared to those in tertile 1 of mDASH. When stratified by sex, little change was observed in females while male odds ratios were attenuated.

Table 15 looks at the association between mDASH adherence and NAFLD stages, with heavy drinkers included in the sample. Compared to primary analyses the prevalence of steatosis without fibrosis, fibrosis without steatosis, or steatosis with fibrosis barely changed. Individuals in tertile 3 of mDASH had 29% (95% CI: 0.55-0.92) lower odds of steatosis without fibrosis compared to those in tertile 1. No association was seen in individuals with fibrosis without steatosis. In individuals with steatosis with (p-trend: 0.0118) or without fibrosis (p-trend: 0.0036), the odds of NAFLD status declined

across the tertiles of mDASH, with p-trends being statistically significant. Further adjusting for BMI, WsHtR or CMR attenuated results in all NAFLD stages. Based upon this, it can be concluded that adding heavy drinkers back in did not significantly change the results of the primary analysis. mDASH remained protective against NAFLD stages when adding heavy drinkers back into the analysis. Only 118 individuals were heavy drinkers in this study, and therefore it is expected that adding them back into analyses will not drastically affect the primary results.

Table 13. Baseline characteristics by mDASH tertiles in the Framingham VCTE Cohorts with heavy drinkers included in the sample (N=2691)

	Tertile 1 (8.0-21.5) N=878	**Tertile 2 (22.0-26.5) N=911**	**Tertile 3 (27.0-39.5) N=902**
Females, N (%)	471 (53.6)	474 (52.0)	491 (54.4)
Age, mean (±SD)	45.2 (8.8)	46.5 (8.9)	48.0 (9.4)
≥ Bachelor's degree, N (%)	426 (48.5)	550 (60.4)	613 (68.0)
Family income (≥ $100,000), N (%)	331 (37.7)	415 (45.6)	434 (48.1)
Non-Hispanic White, N (%)	830 (94.5)	870 (95.5)	862 (95.6)
BMI (kg/m^2), mean (±SD)	28.2 (5.7)	27.5 (5.4)	27.0 (5.0)
WsHtR (kg/m), median (IQR)	0.6 (0.5-0.6)	0.5 (0.5-0.6)	0.5 (0.5-0.6)
mDASH Score, mean (±SD)	18.2 (2.6)	24.1 (1.4)	30.2 (2.5)
TEE (kcal/d), mean (±SD)	2587 (561)	2586 (546)	2540 (509)
Total calories (kcal/d), mean (±SD)	1690 (578)	1970 (585)	2336 (609)
Moderate-vigorous index (METs/hour), median (IQR)	9.8 (7.2-17.0)	12.0 (7.4-17.0)	12.0 (7.4-17.0)
Current Smoker, N (%)	112 (12.8)	70 (7.7)	31 (3.4)
Cigarettes Per Day, mean (±SD)	1.8 (5.4)	0.7 (3.3)	0.3 (2.1)
T2DM/IFG, N (%)	299 (28.8)	215 (23.6)	225 (24.9)

Hypertension, N (%)	189 (21.6)	184 (20.2)	203 (22.5)
Dyslipidemia at Exam 2, N (%)	405 (46.1)	352 (38.6)	330 (36.6)
Elevated TG:HDL ratio, N (%)	168 (19.1)	117 (12.8)	114 (12.6)

Abbreviations: BMI, body mass index; d, day; IQR, interquartile range; mDASH, modified Dietary Approaches to Stop Hypertension; METs, metabolic equivalents; N, sample size; SD, standard deviation; TEE, total energy expenditure; TG:HDL, Triglyceride to HDL Ratio; T2DM/IFG, Type 2 diabetes mellitus or impaired fasting glucose; WsHtR, waist to height ratio.

Table 14. Adjusted ORs for the association between mDASH tertiles and hepatic steatosis in FHS VCTE cohorts with heavy drinkers included in the sample (N=2691)

mDASH Score tertiles	Cases	Prevalence (%)	N	OR (95% CI)[1]	OR (95% CI)[2]	OR (95% CI)[3]	OR (95% CI)[4]
Overall							
T1 (8.0-21.5)	299	34.05	878	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22-26.5)	235	25.8	911	0.66 (0.53-0.82)	0.73 (0.57-0.92)	0.75 (0.59-0.96)	0.73 (0.58-0.91)
T3 (27-39.5)	236	26.16	902	0.66 (0.52-0.84)	0.79 (0.1-1.03)	0.83 (0.64-1.08)	0.73 (0.57-0.93)
P-trend				0.0009	0.0892	0.1849	0.0127
Females							
T1 (9–21.5)	117	24.84	471	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.5)	96	20.25	474	0.70 (0.50-0.96)	0.80 (0.55-1.15)	0.79 (0.54-1.14)	0.80 (0.57-1.12)
T3 (27–39.5)	100	20.37	491	0.59 (0.41-0.85)	0.80 (0.53-1.21)	0.82 (0.54-1.25)	0.70 (0.48-1.03)
P-trend				0.0056	0.2950	0.3696	0.0729
Males							
T1 (8–21.0)	182	44.72	407	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.0)	139	31.81	437	0.61 (0.45-0.82)	0.65 (0.48-0.90)	0.71 (0.51-0.97)	0.65 (0.48-0.87)
T3 (27–38.0)	136	33.09	411	0.66 (0.48-0.90)	0.73 (0.52-1.03)	0.78 (0.55-1.10)	0.69 (0.50-0.96)
P-trend				0.0134	0.0897	0.1774	0.0356

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day) and drink status (neverdrinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for males).

Model 2: Model 1 + BMI

Model 3: Model 1 + WsHtR

Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels)

Abbreviatons: BMI, body mass index; CI, confidence intervals; CMR, cardiometabolic risk; HTN, hypertension; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; OR, odds ratio; Ref., refernce; T, tertile; T2DM/IFG, type 2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography; WsHtR, waist to height ratio.

Table 15. Adjusted ORs for the association between mDASH tertiles and NAFLD stages in FHS VCTE cohorts with heavy drinkers included in the sample (N=2691)

mDASH Score tertiles	Cases	Prevalence (%)	N	OR (95% CI)[1]	OR (95% CI)[2]	OR (95% CI)[3]	OR (95% CI)[4]
Hepatic Steatosis without Fibrosis							
T1 (8.0-21.5)	240	27.33	878	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22-26.5)	196	21.51	911	0.68 (0.54-0.86)	0.75 (0.58-0.96)	0.78 (0.61-1.01)	0.74 (0.58-0.93)
T3 (27-39.5)	202	22.39	902	0.71 (0.55-0.92)	0.86 (0.65-1.14)	0.90 (0.68-1.19)	0.77 (0.60-1.01)
P-trend				0.0118	0.3364	0.5028	0.0663
Hepatic Fibrosis without Steatosis							
T1 (9–21.5)	29	3.3	878	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.5)	30	3.29	911	0.87 (0.50-1.49)	0.92 (0.53-1.59)	0.94 (0.54-1.62)	0.86 (0.50-1.49)
T3 (27–39.5)	45	4.99	902	1.30 (0.74-2.27)	1.41 (0.80-2.48)	1.45 (0.82-2.56)	1.29 (0.74-2.27)
P-trend				0.2803	0.1764	0.1515	0.2865
Hepatic Steatosis and Fibrosis							
T1 (8–21.0)	59	6.72	878	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
T2 (22–26.0)	39	4.28	911	0.56 (0.36-0.88)	0.70 (0.43-1.16)	0.78 (0.47-1.29)	0.61 (0.39-0.96)
T3 (27–38.0)	34	3.77	902	0.47 (0.29-0.78)	0.65 (0.38-1.13)	0.74 (0.42-1.29)	0.52 (0.31-0.87)
P-trend				0.0036	0.1296	0.2855	0.0132

Model 1 adjusted for age, sex, education, energy intake, cigarettes per day (nonsmokers, 0 cigarettes/day; (b) >0-<10 cigarettes/day; (c) ≥ 10 cigarettes/day) and drink status (neverdrinkers; (b) former drinkers; (c) ≤3 drinks/week; (d) >3-≤14 drinks/week for females or >3-≤21 drinks/week for
males).
Model 2: Model 1 + BMI
Model 3: Model 1 + WsHtR
Model 4: Model 1 + CMR (any of the following: T2DM/IFG, HTN, dyslipidemia or elevated TG:HDL levels)
Abbreviatons: BMI, body mass index; CI, confidence intervals; CMR, cardiometabolic risk; HTN, hypertension; mDASH, modified Dietary Approaches to Stop Hypertension; N, sample size; OR, odds ratio; Ref., refernce; T, tertile; T2DM/IFG, type

2 diabetes mellitus or impaired fasting glucose; VCTE, vibration controlled transient elastography; WsHtR, waist to height ratio.

DISCUSSION

This prospective cohort study is one of few studies assessing an mDASH dietary pattern in association with hepatic steatosis and fibrosis. Overall, we found that adherence to mDASH was associated with lower odds of steatosis with or without fibrosis among females and males aged 24 and older. Males saw greater protective effects from mDASH when further adjusting for BMI, WsHtR, or CMR, with their odds of hepatic steatosis being lower than that of females in this study. However, when adjusting for age, sex, education, energy intake, smoking, and drinking status, females in tertile 3 of mDASH had lower odds of hepatic steatosis than males in tertile 3 of mDASH. There was no significant association between mDASH adherence and odds of hepatic fibrosis without steatosis. Higher adherence to mDASH also had protective effects on CAP, a measure of liver fat, in females and males aged 24 and older. This is one of few studies that demonstrates a protective effect of an mDASH dietary pattern on distinct stages of NAFLD. Similar findings were seen in a paper by Gao et al[32], where increased adherence to a DASH dietary pattern was associated with reduced odds of hepatic steatosis with or without fibrosis however there was no association seen with hepatic fibrosis without steatosis. Another study by Yiannakou[104] had also shown that individuals in tertile 3 of mDASH had 29% (95% CI:0.53-0.95) lower risk of NAFLD than those in tertile 1 when adjusting for age, sex, energy intake, sodium, current smokers, education, multivitamin use, and alcohol intake.

We found that participants in the highest category of mDASH were older adults with higher education, income, and healthier lifestyle characteristics such as lower BMI and lower amounts of current smokers and cigarettes per day. Interestingly, we also observed that the baseline characteristics of those in each stage of NAFLD were relatively different from one another. Specifically, those with fibrosis without any steatosis appeared to be more cardiometabolically healthy, with lower BMIs and CMR than other stages. Our unhealthiest stage was those with hepatic steatosis and fibrosis, where increased BMI, waist circumference, and CMR were observed. However, in all stages of NAFLD and all tertiles of mDASH adherence, there were cardiometabolically unhealthy individuals. Cardiometabolic dysfunction is an expected occurrence amongst individuals with steatosis or fibrosis, as NAFLD has been described as the hepatic manifestation of MetS[60]. Another key characteristic to point out is that the mean age in all stages of NAFLD for females varied from 44-53 years old, which is around the age of menopause. It has been found across many papers[20,63], that the risk of NAFLD significantly increases to similar rates or greater rates than that of males their age in post-menopausal women. Part of this association occurs due to the decreased estrogen production in post-menopausal women which alters their visceral fat and can put them at risk for NAFLD[20]. It could be possible that menopause could explain some of the reasons as to why the odds of NAFLD are slightly higher in females than males in this study, however, we did not look into menopause.

When assessing adherence to mDASH and its association with VCTE measurement of liver fat (CAP) and liver stiffness/fibrosis (LSM), we found that mDASH was significantly associated with lowering CAP and LSM. CAP lowered across the tertiles of mDASH, with females having lower CAP values than males. However, mDASH had lowered CAP more from tertile 1 to tertile 3 of mDASH in males than females. Similar results were seen by Gao et al[32] when assessing the association between DASH and CAP/LSM. They found that a one standard deviation increase in DASH led to 10.9 dB/m lower CAP and 4.6% lower LSM. Although mDASH had no association with hepatic fibrosis without steatosis, mDASH did effectively lower LSM as well as the odds of steatosis with fibrosis. Multiple studies have shown that improved diet quality results in improvements or decreased odds of fibrosis[15,85,91,96]. Individuals in our study with hepatic fibrosis without steatosis had distinctly different baseline characteristics and appeared to have different odds of disease than all others. It may be possible that their disease may be an effect of viral causes such as hepatitis C, however this cannot be confirmed. It should also be noted that there were only 96 individuals in this stage and therefore the power was insufficient to detect differences.

Overall, mDASH is successful as a dietary pattern for NAFLD not because of specific score components but because of the collective effect of the diet as a whole. Many studies have found that improved diet quality across a variety of dietary interventions (MED, DASH, HEI, AHEI) is associated with lower risk or prevalence of NAFLD[11,57,58,67,91]. Specifically, improving diet quality includes increased consumption

of fruits, vegetables, and healthy fats and decreased consumption of processed foods and sugars. mDASH promotes increased consumption of vegetables, fruits, dairy, whole grains, legumes, nuts and seeds, and fish and decreased consumption of red and processed meats, and sugar-sweetened beverages. Dietary recommendations by both the AASLD[79] and the EASL-EASD-EASO[23] include avoiding foods and beverages high in fructose, excess saturated fats, and refined carbohydrates. Furthermore, a systematic review of studies on dietary components and their role in the development of NAFLD[97] suggests that dietary patterns to prevent or treat NAFLD may wish to promote the exclusion of fructose and SSBs, reduce red and processed meats, and increase consumption of total dairy, complex carbohydrates, vegetables, fruits, whole grains, legumes, nuts & seeds, and fish. An extensive amount of research has shown the negative effects of high fructose consumption via foods or SSBs. The negative effects include[4,80,84] insulin resistance, intrahepatic lipid accumulation, hypertriglyceridemia, DNL, dyslipidemia, and increased visceral adipose tissue which all lead to the increased risk and prevalence of NAFLD as seen with fructose and SSB consumption. Diets high in ultra-processed foods were found to be associated with increased risks of NAFLD and insulin resistance[52,] most likely due to the added sugars in these foods but also their association with higher calorie consumption and obesity. As mDASH promotes reduced consumption of SSBs and the consumption of whole foods instead of processed foods, it is likely that part of the protective effect seen comes from the lack of negative cardiometabolic effects described above, as well as the promotion of a healthy weight.

Effect modification analysis revealed that mDASH was most protective against hepatic steatosis in those with lower BMIs (≤ 25 F; ≤ M) and waist circumferences (≤ 88 F; ≤ 102 M), however it remained protective in those with high BMI and waist circumference. Although lower adiposity measures alone were protective, the odds further decreased when comparing high mDASH adherence to low mDASH adherence. It has been understood that visceral adipose tissue (VAT) is a strong risk factor for NAFLD[58], with weight reductions of 3-5% improving steatosis while reductions of 7-10% leading to improvements in NASH and fibrosis [28,79,80,84]. Weight loss is effective as NAFLD itself is a disease of hepatic fat accumulation, and reducing weight can affect other cardiometabolic factors closely related to obesity. When assessing diet quality and NAFLD, both BMI and waist circumference are important factors in the effect being seen. A study by Maskarinec[58] found that HEI-2010, AHEI-2010, MED, and DASH dietary patterns were all inversely associated with decreased adiposity measures. Ma et al[57] found that the effects of MED and AHEI scores were attenuated by BMI. We also saw that mDASH was most protective against steatosis in those with higher physical activity. In regards to smoking, mDASH was protective against steatosis in current and former smokers but was most protective in those who were nonsmokers. A significant finding was that although mDASH was most protective when no CMR was present, it was still protective against steatosis with CMR present. This is significant as NAFLD often occurs in conjunction with a variety of cardiometabolic traits, including T2DM, MetS, and CVD. Lonardo et al[53] assessed the differences amongst metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO) individuals and found that

MHO have an increased overall risk of NAFLD and disease progression compared to normal weight healthy individuals however this risk is much lower than that of MUO individuals. Furthermore, MUO individuals have a higher risk of mortality compared to MHO individuals suggesting that cardiometabolic comorbidities may impose greater risk on NAFLD individuals than obesity but both play crucial roles in NAFLD and its progression. Successful dietary interventions are those that treat both factors and mDASH appears to do so.

Studies assessing alcohol and its effects on NAFLD have had mixed findings. In this study, we saw that mDASH was most protective against steatosis in moderate drinkers (>3-≤14 drinks/week in females; >3-≤21 drinks/week in males) followed by light drinkers; mDASH was least protective in our non-drinkers. These findings are contradictory to NAFLD guidelines from both the AASLD[79] and EASL-EASD-EASO[23], which recommend alcohol consumption being kept to a minimum (<20 g/day for females; <30 g/day for males) and mention that alcohol consumption above this can increase risk of NAFLD. Similar to our findings, studies by Hiramine[37] and Sookian et al[86], found that moderate alcohol consumption had reduced odds of fatty liver[37] or NAFLD[86] compared to non-drinkers. A cross-sectional study assessing alcohol use in FHS subjects with hepatic steatosis found that the total number of drinks per week as well as the maximum number of drinks in one sitting were associated with increased odds of hepatic steatosis (OR:1.15, 95% CI: 1.02-1.29 and OR: 1.15, 95% CI: 1.02-1.98)[3]. A review by Kulkarni and Sarin[47] summarizes the various detrimental effects alcohol consumption can have including increased risk of insulin resistance, IFG, HCC, and progression of NAFLD.

Studies have shown that modest alcohol consumption can lead to fatty liver, with 20 g/day of alcohol worsening disease and liver-related mortality[14]. Alcohol may have such mixed findings due to its association with extrahepatic outcomes, including CVD and cancer. Previous studies on the association of alcohol and CVD have suggested that alcohol may be protective and reduce overall risk[14], while studies on cancer show that alcohol increases incidence and risk[14]. The difficulties with studying alcohol consumption and NAFLD have been discussed before and were featured in an editorial in *Gastroenterology & Hepatology*[2]. Dr. Michelle Long explains that the characteristics of moderate drinkers as well as the comparison groups when assessing alcohol can lead to inaccurate findings[2]. Oftentimes, moderate drinkers can be more highly educated, of a higher income status, exercise more, or have leaner BMIs than nondrinkers. Furthermore, moderate drinkers may be compared to nondrinkers which are comprised of former and never drinkers. In this instance, former drinkers may have poorer outcomes as they could have possibly been problematic or heavy drinkers in the past and therefore may lead to current drinkers appearing healthier than them. The alcohol findings in this study should be carefully considered. Although it appeared that adding heavy drinkers back into the analysis had little effect, it is important to note that they were excluded based on drinking patterns reported at one examination and therefore it is not an accurate representation of lifetime drinking behaviors.

This study has several strengths and limitations. One strength of this study is its prospective design which allowed us to assess risk factors and covariates up to eight

years before the VCTE measurements of the liver. All measures of adiposity were direct and therefore more accurate than self-reported data. Many limitations exist with the VCTE measurements of the liver, including that measurements can be inaccurate in the presence of ascites, extra fat in the chest wall, or morbid obesity. FHS investigators had excluded any participants with known liver problems, ascites, or wounds to their chest wall which eliminates this potential bias. Furthermore, an extra-large probe exists for the VCTE that addresses the limitations in measuring those who have greater adiposity[24,25,54]. As a highly accurate non-invasive measure with multiple measurements obtained at the examination, VCTE can be seen as a strength in this study rather than a limitation. Potential confounding variables were thoroughly examined which may minimize the confounding effects in this study.

A major limitation of this study is the use of FFQs. Two concerns include the lack of validation for key nutrients of the components of the mDASH score (calcium, magnesium, and potassium), as well as recall bias. Energy intake is a huge source of error in FFQs and is likely one in this study. Another limitation is the self-reporting of various lifestyle characteristics where there could be misclassification errors as participants may have misreported. A major misclassification error present in this study is alcohol and the exclusion of heavy drinkers. We did not have lifetime alcohol information and therefore it cannot be said that drinking patterns as reported (at exam 2) in this study were followed over the lifetime. Heavy drinkers were excluded based on drinking patterns reported at exam 2, where the rest of our exposures came from. It may be possible that these

individuals were not heavy drinkers prior to or following exam 2. We did have insufficient numbers of cases of fibrosis, especially fibrosis without steatosis. We only had 96 participants who had fibrosis without steatosis, which affects the power of our analysis. Finally, this study is generalizable to non-Hispanic white males and females aged 24 and older, as our cohorts were predominantly non-Hispanic whites. More research should be done on a more ethnically diverse population.

CONCLUSIONS

This is one of few studies looking into a DASH dietary pattern as measured by mDASH in association with NAFLD. Overall, we found that an mDASH dietary pattern was protective against steatosis with or without fibrosis and both moderate and higher adherences were able to lower measures of steatosis and risk of steatosis in non-Hispanic white females and males aged 24 and older. mDASH remained protective in high-risk individuals, including those with higher BMI or waist circumference, lower physical activity, or prevalent cardiometabolic dysfunction. As a disease with no current therapeutics where dietary interventions or patterns are key, such findings add to this literature and suggest that mDASH may be a successful dietary pattern in those with NAFLD. However, further studies should be done to confirm the effectiveness of mDASH and further assess its effectiveness on more advanced stages of disease, such as fibrotic NASH or cirrhosis.

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