The Association of Metabolic Syndrome Severity with Outcomes and Survival in Non-Alcoholic Fatty Liver Disease: A Population Study of Adults in the United States

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ABSTRACT OF THE DISSERTATION

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Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common cause of chronic liver disease. The current global prevalence of NAFLD is 25%, while 26% of adults in the United States (US) are estimated to have NAFLD. At Present, NAFLD related fibrosis is projected to be the leading cause of liver transplantation in the coming years. NAFLD patients have a 1.7-fold increased risk of mortality, yet there are no pharmacologic or other modalities of treatment for this disease. Due to the primary function obesity-induced insulin resistance plays in promoting liver steatosis, NAFLD is regarded as the hepatic manifestation of Metabolic Syndrome (MetS). Despite knowledge of the association between MetS related metabolic abnormalities and NAFLD, it is not known why only some MetS patients develop NAFLD. It is also unknown as to why some NAFLD patients progress to more severe hepatic manifestation, while others do not. Furthermore, the impacts of MetS severity on the risk of mortality in NAFLD are not fully explained. Previously conducted research has solely focused on recognizing the presence of MetS as a risk factor for disease progression without accounting for the effects of its severity on the increased risk of

morbidity and mortality in NAFLD. The limitations of the dichotomous definition of MetS in relation to outcomes in NAFLD could be fully addressed by using a continuous measure of MetS severity that encapsulates the effects of all five metabolic features in one summary risk score.

Specific Aims: The main objectives of this dissertation were to utilize the MetS severity score, a validated gender-race specific Z-score, to assess the association between MetS severity and 1) the odds of NAFLD occurrence, 2) the odds of advanced fibrosis presence in NAFLD, and the risks of all-cause mortality, heart disease-related mortality, diabetes-related mortality and hypertension-related mortality in NAFLD.

Methods and Materials: The study included 10,605 adults ages 20 to 74 years who participated in the Third National Health and Nutrition Examination Survey and met all inclusion and exclusion criteria. All five metabolic features (*i.e.*, high-density lipoprotein, systolic blood pressure, waist circumference, triglycerides, and blood glucose) were used to calculate gender-race specific MetS severity Z-scores, which were then transformed into four percentiles-based categories [mild (0^{th}-50^{th}), moderate (>50^{th}-75^{th}), high (>75^{th}-90^{th}), very high (>90^{th}+)]. NAFLD was defined as mild, moderate, or severe hepatic steatosis on ultrasound in the absence of hepatitis B virus, hepatitis C virus, iron overload, and excessive alcohol intake. The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) was used to estimate the probability of advanced fibrosis presence. An individual NFS value of ≥ -1.455 was used to define an intermediate to high advanced fibrosis probability, while a score of >0.676 was classified as a high probability of advanced fibrosis. A validated matching algorithm was used to link participants' baseline characteristics with mortality outcomes obtained from the National Death Index database. Both adjusted multivariable logistic

regression models and Cox proportional hazard models were used to examine the associations between MetS severity and the odds of NAFLD, odds of advanced fibrosis in NAFLD, and the risk of mortality in NAFLD.

Results: At baseline, the prevalence of NAFLD was 26.7% (95% CI; 24.3% – 29.1%). Stratified by race/ethnicity, NAFLD prevalence was 26.7%, 23.3%, and 33.7% amongst White non-Hispanics, Black non-Hispanics, and Mexican Americans, respectively. We observed racial/ethnic disparities in age adjusted prevalence of all five metabolic abnormalities for both male and female patients with NAFLD. The prevalence of the traditionally defined MetS was higher in NAFLD patients compared to those without NAFLD (44.0% vs. 20.4%; P-value <0.001). Both the mean and median MetS severity scores were significantly higher in NAFLD relative to those without [mean MetS severity Z-score (percentile), 0.48 (61^{st}) vs. -0.14 (46^{th}); median MetS severity Z-score (percentile), 0.48 (69^{th}) vs. -0.23 (41^{st})]. In those with mild, moderate, high, and very high MetS severity, the age adjusted NAFLD prevalence was 17.4%, 25.7%, 42.5, and 54.9% (P-trend <0.001), respectively. The MetS severity score was a significant predictor of NAFLD occurrence in all crude and adjusted models. In the adjusted models with the severity score included as a categorical variable, adults with high MetS severity had adjusted Odds Ratio (aOR) 2.27 (95% CI; 1.70 – 3.03) times the odds of NAFLD presence relative to those with mild MetS severity score. A very high MetS severity was associated with 3.12 (95% CI; 2.20 – 4.42) higher adjusted odds of NAFLD relative to adults with mild MetS severity.

Amongst all NAFLD patients, 65.2%, 29.6%, and 5.2% had a low, intermediate, and high probability of advanced fibrosis. The proportions of NAFLD adults with a high probability of advanced fibrosis was highest amongst Black non-Hispanics (8.0%) and

lowest in Mexican Americans (2.6%). The mean MetS severity Z-scores (percentile) for NAFLD patients with low, intermediate, and high probabilities of advanced fibrosis were 0.184 (55^{th}), 0.965 (73^{rd}), and 1.538 (81^{st}), respectively. NAFLD adults with very high MetS severity had aOR 2.29 (95% CI; 1.65 – 3.19) times the odds of intermediate to high advanced fibrosis probability relative to NAFLD patients with low MetS severity score. A very high MetS severity remained a significant predictor of high advanced fibrosis probability compared to low MetS severity aOR 2.10 (95% CI; 1.02 – 4.34).

In NAFLD, the incidence rate of all-cause mortality was 13.5 per 1,000 person-years, while the cause-specific mortality incidence rates associated with heart disease, diabetes, and hypertension were 3.2 per 1,000 person-years, 2.3 per 1,000 person-years, and 2.1 per 1,000 person-years, respectively. The MetS severity score was a significant predictor for all-cause and cause-specific adjusted mortalities in NAFLD. A quartile increase in MetS severity score was associated with increased in the risk of all-cause mortality adjusted Hazard Ratio (aHR) 1.36 (95% CI; 1.17 – 1.57), heart disease related mortality aHR 1.70 (95% CI; 1.17 – 2.47), diabetes-related mortality aHR 3.64 (95% CI; 2.27 – 5.83), and hypertension-related mortality aHR 1.87 (95% CI; 1.14 – 3.04). Significant non-linear dose-response trends were observed in the relationship between increased risk of mortality, and higher MetS severity score in all adjusted models.

Conclusions: In NAFLD, MetS severity is a significant predictor of disease occurrence, advanced fibrosis presence, and increased risks of mortality. Accounting for the combined effects of MetS severity rather than occurrence help to explain why only some NAFLD patients progress to advanced fibrosis. The MetS severity score

could be used as a screening tool to identify and monitor NAFLD patients at the highest risks of hepatic progressions and mortality.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 Background

1.1 Non-Alcoholic Fatty Liver Disease Definition

Non-Alcoholic Fatty Liver Disease (NAFLD) is defined as the presence of hepatic steatosis, by imaging or histology, in the absence of a secondary cause such as hepatic viral infection(s), drug-induced hepatotoxicity, excessive alcohol consumption, or liver-related hereditary disorders.[1-3] NAFLD includes a spectrum of histological states ranging in severity from simple intrahepatic fat accumulations, non-alcoholic fatty liver (NAFL), to necrotic inflammations in the presence of ballooned hepatocytes — non-alcoholic steatohepatitis (NASH). NAFL is defined as steatosis in greater than five percent of hepatocytes, without additional hepatocellular damage (*i.e.*, ballooning of hepatocytes or cirrhosis).[2-4] NASH encompasses more advanced hepatic damage: steatosis in greater than five percent of hepatocytes, inflammation, and hepatocyte injury with or without fibrosis. Patients with NASH can further develop NASH-cirrhosis, which is recognized by regenerative nodules enclosed by fibrous bands that results in portal hypertension and end stage liver disease.[5]

1.2 Epidemiology of Non-Alcoholic Fatty Liver Disease

The World Gastroenterology Organization (WGO), a large multiethnic cohort study, and additional sources have independently declared NAFLD as the most common cause of chronic liver disease.[6-10] Recent increases in obesity-induced Metabolic Syndrome (MetS) are connected to an upsurge in incidence, prevalence and economic burden of NAFLD, especially in the US, where an estimated 39.8% of all adults are considered obese.[10-13] The current economic burden of NAFLD/NASH on the health care system is

substantial. A recent study analyzed outpatient resource utilization in the United State (US) observed an increase in annual inflation adjusted total NAFLD/NASH per patient charges from $2,624 in 2005 to $5,132 in 2010.[14] In the US, the total annual cost of NAFLD is estimated to be $292.2 billion, of which, $103.3 billion were direct costs.[15]

1.2.1 Prevalence of Non-Alcoholic Fatty Liver Disease

The current global prevalence of NAFLD is 25.2% (95% CI; 22.1-28.7).[2,12,13] The highest NAFLD prevalence is reported in the Middle East 31.8% (95% CI; 22.2-28.7), while the lowest burden for disease is in Africa 13.5% (95% CI; 5.7-28.7).[12,13] In the US, the most recent prevalence estimates of NAFLD puts it at 26.4% (95% CI; 23.8-29.1).[10] Due to the need of liver biopsy for disease diagnosis, data on NASH prevalence have been limited. A study of the natural history of NAFLD puts the global prevalence of NASH between 3-5%.[16,17] In 2016, the prevalence of NASH in the US was estimated to be between 1.5-6.5 %.[12] The prevalence of NASH in NAFLD positive US military personnel and their dependents is 29.9%.[13,18] Other studies put the prevalence of NASH amongst patients with NAFLD positive biopsies at 59.1% (95% CI; 47.6-69.7).[2,12]

1.2.2 Incidence of Non-Alcoholic Fatty Liver Disease

Data on NAFLD incidence in the literature have been scant.[2,12,13,16] A prospective study with five years of follow-up found the incidence of NAFLD on ultrasound to be 12%.[2] Another study conducted in Israel reported a NAFLD incidence rate of 28 per 1,000 person-years.[2,12] A Japanese study of 11,500 adults reported a five years NAFLD cumulative incidence of 10%.[19] Another study conducted in England estimated the incidence rate of NAFLD to be 29 per 1,000 person-years. The pooled regional NAFLD

incidence rate was recently quantified to be 52.3 per 1,000 person-years (95% CI; 28.3-96.8) and 28.0 per 1,000 person-years (95% CI; 19.3-40.6) in Asia and Western countries, respectively.[2,12]

1.2.3 Risk Factors for Non-Alcoholic Fatty Liver Disease

Multiple risk factors, including race, age, gender, and metabolic disorders, have been associated with NAFLD occurrence.[20] Several epidemiological studies have shown that global prevalence varies considerably with the Middle East at 32%, South America at 30%, and Asia, Europe, and Africa at 27%, 24%, and 13%, respectively.[21] According to a recent meta-analysis of 34 studies and more than 360,000 unique patients, substantial disparities in NAFLD prevalence, severity, and prognosis exist between race and ethnic groups within the US.[22] As such, several studies suggest that Hispanics have the highest incidence of NAFLD, with obesity emerging as a central factor in this population. In these studies, African Americans had the lowest incidence.[23-28] Genetic and metabolic factors have been suggested to underlie such racial disparities,[29-32] as have incidence rates of insulin resistance and serum triglyceride concentrations,[33] but conclusive evidence in support of any model explaining those disparities has yet to be uncovered.

Age has been found in several studies to be associated with increased risk of NALFD, NASH, and advanced fibrosis.[20,34] The relationship between age and increased risks of advanced hepatic outcomes is attributed to a longer duration of disease amongst older patients. Namely, a study on age and the risk of liver outcomes found the prevalence of NAFLD to be less than 20% amongst those younger than 20 years and higher than 40% amongst those 60 years or older.[20]

Data on the role of gender as a risk factor for NAFLD have been inconsistent with some studies suggesting males at higher risk, while others show NAFLD to be more common in females. Such conflicting results could be due to racial disparities in the relation between gender and NAFLD occurrence. The evidence for the connections between metabolic disorders such as type 2 diabetes mellitus (T2DM) and obesity are well established in the literature.[30,35-38] A population study of US adults found the prevalence of NAFLD amongst those with T2DM, high triglycerides, and increased waist circumference to be 41%, 35%, and 31%, respectively.[39] Amongst obese patients undergoing bariatric surgery, the prevalence of NAFLD and NASH was found to be 91% and 37%, respectively.[40]

1.3 Diagnosis of Non-Alcoholic Fatty Liver Disease

According to the American Association for the Study of Liver Disease (AASLD) guidelines, the four clinical features for NAFLD diagnosis include four 1) presence of either histological or imagining evidence for hepatic steatosis, 2) lack of excessive alcohol consumption, 3) absent of alternative etiologies for hepatic steatosis and 4) no co-occurring causes of chronic liver disease.[2,4] The AASLD defines suspected NAFLD patients as those with comorbidities such as obesity, insulin resistance, MetS, T2DM, *dyslipidemia*, *hypothyroidism,* sleep apnea, abnormal liver enzymes and imaging.[2] The gold standard for diagnosing NAFLD/NASH is a liver biopsy. Due to its invasive nature and increased risk of infections, performing a liver biopsy is not a feasible option for many suspected NAFLD patients.[41] In turn, initial evaluation of suspected NAFLD patients incorporates three categories of diagnostic tools liver biopsies, noninvasive serum tests and imagining.[2,4,41-45]

The simplest noninvasive serum method to predict fibrosis in NAFLD is the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT).[41] Current guidelines suggest an AST/ALT ratio of above one is indicative of advanced fibrosis. Aside from being used as a standalone method, the AST/ALT ratio has been integrated into more sophisticated predictive models such as the BARD Score and the APRI score.[4,44] The BARD method refers to the use of the Body Mass Index (BMI), the AST/ALT ratio, and T2DM status to quantify a combined weighted score that is prognostic of fibrosis. A BRAD score of two or higher has been shown to increase the likelihood of fibrosis detection by 9 to 31 folds compared to those with a score of less than two.[4,41] The APRI score combines the AST/ALT ratio with platelet counts to predict advanced fibrosis. An APRI score of greater than one has an Area Under Receiver Operator Characteristic Curve (AUROC) of 0.80 for predicting fibrosis.[4]

The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) makes use of all the variables used in the BRAD Score in addition to platelet count and albumin levels. An NFS score of 0.676 or above has an AUROC of 0.82 and 97% specificity for detecting advanced fibrosis.[2,4] The FIB-4 Index was developed to accurately identify both the occurrence and grade of fibrosis amongst patients with Hepatitis C Virus (HCV). A FIB-4 score of greater than 2.67 has an AUROC of 0.82 for predicting advanced fibrosis in NAFLD.[46] Amongst all currently used noninvasive serum methods, the FIB-4 Index and NFS have the highest degree of precision when it gets to detecting and classifying fibrosis.[2]

1.4 Pathogenesis of Non-Alcoholic Fatty Liver Disease

The presence of detectable hepatic steatosis is the defining component of the NAFL spectrum. Four hepatic steatosis development mechanisms are currently known.[47,48] First, elevations in lipolysis from adipose tissue and/or high dietary fat intake result in an increased supply of free fatty acids. Second, steatosis builds up due to an increase in *de novo* hepatic lipogenesis that is triggered by high dietary intakes of saturated fat and simple sugar.[49,50] Third, a decrease in free fatty *β*-oxidation due to adiponectin secretion abnormalities that are induced by insulin resistance in extrahepatic adipocytes results in steatosis accumulates. Finally, a fatty liver could develop due to a reduction in hepatic very low lipoprotein-triglyceride secretions.

NAFL rarely progresses to severe hepatic conditions, such as advanced fibrosis or cirrhosis. To explain such progression patterns, initial studies on the pathogenesis of NAFLD postulated a “two-hit” process for disease development.[48,50,51] In this model, the first hit is characterized by accumulations of triglycerides deposits in hepatocytes that result in hepatic steatosis built-up. Such build-up results in an increase in hepatocytes’ vulnerabilities to the effects of further accumulations. The second hit is then marked by “oxidative stress, endoplasmic reticulum stress, proinflammatory cytokines, and gut-derived bacterial endotoxin”.[50] In turn, the cumulative effects of the second hit were proposed as an explanation for NASH progression in some but not all NAFLD patients.

Antagonists of the two-hit model cite recent findings on the protective effects of triglycerides inhibition against steatosis accumulation, as a major shortcoming in the two-hit model.[50,52] Evidence from animal models suggests that fatty acid inhibition decreases

hepatic steatosis and increases hepatocyte damage.[52,53] Accordingly, more recent studies have proposed a “multi-hit” NAFLD pathogenesis process whereby hepatic inflammations both precede steatosis built-up and are responsible for steatosis accumulations.[48] In that sense, NASH is understood to be the result of the lack of antilipo-toxic protection in the presence of hepatic steatosis.[48]

The multi-hit model has been gaining ground in recent years, as it partially explains the progression from simple steatosis to NASH via “lipo-toxicity, oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress”.[50] In this model, accumulations of both hepatic free fatty acids and cholesterol result in hepatocyte damage.[54] Such damage, in turn, is mediated by lipo-toxicity and oxidative stress as to initiate mitochondrial dysfunction.[55] The currently accepted pathogenic model of NAFLD recognizes hepatic inflammation as the result of multiple simultaneous hits that originate from the gut and/or adipose tissue.[13,48,50,54,55] In this model, NASH is identified as a mitochondrial dysfunction condition.[55]

1.5 Natural History of Non-Alcoholic Fatty Liver Disease

1.5.1 Intrahepatic Outcomes

The progression from mild NAFLD to more severe disease is not fully understood. Over a period of 2145.5 person-years of follow up, an estimated 37%-44% of patients with NAFLD developed NASH, of which 43% progressed to fibrosis.[56] The progression rate from fibrosis to hepatocellular carcinoma (HCC) in the same study was 38%. HCC is understood to occur mainly in the background of liver cirrhosis. However, studies suggest that 54% of HCC cases arise in non-cirrhotic patients, especially amongst those with

NAFLD.[56] Other studies have shown dissimilarities in the pathogenesis of cirrhotic versus non-cirrhotic HCC in the context of NAFLD. Such distinction amongst non-cirrhotic NAFLD-HCC patients is hypothesized to result from unique hepatocarcinogenesis promoting mechanisms related to obesity and MetS, but the exact mechanisms are unknown. Compared with cirrhotic HCC patients, those with non-cirrhotic NAFLD-HCC are more likely to be older, have larger tumors, and experience higher rates of tumor relapse.[57] The 10-years risk of mortality for patients with NAFLD and advanced fibrosis is 16%, and 60% of all those deaths were liver-related.[58] Amongst those with NAFLD and no advanced fibrosis, the 15-years liver-related mortality risk is 9%.[58]

1.5.2 Extrahepatic Outcomes

A common cause of death in NAFLD patients is cardiovascular disease [2,59], and a large body of clinical and population-based studies have demonstrated a positive association between NAFLD and incident cardiovascular disease (CVD), pooled Odds Ratio (OR) 1.6 (95% CI; 1.2, 2.1).[59,60] In particular, NAFLD is associated with coronary artery disease [61] and high-risk coronary atherosclerotic plaques [62], independent of traditional cardiovascular risk factors. Many retrospective and prospective studies [63] show evidence that NAFLD patients have higher rates of CVD-related mortality [64-66] and nonfatal CVD events [65,67] than the general population. A meta-analysis of 27 cross-sectional studies reported an association between NAFLD and markers of atherosclerosis, including increased carotid intima-media thickness, coronary calcification, endothelial dysfunction, and arterial stiffness, independent of traditional cardiometabolic risk factors and metabolic syndrome.[68] An association between NAFLD and cardiovascular disease outcomes was similarly reported in an analysis of the Framingham Heart Study.[69]

1.6 Metabolic Syndrome and Non-Alcoholic Fatty Liver Disease

The MetS describes a group of metabolic abnormalities that are associated with an increased risk of insulin resistance and cardiovascular disease.[53] The clinical features of MetS are atherogenic dyslipidemia, hyperglycemia, hypertension, and visceral obesity.[70] The current guidelines for diagnosing MetS put forward by the American Heart Association, and the National Heart Lung and Blood Institute relies on a "harmonization definition" of this syndrome. Currently, MetS is diagnosed as the presence of three of the following five risk factors: (1) *hyperglycemia* (*i.e.*, fasting blood glucose over 100 mg/dl), (2) *dyslipidemia* (i.e. fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl, women) (3) *hypertriglyceridemia* (i.e. fasting triglyceride (TG) level over 150 mg/dl), (4) central obesity (*i.e.*, waist circumference over 40 inches, men, or 35 inches, women), or (5) hypertension (*i.e.*, systolic blood pressure over 130 mmHg).[6,53]

Obesity is marked by a high accumulation of TG throughout the body. In the hepatocytes, increased uptake of TG results in cell-specific lipo-toxicity, which raises the risk of comorbidities such as NAFLD.[71] Individuals with high visceral adiposity may suffer from increased plasma free fatty acids, which is due to impaired insulin function related to peripheral Insulin resistance.[72] Due to the central role of obesity-induced insulin resistance plays in promoting hepatic steatosis, NAFLD is regarded as the hepatic manifestation of MetS.[2,12,13,17,19,50,73] The prevalence of obesity amongst NAFLD and NASH patients is 51% and 82%, respectively, and the prevalence of NAFLD in patients with MetS and T2DM are particularly high.[32,74,75] This is exacerbated in North America, where the prevalence of MetS amongst NAFLD patients is 66.69% (95% CI; 51.05-

79.21).[38] Studies have shown that the prevalence of NAFLD amongst patients with all five MetS criteria is 91%.[76]

A recent US-based study revealed significant associations between individual components of MetS and NAFLD. In individuals with increased waist circumference, the prevalence of NAFLD (31%, 8.7% with advanced fibrosis) greatly exceeded controls.[39] NAFLD patients with increased waist circumference were predominantly female, older, and less educated. The prevalence of NAFLD in subjects with T2DM (41%, 18% with advanced fibrosis), also greatly exceeded control prevalence, and NAFLD in this population was associated with advanced age and lower education. The prevalence of NAFLD in subjects with high triglyceride levels was 35% (8% with advanced fibrosis). This same level of fibrosis was found in subjects with low HDL, though the prevalence of NAFLD was significantly lower (28%).[39] High triglycerides and low HDL were determined to be independent predictors of NAFLD. In individuals with high blood pressure, NAFLD prevalence was 29% (11% with advanced fibrosis) and not considered an independent predictor. This study also revealed that the presence of NAFLD in subjects with MetS increased according to the number of metabolic abnormalities present, exceeding 65% in patients with all five abnormalities. In the absence of MetS or any of its components, the prevalence of NAFLD is 6.1%.[39] In the absence of metabolic abnormalities, Mexican American race was determined to be an independent predictor of NAFLD.[39]

1.7 Treatments and Prevention of Non-Alcoholic Fatty Liver Disease

Compared to the total population, NAFLD patients have a 1.7-fold increased risk of mortality adjusted for age and gender,[66] yet there are no pharmacologic or other

modalities of treatment for the condition. Lifestyle modifications recommended as a treatment for NAFLD follow those recommended for MetS and include increasing physical activity and weight loss.[77] A meta-analysis of randomized trials showed that weight loss, meeting or exceeding 7%, can improve hepatic histological markers of the disease. However, fewer than 50% of subjects across several trials were able to achieve this level of weight loss.[78] Furthermore, greater weight loss (10%) is needed to improve inflammatory markers of more severe disease.[79] For those patients who can not lose weight due to lifestyle changes, bariatric surgery has been shown to reduced mortality and improve some NAFLD disease markers; however, long-term data are limited.[80,81]

1.8 Rationale

Despite knowledge of the metabolic abnormalities associated with NAFLD and the known association with MetS, it is not known why only some MetS patients develop NAFLD. It is also unknown as to why some NAFLD patients progress to NASH and more severe disease while others do not. Furthermore, the individual contribution of different combinations of metabolic abnormalities and the severity of each is not known in relation to the risk of NAFLD development. Most of the previously conducted research on the natural history of MetS has solely been focused on identifying biomarkers, risk factors, and clinical outcomes [73,82] and have included MetS diagnosis as a dichotomous outcome (*i.e.*, present, or absent).

The current dichotomous classification of MetS creates a knowledge gap about NAFLD incidence and progression. As the natural history of NAFLD is closely related to the component features of MetS, three main shortcomings in the current dichotomous

MetS classification exist. First, current guidelines for diagnosing MetS entail the occurrence of any three of the five risk factors (*i.e.*, ten different metabolic combinations). The dichotomous nature of this classification treats, equally, the effects of any of the ten metabolic combinations on outcomes and survival in NAFLD. Second, MetS diagnosis involves meeting at least three predefined cutoff points on any of the five metabolic abnormalities, and it neglects both the sole and combined values of all MetS features. In turn, the current disease definition creates a gap in knowledge about the impact of overall MetS severity and intrahepatic natural history of NAFLD. Third, a dichotomous MetS categorization makes it challenging to study and monitor the clinical implications of worsening in the severity of MetS over time. The current shortcoming associated with the dichotomous definition of MetS could be fully addressed using a continuous measure of MetS severity that encapsulates the statuses of all five metabolic features in one summary risk score. Such a score could, in turn, be used to assess the association of MetS severity with outcomes and survival in NAFLD.

1.9 Public Health Perspective

Patients with NAFL do not typically require medical therapy; however, NASH can progress to fibrosis, cirrhosis, and, ultimately, HCC.[64,79,83] Lack of awareness and reliable screening approaches contribute to delayed diagnosis and disease progression. Though liver-related death is currently the third leading cause of mortality in NAFLD patients, [35,66] NAFLD/NASH related fibrosis is on the rise and is projected to be the leading cause of liver transplantation in coming years.[7] Current five-year survival of patients with HCC is 17.60%, and NAFLD/NASH is expected to be the main risk factor for liver-related mortality in the coming years.[8,10,17] While already a serious public health issue, the threat of NAFLD

is expected to grow as the prevalence of obesity continues to increase.[37] Current treatment options for patients with NAFLD are limited and indirect, only targeting associated conditions.

The gold standard of diagnosing NAFLD is liver biopsy, which is not a feasible option for most patients. Quantifying the relationship between MetS severity and outcomes and survival in NAFLD is a step towards tackling the current public health burden of this disease. Results from the underlined dissertation will aid both clinicians and public health practitioners in planning and executing secondary and tertiary prevention efforts related to NAFLD. Secondary prevention efforts could take place by using the MetS severity score as a screening tool to identify patients at the highest risk of NAFLD. The MetS severity score could also be used as a tertiary prevention tool whereby the progression of severity is monitored with the aim of designing interventions to mitigate the chances of more advanced hepatic manifestations in NAFLD.

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Chapter 2 The association between Metabolic Syndrome Severity and Non-alcoholic Fatty Liver Disease Occurrence in United States Adults

ABSTRACT

Background and Objectives: Non-Alcoholic Fatty Liver Disease (NAFLD) is currently recognized as the most common cause of Chronic Liver Disease (CLD) globally. Despite knowledge of the association between Metabolic Syndrome (MetS) related metabolic abnormalities and NAFLD, it is not known why only some MetS patients develop NAFLD. It is also unknown as to why some NAFLD patients progress to more severe hepatic manifestation, while others do not. Previously conducted studies on the natural history of NAFLD have accounted for the effects of MetS as a dichotomous risk factor (*i.e.*, present, or absent). Such a dichotomous account of MetS creates a knowledge gap concerning NAFLD incidence and progression. The limitations of the dichotomous definition of MetS in relation to NAFLD occurrence could be fully addressed by using a continuous measure of MetS severity that encapsulates the statuses of all five metabolic features in one summary risk score. The main objective of this chapter was to utilize the MetS severity score, a validated gender-race specific Z-score, to assess the association between MetS severity and the odds of NAFLD occurrence.

Methods & Materials: The study included 10,605 adults ages 20 to 74 years who participated in the Third National Health and Nutrition Examination Survey (NHANES III) and met all inclusion and exclusion criteria. The primary outcome was NAFLD occurrence, which was defined as mild, moderate, or severe hepatic steatosis on ultrasound in the absence of hepatitis B, hepatitis C, iron overload, and excessive alcohol intake. All five metabolic features (*i.e.*, high-density lipoprotein, systolic blood pressure,

waist circumference, triglycerides, and blood glucose) were used to calculate gender-race specific MetS severity Z-score, which was then transformed into four percentiles-based categories [low (0^{th}-50^{th}), moderate (>50^{th}-75^{th}), high (>75^{th}-90^{th}), very high (>90^{th}+)]. Multivariable adjusted logistic regression models were used to test the associations between increases in MetS severity and the odds of NAFLD. The dose-response relationships between MetS severity and the odds of NAFLD were evaluated using the MetS severity score percentiles as a continuous variable with a three-knot restricted cubic spline (RCS) in the adjusted logistic regression models. Complex survey methods using sampling weights, strata, and clusters were used to yield nationally representative prevalence and effect estimates.

Results: At baseline, the prevalence of NAFLD was 26.7% (95% CI; 24.3% – 29.1%). Stratified by race/ethnicity, NAFLD prevalence was 26.7%, 23.3%, and 33.7% amongst White non-Hispanics, Black non-Hispanics, and Mexican Americans, respectively. Amongst male participants, the age adjusted prevalence of NAFLD was significantly higher for Mexican Americans when compared to White non-Hispanics (25.9% vs. 31.5%; P-value 0.04). Similarly, Mexican American females had a significantly higher age adjusted NAFLD prevalence compared to White non-Hispanics females (27.6% vs. 41.1%; P<0.001). We observed racial/ethnic disparities in age adjusted prevalence of all five metabolic abnormalities for both male and female patients with NAFLD. The prevalence of the traditionally defined MetS was higher in NAFLD patients compared to those without NAFLD (44.0% vs. 20.4%; P-value <0.001). An estimated 82.1% of adults with NAFLD had at least one feature of the traditionally defined MetS, while 9.2% met the criteria for all five metabolic components. The distribution of the MetS severity score was

normally distributed in both the NAFLD and no NAFLD groups. Both the mean and median MetS severity scores were significantly higher in NAFLD relative to those without [mean MetS severity Z-score (percentile), 0.48 (61st) vs. -0.14 (46th); median MetS severity Z-score (percentile), 0.48 (69th) vs. -0.23 (41st)]. The age adjusted prevalence of NAFLD increased significantly with higher MetS severity (P-trend <0.001). In those with mild, moderate, high, and very high MetS severity, the age adjusted NAFLD prevalence was 17.4%, 25.7%, 42.5, and 54.9%, respectively. The MetS severity score was a significant predictor of NAFLD occurrence in all crude and adjusted models. In the adjusted models with the severity score included as a categorical variable, adults with high MetS severity had aOR 2.27 (95% CI; 1.70 – 3.03) times the odds of NAFLD presence relative to those with mild MetS severity score. A very high MetS severity was associated with 3.12 (95% CI; 2.20 – 4.42) higher adjusted odds of NAFLD relative to adults with mild MetS severity. In the RCS analysis, compared to those with median severity score, the aOR of NAFLD were 1.17 (95% CI; 1.11 – 1.23), 2.05 (95% CI; 1.72 – 2.43), 2.85 (95% CI; 2.23 – 3.65), and 3.38 (95% CI; 2.55 – 4.49), for adults in the 60th, 80th, 90th, and 95th severity percentiles, respectively. Relative to those in the 90th severity score, the aOR of NAFLD were 0.72 (95% CI; 0.66 – 0.77), and 0.35 (95% CI; 0.27 – 0.45), respectively, for adults in the 80th, and 50th severity percentiles.

Conclusions: Our findings demonstrate the utility of MetS severity as a driving force of NAFLD occurrence in US adults. Non-linear dose-response trends were observed in the relationship between increased odds of NAFLD occurrence and MetS severity. Factoring for the effects of MetS severity rather than occurrence help to explain why some but not all MetS patients develop NAFLD. While current treatment options for patients with

NAFLD are limited and indirect, the MetS severity score could be used as a screening tool in both primary and secondary prevention efforts to tackle this hepatic epidemic.

Keywords: NAFLD, NASH, Dose-response, metabolic syndrome severity, NHANES III

2.1 Introduction

Recent increases in the prevalence of obesity-induced metabolic syndrome resulted in an upsurge in the global incidences of both Nonalcoholic fatty liver disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH).[1,2] Currently, NAFLD is recognized as the most common cause of Chronic Liver Disease (CLD) globally.[3] The onsets of NAFLD are characterized by the presence of detectable fatty deposits in the liver in the absence of hepatic viral infections, other CLD etiologies, the use of steatosis inducing medications, or excessive alcohol consumption. NASH is the advanced progression of NAFLD, as it describes premiant damage to hepatocytes due to the presence of hepatic steatosis.[4-6]

The current global prevalence of NAFLD is 25%, while 26% of adults in the United States (US) are estimated to have NAFLD.[5,7,8] The progression from NAFLD to more severe disease is not fully understood. Over a period of 2145.5 person-years of follow up, an estimated 37% to 44% of patients with NAFLD developed NASH, of which, 43% progressed to advanced fibrosis.[9] In turn, advanced fibrosis progresses to decompensated cirrhosis followed by hepatocellular carcinoma (HCC) or directly to HCC.[5,10,11] Some studies have also suggested that 54% of HCC cases arise in non-cirrhotic patients, especially among those with NAFLD.[9]

Long-term observational studies involving liver biopsies have defined the course of NAFLD, but considerable gaps remain in understanding disparities between individuals and in progression, complications, and regression patterns of disease.[12-16] Namely, epidemiological studies have shown that global prevalence of NAFLD varies considerably with the Middle East at 32%, South America at 30%, and Asia, Europe, and Africa at 27%,

24%, and 13%, respectively.[17] According to a meta-analysis of 34 studies with more than 360,000 unique patients, strong disparities in NAFLD prevalence, severity, and prognosis exist between race and ethnic groups within the US.[18] Several studies suggest Hispanics have the highest incidence of NAFLD, with obesity emerging as a central Metabolic Syndrome (MetS) factor in this population. In these studies, African Americans had the lowest incidence of NAFLD.[19-24]

Due to the primary function obesity-induced insulin resistance plays in promoting hepatic steatosis, NAFLD is considered to be the hepatic manifestation of MetS.[3,5,7,8,25-27] MetS encompasses a group of five metabolic abnormalities that are associated with an increased risk of insulin resistance and cardiovascular disease.[28] The five clinical features of MetS are atherogenic hyperglycemia, *dyslipidemia*, *hypertriglyceridemia,* hypertension, and central obesity.[29] The current guidelines for diagnosing MetS utilize a "harmonization definition" of this syndrome. As such, MetS is characterized based on a patient presenting abnormalities that exceed pre-specified cutoff values for three of the five clinical features of MetS.[28,30]

Despite knowledge of the association between MetS related metabolic abnormalities and NAFLD, it is not known why only some MetS patients develop NAFLD. It is also unknown as to why some NAFLD patients progress to NASH and more severe disease while others do not. The individual contribution of different combinations of metabolic abnormalities of varying severities to the overall risk of NAFLD development are also not known. Furthermore, the racial and ethnic disparities in NAFLD incidence and prevalence remain unexplained.

The majority of previously conducted research on the natural history of NAFLD in relation to MetS has solely been focused on identifying disease biomarkers, risk factors, and clinical outcomes [25,31] and have included MetS diagnosis as a dichotomous outcome (*i.e.*, present or absent). The being said, the current dichotomous classification of MetS creates a main knowledge gap concerning NAFLD incidence and progression.

As the natural history of NAFLD is closely related to the component features of MetS, four main shortcomings in the current dichotomous MetS classification exist. First, current guidelines for diagnosing MetS entail the occurrence of any three of the five risk factors (*i.e.*, ten possible different metabolic combinations). The dichotomous nature of this classification treats, equally, the effects of any of the ten metabolic combinations on outcomes and survival in NAFLD. Second, MetS diagnosis involves meeting at least three predefined cutoff points on any of the five metabolic abnormalities, and it neglects both the sole and combined values of all MetS features. Third, a dichotomous MetS categorization makes it challenging to study and monitor the clinical implications of worsening in the severity of MetS over time. Fourth, a binary system for MetS definition does not account for the racial and gender disparities in MetS severity and their corresponding effects on the risk of NAFLD. In turn, the current disease definition creates a gap in knowledge about the impact of overall MetS severity in relation to the intrahepatic natural history of NAFLD.

The shortcoming associated with the dichotomous definition of MetS in relation to NAFLD occurrence could be fully addressed by using a continuous measure of MetS severity that encapsulates the statuses of all five metabolic features in one summary risk score. Such score could, in turn, be used to assess the association of MetS severity with

outcomes and survival in NAFLD. The MetS Severity Score is a validated clinically-accessible gender-race specific Z-score that encapsulates the combined effects of the nature and severity of all five metabolic abnormalities amongst US adults.[32] Therefore, the MetS severity score is a continuous representation of the traditional MetS classification, while adjusting for gender and racial/ethnic disparities in the relationship between MetS and cardiometabolic outcomes. The MetS severity score is significantly correlated with pathophysiological biomarkers of MetS, including the Homeostasis Model for Insulin Resistance (HOMA-IR), C-Reactive protein (CRP), uric acid, and adiponectin.[32,33] Multiple studies have also shown the MetS severity score to be a significant predictor of long-term risks of cardiovascular disease, type 2 diabetes mellitus, and coronary heart disease.[33-36]

The main objective of this chapter is to utilize the continuous measure of MetS to examine the association between increased MetS severity and NAFLD occurrence. Quantifying the relationship between MetS severity and NAFLD is a step towards tackling the current public health burden of this disease. Results from this study could aid prevention efforts by examining the utility of the MetS severity score as a screening tool to identify patients with the highest probability of NAFLD presence.

2.2 Materials and Methods

2.2.1 Data Source

The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) conducted the third National Health and Nutrition Examination Survey (NHANES III) between 1988 and 1994. The objective of NHANES III was to investigate

the health status of the US population with a focus on non-Hispanic Blacks, Mexican Americans, and individuals sixty years of age or older. The NHANES III utilized a stratified multistage clustered probability design to sample noninstitutionalized members of the US population ages two months or older. The survey incorporated cross-sectional examinations, interview questionnaires, and laboratory sample collections. Of all the interviewed participants, 78% took part in the physical examination phase of the survey.[37] The ethics review board of the CDC approved the NHANES III survey protocol.

2.2.2 Study Sample

Hepatic steatosis was assessed by three trained ultrasound readers using gallbladder ultrasounds video recorded during the physical examinations for all NHANES III participants 20 to 74 years of age. Following the initial assessments, all ultrasound readings were reevaluated and validated by a certified radiologist specialized in hepatic imaging. Hepatic steatosis images were classified into normal, mild, moderate, or severe. Criteria for grading hepatic steatosis included gallbladder walls definition, liver parenchyma degree of brightness, the occurrence of deep beam attenuation, the presence of liver to kidney contrast, and echogenic walls in the small intrahepatic vessels.[38]

Participants were excluded from the study if they had missing values for exposure, outcome, alcohol intake, ultrasound images, or any of the covariates included in the adjusted analyses. Participants who identified as “Other” race/ethnicity were also excluded, as the exposure assessment is only applicable to Non-Hispanic Whites, Non-

Hispanic Blacks, and Mexican Americans. After implementing all inclusion and exclusion criteria, the final study sample included 10,605 adult participants.

2.2.3 Exposure

The MetS severity score was the primary exposure variable. The MetS severity score is a validated gender- and race/ethnicity- specific Z-score that captures the relative MetS severity of all five metabolic abnormalities.[32] The score was quantified using Confirmatory Factor Analysis (CFA) with data from the 1999-2010 NHANES.[32] All five metabolic features were used in the CFA to construct a summary score that is a continuous representation of the conventional metabolic syndrome characterization.

Participants in the 1999-2010 NHANES were divided into six groups based on gender and self-identified race/ethnicity, including Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics. Different loading coefficients were quantified to determine a single latent MetS factor for all six sup-groups. Individual-level data for HDL, SBP, waist circumference, TG, and fasting blood glucose were used to calculate gender- and race/ethnicity-specific MetS severity Z-scores according to the score's standardized equations.[32] In turn, the MetS severity Z-score was transformed into four percentiles-based categories [low (0^{th}-50^{th}), moderate (>50^{th}-75^{th}), high (>75^{th}-90^{th}), very high (>90^{th}+)].

The ATP-III guidelines were used to define the traditional MetS classification. As such, MetS is defined by the presence of three of the five metabolic factors: (1) *hyperglycemia* (*i.e.*, fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) *dyslipidemia* (*i.e.*, fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl,

women, or pharmacological treatment) (3) *hypertriglyceridemia* (*i.e.*, fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (*i.e.*, waist circumference over 40 inches for men, or 35 inches for women), or (5) hypertension (*i.e.*, systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment).[29]

2.2.4 Outcome

The study's primary outcome was NAFLD status. NAFLD was identified by the presence of mild, moderate, or severe hepatic steatosis in the absence of excessive drinking (*i.e.*, more than three alcoholic beverages per day for males and more than two alcoholic beverages per day for females), binge drinking (*i.e.*, frequent consumption of five or more alcoholic beverages per day), alcohol consumption restrictions due to illness, positive Hepatitis B virus (HBV) surface antigen test, positive Hepatitis C virus (HCV) RNA Test, or Iron overload (*i.e.*, transferrin saturation of ≥ 50%).

2.2.5 Covariates

During the interview and examination phases, data were gathered on multiple covariates, including confounding variables and other factors used in the secondary statistical analyses. Confounder selection was based on both *a priori* knowledge, form the literature, and theoretical rationale. Confounders used in the adjusted multivariate analyses included age, gender, race/ethnicity (White non-Hispanics, Black non-Hispanics, or Mexican Americans), education level (< high school, high school, or GED; some college or college degree or higher), access to health insurance (yes or no), alcohol intake (never, former, > 0-1 drinks/day, or > 1 drinks/day), smoking status (never, former, or current), body mass index (Kg/m^2) [underweight (<18.5), healthy weight (≥18.5 - 25.0<),

overweight (≥25.0 - 30.0<), or obese (≥30)], abdominal obesity, physical activity (metabolic equivalents/month), healthy eating index percentile, HOMA-IR, aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio and total cholesterol.

2.2.6 Statistical Analyses

The study sample was restricted to participants with non-missing values on exposure, outcome, or any of the variables used in the adjusted multivariate analyses. Complex survey methods, using sampling weights, strata, and clusters were used to yield nationally representative estimates. In order to account for the effects of the survey design, Taylor series linearization was used to quantify all variance values. Missing values related to variance estimation were assumed not to be missing completely at random.

Participants' characteristics stratified by NAFLD status were examined by testing the difference in means for continuous variables, using weight adjusted analysis of variance, and using Rao Scott Chi-Square for categorical variables. In a sensitivity analysis, we stratified the study sample by race/ethnicity and evaluated the distributions of baseline characteristics by NAFLD status. We accounted for age, gender, and race/ethnicity in all further analyses to account for their biological impacts on the values of metabolic features. To assess the association between MetS and NAFLD occurrence, age adjusted mean estimates for clinical characteristics related to MetS for NAFLD patients were quantified by gender and race/ethnicity. We also evaluated the age adjusted distributions of the MetS severity score by gender and race/ethnicity in relation to NAFLD status. We also estimated the age adjusted NAFLD prevalence by race/ethnicity, gender, and MetS severity Z-score quartile to understand disease

distribution better. Disease prevalence by MetS severity distribution was quantified for the US adult population, as to evaluate different potential NAFLD prevention strategies.

Multivariable logistic regression models were used to test the dose-response between MetS severity and the odds of NAFLD. The relationships between MetS severity and the odds of NAFLD were estimated using the MetS severity score percentiles as a continuous variable with a three-knot restricted cubic spline (RCS) in the adjusted logistic regression models. As recommended by Harrell, 2015, the three-knots were places at the 10^{th}, 50^{th}, and 90^{th} of the weighted MetS severity score values for US adults.[39] Wald-Chi Square tests were used to assess the overall and non-linear associations between the MetS severity score percentiles and the odds of NAFLD. A P-value of less than 0.05 was considered statistically significant. All analyses were performed using the SAS 9.4 software (SAS Institute, NC, USA).

2.3 Results

2.3.1 Participants Baseline Characteristics

Baseline characteristics of adults with and without NAFLD are summarized in Table (2-1). The study sample included 10,605 adult participants in the NHANES III, who met the inclusion and exclusion criteria. The distribution of race/ethnicity differed by NAFLD status. Adults with NAFLD were more likely to be females (54.7% vs. 51.0%; P-value <0.047), older (mean age in years, 45.3 vs. 41.6; P-value <0.001) and had a higher percentage of Mexican Americans (7.0% vs. 5.0%) and a lower proportion of Black non-Hispanics (9.6% vs. 11.5%), relative to those without. The distributions of education level, marital status, and smoking status also differenced by NAFLD status.

An estimated 71.3% of NAFLD patients were overweight or obese, compared to 49.9% in those without NAFLD. Similarity, adults with NAFLD had 3.0 kg/m^2 higher average BMI when compared to those without NAFLD. The prevalence of abdominal obesity was also significantly associated with NAFLD status (with vs. without; 75.5% vs. 62.4%; P-value <0.001). In contrast, the proportion of physically active participants was lower in NAFLD compared to no NAFLD (84.2% vs. 88.8%; P-value <0.001). This translated into significantly higher levels of both total cholesterol and HOMA-IR in NAFLD versus no NAFLD.

In a sensitivity analysis, we stratified the sample by race/ethnicity and compared baseline characteristics by NAFLD status (Appendix 2-1). Compared to those without, Black non-Hispanics, and Mexican Americans adults with NAFLD were more likely to be females. Lower education levels were associated with NAFLD status in White non-Hispanics and Hispanics but not amongst Black non-Hispanics. The distributions of alcohol intake and smoking status also differed by NAFLD status independent of race/ethnicity.

The prevalence of being overweight or obese was 71.0%, 70.0%, and 77.2, respectively, in White non-Hispanics, Black non-Hispanics, and Mexican American NAFLD patients, compared to 48.0%, 60.5%, and 58.3%, respectively in those without NAFLD of the same race/ethnicity. Similarly, all adults with NAFLD had 3.1 kg/m^2 higher average BMI when compared to those without NAFLD. The prevalence of abdominal obesity was also significantly associated with NAFLD status in all racial/ethnic groups.

Table 2-1 Sample Characteristics by Non-Alcoholic Fatty Liver Disease (NAFLD) Status, United States Adults, The National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=10,605)

Characteristics	NAFLD (n=3,080)	No NAFLD (n=7,530)	P-value*
Gender, % (SE)			0.040
Male	45.2 (1.3)	48.9 (0.8)	
Female	54.8 (1.3)	51.1 (0.8)	
Age, (years)			<0.001
Median (25th, 75th Percentile)	42.9 (32.7, 57.0)	36.6 (29.0, 51.7)	
Mean (SE)	45.3 (0.49)	41.6 (0.45)	
Age Group, % (SE)			<0.001
18-34	28.4 (2.0)	38.8 (1.3)	
35-49	32.9 (2.2)	32.5 (0.9)	
49-64	24.5 (1.3)	18.4 (0.7)	
65+	14.2 (1.0)	10.2 (0.7)	
Race/ethnicity, % (SE)			<0.001
White, non-Hispanics	83.4 (1.3)	83.5 (0.9)	
Black, non-Hispanics	9.6 (0.8)	11.5 (0.8)	
Mexican Americans	7.0 (0.9)	5.0 (0.4)	
Education Level, % (SE)			0.001
< High School	22.2 (1.3)	20.1 (1.0)	
High School or GED	39.2 (1.5)	34.2 (0.9)	
Some College	19.4 (1.4)	22.6 (0.9)	
College degree or Higher	19.1 (1.6)	23.1 (1.0)	
Have Health Insurance, % (SE)	89.0 (0.9)	87.4 (0.9)	0.159
Alcohol Intake, % (SE)			<0.001
Never	14.8 (1.1)	9.0 (0.7)	
Former	34.8 (1.4)	31.1 (1.4)	
> 0 - 1 drinks/day	40.4 (1.8)	41.8 (1.4)	
> 1 drinks/day**	10.0 (1.1)	18.1 (0.9)	
Smoking Status, % (SE)			<0.001
Never	47.4 (1.3)	42.9 (1.1)	
Former	29.8 (1.5)	25.3 (0.8)	
Current	22.9 (1.2)	31.7 (1.0)	
Body Mass Index (Kg/M^2)			<0.001
Median (25th, 75th Percentile)	27.9 (24.2, 32.2)	24.9 (22.3, 28.0)	
Mean (SE)	28.8 (0.30)	25.8 (0.11)	
Body Mass Index Category† (Kg/M^2)**,** % (SE)			<0.001
Underweight	1.9 (0.4)	2.4 (0.3)	
Healthy Weight	26.8 (1.8)	47.7 (0.9)	
Overweight	33.6 (1.4)	32.9 (0.8)	
Obese	37.7 (2.0)	17.0 (0.8)	
Waist to Hip Ratio			<0.001
Median (25th, 75th Percentile)	0.93 (0.85, 0.99)	0.89 (0.83, 0.95)	
Mean (SE)	0.93 (0.004)	0.90 (0.002)	
Abdominal Obesity ‡, % (SE)	75.5 (1.6)	62.4 (1.2)	<0.001
Physical Activity (METs/month)			<0.001
Median (25th, 75th Percentile)	58.0 (14.3, 142.1)	73.0 (19.9, 164.0)	
Mean (SE)	97.8 (4.0)	115.8 (3.5)	
Physically Active, % (SE)	84.2 (1.2)	88.8 (0.7)	<0.001

Healthy Eating Index			0.219
Median (25th, 75th Percentile)	64.0 (53.9, 73.6)	63.1 (54.1, 72.5)	
Mean (SE)	63.7 (0.4)	63.2 (0.3)	
Healthy Eating Index §**,** % (SE)			0.321
Poor	17.8 (1.1)	17.8 (0.8)	
Fair	70.1 (1.5)	71.5 (0.6)	
Good	12.1 (1.2)	10.7 (0.6)	
Total Cholesterol (mg/dL)			0.001
Median (25th, 75th Percentile)	204.3 (176.1, 234.7)	198.1 (172.3, 226.7)	
Mean (SE)	207.6 (1.4)	201.8 (0.9)	
HOMA-IR			<0.001
Median (25th, 75th Percentile)	2.5 (1.6, 4.1)	1.7 (1.2, 2.4)	
Mean (SE)	4.13 (0.3)	2.4 (0.1)	
AST/ALT Ratio			<0.001
Median (25th, 75th Percentile)	1.2 (0.9, 1.6)	1.4 (1.1, 1.7)	
Mean (SE)	1.31 (0.03)	1.5 (0.02)	

* Rao-Scott Chi Square P-values for difference in proportions and T-tests P-values for difference in means between adults with versus without Non-Alcoholic Fatty Liver Disease of the same race/ethnicity

** In NAFLD up to 2 drink per days for females and 3 drinks per day for males

† Underweight (< 18.50), Healthy Weight (≥ 18.50 - 25.00 <), Overweight (≥ 25.00 - 30.00 <) and Obese (≥ 30)

‡ Waist to Hip Ratio ≥ 0.90 for males or ≥ 0.85 for females

§ Poor < 51%, Fair < 80%, Good ≥ 80%

MET= Metabolic equivalent; AST= Aspartate Aminotransferase; ALT= alanine aminotransferase; % = Weighted Proportion; SE= Standard Error

2.3.2 NAFLD Prevalence in the United States

At baseline, the prevalence of NAFLD was 26.7% (95% CI; 24.3% – 29.1%). Stratified by race/ethnicity, NAFLD prevalence was 26.7%, 23.3%, and 33.7% amongst White non-Hispanics, Black non-Hispanics, and Mexican Americans, respectively. The prevalence of NAFLD was not statistically different for males compared to females (25.6% vs. 28.32%; P-value 0.055). However, amongst male participants, the age adjusted prevalence of NAFLD was significantly higher for Mexican Americans when compared to White non-Hispanics (25.9% vs. 31.5%; P-value 0.04). Similarly, Mexican American females had a significantly higher age adjusted NAFLD prevalence compared to White non-Hispanics females (27.6% vs. 41.1%; P<0.001).

2.3.3 Metabolic Syndrome Severity in NAFLD

The prevalence of the traditionally defined MetS was higher in NAFLD patients compared to those without NAFLD (44.0% vs. 20.4%; P-value <0.001)(Table 2-2). Amongst females with NAFLD, Mexican Americans had a significantly higher MetS prevalence compared to White non-Hispanics (55.0% vs. 42.0%; P-value <0.001). The age adjusted mean number of metabolic abnormalities was highest in Mexican American females with NAFLD (2.7) and lowest amongst Black non-Hispanics males (2.0).

An estimated 82.1% of adults with NAFLD had at least one feature of the traditionally defined MetS, while 9.2% met the criteria for all five metabolic components. When NAFLD patients were stratified by race/ethnicity, the prevalence of having at least one metabolic abnormality was 82.3%, 77.2% and 86.9% of White non-Hispanics, Black non-Hispanics, and Mexican Americans, respectively. In males with NAFLD, the age

adjusted prevalence of hypertriglyceridemia and dyslipidemia was lower for Black non-Hispanics compared to White non-Hispanics. In contrast, the age adjusted systolic blood pressure was higher for Black non-Hispanics male patients with NAFLD compared to their White non-Hispanics counterparts. Mexican American males with NAFLD had significantly higher age adjusted plasma glucose relative to White non-Hispanics males with NAFLD.

We observed racial/ethnic disparities in age adjusted prevalence of all five metabolic abnormalities for female patients with NAFLD (Table 2-2). Mexican American females with NAFLD had higher age adjusted prevalence of central obesity, hypertriglyceridemia, dyslipidemia, and hyperglycemia when compared to White non-Hispanics females with NAFLD. Black non-Hispanics females had higher prevalence of central obesity, hypertension, and hyperglycemia relative to White non-Hispanics females with NAFLD. In contrast, Black non-Hispanics females with NAFLD lower prevalence of hypertriglyceridemia compared to White non-Hispanics females with NAFLD.

Table 2-2 Age Adjusted Estimates for Clinical Characteristics Related to Metabolic Syndrome by Gender and Race/Ethnicity, Adults with NAFLD, The National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=3,080)

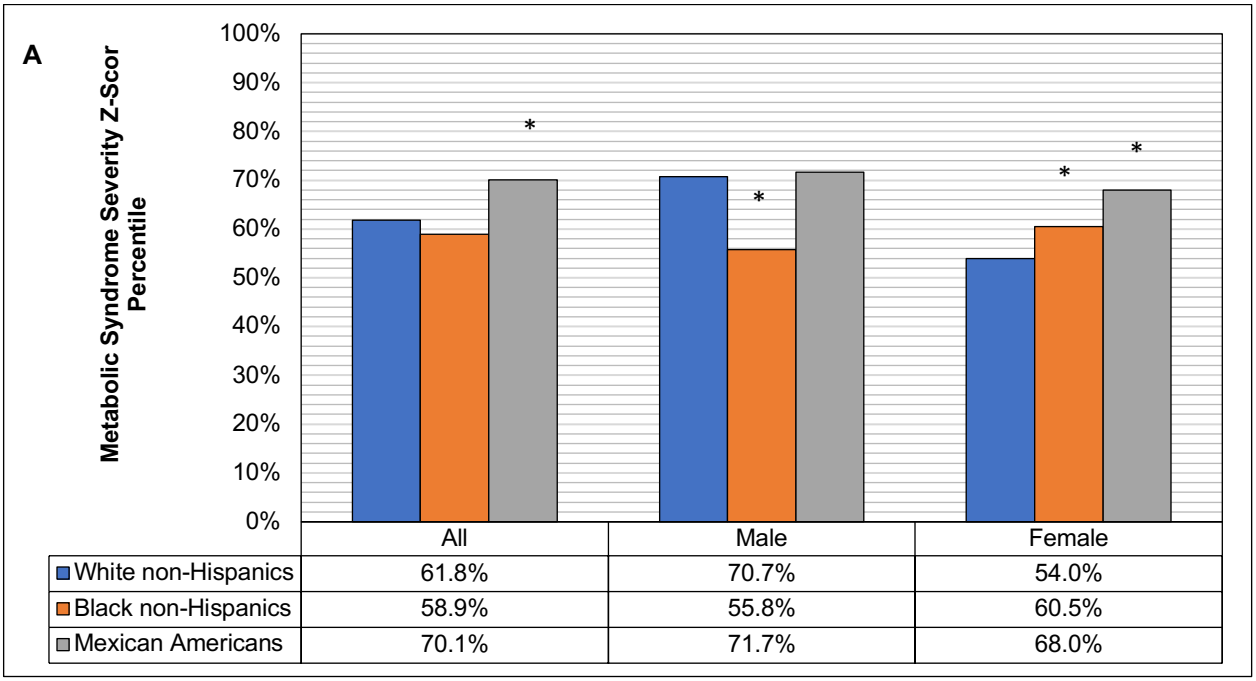
Clinical Characteristics	Males			Females		
	White, non-Hispanics	Black, non-Hispanics	Mexican-Americans	White, non-Hispanics	Black, non-Hispanics	Mexican-Americans
	(n=516)	(n=282)	(n=457)	(n=693)	(n=474)	(n=657)
Number of Metabolic Abnormalities†, Mean (SE)	2.5 (0.08)	2.0 (0.09) *	2.4 (0.14)	2.1 (0.09)	2.4 (0.06) *	2.7 (0.07) *
Metabolic Syndrome‡, % (SE)	48.0 (3.0)	40.0 (4.0)	51.0 (4.0)	42.0 (2.0)	48.0 (2.0)	55.0 (2.0) *
Central Obesity, % (SE)	51.0 (2.0)	45.0 (3.0)	44.0 (4.0)	57.0 (3.0)	70.0 (2.0) *	77.0 (3.0) *
Waist Circumference (inches), Mean (SE)	102.2 (0.64)	98.8 (1.0) *	100.1 (1.2)	93.2 (1.4)	100.5 (1.0) *	97.63 (0.83) *
Hypertriglyceridemia, % (SE)	58.0 (3.0)	32.0 (3.0) *	59.0 (4.0)	40.0 (2.0)	29.0 (2.0) *	52.0 (2.0) *
Triglyceridemia (mg/dL), Mean (SE)	205.01 (8.26)	146.1 (7.49) *	210.81 (11.77)	156.17 (5.43)	131.3 (4.37) *	179.33 (5.79) *
Hypertension, % (SE)	49.0 (3.0)	57.0 (3.0)	46.0 (4.0)	37.0 (2.0)	53.0 (3.0) *	40.0 (2.0)
Systolic Blood Pressure (mmHg), Mean (SE)	127.2 (0.7)	131.3 (1.02) *	128.6 (1.04)	121.3 (0.52)	126.4 (1.08) *	123.5 (0.47) *
Dyslipidemia, % (SE)	52.0 (3.0)	27.0 (3.0) *	50.0 (3.0)	48.0 (3.0)	45.0 (2.0)	59.0 (2.0) *
High-Density Lipoprotein Cholesterol (mg/dL), Mean (SE)	40.9 (0.67)	50.0 (1.19) *	41.9 (0.71)	52.6 (0.74)	53.2 (0.78)	49.2 (0.71) *
Hyperglycemia, % (SE)	37.0 (3.0)	39.0 (3.0)	44.0 (3.0)	26.0 (2.0)	39.0 (3.0) *	41.0 (2.0) *
Plasma Glucose (mg/dL), Mean (SE)	103.38 (1.0)	112.15 (3.5) *	111.66 (3.0) *	101.18 (1.5)	113.01 (3.3) *	108.99 (1.9) *

* P-value <0.05 for adults of the same gender in reference to white non-Hispanics of the same gender

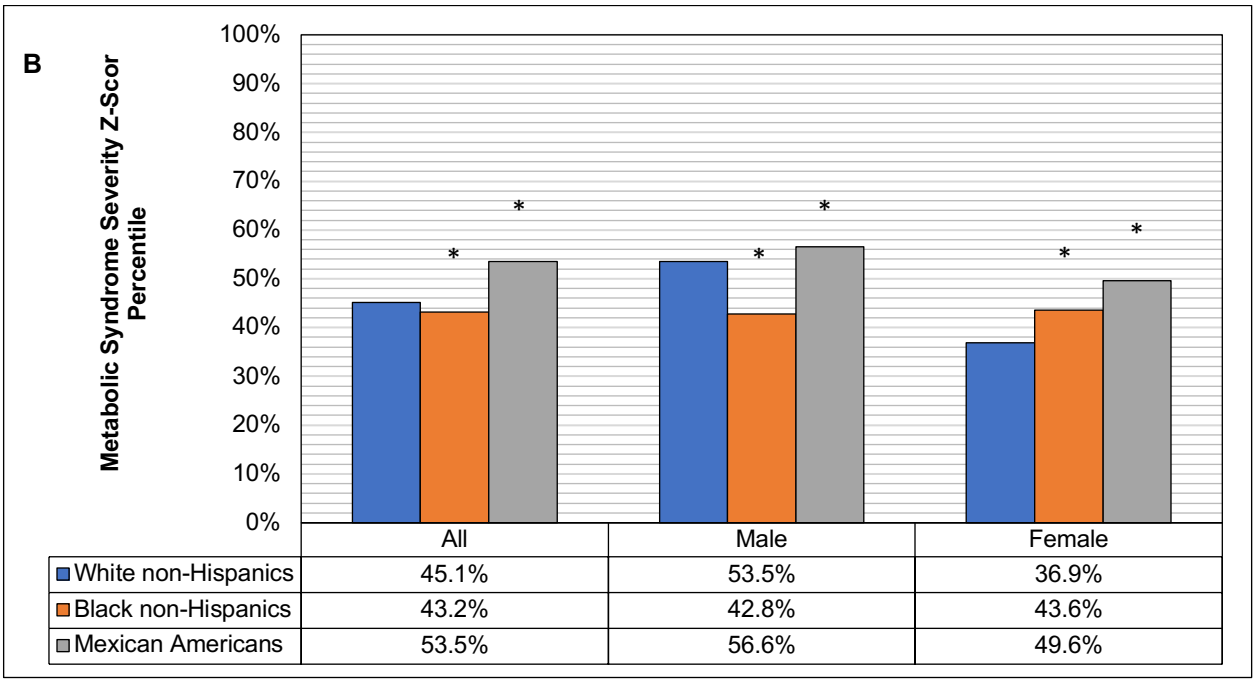
† (1) hyperglycemia (i.e., fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) dyslipidemia (i.e., fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl, women, or pharmacological treatment) (3) hypertriglyceridemia (i.e., fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (i.e., waist circumference over 40 inches ,men, or 35 inches, women), or (5) hypertension (i.e., systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment)

The distribution of the MetS severity score was normally distributed in both the NAFLD and no NAFLD groups. However, the severity of MetS was significantly higher in NAFLD patients. The overall mean and median MetS severity Z-scores and their corresponding percentiles were 0.03 (49^{th}) and -0.07 (47^{th}), respectively. Both the mean and median MetS severity scores were significantly higher in NAFLD relative to those without [mean MetS severity Z-score, 0.48 (61^{st}) vs. -0.14 (46^{th}); median MetS severity Z-score (percentile), 0.48 (69^{th}) vs. -0.23 (41^{st})].

When we stratified the sample by race/ethnicity and gender, NAFLD patients had significantly higher MetS severity in all comparison groups (Figure 2-1). In adults without NAFLD, Mexican Americans had the highest severity (53^{rd}) compared respectively to both White non-Hispanics (45^{th}) and Black non-Hispanics (43^{rd}). Similarly, amongst those with NAFLD, Mexican American male and female adults had the highest MetS severity compared to White non-Hispanic male and females NAFLD patients. Black non-Hispanics male adults with NAFLD had significantly lower MetS severity (56^{th}) compared to White non-Hispanics males with NAFLD (70^{th}). In contrast, Black non-Hispanics females with NAFLD had higher MetS severity than their White non-Hispanic counterparts.



	All	Male	Female
White non-Hispanics	61.8%	70.7%	54.0%
Black non-Hispanics	58.9%	55.8%	60.5%
Mexican Americans	70.1%	71.7%	68.0%



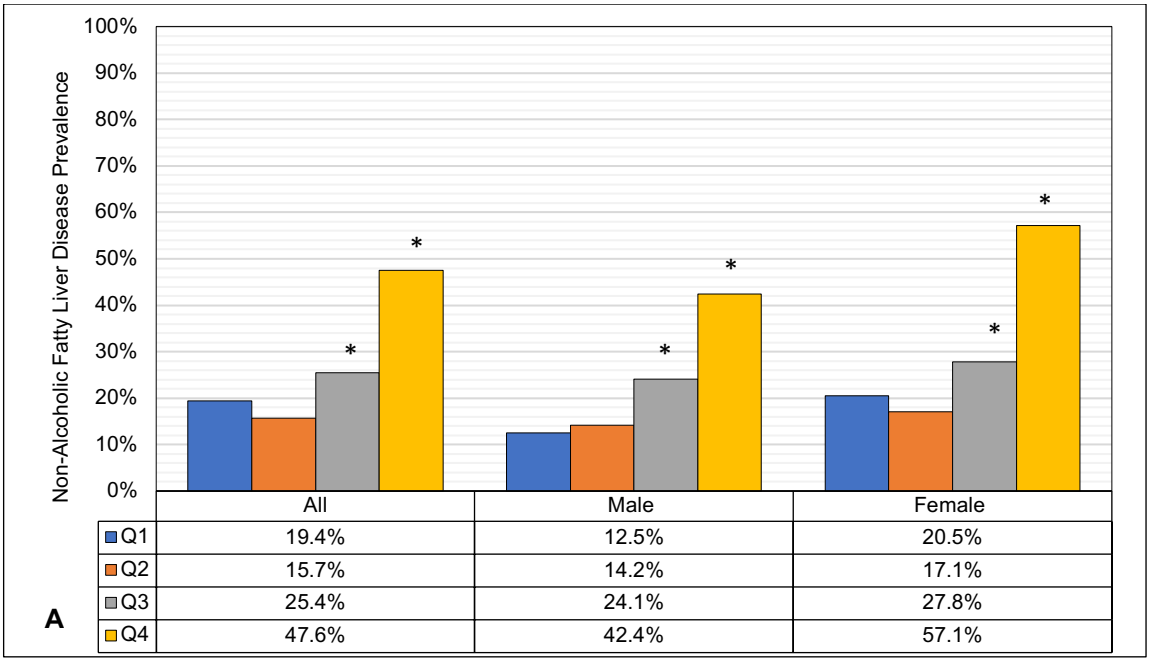
	All	Male	Female
White non-Hispanics	45.1%	53.5%	36.9%
Black non-Hispanics	43.2%	42.8%	43.6%
Mexican Americans	53.5%	56.6%	49.6%

* P-value <0.05 for the difference in metabolic syndrome percentile in reference to white non-Hispanics of the same gender

Figure 2-1 Age adjusted Mean Metabolic Syndrome Severity Z-Score Percentile by Race/Ethnicity and Gender in A) Adults with Non-Alcoholic Fatty Liver Disease (n=3,080) and B) Adults without Non-Alcoholic Fatty Liver Disease, US Adults, (NHANES III), (n=7,525)

2.3.4 NAFLD Prevalence and Metabolic Syndrome Severity

The age adjusted prevalence of NAFLD increased significantly with higher MetS severity group (P-trend <0.001). In those with mild, moderate, high, and very high MetS severity, the age adjusted NAFLD prevalence was 17.4%, 25.7%, 42.5, and 54.9%, respectively. When the MetS severity Z-score was divided into quartiles, the age adjusted NAFLD prevalence was not significantly different between those in the first and second MetS severity quartiles (Figure 2-2). Amongst both White non-Hispanic and Mexican American males and females, the second and third severity quartiles were respectively higher than the first quartiles. In both Black non-Hispanic males and females, only the third severity quartile was significantly higher than the first quartile.



	All	Male	Female
Q1	19.4%	12.5%	20.5%
Q2	15.7%	14.2%	17.1%
Q3	25.4%	24.1%	27.8%
Q4	47.6%	42.4%	57.1%

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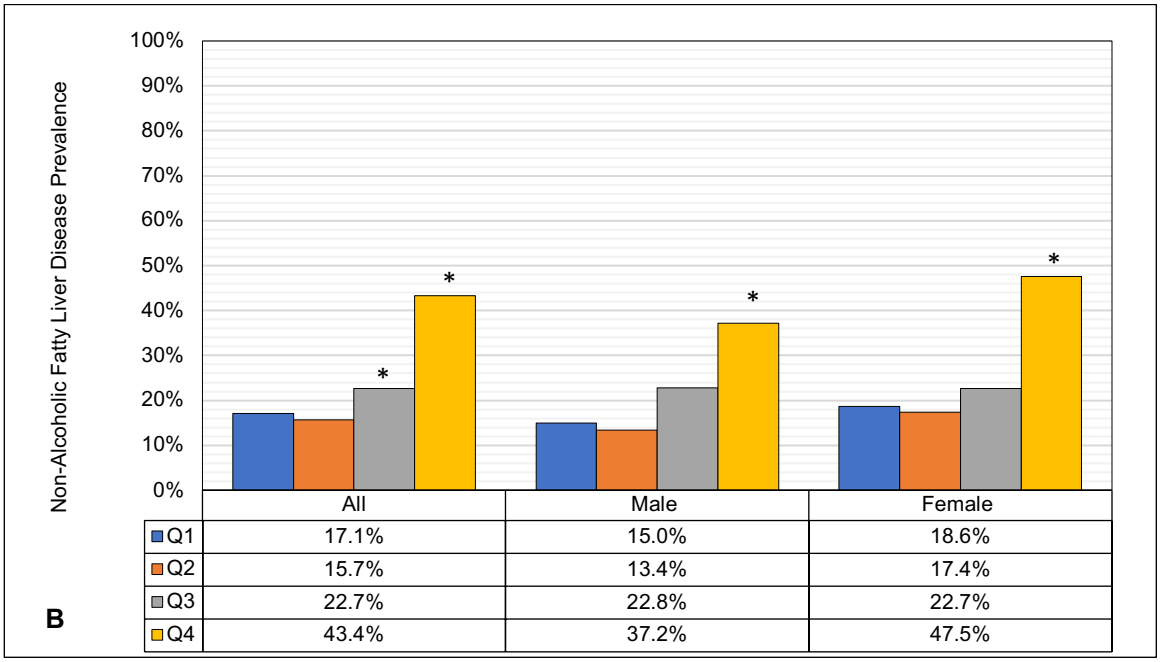
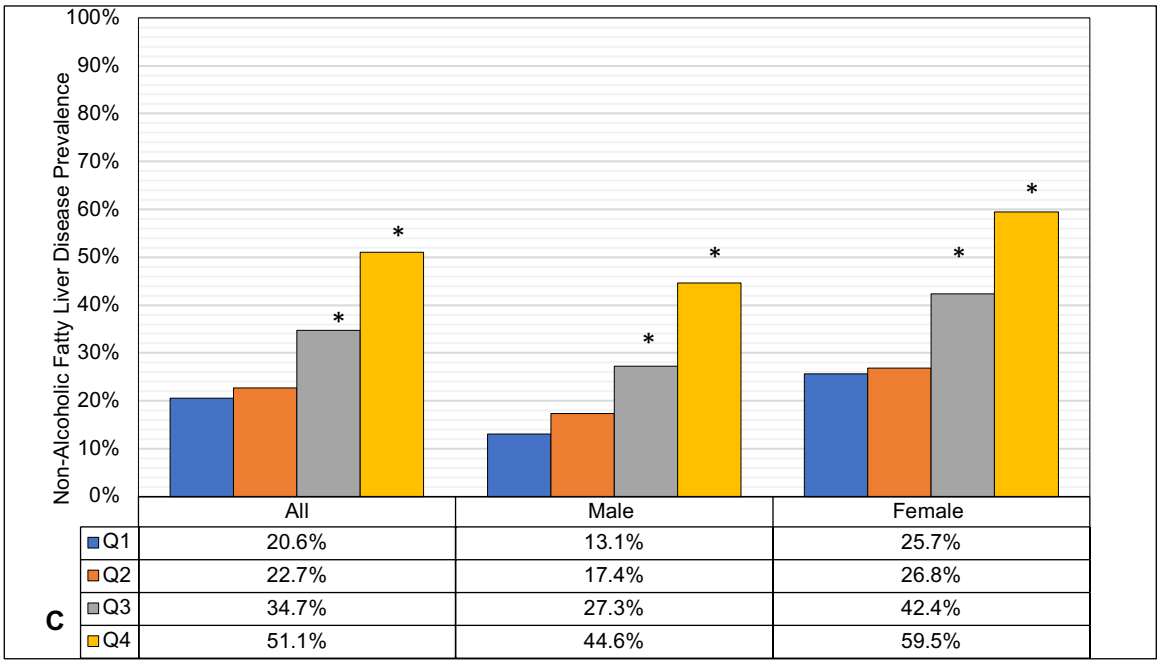


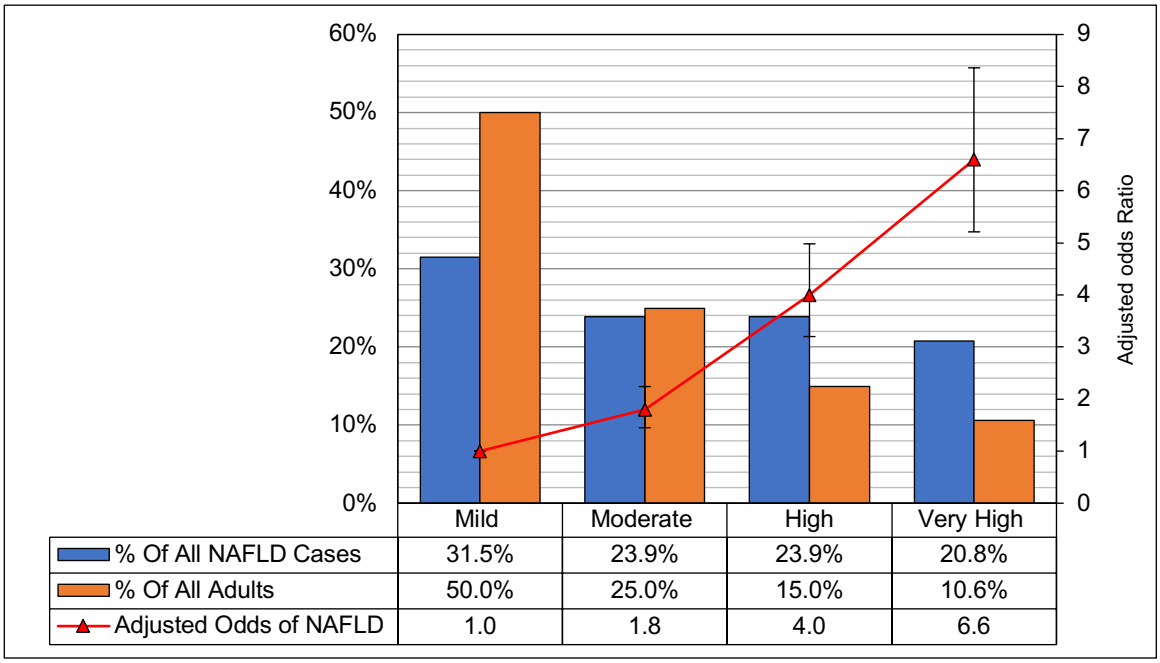
	All	Male	Female
Q1	17.1%	15.0%	18.6%
Q2	15.7%	13.4%	17.4%
Q3	22.7%	22.8%	22.7%
Q4	43.4%	37.2%	47.5%



	All	Male	Female
Q1	20.6%	13.1%	25.7%
Q2	22.7%	17.4%	26.8%
Q3	34.7%	27.3%	42.4%
Q4	51.1%	44.6%	59.5%

Figure 2-2 Age adjusted Non-Alcoholic Fatty Liver Disease Prevalence by Gender and Metabolic Syndrome Severity Z-Score Quartile (Q) for A) White, non-Hispanics B) Black, non-Hispanics and C) Mexican-Americans, US Adult, (NHANES III), (n=10,605)

The proportion of adult participants with mild, moderate, high, and very high MetS severity scores were 50%, 25%, 15%, and 10%, respectively. Approximately 31% of all NAFLD patients had low MetS severity, while an estimated 21% had very high severity scores (*i.e.*, 90th or above) (Figure 2-3). A dose-response was observed whereby an increase in the MetS severity group, in reference to mild severity, was associated with higher odds of NAFLD presence (Figure 2-3). The highest odds of NALFD presence were observed for those with very high versus low MetS severity adjusted Odds Ratio (aOR) 6.60 (95% CI; 5.21, 8.36).



	Mild	Moderate	High	Very High
% Of All NAFLD Cases	31.5%	23.9%	23.9%	20.8%
% Of All Adults	50.0%	25.0%	15.0%	10.6%
Adjusted Odds of NAFLD	1.0	1.8	4.0	6.6

* To construct the Metabolic Syndrome Severity Group, the Metabolic Syndrome Z-score was transformed into four percentiles-based categories [low (0 – 50th), Moderate (>50th – 75th), High (>75th – 90th), and very-high (>90th)]
** NAFLD odds rations were adjusted for age, gender, and race/ethnicity.

Figure 2-3 The Distribution of Metabolic Syndrome Severity, Nonalcoholic Fatty Liver Disease (NAFLD) Prevalence and the Adjusted Odds of NAFLD, US Adults, (NHANES III), (n=10,605)

2.3.5 Metabolic Syndrome Severity and the Odds of NAFLD Occurrence

The MetS severity score was a significant predictor for NAFLD occurrence in all crude and adjusted models. A quartile increase in MetS severity score was associated with an increase in the adjusted odds for NAFLD aOR 1.36 (95% CI; 1.17 – 1.57) (Data not shown). Similarly, a ten percentile increase in MetS severity was associated with 1.15 (95% CI; 1.09 – 1.20) higher adjusted odds of NAFLD. In the adjusted models with the severity score included as a categorical variable, adults with high MetS severity had aOR 2.27 (95% CI; 1.70 – 3.03) times the odds of NAFLD presence relative to those with mild MetS severity score (Table 2-3). Very high MetS severity was associated with 3.12 (95% CI; 2.20 – 4.42) higher adjusted odds of NAFLD relative to adults with mild MetS severity.

Table 2-3 The Association between Metabolic Syndrome Severity and Odds of Non-Alcoholic Fatty Liver Disease Occurrence in United States Adults, the National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=10,605)

Metabolic Syndrome Severity	Unadjusted	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Mild	Reference	Reference	Reference
Moderate	1.69 (1.36 – 2.11)	1.80 (1.45 – 2.24)	1.26 (0.97 – 1.64)
High	3.67 (2.96 – 4.54)	3.99 (3.20 – 4.98)	2.27 (1.70 – 3.03)
Very High	6.08 (4.79 – 7.72)	6.60 (5.21 – 8.36)	3.12 (2.20 – 4.42)

Metabolic Syndrome Severity Group, the Metabolic Syndrome Z-score was transformed into four percentiles-based categories [mild (0 – 50th), Moderate (>50th – 75th), High (>75th – 90th), and very-high (>90th)]

Model 1 = adjusted for age, gender, and race/ethnicity

Model 2 = Adjusted for age, gender, race/ethnicity, education level, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index percentile, HOMA-IR, total cholesterol, and Aspartate Aminotransferase (AST)/alanine aminotransferase (ALT) Ratio

OR; odds ratio, CI; Confidence interval

In the RCS analysis, a significant non-linear dose-response trend was observed in the relationship between increased odds of NAFLD occurrence and higher MetS severity score in all adjusted models. Generally, the adjusted odds of NAFLD presence increased with higher MetS severity scores relative to the median severity value. Namely, compared to those with median severity score, the aOR of NAFLD were 1.17 (95% CI; 1.11 – 1.23), 2.05 (95% CI; 1.72 – 2.43), 2.85 (95% CI; 2.23 – 3.65), and 3.38 (95% CI; 2.55 – 4.49), respectively for adults in the 60th, 80th, 90th, and 95th severity percentiles. In contrast, the adjusted odds of NAFLD were significantly lower for those with MetS severity scores below the median value up to the 43rd severity percentile (Figure 2-4).

Next, the dose-response relationship between MetS severity and the odds of NAFLD were estimated in reference to the 90th severity score percentile (Figure 2-4.b). The adjusted odds of NAFLD presence were significantly higher for adults with severity above 90th percentile relative to those in the 90th percentile. For example, adults with 95th percentile had aOR 1.18 (95% CI; 1.14 – 1.23) higher odds of NAFLD compared to those in the 90th percentile. In contrast, adults with values below the 90th severity percentile had significantly lower odds of NAFLD occurrence. Relative to those in the 90th severity score, the aOR of NAFLD were 0.72 (95% CI; 0.66 – 0.77), 0.35 (95% CI; 0.27 – 0.45), and 0.48 (95% CI; 0.32 – 0.73), respectively for adults in the 80th, 50th, and 10th severity percentiles.

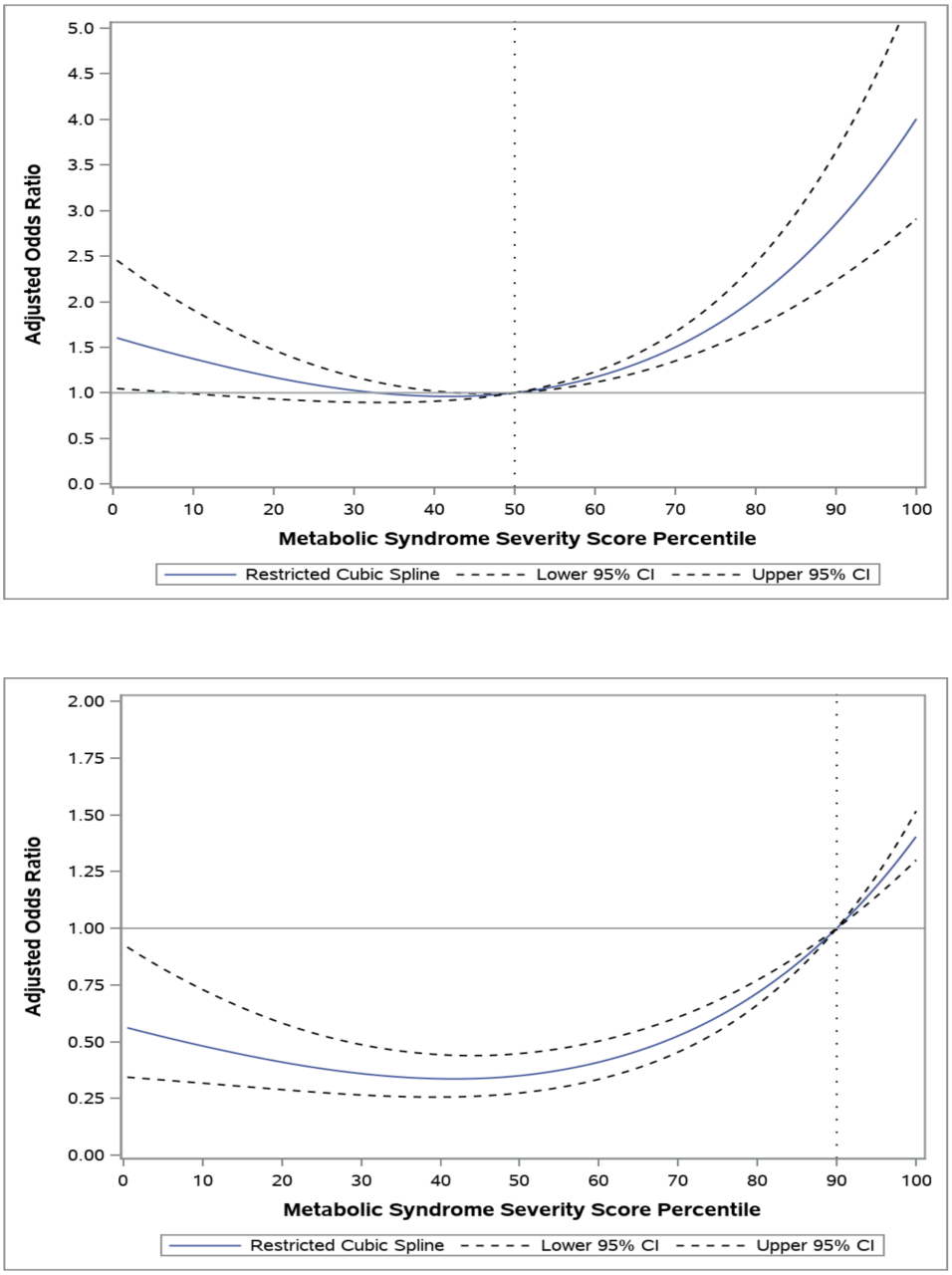


Figure 2-4 Adjusted Odds Ratios of Non-Alcoholic Fatty Liver Disease for Different Metabolic Syndrome Severity Score Percentiles Relative to A) 50th and B) 90th Severity Percentile as the Reference Levels, (NAHNES III), (n=10,605)

2.4 Discussion

Studies conducted to date on the association between MetS and NAFLD have solely utilized a harmonization disease definition, which entails exceeding pre-specified cut-off values for three out of five metabolic features. While this definition factors in disease occurrence, it equally treats the impacts of any combination of metabolic abnormalities on the risk of NAFLD development. Additionally, the traditional definition of MetS does not account for the racial and gender disparities in MetS severity and the effects of those disparities on the risk of NAFLD. To address this knowledge gap, we used the MetS severity score, a validated gender-race specific Z-score, to evaluate the association between MetS severity and NAFLD occurrence.

In this representative sample, stratified by race/ethnicity, NAFLD patients were older, obese with low levels of physical activities compared to the general US adult population. In NAFLD, racial/ethnic disparities were detected for baseline characteristics related to MetS. The prevalence of both NAFLD and traditionally defined MetS differed by race/ethnicity, with the highest estimates in Mexican American females. The MetS severity was significantly higher in NAFLD patients relative to participants without NAFLD. Amongst those with NAFLD, both Mexican American male and female adults had the highest MetS severity compared respectively to White non-Hispanic male and females NAFLD patients. The MetS severity score was a significant predictor for NAFLD occurrence in all crude and adjusted models. We also found non-linear dose-response relationships between increased adjusted NAFLD odds and higher MetS severity score.

Stratified by race/ethnicity, NAFLD prevalence was highest amongst Mexican Americans and lowest for Black non-Hispanics. Furthermore, amongst male participants, the age adjusted prevalence of NAFLD was significantly higher for Mexican Americans when compared to White non-Hispanics (25.9% vs. 31.5%; P-value 0.04). Mexican American females had a significantly higher age adjusted NAFLD prevalence when compared respectively to White non-Hispanics females (27.6% vs. 41.1%; P<0.001). Those findings are consistent with results from a meta-analysis of 343,393 individuals and two large observational studies, where NAFLD prevalence was highest in Hispanics and lowest in Black non-Hispanics.[18,40,41]

In our study, stratified by race/ethnicity and gender, NAFLD patients had significantly higher MetS severity in all comparison groups. Amongst those with NAFLD, both Mexican American male and female adults had the highest MetS severity compared to respectively to White non-Hispanic male and females NAFLD patients. Black non-Hispanics male adults with NAFLD had significantly lower MetS severity (56^{th}) compared to White non-Hispanics males with NAFLD (70^{th}). In contrast, Black non-Hispanics females with NAFLD had higher MetS severity than their White non-Hispanic counterparts.

Several studies aimed at explaining the race/ethnic disparities in relation to both the prevalence and severity of the individual components of MetS. Walker *et al.* studied waist circumference, blood pressure, triglycerides, HDL cholesterol, and fasting glucose measurements in adolescents and adults and found that the odds of MetS varied considerably among White non-Hispanics, Black non-Hispanics, and Mexican American groups.[42] Prevalence of high blood pressure, elevated fasting glucose, and insulin

resistance were significantly higher in male African Americans versus whites. In females, triglycerides associated with waist circumference in whites but not African Americans, while African American women displayed higher prevalence rates of elevated blood pressure, low HDL, and elevated fasting glucose. The study findings took into account demographic and other differences.[43] Genetic and metabolic factors have been suggested to underlie disparities,[14,44-46] as have incidence rates of insulin resistance and serum triglyceride concentrations,[47] but conclusive evidence in support of any model explaining disparities has yet to be uncovered.

Approximately 55% of all NAFLD patients in our weighted sample had low-to-moderate MetS severity, while 21% had very high severity scores (*i.e.*, 90th or above). With that in mind, any effective prevention measures must deploy a combination of two distinct strategies one for the high-risk individuals and another for the general population. Patients with very high MetS are likely to seek medical care due to their increased disease severity. Furthermore, high-risk adults are prone to having advanced symptomatic hepatic conditions such as NASH, fibrosis, cirrhosis, or HCC. Hence preventative measures for this group are expected to be of the secondary and tertiary forms of preventions. Early detection and diagnosis of those at the highest risk and providing them with medical attention will ensure an increase in survival by mitigating hepatic progression.

A population-wide preventive strategy should employ primary prevention measures such as a healthy lifestyle and dietary changes aimed at reducing MetS. From a policy perspective, primary interventions should attempt to shift, downwards, the mean MeS severity score for those with NAFLD. Since MetS predicts a wide array of cardiovascular outcomes, a decrease in severity will provide significant public health

benefits that extend beyond the NAFLD spectrum. While the natural history of NALFD might extend outside the MetS spectrum, those with low-to-moderate severity are less likely to seek medical care. Thus, regardless of the causal mechanisms linking low-to-moderate severity with NAFLD, having low severity could be viewed as a proxy for a lower probability of seeking medical care, which in turn, will result in slow disease progression.

Our findings show that an increase in MetS severity was associated with higher odds of NAFLD. Adults with high MetS severity had aOR 2.27 (95% CI; 1.70 – 3.03) times the odds of NAFLD presence relative to those with mild MetS severity score, while those with very high MetS had 3.12 (95% CI; 2.20 – 4.42) higher adjusted odds of NAFLD relative to adults with mild MetS severity. Furthermore, we observed a non-linear dose-response relationship between increase MetS severity and higher odds of NAFLD. Namely, the adjusted odds of NAFLD presence increased with higher MetS severity scores relative to the median severity value. In reference to adults with 90th percentile severity score had significantly lower odds of NAFLD occurrence.

The onsets of obesity are associated with excess accumulations of TG through the body. In the hepatocytes, increased uptake of TG results in cell-specific lipotoxicity, which elevates the risk of comorbidities such as NAFLD.[48] Individuals with high visceral adiposity may suffer from increased plasma free fatty acids, which is due to impaired insulin function related to peripheral Insulin resistance.[49] Due to the central role of obesity-induced insulin resistance plays in promoting hepatic steatosis, NAFLD is regarded as the hepatic manifestation of MetS.[3,5,7,8,25-27] The prevalence of obesity amongst NAFLD and NASH patients is 51% and 82%, respectively, and the prevalence of NAFLD in patients with MetS and diabetes is particularly high.[46,50,51]

A recent US-based study revealed significant associations between individual components of MetS and NAFLD. In individuals with increased waist circumference, the prevalence of NAFLD (31%, 8.7% with advanced fibrosis) greatly exceeded controls.[38] NAFLD patients with increased waist circumference were predominantly female, older, and less educated. Prevalence of NAFLD in subjects with diabetes (41%, 18% with advanced fibrosis), also greatly exceeded control prevalence, and NAFLD in this population was associated with advanced age and lower education.

The prevalence of NAFLD in subjects with high triglyceride levels was 35% (8% with advanced fibrosis). This same level of fibrosis was found in subjects with low HDL, though the prevalence of NAFLD was significantly lower (28%).[38] High triglycerides and low HDL were determined to be independent predictors of NAFLD. In individuals with high blood pressure, NAFLD prevalence was 29% (11% with advanced fibrosis) and not considered an independent predictor. This study also revealed that the presence of NAFLD in subjects with MetS increased according to the number of metabolic abnormalities present, exceeding 65% in patients with all five abnormalities. In the absence of MetS or any of its components, the prevalence of NAFLD is 6.1%.[38]

Our findings are not without limitations. Data used in the study were collected cross-sectionally, which did not allow for the risk estimates to be quantified. The prevalence of NAFLD in the US population exceeds the 10% threshold for the odds ratio to estimate relative risk (RR), indicating that the quantified odds ratio may over-estimate the association. NAFLD assessment was done using ultrasonography, which could result in misclassifications. Ascertainments of exposure and baseline characteristics were

conducted cross-sectionally. Alcohol intake was assessed based on self-reporting, which might result in underestimation.

2.5 Conclusion

Despite knowledge of the association between MetS related metabolic abnormalities and NAFLD, it is not known why only some MetS patients develop NAFLD. It is also unknown as to why some NAFLD patients progress to NASH and more severe disease while others do not. To address this knowledge gap, we utilized the MetS severity score to examine the association increased MetS severity and the odds of NAFLD occurrence. In this representative sample, NAFLD occurrence was associated with being older, obese, and having low levels of physical activities relative to the general US adult population. Racial/ethnic disparities in the relationship between factors related to MetS and NAFLD were detected at baseline. The proportions of both NAFLD and traditionally defined MetS differed by race/ethnicity, with Mexican American females having the highest disease burden. The MetS severity was significantly higher in NAFLD patients relative to participants without NAFLD. Amongst those with NAFLD, both Mexican American male and female adults had the highest MetS severity compared respectively to White non-Hispanic male and females NAFLD patients. The MetS severity score was a significant predictor for NAFLD occurrence in all crude and adjusted models. Adults with high and very high MetS severity had aOR 2.27 (95% CI; 1.70 – 3.03) and 3.12 (95% CI; 2.20 – 4.42) times the odds of NAFLD presence when compared respectively to those with mild MetS severity score. We also found non-linear dose-response relationships between increased adjusted NAFLD odds and higher MetS severity score. Those findings demonstrate the utility of MetS severity as a driving force of NAFLD occurrence.

Accounting for the effects of MetS severity rather than occurrence help to explain why some NAFLD patients progress to NASH and more severe disease manifestation while others do not. While current treatment options for patients with NAFLD are limited and indirect, the MetS severity score could be used as a screening tool in both primary and secondary prevention efforts to tackle this hepatic epidemic.

2.6 References

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Appendix 2.1 Sample Characteristics by Race/Ethnicity and Non-Alcoholic Fatty Liver Disease (NAFLD) Status, United States Adults, The National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=10,605)

Characteristics	White, non-Hispanics			Black, non-Hispanics			Mexican Americans		
	NAFLD (n=1,029)	No NAFLD (n=3,075)	P-value*	NAFLD (n=756)	No NAFLD (n=2,385)	P-value*	NAFLD (n=1,115)	No NAFLD (n=2,065)	P-value*
Gender, % (SE)			0.168			0.008			<0.001
Male	46.2 (1.5)	49.1 (0.9)		38.1 (2.2)	44.7 (1.3)		43.3 (1.4)	55.5 (1.2)	
Female	53.8 (1.5)	50.9 (0.9)		61.9 (2.2)	55.3 (1.3)		56.7 (1.4)	44.5 (1.2)	
Age, (years)			<0.001			<0.001			<0.001
Median (25th, 75th Percentile)	44.2 (33.6, 58.1)	39.4 (29.6, 52.7)		38.1 (28.7, 51.6)	35.7 (27.4, 46.6)		36.7 (28.0, 47.2)	31.6 (24.6, 41.6)	
Mean (SE)	46.3 (0.6)	42.3 (0.5)		41.2 (0.5)	39.1 (0.4)		39.4 (0.6)	35.4 (0.4)	
Age Group, % (SE)			<0.001			0.006			<0.001
18-34	26.2 (2.4)	36.9 (1.4)		38.7 (2.1)	44.8 (1.3)		41 (2.1)	57.2 (1.7)	
35-49	32.6 (2.5)	32.8 (1.1)		32.9 (2.1)	32.9 (1.1)		36.6 (1.7)	27.6 (1.5)	
49-64	25.8 (1.6)	19.3 (0.8)		19.1 (1.5)	15.4 (1.1)		15.7 (1.3)	11 (1)	
65+	15.4 (1.1)	11.1 (0.8)		9.3 (1.0)	6.9 (0.7)		6.7 (0.7)	4.2 (0.5)	
Education Level, % (SE)			0.001			0.727			0.042
< High School	18.3 (1.4)	16.9 (1.1)		29.2 (2.7)	28.4 (1.5)		59.6 (1.8)	53.5 (1.9)	
High School or GED	40.5 (1.7)	34.0 (1.1)		39.1 (2.7)	39.1 (1.4)		23.7 (1.6)	26.4 (1.2)	
Some College	19.8 (1.7)	23.4 (1.1)		21.9 (1.9)	20.8 (1.1)		11.3 (1.1)	14.6 (1.3)	
College degree or Higher	21.4 (2.0)	25.7 (1.2)		9.8 (1.3)	11.7 (1.2)		5.4 (0.8)	5.4 (0.8)	
Have Health Insurance, % (SE)	91.7 (0.9)	89.5 (1.0)	0.088	84.7 (2.1)	83.6 (1.6)	0.428	62.8 (1.8)	61.3 (1.9)	0.443
Alcohol Intake, % (SE)			<0.001			<0.001			<0.001
Never	13.6 (1.3)	7.7 (0.8)		21.4 (1.6)	16.1 (1.1)		20.5 (1.7)	15.2 (1.3)	
Former	33.9 (1.7)	30.5 (1.7)		42.1 (1.9)	35.4 (1.5)		34.8 (1.3)	30.8 (1)	
> 0 - 1 drinks/day	42.1 (2.0)	43.4 (1.6)		29 (2.3)	32 (1.4)		36 (2.9)	36.4 (1.1)	
> 1 drinks/day**	10.4 (1.3)	18.4 (1.1)		7.6 (0.9)	16.5 (0.8)		8.7 (1.3)	17.6 (1)	
Smoking Status, % (SE)			<0.001			<0.001			0.017
Never	44.9 (1.6)	41.2 (1.3)		58.6 (2.3)	49.8 (1.1)		61.6 (1.5)	56.2 (1.1)	
Former	31.8 (1.7)	27.1 (0.9)		18.7 (1.5)	14.9 (0.9)		20.4 (1.5)	20.1 (1.2)	
Current	23.3 (1.5)	31.7 (1.2)		22.7 (1.9)	35.3 (1.1)		18.0 (1.3)	23.7 (1.3)	
Body Mass Index (Kg/M^2)			<0.001			<0.001			<0.001
Median (25th, 75th Percentile)	27.8 (24.2, 32.0)	24.7 (22.2, 27.8)		28.9 (23.7, 34.6)	26.2 (23.0, 30.1)		28.6 (25.4, 32.2)	25.8 (23.2, 28.7)	
Mean (SE)	28.6 (0.4)	25.5 (0.1)		30.0 (0.4)	27.1 (0.2)		29.3 (0.3)	26.5 (0.1)	
Body Mass Index Category† (Kg/M^2)**,** % (SE)			<0.001			<0.001			<0.001
Underweight	1.9 (0.4)	2.5 (0.4)		2.6 (0.6)	2.2 (0.3)		0.7 (0.3)	1.3 (0.4)	
Healthy Weight	27.1 (2.2)	49.6 (1.1)		27.2 (1.8)	37.3 (1.3)		22.1 (2.3)	40.5 (1.1)	
Overweight	34.1 (1.7)	32.4 (1.0)		26.6 (1.3)	34.2 (0.9)		36.9 (1.7)	38.8 (1.1)	
Obese	36.9 (2.3)	15.6 (1.0)		43.6 (1.9)	26.3 (1.2)		40.3 (2.3)	19.5 (1.1)	
Waist to Hip Ratio			<0.001			<0.001			<0.001

Median (25th, 75th Percentile)	0.93 (0.85, 0.99)	0.89 (0.83, 0.95)		0.91 (0.85, 0.97)	0.88 (0.82, 0.94)		0.94 (0.88, 0.99)	0.91 (0.85, 0.96)	
Mean (SE)	0.93 (0.004)	0.90 (0.002)		0.89 (0.004)	0.91 (0.002)		0.94 (0.003)	0.92 (0.002)	
Abdominal Obesity ‡, % (SE)	75.5 (1.8)	62.6 (1.3)	<0.001	70.3 (1.9)	57.6 (1.6)	<0.001	83.4 (1.2)	70.1 (1.2)	<0.001
Physical Activity (METs/month)			0.002			0.005			0.100
Median (25th, 75th Percentile)	62.9 (17.5, 149.5)	78.8 (23.7, 167.5)		39.5 (1.1, 124.5)	52.0 (7.7, 148.8)		22.6 (0, 104.9)	34.7 (4.1, 124.8)	
Mean (SE)	100.6 (4.4)	118.5 (3.9)		88.3 (5.3)	107.9 (4.7)		78.5 (6.1)	89.9 (3.8)	
Physically Active, % (SE)	86.2 (1.3)	90.7 (0.7)	<0.001	75.7 (2.5)	80.5 (1.2)	0.035	72.1 (2.1)	76.9 (1.5)	0.008
Healthy Eating Index			0.470			0.390			0.102
Median (25th, 75th Percentile)	64.5 (54.1, 74.0)	63.7 (54.5, 73.2)		60.2 (50.6, 68.7)	58.7 (50.2, 67.8)		65.3 (56.0, 73.9)	63.8 (55.7, 72.2)	
Mean (SE)	64.1 (0.5)	63.8 (0.4)		59.5 (0.6)	58.9 (0.2)		64.8 (0.6)	63.8 (0.6)	
Healthy Eating Index §, % (SE)			0.369			0.517			0.449
Poor	17.2 (1.3)	16.6 (0.9)		25.4 (2.1)	27.4 (0.7)		14.2 (1.6)	15.3 (1.2)	
Fair	69.8 (1.8)	71.9 (0.7)		70 (2.0)	67.6 (0.8)		73.7 (1.6)	74.2 (1.0)	
Good	13.0 (1.4)	11.5 (0.7)		4.6 (0.7)	5.1 (0.5)		12.1 (1.3)	10.5 (1.2)	
Total Cholesterol (mg/dL)			0.006			0.002			0.015
Median (25th, 75th Percentile)	204.7 (176.9, 235.6)	199.0 (173.3, 227.7)		202.6 (172.8, 231.5)	192.5 (168.2, 220.8)		198.2 (170.8, 228.2)	190.9 (166.2, 219.7)	
Mean (SE)	208.4 (1.7)	202.8 (1.0)		205.6 (1.8)	197.2 (1.0)		201.9 (2.4)	195.2 (1.7)	
HOMA-IR			<0.001			<0.001			<0.001
Median (25th, 75th Percentile)	2.39 (1.58, 3.90)	1.62 (1.19, 2.32)		3.09 (1.80, 5.38)	2.09 (1.41, 3.18)		3.06 (1.97, 5.0)	1.92 (1.32, 3.03)	
Mean (SE)	3.9 (0.3)	2.2 (0.08)		5.2 (0.3)	3.4 (0.2)		4.9 (0.4)	2.8 (0.1)	
AST/ALT Ratio			<0.001			0.004			<0.001
Median (25th, 75th Percentile)	1.20 (0.92, 1.54)	1.35 (1.06, 1.69)		1.38 (1.09, 1.78)	1.49 (1.18, 1.89)		1.05 (0.79, 1.35)	1.23 (0.95, 1.54)	
Mean (SE)	1.3 (0.04)	1.5 (0.08)		1.5 (0.04)	1.6 (0.04)		1.1 (0.02)	1.3 (0.02)	

* Rao-Scott Chi Square P-values for difference in proportions and T-tests P-values for difference in means between adults with versus without Non-Alcoholic Fatty Liver Disease of the same race/ethnicity

** In NAFLD up to 2 drink per days for females and 3 drinks per day for males

† Underweight (< 18.50), Healthy Weight (≥ 18.50 - 25.00 <), Overweight (≥ 25.00 - 30.00 <) and Obese (≥ 30)

‡ Waist to Hip Ratio ≥ 0.90 for males or ≥ 0.85 for females

§ Poor < 51%, Fair < 80%, Good ≥ 80%

MET= Metabolic equivalent; AST= Aspartate Aminotransferase; ALT= alanine aminotransferase; % = Weighted Proportion; SE= Standard Error

Chapter 3 The Relationship between Increased Metabolic Syndrome Severity and the Presence of Advanced Fibrosis amongst Adults with Non-alcoholic Fatty Liver Disease.

ABSTRACT

Background and Objectives: The presence of advanced fibrosis is a significant predictor of increased risk of mortality in Non-Alcoholic Fatty Liver Disease (NAFLD). While Metabolic Syndrome (MetS) is a significant risk factor for hepatic degeneration, it is unknown why only some NAFLD patients with MetS progress to advanced fibrosis. This chapter aimed to use the MetS severity score, a clinically validated continuous measure of MetS, to investigate the association between increased MetS severity and the probability of advanced fibrosis occurrence in NAFLD.

Methods & Materials: The study included 3,036 NAFLD patients ages 20 to 74 years who participated in the Third National Health and Nutrition Examination Survey (NHANES III) and met the study's inclusion and exclusion criteria. NAFLD was defined as mild, moderate, or severe hepatic steatosis on ultrasound in the absence of hepatitis B, hepatitis C, iron overload, and excessive alcohol intake. The study's primary outcome was the probability of advanced fibrosis in adults with NAFLD. The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) was used to estimate the probability of advanced fibrosis presence. An individual NFS value between -1.455 and 0.676 was used to define an intermediate to high advanced fibrosis probability, while a score of >0.676 was classified as a high probability of advanced fibrosis. The primary exposure was the MetS severity score. All five metabolic features (*i.e.*, high-density lipoprotein, systolic blood

pressure, waist circumference, triglycerides, and blood glucose) were used to calculate gender-race specific MetS severity Z-score, which was then transformed into four percentiles-based categories [low (0^{th}-50^{th}), moderate (>50^{th}-75^{th}), high (>75^{th}-90^{th}), very high (>90^{th}+)]. Multivariable adjusted logistic regression models were used to test the associations between increases in MetS severity and the odds of advanced fibrosis in NAFLD. The dose-response relationships between MetS severity and the odds of advanced fibrosis in NAFLD were evaluated using the MetS severity score percentiles as a continuous variable with a three-knot restricted cubic spline (RCS) in the adjusted logistic regression models. Complex survey methods using sampling weights, strata, and clusters were used to yield nationally representative prevalence and effect estimates.

Results: A higher MetS severity in NAFLD was associated with older age, increases in BMI, central obesity, total cholesterol, and HOMA-IR, along with a decrease in physical activities. An estimated 95.3% of those in the fourth severity quartile were overweight or obese compared to 21.9% of those in the first quartile. Similarly, the proportion of those with abdominal obesity increased significantly with higher MetS quartile (Q_1 vs. Q_4; 29.7% vs. 97.4%; P-value <0.001). Amongst all NAFLD patients, 65.2%, 29.6%, and 5.2% had a low, intermediate, and high probability of advanced fibrosis. The proportions of NAFLD adults with a high probability of advanced fibrosis was highest amongst Black non-Hispanics (8.0%) and lowest in Mexican Americans (2.6%). A higher probability of advanced fibrosis was associated with increased MetS severity in all five metabolic features. In those with a low, intermediate, and high probability of advanced fibrosis, 76.0%, 93.0%, and 97.0% had at least one feature of the traditionally defined MetS, respectively. The severity of MetS increased significantly with a higher probability of

advanced fibrosis. The mean MetS severity Z-scores for NAFLD patients with low, intermediate, and high advanced fibrosis were 0.184 (55th), 0.965 (73rd), and 1.538 (81st), respectively. The prevalence of intermediate to high advanced fibrosis probability was 39.0% and 58%.0 amongst NAFLD adults with high and very high MetS severity scores, respectively. In NAFLD, the MetS severity score was a significant predictor for both intermediate to high and high probabilities of advanced fibrosis in all crude and adjusted models. NAFLD adults with very high MetS severity had aOR 2.29 (95% CI; 1.65 – 3.19) times the odds of intermediate to high advanced fibrosis probability relative to NAFLD patients with low MetS severity score. A very high MetS severity remained a significant predictor of high advanced fibrosis probability compared to low MetS severity aOR 2.10 (95% CI; 1.02 – 4.34). A significant non-linear dose-response trend was observed in the relationship between increased odds of advanced fibrosis probability and higher MetS severity score in NAFLD. Compared to those in the 90th severity score percentile, the aOR of intermediate to high advanced fibrosis probability was 1.24 (95% CI; 1.13 – 1.36) for adults with NAFLD in 95th severity percentiles. In contrast, the adjusted odds of NAFLD were significantly lower for those with MetS severity scores below the 90th percentiles and up to those in the 34th severity percentile

Conclusions: In NAFLD, advanced fibrosis is associated with higher MetS severity that is triggered by obesity-induced insulin resistance. Accounting for the combined effects of MetS severity rather than occurrence help to explain why only some NAFLD patients progress to advanced fibrosis. The MetS severity score could be used as a screening tool to monitor hepatic progressions in NAFLD.

Keywords: NAFLD, NFS, Advanced fibrosis, dose-response, metabolic syndrome severity

3.1 Introduction

Non-alcoholic Fatty Liver Disease (NAFLD) is the most common cause of Chronic Liver Disease (CLD) globally, with a prevalence of 25%.[1,2] Recent increases in obesity-induced metabolic syndrome (MetS) are connected to an upsurge in the incidence of NAFLD [3], especially in the United States (US), where an estimated 39.8% of all adults are considered to be obese.[4,5] Accordingly, the current prevalence of NAFLD in the US adults is estimated to be 26%.[6] Aside from its high prevalence, the economic burden of NAFLD has increased dramatically in recent years[7], with the total annual cost of NAFLD estimated at $292.19 billion, of which $103.31 billion are direct costs.[8]

NAFLD is defined as the presence of hepatic steatosis, by imaging or histology, in the absence of a secondary cause such as hepatotoxic drug use, excessive alcohol consumption, or hereditary disorders.[9,10] NAFLD includes a spectrum of histological states ranging in severity from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH). NASH encompasses more widespread disease including steatosis in greater than five percent of hepatocytes, inflammation, and hepatocyte injury with or without fibrosis. An estimated 59.1% of patients with NAFLD develop NASH, of which, 10%-25% progress to fibrosis and a further 2%-13% progress to hepatocellular carcinoma (HCC).[6] In 2009-2012, the prevalence of NAFLD related advance fibrosis in the US increased 2-fold over the 1999-2002 levels.[11]

Patients with NAFL do not typically require medical therapy; however, NASH, especially NASH-fibrosis, cirrhosis, and HCC, are targets for innovative interventions.[12,13] Although liver-related death is currently the third leading cause of death in NAFLD patients[14-16], NASH related fibrosis is the most rapidly rising cause of liver transplantation and is projected to be the leading cause in the coming years.[17] Furthermore, fibrosis in NAFLD/NASH is expected to become the main risk factor for liver-related mortality.[17]

Obesity results in increased accumulations of triglyceride (TG) throughout the body. In the hepatocytes, increased uptake of TG results in cell-specific lipo-toxicity, which raises the risk of comorbidities such as NAFLD.[18] Individuals with high visceral adiposity may suffer from increased plasma free fatty acids, which is due to impaired insulin function related to peripheral Insulin resistance.[19] Due to the essential role of obesity-induced insulin resistance plays in promoting hepatic steatosis, NAFLD is regarded as the hepatic manifestation of MetS.[1,6,20-24]

The presence of advanced fibrosis is a significant predictor of increased risk of mortality in CLD.[25] Patients with NAFLD related fibrosis have a higher risk of developing cirrhosis compared to those with simple steatosis (10.8 vs. 0.7).[16] Furthermore, NASH fibrosis patients have an 8-fold increase in liver-related mortality relative to NAFLD patients without fibrosis.[16] While insulin resistance has been suggested as the main risk factor for fibrosis progression in NAFLD, little is known about the pathophysiological (*i.e.*, regression and progression) relationships between MetS and hepatic steatosis stages (*i.e.*, NASH, fibrosis, cirrhosis, and HCC) in NAFLD.[3,26]

MetS describes a group of metabolic abnormalities that are associated with an increased risk of insulin resistance and cardiovascular disease.[3] The clinical features of MetS are atherogenic dyslipidemia, hyperglycemia, hypertension, and visceral obesity.[27] The diagnosis of MetS involves the presence of three of the following five risk factors: 1) hyperglycemia (fasting glucose ≥ 100 mg/dL), 2) low concentrations of high-density lipoprotein (HDL) cholesterol (< 40 mg/dL for males and < 50 mg/dL for females), 3) hypertriglyceridemia, (triglycerides > 150 mg/dL), 4) increased waist circumference (102 cm for men and 89 cm for females) or hypertension (> 85 mm/Hg diastolic blood pressure).[27]

The current dichotomous classification of MetS diagnosis has three main shortcomings concerning understanding hepatic occurrence and progression in NAFLD. First, the diagnosis of the MetS entails the occurrence of any three of the mentioned five risk factors (*i.e.*, ten potential different combinations). However, the rates of each of the five metabolic syndrome features (MSFs) differ among NAFLD patients. Namely, the prevalence of hyperlipidemia among European NAFLD patients is 81.3%, while the prevalence of MetS is 38.3%.[28] In addition, the rates of progression and regression between each MSF and NAFLD are not fully understood, as natural history studies, to date, make use of the binary MetS classification. Second, MetS diagnosis involves meeting at least three predefined cutoff points, and it neglects both the sole and combined severity of MSFs. Such neglect creates a gap in knowledge about the impact of overall MetS severity in relation to the natural history of NAFLD. Third, a dichotomous MetS categorization makes it difficult to study the clinical implications of worsening in the severity of MetS over time.

There is currently a need for a better understanding of the combined role of all five MSFs, as oppose to MetS, in analyzing the hepatic manifestation and progressions of NAFLD. The current shortcomings in the definition of MetS in relation to the NAFLD spectrum could be overcome by using a continuous measure of MetS severity that encapsulates the statuses of all five MSF in one summary risk score. The MetS severity score is a validated clinically-accessible gender-race specific Z-score that summarizes the combined severity of all five MSFs amongst US adults.[29] As such, the MetS severity score is a continuous representation of the traditional MetS classification, while adjusting for gender and racial/ethnic disparities in the relationship between MetS and cardiometabolic outcomes.

The main objective of this chapter is to use the continuous measure of MetS to investigate the association between increased MetS severity and the probability of advanced fibrosis occurrence in NAFLD. Evaluating the relationship between MetS severity and fibrosis occurrence in NAFLD is a step toward a better understanding of the risk of hepatic progression in NAFLD. Such information could aid the ongoing public health efforts devoted to addressing the burden of this disease. Results from this study could aid prevention efforts by examining the utility of the MetS severity score as a screening tool to identify patients with the highest probability fibrosis in NAFLD.

3.2 Materials and Methods

3.2.1 Data Source

The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) conducted the third National Health and Nutrition Examination Survey

(NHANES III) between 1988 and 1994. The survey aimed to evaluate the health status of the US population with a focus on minority groups such as non-Hispanic Blacks, Mexican Americans, and individuals sixty years of age and older. Stratified multistage clustered probability design was employed to construct a representative sample of noninstitutionalized members of the US population ages two months or older. The survey incorporated cross-sectional examinations, interview questionnaires, and laboratory sample collections. Of all the interviewed participants, 78% took part in the physical examination phase of the survey.[30] The ethics review board of the CDC approved the NHANES III protocol.

3.2.2 Study Sample

For all NHANES III participants 20 to 74 years of age, hepatic steatosis was assessed by three trained ultrasound readers, using gallbladder ultrasound videos recorded during the physical examinations. Following the initial assessments, all ultrasound readings were reevaluated and validated by a certified radiologist specialized in hepatic imaging. Upon reevaluations, hepatic images were classified into four steatosis-based groups normal, mild, moderate, or severe. Criteria for grading hepatic steatosis included gallbladder walls definition, the liver parenchyma degree of brightness, occurrence of deep beam attenuation, the presence of liver to kidney contrast, and echogenic walls in the small intrahepatic vessels.[31]

NAFLD was identified by the presence of mild, moderate, or severe hepatic steatosis in the absence of excessive drinking (*i.e.*, more than three alcoholic beverages per day for males and more than two alcoholic beverages per day for females), binge

drinking (*i.e.*, frequent consumption of five or more alcoholic beverages per day), alcohol consumption restrictions due to illness, positive Hepatitis B virus (HBV) surface antigen test, positive Hepatitis C virus (HCV) RNA Test, or Iron overload (*i.e.*, transferrin saturation of ≥50%).

Participants were excluded from the study if they had missing values for exposure, outcome, alcohol intake, ultrasound images, or any of the covariates included in the adjusted analyses. Participants who self-identified as "Other" race/ethnicity were also excluded, as the exposure assessment is only applicable to Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics. Of the total 16,573 individuals ages twenty years or older who attended the examination phase of the survey, 14,707 qualified for the gallbladder ultrasound readings, of which, 13,856 participants had readable images.[32] Accordingly, a total of 5,484 participants had mild, moderate, or severe hepatic steatosis on ultrasound, of which, 3,036 NAFLD patients met the study's inclusion and exclusion criteria.

3.2.3 Exposure

The MetS severity score was the primary exposure variable. The MetS severity score is a clinically validated gender- and race/ethnicity- specific Z-score that captures the relative MetS severity of all five metabolic abnormalities.[29] The score was quantified using Confirmatory Factor Analysis (CFA) with data from the 1999-2010 NHANES.[29] All five metabolic features were used in the CFA to construct a summary score that is a continuous representation of the conventional metabolic syndrome characterization.

Participants in the 1999-2010 NHANES were divided into six groups based on gender and self-identified race/ethnicity, including Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics. Different loading coefficients were quantified to determine a single latent MetS factor for all six sup-groups. Individual-level data for HDL, SBP, waist circumference, TG, and fasting blood glucose were used to calculate gender- and race/ethnicity-specific MetS severity Z-scores according to the score's standardized equations.[29] In turn, the MetS severity Z-score was transformed into four percentiles-based categories [low (0 – 50th), Moderate (>50th – 75th), High (>75th – 90th), and very-high (>90th)].

The MetS severity score is significantly correlated with pathophysiological biomarkers of MetS, including the Homeostasis Model for Insulin Resistance (HOMA-IR), C-Reactive protein (CRP), uric acid, and adiponectin.[29,33] Multiple studies have also shown the MetS severity score to be a significant predictor of long-term risks of cardiovascular disease, type 2 diabetes mellitus, and coronary heart disease.[33-36]

The ATP-III guidelines were used to define the traditional MetS classification. As such, MetS is defined by the presence of three of the five metabolic factors: (1) *hyperglycemia* (*i.e.*, fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) *dyslipidemia* (*i.e.*, fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl, women, or pharmacological treatment) (3) *hypertriglyceridemia* (*i.e.*, fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (*i.e.*, waist circumference over 40 inches for men, or 35 inches for women), or (5) hypertension (*i.e.*, systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment).[27]

3.2.4 Outcome

The study's main outcome was the presence of advanced fibrosis in adults with NAFLD. The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) is a clinically validated measure of advanced fibrosis probability in NAFLD.[25] The NFS makes use of the Body Mass index (BMI), the aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio, platelet count, albumin levels, and diabetes status.[37]

> NFS = -1.675 + 0.307 age (years) + 0.094 BMI (kg/m^2) +1.13 prediabetes /diabetes (yes =1, no =0) + 0.99 (AST [IU/L]/ALT [IU/L]) – 0.013 platelet count (10^9/L) – 0.66 albumin (g/dL).

An NFS score of 0.676 or above has 0.82, 97%, and 82% Area Under Receiver Operator Characteristic Curve (AUROC), specificity, and positive predictive value, for detecting bridging fibrosis in NAFLD, respectively.[6,38] A cutoff point of less than -1.455 has a negative predictive value of 88% for excluding patients with advanced fibrosis. Two different outcome variables were used, an individual NFS value of -1.455 through 0.676 was used to define an intermediate to high advanced fibrosis probability, while a score of >0.676 was classified as a high probability of advanced fibrosis. Two distinctive outcome variables were used to assess the robustness of the relationship between MetS severity and probabilities of advanced fibrosis.

3.2.5 Covariates

During the interview and examination phases, data were gathered on multiple covariates, including confounding variables and other factors used in the secondary statistical analyses. Confounder selection was based on both *a priori* knowledge, form the literature,

and theoretical rationale. Confounders used in the adjusted multivariate analyses included age, gender, race/ethnicity (White non-Hispanics, Black non-Hispanics, or Mexican Americans), education level (< high school, high school, or GED; some college or college degree or higher), access to health insurance (yes or no), alcohol intake (never, former, > 0-1 drinks/day, or > 1 drinks/day), smoking status (never, former, or current), BMI (Kg/m^2) [healthy weight (25.0<), overweight (≥25.0 - 30.0<), or obese (≥30)], abdominal obesity, physical activity (metabolic equivalents/month), healthy eating index percentile, HOMA-IR, AST/ALT ratio and total cholesterol.

3.2.6 Statistical Analyses

Complex survey methods, using sampling weights, strata, and clusters were used to yield nationally representative estimates. In order to account for the effects of the survey design, Taylor series linearization was used to quantify all variance values. Missing values related to variance estimation were assumed not to be missing completely at random.

Baseline characteristics of NAFLD patients stratified by MetS severity quartile were examined by testing the difference in means for continuous variables, using weight adjusted analysis of variance, and using Rao Scott Chi-Square for categorical variables. To assess the association between MetS and the probability of advanced fibrosis in NAFLD, age, gender, and race/ethnicity adjusted mean estimates for clinical characteristics related to MetS were quantified. We also evaluated the probability distribution of advanced fibrosis amongst NAFLD patients by race/ethnicity. Furthermore,

we quantified the advanced fibrosis probability distribution by MetS severity group for all adults with NAFLD.

Multivariable logistic regression models were used to test the associations between an increase in MetS severity and the odds of 1) intermediate to high advanced fibrosis probability, and 2) high advanced fibrosis probability in NAFLD. The dose-response relationships between MetS severity and the odds of both intermediate to high, and high advanced fibrosis probabilities in NAFLD were evaluated using the MetS severity score percentiles as a continuous variable with a three-knot restricted cubic spline (RCS) in the adjusted logistic regression models. As recommended by Harrell, 2015,[39] the three-knots were places at 10^{th}, 50^{th}, and 90^{th} of the weighted MetS severity score percentile values for US adults. Wald-Chi Square tests were used to assess the overall and non-linear associations between the MetS severity score percentiles and the odds of advanced fibrosis in NAFLD. A P-value of less than 0.05 was considered statistically significant. All analyses were performed using the SAS 9.4 software (SAS Institute, NC, USA).

3.3 Results

3.3.1 Participants Baseline Characteristics

Baseline characteristics of adults with NAFLD by MetS severity quartile are outlined in Table (3-1). The study sample included 3,036 adult participants in the NHANES III, who met the inclusion and exclusion criteria for NAFLD classification. The male gender was significantly associated with an increase in the MetS severity quartile. The mean age of NAFLD patients in the fourth quartile was 14.4 years higher than those in the first quartile.

The distributions of both race/ethnicity and education level were associated with increases in MetS severity. The proportions of NAFLD patients who were current smokers and those who reported average alcohol intake of >1 drinks/day decreased by an increase in MetS severity. Both the healthy eating index and health insurance status were not associated with MetS severity increase.

Obesity-related factors were more prevalent amongst NAFLD patients with higher MetS severity quartiles relative to those with low MetS severity. An estimated 95.3% of those in the fourth severity quartile were overweight or obese compared to 21.9% of those in the first quartile. Similarly, the proportion of those with abdominal obesity increased significantly with higher MetS quartile (Q_1 vs. Q_4; 29.7% vs. 97.4%; P-value <0.001). NAFLD patients in the first severity quartile had 42.8 mg/dL lower average total cholesterol compared to those in the fourth quartile. NAFLD patients in the fourth severity quartile had the highest mean HOMA-IR with 9.0 compared to 1.5 for individuals in the first quartile.

Table 3-1 Sample Characteristics by Metabolic Syndrome Severity Score Quartiles, United States Adults with Non-Alcoholic Fatty Liver Disease (NAFLD), The National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=3,036)

Characteristics	Q1 (n=688)	Q2 (n=775)	Q3 (n=757)	Q4 (n=816)	P-Value*
Gender, % (SE)					<0.001
Male	25.9 (2.8)	45.4 (2.6)	55.2 (2.6)	55.1 (2.8)	
Female	74.1 (2.8)	54.6 (2.6)	44.8 (2.6)	44.9 (2.8)	
Age, (years)					<0.001
Median (25th, 75th Percentile)	32.9 (26.4, 40.9)	43.3 (33.5, 56.2)	47.1 (36.6, 61.2)	50.8 (40.2, 61.1)	
Mean (SE)	36.1 (0.91)	45.6 (0.73)	48.9 (0.88)	50.5 (0.72)	
Age Group, % (SE)					<0.001
18-34	53.6 (4.5)	26.6 (2.8)	18.6 (2.3)	15.2 (2.5)	
35-49	33.4 (3.7)	34.4 (3.1)	33.5 (3)	30.5 (3.4)	
49-64	7.2 (1.3)	26.3 (2.4)	28.7 (2.1)	35.9 (2.6)	
65+	5.9 (1.3)	12.7 (1.5)	19.3 (2.1)	18.4 (1.7)	
Race/ethnicity, % (SE)					<0.001
White, non-Hispanics	80.9 (2.1)	82.0 (2.0)	84.8 (1.5)	85.1 (1.1)	
Black, non-Hispanics	13.5 (1.6)	10.0 (1.5)	7.3 (0.83)	7.9 (0.73)	
Mexican Americans	5.6 (1.0)	8.0 (1.2)	7.9 (1.1)	7.0 (0.87)	
Education Level, % (SE)					0.002
< High School	16.7 (2.4)	21.5 (2.2)	23.0 (2.5)	27.7 (2.3)	
High School or GED	34.8 (3.4)	40.6 (2.7)	40.2 (2.6)	42.5 (3.0)	
Some College	25.7 (2.9)	19.0 (2.7)	15.5 (1.8)	16.9 (2.2)	
College degree or Higher	22.8 (3.6)	18.9 (3.0)	21.2 (2.9)	13.0 (1.9)	
Have Health Insurance, % (SE)	88 (1.5)	88.2 (1.5)	87 (2.7)	92.5 (1.3)	0.142
Alcohol Intake, % (SE)					<0.001
Never	11.6 (1.4)	14.0 (2.1)	16.1 (2)	18.0 (1.8)	
Former	23.1 (3.1)	32.2 (2.0)	37.6 (2.4)	46.7 (2.8)	
> 0 - 1 drinks/day	51.8 (3.1)	42.4 (3.0)	38.0 (2.9)	29.8 (2.7)	
> 1 drinks/day**	13.4 (1.9)	11.4 (2.0)	8.3 (1.9)	5.5 (1.6)	
Smoking Status, % (SE)					<0.001
Never	55.3 (3.2)	47.8 (2.8)	45.9 (3.0)	40.1 (2.8)	
Former	18.2 (2.1)	28.5 (2.6)	32.8 (3.0)	39.6 (2.6)	
Current	26.5 (2.4)	23.7 (2.5)	21.3 (3.0)	20.4 (2.0)	
Body Mass Index (Kg/M^2)					<0.001
Median (25th, 75th Percentile)	22.0 (20.2, 24.6)	27.4 (24.9, 30.7)	30.2 (27.0, 33.4)	32.0 (28.9, 36.9)	
Mean (SE)	22.6 (0.18)	28.0 (0.25)	30.9 (0.32)	33.6 (0.55)	

Body Mass Index Category† (Kg/M^2), % (SE)					<0.001
Healthy Weight	78.1 (2.5)	25.0 (2.6)	7.1 (1.3)	4.7 (0.9)	
Overweight	17.8 (2.4)	43.0 (2.5)	41.7 (2.9)	31.0 (2.8)	
Obese	4.1 (0.9)	32.0 (2.3)	51.2 (3.1)	64.3 (3.0)	
Waist to Hip Ratio					<0.001
Median (25th, 75th Percentile)	0.81 (0.76, 0.87)	0.92 (0.87, 0.97)	0.96 (0.92, 1.0)	0.99 (0.94, 1.03)	
Mean (SE)	0.83 (0.005)	0.93 (0.004)	0.97 (0.003)	0.99 (0.003)	
Abdominal Obesity ‡, % (SE)	29.7 (2.7)	82.2 (2.2)	93.5 (1.4)	97.4 (1.0)	<0.001
Physical Activity (METs/month)					<0.001
Median (25th, 75th Percentile)	79.1 (21.8, 170.5)	51.5 (9.1, 134.7)	60.1 (19.6, 141.6)	34.8 (9.7, 110.5)	
Mean (SE)	126.2 (9.5)	90.6 (6.1)	99.2 (7.4)	74.6 (6.1)	
Physically Active, % (SE)	88.2 (1.7)	82.0 (2.6)	85.1 (2.1)	81.6 (2.0)	0.071
Healthy Eating Index					<0.001
Median (25th, 75th Percentile)	62.7 (52.9, 71.2)	64.8 (55.0, 75.3)	65.0 (54.9, 74.6)	63.8 (53.1, 73.4)	
Mean (SE)	62.2 (1.1)	64.7 (0.76)	64.6 (0.68)	63.7 (0.77)	
Healthy Eating Index §, % (SE)					0.222
Poor	20.8 (3.5)	15.0 (2.4)	17.4 (2.1)	17.8 (2.1)	
Fair	70.1 (3.1)	69.2 (3.6)	69.4 (2.3)	71.2 (2.3)	
Good	9.1 (1.7)	15.8 (2.5)	13.2 (1.6)	11.0 (1.7)	
Total Cholesterol (mg/dL)					<0.001
Median (25th, 75th Percentile)	176.1 (154.8, 200.1)	208.0 (180.5, 233.8)	211.9 (189.9, 239.1)	219.4 (192.7, 249.4)	
Mean (SE)	180.8 (2.2)	209.3 (2.1)	216.5 (1.8)	223.6 (3.0)	
HOMA-IR					<0.001
Median (25th, 75th Percentile)	1.3 (1.0, 1.7)	2.2 (1.7, 3.0)	4.1 (3.1, 4.1)	5.4 (3.3, 9.0)	
Mean (SE)	1.5 (0.05)	2.6 (0.08)	3.5 (0.12)	9.0 (0.73)	
AST/ALT Ratio					<0.001
Median (25th, 75th Percentile)	1.5 (1.3, 1.9)	1.2 (0.94, 1.49)	1.1 (0.85, 1.39)	1.0 (0.82, 1.28)	
Mean (SE)	1.6 (0.05)	1.3 (0.04)	1.2 (0.03)	1.1 (0.03)	

* Rao-Scott Chi Square P-values for difference in proportions and T-tests P-values for difference in means between adults with versus without Non-Alcoholic Fatty Liver Disease of the same race/ethnicity

** In NAFLD up to 2 drink per days for females and 3 drinks per day for males

† Healthy Weight = (25.0 <), Overweight = (≥ 25.0 - 30.0 <) and Obese = (≥ 30.0)

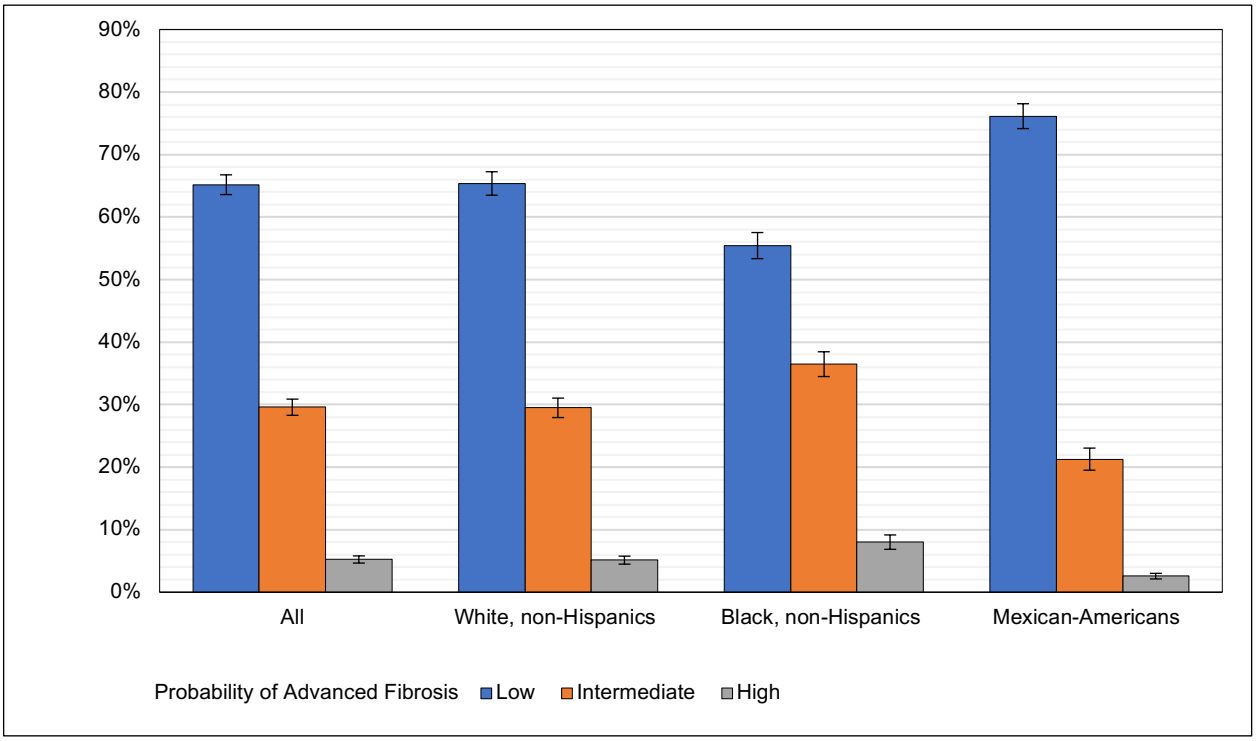
‡ Waist to Hip Ratio ≥ 0.90 for males or ≥ 0.85 for females

§ Poor < 51%, Fair < 80%, Good ≥ 80%

MET= Metabolic equivalent; AST= Aspartate Aminotransferase; ALT= alanine aminotransferase; % = Weighted Proportion; SE= Standard Error

3.3.2 Advanced Fibrosis Probability in NAFLD

Figure 3-1 depicts the distribution of the advanced fibrosis probability status for adults with NAFLD by race/ethnicity. Amongst all NAFLD patients, 65.2%, 29.6%, and 5.2% had a low, intermediate, and high probability of advanced fibrosis. This distribution differed significantly by race/ethnicity. The proportions of NAFLD adults with a high probability of advanced fibrosis was highest amongst Black non-Hispanics (8.0%) and lowest in Mexican Americans (2.6%). The prevalence of intermediate to high probability of advanced fibrosis was 44.6% and 23.9% in Black non-Hispanics and Mexican Americans NAFLD patients, respectively.



* Fibrosis probability based on the Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS); Low (< -1.455); Intermediate (-1.455 to 0.676); High (> 0.676)

Figure 3-1 Advanced Fibrosis Probability Distribution by Race/Ethnicity for Adults with Nonalcoholic fatty liver disease (NAFLD), (NHANES III), (n=3,036)

3.3.3 MetS Severity and the Probability of Advanced Fibrosis in NAFLD

Adjusted clinical characteristics related to MetS stratified by advanced fibrosis probability are outlined in table (3-2). A higher probability of advanced fibrosis was associated with increased MetS severity in all five metabolic features. The prevalence of the traditionally defined MetS increased in NAFLD patients with higher probabilities of fibrosis. In those with a low, intermediate, and high probability of advanced fibrosis, 76.0%, 93.0%, and 97.0% had at least one feature of the traditionally defined MetS, respectively. An estimated 33.0% of NALFD patients with a high probability of advanced fibrosis met the criteria for all five metabolic components.

Table 3-2 Age, Gender and Race/ethnicity Adjusted Estimates for Clinical Characteristics Related to Metabolic Syndrome by Advanced Fibrosis Probability, Adults with Non-Alcoholic Fatty Liver Disease (NAFLD), (NHANES III), (n=3,036)

Clinical Characteristics	Advanced Fibrosis Probability ‡			P-Trend**
	Low	Intermediate	High	
	(n=1,818)	(n=1,001)	(n=217)	
Metabolic Syndrome Severity Score, Mean (SE)	0.42 (0.04)	0.90 (0.07) *	1.4 (0.15) *	<0.001
Number of Metabolic Abnormalities†, Mean (SE)	2.1 (0.06)	2.7 (0.09) *	3.1 (0.15) *	<0.001
Metabolic Syndrome‡, % (SE)	40.7 (0.02)	57.4 (0.03) *	65.6 (0.05) *	<0.001
Central Obesity, % (SE)	40.4 (0.02)	72.4 (0.02) *	76.4 (0.04) *	<0.001
Waist Circumference (inches), Mean (SE)	94.6 (0.76)	104.5 (1.10) *	113.5 (2.42) *	<0.001
Hypertriglyceridemia, % (SE)	41.9 (0.02)	54.4 (0.03) *	64.1 (0.05) *	<0.001
Triglyceridemia (mg/dL), Mean (SE)	169.2 (4.9)	178.2 (8.6)	182.9 (15.3)	0.584
Hypertension, % (SE)	42.8 (0.02)	52.6 (0.03) *	58.0 (0.05) *	0.031
Systolic Blood Pressure (mmHg), Mean (SE)	125.4 (0.05)	128.0 (0.73) *	129.1 (1.6) *	0.009
Dyslipidemia, % (SE)	44.2 (0.02)	47.9 (0.03)	51.3 (0.05)	0.379
High-Density Lipoprotein Cholesterol (mg/dL), Mean (SE)	48.7 (0.51)	46.0 (0.67) *	44.6 (1.98) *	0.005
Hyperglycemia, % (SE)	32.2 (0.02)	44.4 (0.02) *	60.1 (0.04) *	<0.001
Plasma Glucose (mg/dL), Mean (SE)	103.6 (1.4)	113.62 (2.21)*	126.5 (4.7) *	<0.001

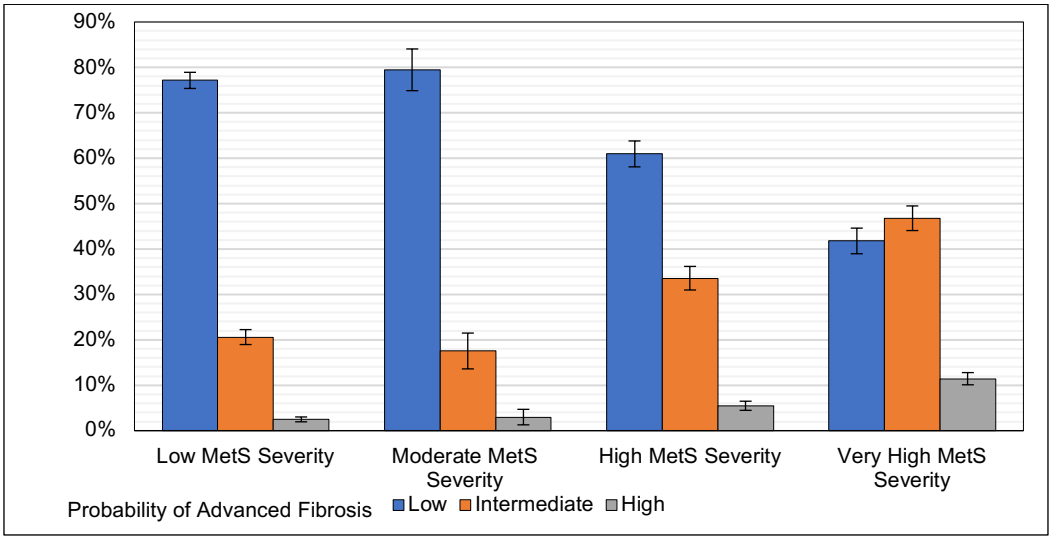
* P-value <0.05 in reference low fibrosis probability

** P-values for the trend in the association between metabolic factor and fibrosis probability

† (1) hyperglycemia (i.e., fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) dyslipidemia (i.e., fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl, women, or pharmacological treatment) (3) hypertriglyceridemia (i.e., fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (i.e., waist circumference over 40 inches ,men, or 35 inches, women), or (5) hypertension (i.e., systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment)

‡ The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS); Low (< -1.455); Intermediate (-1.455 to 0.676); High (> 0.676)

The distribution of the MetS severity score was normally distributed in all three advanced fibrosis probability groups. In NAFLD, the overall mean and median MetS severity scores and their corresponding percentiles were 0.4862 (61st) and 0.4895 (69th), respectively. The severity of MetS increased significantly with a higher probability of advanced fibrosis. The mean MetS severity Z-scores (percentile) for NAFLD patients with low, intermediate, and high advanced fibrosis were 0.184 (55th), 0.9653 (73rd), and 1.538 (81st), respectively. In turn, the prevalence of advanced fibrosis probability status differed significantly by MetS severity category (Figure 3-2). The prevalence of intermediate to high advanced fibrosis probability was similar for NAFLD adults with low to moderate MetS severity. However, the prevalence of intermediate to high advance fibrosis probability was 39.0% and 58%.0 amongst NAFLD adults with high and very high MetS severity scores.



* Metabolic Syndrome Severity Group, the Metabolic Syndrome Z-score was transformed into four percentiles-based categories [low (0 – 50th), Moderate (>50th – 75th), High (>75th – 90th), and very-high (>90th)]
** Fibrosis probability based on the Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS); Low (< -1.455); Intermediate (-1.455 to 0.676); High (> 0.676)

Figure 3-2 Advanced Fibrosis Probability Distribution by Metabolic Syndrome (MetS) Severity Group for Adults with Nonalcoholic fatty liver disease (NAFLD), (NHANES III), (n=3,036)

3.3.4 MetS Severity and the Odds of Advanced Fibrosis Occurrence in NAFLD

In NAFLD, the MetS severity score was a significant predictor for both intermediate to high and high probabilities of advanced fibrosis in all crude and adjusted models. A one standard deviation increase in MetS severity score was associated with an increase in the adjusted odds of adjusted Odds Ratio (aOR) 1.45 (95% CI; 1.18 – 1.77) and 1.49 (95% CI; 1.30 – 1.71), respectively for intermediate to high and high advanced fibrosis probabilities (Data not shown). Similarly, a one quartile increase in MetS severity score was associated with 1.30 (95% CI; 1.17 – 1.45) and 1.30 (95% CI; 1.17 – 1.45) higher adjusted odds, respectively, for intermediate to high and high advanced fibrosis probabilities amongst NAFLD patients.

In the adjusted models with the severity score included as a categorical variable, adults with very high MetS severity had aOR 2.29 (95% CI; 1.65 – 3.19) times the odds of intermediate to high advanced fibrosis probability relative to NAFLD patients with low MetS severity score (Table 3-3). In contrast, moderate MetS severity was associated with 0.32 (95% CI; 0.18 – 0.57) lower adjusted odds of intermediate to high advanced fibrosis probability compared to NAFLD adults with low MetS severity.

In the adjusted model with a high probability of advanced fibrosis as the outcome, an increase in the severity category was associated with higher odds of fibrosis presence (Table 3-4). Adjusted for age, gender, and race/ethnicity, having very high MetS severity was associated with aOR 3.97 (95% CI; 2.30 – 6.86) relative to NAFLD adults with low MetS severity. In the fully adjusted model, very high MetS severity remained a significant

predictor of high advanced fibrosis probability compared to low MetS severity aOR 2.10 (95% CI; 1.02 – 4.34).

Table 3-3 The Association between Metabolic Syndrome Severity Level and Odds of Intermediate to High Probability of Advanced Fibrosis‡ Adults with Non-Alcoholic Fatty Liver Disease Occurrence, (NHANES III), (n=3,036)

Metabolic Syndrome Severity	Unadjusted	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Low	Reference	Reference	Reference
Moderate	0.87 (0.48 – 1.60)	0.57 (0.31 – 1.04)	0.32 (0.18 – 0.57)
High	2.16 (1.57 – 2.98)	1.39 (0.95 – 2.04)	0.93 (0.59 – 1.46)
Very High	4.70 (3.50 – 6.31)	3.76 (2.64 – 5.36)	2.29 (1.65 – 3.19)

Metabolic Syndrome Severity Group, the Metabolic Syndrome Z-score was transformed into four percentiles-based categories [low (0 – 50th), Moderate (50th – 75th), High (75th – 90th), and very-high (90th)]

‡ The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) ≥ -1.455
Model 1 = adjusted for age, gender, and race/ethnicity
Model 2 = Adjusted for age, gender, race/ethnicity, education level, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index percentile, HOMA-IR, total cholesterol, and Aspartate Aminotransferase (AST)/alanine aminotransferase (ALT) Ratio

OR; odds ratio, CI; Confidence interval

Table 3-4 The Association between Metabolic Syndrome Severity Level and Odds of High Probability of Advanced Fibrosis‡ Adults with Non-Alcoholic Fatty Liver Disease Occurrence, (NHANES III), (n=3,036)

Metabolic Syndrome Severity	Unadjusted	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Low	Reference	Reference	Reference
Moderate	1.34 (0.36 – 4.98)	1.04 (0.28 – 3.84)	0.70 (0.18 – 2.89)
High	2.52 (1.42 – 4.48)	1.61 (0.90 – 2.87)	0.85 (0.43 – 1.70)
Very High	5.62 (3.41 – 9.26)	3.97 (2.30 – 6.86)	2.10 (1.02 – 4.34)

Metabolic Syndrome Severity Group, the Metabolic Syndrome Z-score was transformed into four percentiles-based categories [low (0 – 50th), Moderate (50th – 75th), High (75th – 90th), and very-high (90th)]

‡ The Nonalcoholic Fatty Liver Disease Fibrosis Score (NFS) ≥ 0.676

Model 1 = adjusted for age, gender, and race/ethnicity

Model 2 = Adjusted for age, gender, race/ethnicity, education level, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index percentile, HOMA-IR, total cholesterol, and Aspartate Aminotransferase (AST)/alanine aminotransferase (ALT) Ratio

OR; odds ratio, CI; Confidence interval

In the RCS analysis, a significant non-linear dose-response trend was observed in the relationship between increased odds of advanced fibrosis probability and higher MetS severity score in NAFLD. Compared to those in the 90th severity score, the aOR of intermediate to high advanced fibrosis probability was 1.24 (95% CI; 1.13 – 1.36) for adults with NAFLD in 95th severity percentiles. In contrast, the adjusted odds of NAFLD were significantly lower for those with MetS severity scores below the 90th value up to the 34th severity percentile (Figure 3-3a). The adjusted odds of intermediate to high advanced fibrosis for those with NAFLD in the 40th, 50th and 75th percentiles were 0.53 (95% CI; 0.35 – 0.82), 0.46 (95% CI; 0.31 – 0.70), and 0.58 (95% CI; 0.46 – 0.74), relative respectively to those in the 90th severity score. Similar dose-response trends were observed when we restricted to the outcome to a high probability of advanced fibrosis in NAFLD (Figure 3-3b).

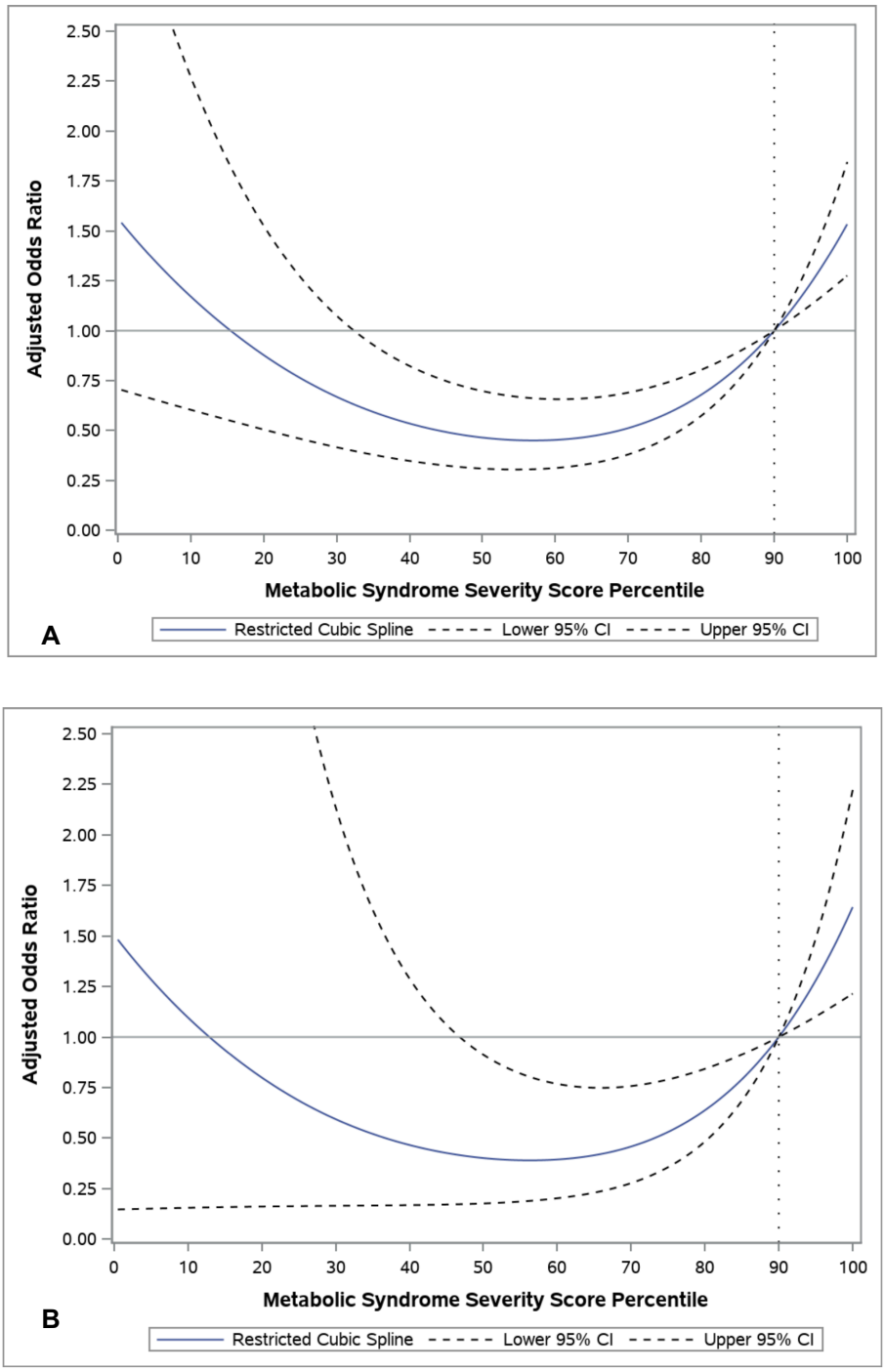


Figure 3-3 Adjusted Odds Ratios of A) Intermediate to High Probability of Advanced Fibrosis and B) High Probability of Advanced Fibrosis for Different Metabolic Syndrome Severity Score Percentiles Relative to the 90th Severity Percentile as the Reference Level

3.4 Discussion

Patients with NAFLD related fibrosis have a higher risk of developing cirrhosis and more severe CLD compared to those with simple steatosis. In addition, advanced fibrosis is a significant predictor of increased risk of mortality in CLD. Despite the knowledge of the role MetS plays as a risk for hepatic degeneration, it is unknown why only some NAFLD patients with MetS progress to advanced fibrosis. Research conducted to date on the relationship between MetS and advanced fibrosis in NAFLD has solely utilized a harmonization disease definition. Such a definition neglects both the sole and combined severity of metabolic abnormalities in relation to advanced fibrosis development. To tackle this knowledge gap, we used a continuous representation of MetS, the MetS severity score, to examine the association between increased MetS severity and the probability of advanced fibrosis occurrence in NAFLD.

Our findings show that higher MetS severity in NAFLD was associated with older age, increases in BMI, central obesity, total cholesterol, and HOMA-IR, along with a decrease in physical activities. The proportion of NAFLD adults with high probability of advanced fibrosis was a highest amongst Black non-Hispanics (8.0%) and lowest in Mexican Americans (2.6%). A higher probability of advanced fibrosis was associated with increased MetS severity in all five metabolic features. The prevalence of intermediate to high advanced fibrosis probability was 39.0% and 58%.0 amongst NAFLD adults with high and very high MetS severity scores, respectively. An increase in the MetS severity score was a significant predictor for both intermediate to high and high probabilities of advanced fibrosis in all crude and adjusted models. We also observed significant non-linear dose-response trends in the relationship between higher MetS severity score and

increased odds of both intermediate to high and high probabilities of advanced fibrosis in NAFLD.

In our sample, increases in MetS severity quartiles were linked to significant increases in BMI, central obesity, total cholesterol, and HOMA-IR. Those findings are consistent with prior research that utilized the severity score. In those studies, the MetS severity score is significantly correlated with biomarkers of MetS, including the HOMA-IR, C-Reactive protein (CRP), uric acid, and adiponectin.[29,33] Multiple studies have also found the MetS severity score to be a significant predictor of long-term risk of cardiovascular disease, T2DM, and coronary heart disease.[33-36]

We detected a significant association between age and increased MetS severity in NAFLD. Age has been associated with increased risk of NALFD, NASH, and advanced fibrosis in multiple studies.[6] In a cross-sectional study using NHANES 2011-2014 data, an increase in age was associated with 1.08 (95% CI; 1.03 – 1.13) higher adjusted odds of advanced fibrosis.[40] In another observational study on age and the risk of liver outcomes, NAFLD prevalence was less than 20% in patients younger than age 20 and 40% amongst those ages 60 or older.[41] The relationship between age and increased risks of advanced hepatic outcomes is attributed to longer disease duration in older patients.

Obesity is a known risk factor for NAFLD development.[42] In the hepatocytes, higher uptake of TG results in cell-specific lipo-toxicity, which results in an increased risk of comorbidities such as NAFLD.[18] Individuals with high visceral adiposity may suffer from increased plasma free fatty acids, which is due to impaired insulin function related to peripheral insulin resistance.[19] Accordingly, studies have reported the prevalence of

simple steatosis and NASH to be 65% and 20%, respectively, in individuals with BMI 30.0–39.9 kg/m^2, and 85% and 40%, respectively, in morbidly obese persons (BMI ≥40 kg/m^2).[43] Similarly, in NAFLD patients with BMI ≥40 kg/m^2 have 9-fold increased adjusted odds of advanced fibrosis. Those findings support the notion that higher obesity-induced MetS is the main driver for onsets of advanced fibrosis in NAFLD.[40]

In NAFLD, we found the prevalence of advanced fibrosis to be highest amongst Black non-Hispanics (8.0%) and lowest in Mexican Americans (2.6%). Those findings are consistent with results from a recent study using NAHNES 2005-2016 data.[44] In our sample, the age and gender adjusted prevalence of T2DM in NAFLD was 11.5%, 22.1%, and 18.5% amongst White, non-Hispanics, Black, non-Hispanics, and Mexican Americans, respectively. This translated into 10% and 3.7% higher prevalence of T2DM in Blacks with NAFLD compared respectively to Whites and Mexican Americans.

Insulin resistance plays an essential role as a risk factor for the progression of liver fibrosis in NAFLD.[45-49] In a liver biopsy-based study, an increase in insulin resistance in severely obese patients was associated with 9.3 (95% CI; 3.4 – 26.0) higher adjusted odds of advanced fibrosis.[46] In a cohort study with 7.5 years of median follow-up, the risk of advanced fibrosis in those with T2DM was Hazard Ratio (HR) 1.88 (95% CI; 1.40 – 2.52) times the risk for those without T2DM.[47] Those findings are in line with our results of an increase in the prevalence of advanced fibrosis with higher MetS severity, especially amongst Black, non-Hispanics, due to the higher burdens of diabetes and insulin resistance.

In our adjusted models, relative to NAFLD adults with low MetS severity, having very high MetS severity was associated with aOR 3.97 (95% CI; 2.30 – 6.86) and aOR 2.10 (95% CI; 1.02 – 4.34) for intermediate to high and high advanced fibrosis probabilities, respectively. Insight into the associations between advanced fibrosis in NAFLD and specific metabolic abnormalities associated with MetS are well established. In a US-based study, the prevalence of intermediate and high advanced probabilities in NAFLD patients with metabolic syndrome was 40.8% and 11.1%, respectively. When the analysis was limited to those with all five metabolic abnormalities, the proportion of high advanced fibrosis probability increased to 30.3%.

In individuals with increased waist circumference, the prevalence of NAFLD (31%, 8.7% with advanced fibrosis) greatly exceeded controls. The prevalence of NAFLD in subjects with diabetes (41%, 18% with advanced fibrosis), and it also greatly exceeded control prevalence. The prevalence of NAFLD in subjects with high triglyceride levels was 35% (8% with advanced fibrosis). This same level of fibrosis was found in subjects with low HDL, though the prevalence of NAFLD was significantly lower (28%). This study also revealed that the presence of NAFLD in subjects with MetS increased according to the number of metabolic abnormalities present, exceeding 65% in patients with all five abnormalities. In the absence of MetS or any of its components, the prevalence of NAFLD was 6%.[31] Our study expands on those findings by accounting for the impacts of the combined effects of all MetS components on the adds of advanced fibrosis in NAFLD. This study is also the first research to date to report on the non-linear relationship between higher MetS severity score and increased odds of advanced fibrosis.

The gold standard of diagnosing advanced fibrosis in NAFLD is liver biopsy. The use of liver biopsy for disease diagnosis is not a feasible option for most patients. Quantifying the relationship between MetS severity and the odds of advanced fibrosis in NAFLD is a step towards tackling the current public health burden of this disease. Results from the underlined chapter could aid both clinicians and public health practitioners in planning and executing secondary and tertiary prevention efforts related to NAFLD. Secondary prevention efforts could take place by using the MetS severity score as a screening tool to identify patients at highest risk of advanced fibrosis in NAFLD. The MetS severity score could also be used as a tertiary prevention tool whereby the progression of severity is monitored with an aim of designing interventions to mitigate the chances of more advanced hepatic manifestations in NAFLD.

Our findings are not without limitations. The study utilized cross-sectional data, which did not allow for the risk estimates to be quantified. However, the prevalence of high probability of advanced fibrosis is well below the 10% threshold for the odds ratio to estimate relative risk. NAFLD assessment was done using ultrasonography, which could result in misclassifications. Assessments of advanced fibrosis probabilities were done using non-invasive scores. However, a >0.676 cutoff was chosen as it has a specificity of 97% for advanced fibrosis.[6,38] This is essential since patients must undergo a liver biopsy to confirm fibrosis diagnosis. Increased specificity will ensure that 97% of those classified as without advance fibrosis will be identified by the score as such. Ascertainments of exposure and baseline characteristics were conducted cross-sectionally. Alcohol intake was assessed based on self-reporting, which might result in underestimation.

3.5 Conclusion

It is unknown as to why some NAFLD patients progress to NASH and advanced fibrosis while others do not. To address this knowledge gap, we used the MetS severity score to investigate the association between increased MetS severity and the odds of advanced fibrosis in patients with NAFLD. Our findings indicate that in NAFLD, advanced fibrosis is associated with higher MetS severity, which is triggered by obesity-induced insulin resistance. Accounting for the effects of MetS severity rather than occurrence help to explain why only some NAFLD patients progress to advanced fibrosis. The MetS severity score could be used as a screening tool to monitor hepatic progressions in NAFLD.

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Chapter 4 The association between Metabolic Syndrome Severity and Survival in Non-alcoholic Fatty Liver Disease: A Population Based Cohort Study of United States Adults

ABSTRACT

Background and Objectives: Previously conducted studies on the association between Metabolic Syndrome (MetS) and the risk of mortality in Non-alcoholic Fatty Liver Disease (NAFLD) have utilized the harmonization definition of this syndrome. This definition creates a knowledge gap as it treats, equally, the effects of any of the possible ten combinations of metabolic abnormalities on survival in NAFLD. The main aim of this chapter was to use the MetS severity score, a validated gender-race specific Z-score, to assess the association between MetS severity and the risk of mortality in NAFLD.

Methods & Materials: The study cohort included 10,638 adults ages 20 to 74 years who participated in the Third National Health and Nutrition Examination Survey (NHANES III). A validated matching algorithm was used to link participants' baseline characteristics with mortality outcomes obtained from the National Death Index database. NAFLD was defined as mild, moderate, or severe hepatic steatosis on ultrasound in the absence of hepatitis B, hepatitis C, iron overload, and excessive alcohol intake. All five metabolic features (i.e., high-density lipoprotein, systolic blood pressure, waist circumference, triglycerides, and blood glucose) were used to calculate gender-race specific MetS severity Z-score, which was then transformed into quartiles. Cox proportional hazard models adjusting for attained age, gender, race/ethnicity, education level, marital status, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index, chronic kidney disease, family history of

diabetes, family history of myocardial infarction, and history of cancer were used to test the association between the MetS severity score and the risk of mortality related to all-cause, heart disease, diabetes, and hypertension. To account for competing risks in the cause-specific proportional hazard models, participants who died from other causes were censored at the time of death. Complex survey methods using sampling weights, strata, and clusters were used to yield nationally representative prevalence and effect estimates.

Results: The prevalence of NAFLD was 26.7% (95% CI; 24.3% – 29.1%). Stratified by race/ethnicity, NAFLD prevalence was 26.6%, 23.2%, and 33.7% amongst White non-Hispanics, Black non-Hispanics, and Mexican Americans, respectively. An estimated 82.2% of adults with NAFLD had at least one feature of the traditionally defined MetS, while 9.3% met the criteria for all five metabolic components. Both the mean and median MetS severity scores were significantly higher in NAFLD relative to those without [mean MetS severity Z-score, 0.49 (69^{th}) vs. -0.14 (46^{th}); median MetS severity Z-score, 0.49 (69^{th}) vs. -0.23 (41^{st})]. An increase in the MetS severity corresponded to a linear rise in biomarkers for cardiovascular disease, insulin resistance, lipid abnormalities, and decreases in both liver and kidney functions. An estimated 46.2% of all deaths occurred amongst NAFLD patients in the fourth MetS severity score quartile, compared to 8.6% for those in the first quartile. In NAFLD, the all-cause mortality incidence rate was 13.5 per 1,000 person-years, while the cause-specific mortality incidence rates associated with heart disease, diabetes, and hypertension were 3.2 per 1,000 person-years, 2.3 per 1,000 person-years, and 2.1 per 1,000 person-years, respectively. The MetS severity score was a significant predictor for all-cause and cause-specific adjusted mortalities in NAFLD. A quartile increase in MetS severity score was associated with increased in the risk of all-

cause mortality adjusted Hazard Ratio (aHR) 1.36 (95% CI; 1.17 – 1.57), heart disease related mortality aHR 1.70 (95% CI; 1.17 – 2.47), diabetes-related mortality aHR 3.64 (95% CI; 2.27 – 5.83), and hypertension-related mortality aHR 1.87 (95% CI; 1.14 – 3.04). Significant non-linear dose-response trends were observed in the relationship between increased risk of mortality and higher MetS severity score in all adjusted models. The risk of mortality from all causes, heart disease, diabetes, and hypertension was aHR 2.16 (1.61 – 2.90), aHR 3.41 (1.70 – 6.81), aHR 18.92 (7.59 – 47.13), and aHR 3.98 (1.30 – 12.18), respectively, times the mortality risk for NAFLD patients in the 99th MetS severity percentile compared to those with median severity.

Conclusions: The MetS severity score predicts mortality in NAFLD. Non-linear dose-response trends were observed in the relationship between increased risk of mortality and higher MetS severity score in adults with NAFLD. Significant increases in adjusted mortality risks amongst adults with NAFLD were observed for all severity estimates above the median MetS severity values. The MetS severity score is a clinically validated tool that could be used to identify and monitor NAFLD patients at the highest risk of mortality.

Keywords: NASH, NAFLD, mortality risk, NHANES

4.1 Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is defined as the presence of hepatic steatosis, by imaging or histology, in the absence of a secondary cause such as hepatic viral infection(s), drug-induced hepatotoxicity, excessive alcohol consumption, or hereditary disorders.[1-3] NAFLD encompasses a spectrum of histological states ranging in severity from simple intrahepatic fat accumulations, non-alcoholic fatty liver (NAFL), to necrotic inflammations in the presence of ballooned hepatocytes—non-alcoholic steatohepatitis (NASH).[2-4] NASH encompasses more advanced hepatic damage: steatosis in greater than five percent of hepatocytes, inflammation, and hepatocyte injury with or without fibrosis. Patients with NASH can further develop NASH-cirrhosis, which is recognized by the presence of regenerative nodules enclosed by fibrous bands that results in portal hypertension and end-stage liver disease.[5]

Due to the principal role obesity induced insulin resistance plays in promoting hepatic steatosis, NAFLD is regarded as the hepatic manifestation of Metabolic Syndrome (MetS).[2,6-11] MetS describes a group of metabolic abnormalities that are associated with increased risks of insulin resistance and cardiovascular disease.[12] The current guidelines for diagnosing MetS put forward by the American Heart Association, and the National Heart Lung and Blood Institute relies on a “harmonization definition” of this syndrome. As such, MetS is characterized based on a patient presenting abnormalities that exceed pre-specified cut-off values for three of five metabolic components: *hyperglycemia*, *dyslipidemia*, *hypertriglyceridemia*, central obesity, or hypertension.[12,13] The prevalence of NAFLD amongst patients with all five MetS criteria is 91%.[14]

The current obesity epidemic is directly connected to an upsurge in the prevalence and economic burden of NAFLD, especially in the United States (US), where an estimated 40% of all adults are considered obese.[6,10,15,16] In turn, the current prevalence of NAFLD amongst US adults is estimated to be 26%.[16], while the prevalence of obesity amongst NAFLD and NASH patients is 51% and 82%, respectively.[17-19] Similarly, the prevalence of NAFLD in patients with diabetes is 41%.[17-20] In individuals with increased waist circumference, the prevalence of NAFLD (31%, 8.7% with advanced fibrosis).[20] In turn, the total annual cost of NAFLD in the US is estimated to be $292.19 billion, of which $103.31 billion were direct costs.[21]

Aside from obesity, additional risk factors, including race and gender, have been associated with the risk of NAFLD development.[22] According to a recent meta-analysis of 34 studies, significant disparities in NAFLD prevalence, severity, and prognosis exist between race and ethnic groups within the US.[23] Several studies suggest Hispanics have the highest NAFLD incidence, with obesity emerging as a central factor in this population.[24] In these studies, African Americans had the lowest NAFLD incidence. Data on the role of gender as a risk factor for NAFLD have been inconsistent with some studies suggesting males at a higher risk while others are showing NAFLD to be more common in females.[25-30] Genetic and metabolic factors have been suggested to underlie racial and gender disparities,[19,31-33] as have incidence rates of insulin resistance and serum triglyceride concentrations.[34]

A large body of clinical and population-based studies have demonstrated a positive association between NAFLD and incident cardiovascular disease (CVD).[35,36] In particular, NAFLD is associated with coronary artery disease [37] and high risk of coronary

atherosclerotic plaques [38], independent of traditional cardiovascular risk factors. Compared to the general population, NAFLD patients have a 1.7-fold increased risk of mortality adjusted for age and gender,[39] yet there are no pharmacologic or other modalities of treatment for this condition. Furthermore, many retrospective and prospective studies show evidence that NAFLD patients have higher rates of CVD-related mortality compared to the general population.[40-42] Such increases in the risks of morbidity and mortality could be attributed to a higher degree of MetS severity amongst NAFLD patients relative to the general population. However, previously conducted research on the natural history of NAFLD has solely focused on identifying the presence of risk factors for disease progression [7,43] without accounting for the effects of MetS severity, rather than disease occurrence, on the increased risk of morbidity and mortality in NAFLD.

The traditional classification of MetS creates four main knowledge gaps in accurately assessing the risk of mortality in NAFLD. First, the dichotomous nature of current classification treats, equally, the effects of any of the possible ten metabolic combinations (i.e., any three out of five metabolic factors) on outcomes and survival in NAFLD. Second, the traditional MetS diagnosis criteria neglects both the sole and combined impacts of all MetS features on the risk of mortality in NAFLD. Third, a dichotomous MetS categorization makes it challenging to study and monitor the clinical implications of worsening in the severity of MetS over time. Four, a binary system for MetS definition does not account for the racial and gender disparities in MetS severity and their corresponding effects on the risk of mortality in NAFLD.

The shortcomings associated with the dichotomous definition of MetS could be fully addressed by using a continuous measure of MetS severity that encapsulates the

statuses of all five metabolic features in one summary risk score. Such a score could, in turn, be used to assess the association of MetS severity with outcomes and survival in NAFLD. The MetS Severity Score is a validated clinically-accessible gender-race specific Z-score that encapsulates the combined effects of the nature and severity of all five metabolic abnormalities amongst US adults.[44] The main objective of this chapter is to utilize the continuous measure of MetS to examine the association between increased MetS severity and the risks of all-cause mortality, heart disease-related mortality, diabetes-related mortality and hypertension-related mortality amongst adults with NAFLD. Quantifying the relationship between MetS severity and survival in NAFLD is a step towards tackling the current public health burden of this disease.

4.2 Materials and Methods

The third National Health and Nutrition Examination Survey (NHANES III) was conducted between 1988 and 1994 by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The survey aimed to assess the health and nutritional status of the US population with oversampling of non-Hispanic Blacks, Mexican Americans, and individuals sixty years of age and older. Participants in NHANES III were noninstitutionalized members of the US population ages two months or older who were selected using a stratified multistage clustered probability design. The survey included cross-sectional physical examinations, interview questionnaires, and laboratory sample collections. The overall examination rate of interviewed participants was 78%.[45] The ethics review board of the CDC approved the NHANES III survey protocol.

Participants in the NHANES III were passively followed from the interview date through December 31st, 2011. Participants' mortality outcomes were linked with the National Death Index (NDI) database using a validated matching algorithm.[46,47] The matching algorithm links together records from the original NHANES III survey with death certificates data in order to obtain the underlying causes of death for all participants. The accuracy of the mortality matching algorithm to correctly determine the status of decedent is 96.10% and 99.40% for deceased and alive participants, respectively.[47] The International Classification of Diseases Ninth Revision (ICD-9) codes were reported for underlying causes of deaths occurring before 1998, while ICD-10 records were used for deaths that took place after 1999.

4.2.1 Study Sample

Gallbladder ultrasounds video images were recorded during the physical examinations for all NHANES III participants 20 to 74 years of age. Three trained ultrasound readers assessed the video images for hepatic steatosis, using standardized reading protocols. A certified radiologist, who specializes in hepatic imaging, trained all three ultrasound readers. Following the initial assessments, all gallbladder ultrasound readings were reevaluated and validated by another certified radiologist. Hepatic steatosis images were classified into normal, mild, moderate, or severe. Criteria for grading hepatic steatosis included 1) gallbladder walls definition, 2) liver parenchyma degree of brightness, 3) occurrence of deep beam attenuation, 4) the presence of liver to kidney contrast, and 5) echogenic walls in the small intrahepatic vessels.[20]

NAFLD was identified by the presence of mild, moderate, or severe hepatic steatosis in the absence of any of the following:

1. Excessive drinking (i.e., more than three alcoholic beverages per day for males and more than two alcoholic beverages per day for females).

2. Binge drinking (i.e., frequent consumption of five or more alcoholic beverages per day).

3. Alcohol consumption restrictions due to illness.

4. Positive Hepatitis B virus (HBV) surface antigen test.

5. Positive Hepatitis C virus (HCV) RNA Test.

6. Iron overload (i.e., transferrin saturation of ≥ 50%).

NAFLD patients were excluded from the study if they met any of the following criteria:

7. The participant had missing values for alcohol intake.

8. The participant had a missing or an unreadable ultrasound image.

9. The participant identified as “Other” in the race-ethnicity, as the exposure assessment is only applicable to Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics.

10. Participant had a missing cause of death

11. Participant had a missing value for exposure, outcomes, or any of the covariates included in the adjusted analyses.

Of the total 16,573 individuals ages twenty years or older who attended the examination phase of the survey, 14,707 qualified for the gallbladder ultrasound reading, of which, 13,856 participants had readable ultrasound images.[48] Accordingly, a total of 5,484 participants had mild, moderate, or severe hepatic steatosis on ultrasound, of which, 3,088 NAFLD patients met the study’s inclusion and exclusion criteria.

4.2.2 Exposure

The MetS severity score is a validated gender- and race/ethnicity- specific Z-score that encapsulates the relative MetS severity of all five metabolic factors.[44] The MetS severity

score was initially derived from a Confirmatory Factor Analysis (CFA) using data from the 1999-2010 NHANES.[44] The CFA aimed to utilized all five metabolic features to construct a summary severity score that is a continuous representation of the conventional metabolic syndrome definition. Participants in the 1999-2010 NHANES were divided into six groups based on gender and self-identified race/ethnicity, which included Non-Hispanic Whites, Non-Hispanic Blacks, and Hispanics. For all five metabolic components of MetS, different loading coefficients were quantified to determine a single latent MetS factor for all six sup-groups.

All the CFA loading coefficients were used to construct equations that can be used to quantify a standardized MetS severity score for all six sup-groups.[44] Accordingly, the MetS severity score is a continuous representation of the traditional MetS classification, while adjusting for gender and racial/ethnic disparities in the relationship between MetS and cardiometabolic outcomes. The MetS severity score is significantly correlated with pathophysiological biomarkers of MetS, including the Homeostasis Model for Insulin Resistance (HOMA-IR), C-Reactive protein (CRP), uric acid, and adiponectin.[44,49] Multiple studies have also shown the MetS severity score to be a significant predictor of long-term risks of cardiovascular disease, type 2 diabetes mellitus, and coronary heart disease.[49-52]

MetS was defined based on the current ATP-III guidelines put forward by the American Heart Association and the National Heart Lung and Blood Institute. As such, MetS is defined by the presence of three of the following five risk factors: (1) *hyperglycemia* (*i.e.*, fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) *dyslipidemia* (*i.e.*, fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl,

women, or pharmacological treatment) (3) *hypertriglyceridemia* (*i.e.*, fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (*i.e.*, waist circumference over 40 inches for men, or 35 inches for women), or (5) hypertension (*i.e.*, systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment).[53] Individual-level data for HDL, SBP, waist circumference, TG, and fasting blood glucose were used to calculate gender- and race/ethnicity-specific MetS severity Z-scores according to the score's standardized equations.[44]

4.2.3 Outcomes

The study's primary outcomes included all-cause mortality and cause-specific mortalities related to heart disease [*i.e.*, ICD-10 codes I00-I09 (Acute rheumatic fever and chronic rheumatic heart diseases), I11 (Hypertensive heart disease), I13 (Hypertensive heart and renal disease), I20-I25 (Ischemic heart diseases), and, I26-I51 (Other heart diseases)], diabetes [*i.e.*, ICD-10 codes (E10-E14)], and hypertension [i.e., ICD-10 codes (I10-I12)]. Follow-up time was defined as the number of person-years from the interview date to either death or end of study (*i.e.*, December 31st, 2011, or last date of follow-up whichever was earlier). During the study period, Individuals were censored if they were lost to follow-up, assumed alive at the end of the study or if they died in accidents [i.e., unintentional injuries (V01-X59, Y85-Y86)].

4.2.4 Covariates

During the interview and examination phases, data were gathered on multiple covariates, including confounding variables and other factors used in the secondary statistical analyses. Confounder selection was based on both *a priori* knowledge, form the literature,

and theoretical rationale. Attained age at the end of follow-up was quantified by adding the follow-up time in years to each participant's baseline age (obtained during the interview phase of the survey). Confounders used in the adjusted multivariate analyses included gender, race/ethnicity (White non-Hispanics, Black non-Hispanics, or Mexican Americans) education level (< high school, high school, or GED; some college or college degree or higher) marital Status (married, widowed, separated, or divorced; or single) access to health insurance (yes or no), alcohol intake (never, former, > 0-1 drinks/day, or > 1 drinks/day), smoking status (never, former, or current), body mass index (Kg/m^2) [underweight (< 18.5), healthy weight (≥ 18.5- 25.0 <), overweight (≥ 25.0-30.0 <), or obese (≥ 30)], abdominal obesity (≥ 0.9 for males or ≥ 0.85 for females), physical activity (metabolic equivalents/month), healthy eating index percentile, chronic kidney disease (glomerular Filtration Rate < 60 ml/min per 1.73 m^2), family history of diabetes (yes or no), family history of myocardial infarction (yes or no), and history of cancer (yes or no).

Venous blood samples were obtained during the physical examinations phase of the survey. Full biochemistry evaluations of all blood samples were performed at the CDC labs using previously described procedures.[54] Data on plasma glucose, Serum Insulin, glycated hemoglobin (HbA1c), total cholesterol, HDL, low-density lipoprotein cholesterol (LDL), TG, alkaline phosphatase, serum albumin, bilirubin, ferritin, gamma-glutamyl transferase, aspartate aminotransferase (AST), alanine, aminotransferase (ALT), urea nitrogen, creatinine, uric acid, and c-reactive protein (CRP) were used in this study.

4.2.5 Statistical Analyses

The study sample was restricted to participants with non-missing values on exposure, outcomes, or any of the variables used in the adjusted multivariate analyses. Complex survey methods, using sampling weights, strata, and clusters were used to yield nationally representative estimates. In order to account for the effects of the survey design, Taylor series linearization was used to quantify all variance values. Missing values related to variance estimation were assumed not to be missing completely at random.

Participants' characteristics stratified by NAFLD status were examined by testing the difference in means for continuous variables, using weight adjusted analysis of variance, and using Rao Scott Chi-Square for categorical variables. Age, gender, and race/ethnicity adjusted mean estimates for biomarkers related to cardiovascular factors, metabolic control, lipid profile, liver function, and kidney function were quantified for all MetS severity score quartiles. Linear trends of all biomarkers across the MetS severity score quartiles were tested using orthogonal polynomial contrasts. Kaplan-Meier analyses were used to quantify unadjusted cumulative mortality by the MetS severity score quartiles during the follow-up period. Incidence mortality rates by MetS severity score quartiles were calculated using the number of deaths divided by 1,000 person-years of follow-up.

Fully adjusted Cox proportional hazard models, with attained age as the survival timescale, were used to test the association between the MetS severity score and the risk of mortality related to all-cause, heart disease, diabetes, and hypertension amongst adults with NAFLD. As such, participants' age at baseline marketed their start of follow-

up, and the attained age (*i.e.*, at evet, or time of censoring) indicated their exit from the study. The use of attained age, as opposes to the time-on-study, fully accounts for the effects of the age-mortality associations at the time of event rather than solely adjusting for the effects of baseline age.[55] All variables included in the Cox models met the proportional hazard assumption through testing the cumulative sums of martingale residuals.[56] Competing risks of mortality were accounted for in all case-specific models by censoring follow-up time at the date of death from other causes.

The dose-response relationships between MetS severity and the risk of mortality were envaulted using the MetS severity score percentiles as a continuous variable with a three-knot restricted cubic spline (RCS) in the adjusted Cox proportional hazard models. As recommended by Harrell, 2015,[57] the three-knots were places at 10th, 50th, and 90th of the weighted MetS severity score percentile values for NAFLD patients. Wald-Chi Square tests were used to assess the overall and non-linear associations between the MetS severity score percentiles and the risk of mortality. A P-value of less than 0.05 was considered statistically significant. All analyses were performed using the SAS 9.4 software (SAS Institute, NC, USA).

4.3 Results

4.3.1 Participants Baseline Characteristics

Table 4-1 summarizes the study cohort by NAFLD status. The study sample included 10,638 adult participants in the NHANES III, who met the inclusion and exclusion criteria. At baseline, the prevalence of NAFLD was 26.7% (95% CI; 24.3% – 29.1%). Stratified by race/ethnicity, NAFLD prevalence was 26.6%, 23.2%, and 33.7% amongst White non-

Hispanics, Black non-Hispanics, and Mexican Americans, respectively. Compared to those without, adults with NAFLD were more likely to be females (54.7% vs. 51.0%; P-value <0.047), older (mean age in years, 45.3 vs. 41.6; P-value <0.001) and had a higher percentage of Mexican Americans (7.0% vs. 5.0%) and a lower proportion of Black non-Hispanics (9.6% vs. 11.5%). The distributions of education level, marital status, and smoking status also differenced by NAFLD status.

An estimated 71.4% of NAFLD patients were overweight or obese, compared to 49.7% in those without NAFLD. Similarity, adults with NAFLD had 3.1 kg/m^2 higher average BMI when compared to those without NAFLD. The prevalence of abdominal obesity was also significantly associated with NAFLD status (with vs. without; 75.5% vs. 62.3%; P-value <0.001). In contrast, the proportion of physically active participants was lower in NAFLD compared to no NAFLD (84.1% vs. 88.8%; P-value <0.001). The prevalence of Chronic Kidney Disease (CKD) and history of T2DM were higher amongst NAFLD patients relative to those without NAFLD (Table 4-1).

Table 4-1 Sample Characteristics by Non-Alcoholic Fatty Liver Disease (NAFLD) Status, The National Health and Nutrition Examination Survey (NHANES III) 1988-1994 (n=10,638)

Characteristics	NAFLD (n=3,088)	No NAFLD (n=7,550)	P-value***
Gender, % (SE)			0.047
Male	45.3 (1.3)	49.0 (0.76)	
Female	54.7 (1.3)	51.0 (0.76)	
Age, (years)			<0.001
Median (25th, 75th Percentile)	42.9 (32.8, 57.0)	38.6 (28.9, 51.7)	
Mean (SE)	45.3 (0.49)	41.6 (0.45)	
Age Group, % (SE)			<0.001
18-34	28.3 (2.0)	38.9 (1.3)	
35-49	33.1 (2.2)	32.5 (0.93)	
49-64	24.4 (1.3)	18.3 (0.73)	
65+	14.2 (0.95)	10.3 (0.70)	
Race/ethnicity, % (SE)			0.001
White, non-Hispanics	83.4 (1.3)	83.5 (0.89)	
Black, non-Hispanics	9.6 (0.84)	11.5 (0.76)	
Mexican Americans	7.0 (0.86)	5.0 (0.43)	
Education Level, % (SE)			0.001
< High School	22.3 (1.3)	20.1 (1.0)	
High School or GED	39.1 (1.5)	34.3 (0.95)	
Some College	19.4 (1.4)	22.6 (0.89)	
College degree or Higher	19.2 (1.6)	23.0 (1.0)	
Marital Status, % (SE)			<0.001
Married*	73.3 (1.3)	67.6 (1.2)	
Widowed, Separated or Divorced	15.5 (0.96)	15.3 (0.71)	
Single	11.2 (0.91)	17.1 (1.2)	
Have Health Insurance, % (SE)	89.0 (0.87)	87.4 (0.94)	0.159
Alcohol Intake, % (SE)			<0.001
Never	14.9 (1.1)	9.0 (0.69)	
Former	34.7 (1.4)	31.0 (1.4)	
> 0 - 1 drinks/day	40.4 (1.85)	41.7 (1.4)	
> 1 drinks/day**	10.0 (1.1)	18.4 (0.97)	
Smoking Status, % (SE)			<0.001
Never	47.4 (1.3)	43.0 (1.2)	
Former	29.8 (1.5)	25.2 (0.78)	
Current	22.9 (1.2)	31.9 (1.0)	
Body Mass Index Group (Kg/M^2)			<0.001
Median (25th, 75th Percentile)	27.9 (24.3, 32.2)	24.9 (22.3, 28.0)	
Mean (SE)	28.8 (0.31)	25.7 (0.11)	
	NAFLD	**No NAFLD**	

Table 4-1 *Continued*

Characteristics	(n=3,088)	(n=7,550)	P-value***
Body Mass Index Category† (Kg/M^2), % (SE)			<0.001
Underweight	1.9 (0.37)	2.4 (0.30)	
Healthy Weight	26.8 (1.8)	47.9 (0.91)	
Overweight	33.6 (1.4)	32.9 (0.77)	
Obese	37.8 (2.0)	16.8 (0.78)	
Waist to Hip Ratio			<0.001
Median (25th, 75th Percentile)	0.93 (0.85, 0.99)	0.89 (0.83, 0.95)	
Mean (SE)	0.93 (0.004)	0.90 (0.002)	
Abdominal Obesity ‡, % (SE)	75.5 (1.6)	62.3 (1.2)	<0.001
Physical Activity (METs/month)			<0.001
Median (25th, 75th Percentile)	58.6 (14.3, 142.3)	73.6 (19.9, 164.4)	
Mean (SE)	98.0 (4.0)	116.3 (3.6)	
Physically Active, % (SE)	84.1 (1.2)	88.8 (0.7)	<0.001
Healthy Eating Index			0.179
Median (25th, 75th Percentile)	64.1 (53.0, 73.6)	63.1 (54.1, 72.5)	
Mean (SE)	63.8 (0.42)	63.2 (0.33)	
Healthy Eating Index §, % (SE)			0.304
Poor	17.7 (1.14)	17.9 (0.82)	
Fair	70.1 (1.51)	71.4 (0.61)	
Good	12.2 (1.19)	10.7 (0.62)	
Glomerular Filtration Rate (ml/min per 1.73 M^2)			0.001
Median (25th, 75th Percentile)	89.5 (76.9, 102.7)	93.1 (80.5, 106.5)	
Mean (SE)	91.5 (0.68)	94.7 (0.64)	
Chronic Kidney Disease ¶, % (SE)	5.0 (0.56)	3.0 (0.27)	0.001
Family History of Diabetes, % (SE)	48.5 (1.73)	44.7 (1.04)	0.026
Family History of Myocardial Infarction, % (SE)	17.8 (1.03)	17.7 (0.74)	0.973
History of Cancer, % (SE)	7.4 (0.65)	6.6 (0.41)	0.365
Follow Up (years)			<0.001
Median (25th, 75th Percentile)	19.2 (17.5, 20.6)	19.5 (18.0, 21.1)	
Mean (SE)	18.2 (0.50)	18.7 (0.21)	

* Including those living with a partner

† Underweight = (< 18.50), Healthy Weight = (≥ 18.50 - 25.00 <), Overweight = (≥ 25.00 - 30.00 <) and Obese = (≥ 30)

‡ Waist to Hip Ratio ≥ 0.90 for males or ≥ 0.85 for females

§ Poor < 51%, Fair < 80%, Good ≥ 80%

¶ Glomerular Filtration Rate < 60 ml/min per 1.73 M^2

** In NAFLD up to 2 drink per days for females and 3 drinks per day for males

*** Rao-Scott Chi Square P-values for difference in proportions and T-tests P-values for difference in means between adults with versus without Non-Alcoholic Fatty Liver Disease

MET= Metabolic equivalent; % = Weighted Proportion; SE= Standard Error

4.3.2 Metabolic Syndrome Severity in NAFLD

An estimated 82.2% of adults with NAFLD had at least one feature of the traditionally defined MetS, while 9.3% met the criteria for all five metabolic components. The mean number of metabolic abnormalities was significantly higher in NAFLD versus no NALFD (2.2 vs. 1.4; P-value <0.001). The prevalence of traditionally defined MetS was higher in NAFLD patients compared to those without NAFLD (44.0% vs. 20.4%; P-value <0.001).

The distribution of the MetS severity score was normally distributed in both the NAFLD and no NAFLD groups. However, the severity of MetS was significantly higher in NAFLD patients (Figure 4-1). The overall mean and median MetS severity scores and their corresponding percentiles were 0.03 (51^{st}) and -0.08 (47^{th}), respectively. Both the mean and median MetS severity scores were significantly higher in NAFLD relative to those without [mean MetS severity Z-score, 0.49 (69^{th}) vs. -0.14 (46^{th}); median MetS severity Z-score, 0.49 (69^{th}) vs. -0.23 (41^{st})]. In a Receiver Operating Characteristic (ROC) analysis, the MetS severity score showed a high ability to predict the ATP-III defined MetS in NAFLD (area under the curve 0.93). In the NAFLD sample, using MetS severity scores cut-off value of 0.43 (67^{th}) yielded a sensitivity of 83% and 87% specificity for identifying the traditionally defined MetS.

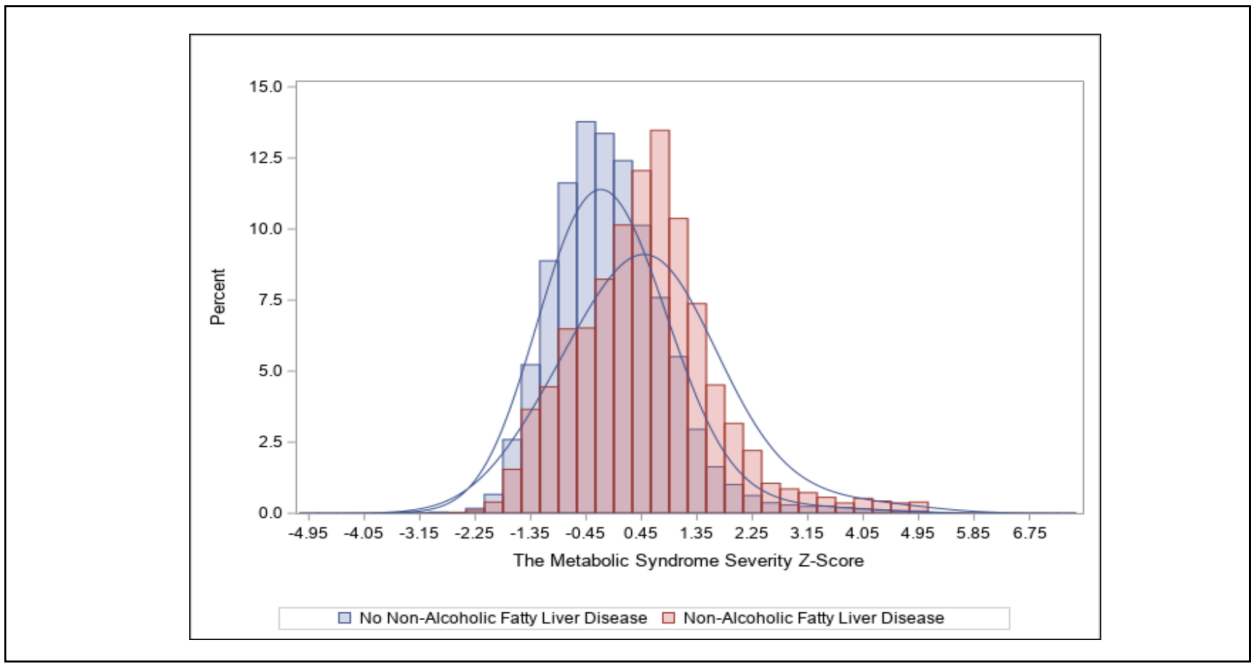

Figure 4-1 The Distribution of the Metabolic Syndrome Severity Z-Score among Adults with Versus Without Non-Alcoholic Fatty Liver Disease, (NHANES III), (n=10,638)

Table 4-2 summarizes the relationship between adjusted clinical characteristics related to cardiovascular factors, metabolic control, lipid profile, liver function, and kidney function by the MetS severity score quartiles in NAFLD. In general, an increase in the MetS severity corresponded to a linear rise in markers for cardiovascular disease, insulin resistance, and lipid abnormalities. Namely, the age, gender, and race/ethnicity adjusted mean values of SBP, diastolic blood pressure, and pulse rate showed significant linear dose-response relationships with increases in MetS severity score. In turn, the adjusted prevalence of hypertension in NAFLD for the adults in the first, second, third, and fourth quartiles was 29.5%, 42.9%, 56.1%, and 68.4%, respectively. Similarly, all metabolic controls biomarkers had significant dose-response relationships with rises in the MetS severity score. In NAFLD, the adjusted prevalence of diabetes and the traditionally

defined MetS for adults in the fourth quartile of the severity score were 38.7% and 91.2%, respectively.

An increase in the MetS severity was also associated with elevations in biomarkers for lipid abnormalities, decreased liver function, and a decline in kidney function. The adjusted values for total cholesterol and LDL both increased significantly with a higher MetS severity score quartile. In contrast, adjusted mean HDL values decreased with a higher MetS severity score. The age, gender, and race/ethnicity adjusted mean values of triglycerides increased from 76.6 mg/dL to 294.7 mg/dL for NAFLD patients in the first and fourth MetS severity score quartiles, respectively. This trend translated into an increase of 74.1% in the prevalence of hyperlipidemia between NAFLD adults in the first and fourth MetS severity score quartiles.

Decreased liver function as marked by increases in alkaline phosphatase, ferritin, gamma glutamyl-transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were significantly associated with higher MetS severity score. The adjusted value of uric acid in adults with NAFLD was 32% higher for those in the first versus fourth MetS severity score quartile. The adjusted prevalence of detectable CRP (*i.e.*, >0.3 mg/dL) was 11.5%, 33.9%, 44.4%, and 53.8% for NAFLD adults in the first, second third, and fourth MetS severity score quartiles, respectively (Data not shown).

Table 4-2 Age, Gender and Race/ethnicity Adjusted Clinical Characteristics Related to Cardiovascular Factors, Metabolic Control, Lipid Profile, Liver function, and kidney function by Metabolic Syndrome Severity Score, Adults with NAFLD, (NHANES III),(n=3,088)

Clinical Characteristics	Quartile 1 (n=698)	Quartile 2 (n=785)	Quartile 3 (n=771)	Quartile 4 (n=834)	P_{trend}
Cardiovascular Factors					
Systolic Blood Pressure (mm Hg), Mean (SE)	120.2 (0.79)	126.2 (0.72)	130.7 (0.93)	133.5 (0.69)	<0.001
Diastolic Blood Pressure (mm Hg), Mean (SE)	71.3 (0.53)	75.7 (0.46)	78.3 (0.52)	78.9 (0.53)	<0.001
Pulse Rate (beats/min), Mean (SE)	70.5 (0.83)	74.8 (0.67)	76.5 (0.80)	79.5 (0.74)	<0.001
Hypertension, % (SE)	29.5 (3.0)	42.9 (2.1)	56.1 (3.2)	68.4 (2.3)	<0.001
Metabolic Control					
Plasma Glucose (mg/dL), Mean (SE)	96.3 (1.1)	100.3 (0.86)	102.9 (1.1)	136.6 (2.5)	<0.001
Fasting Plasma Glucose* (mg/dL), Mean (SE)	100.5 (1.3)	103.8 (1.1)	112.6 (2.3)	116.2 (2.6)	<0.001
Serum Insulin (μU/mL), Mean (SE)	6.9 (0.87)	12.2 (0.61)	16.4 (0.53)	27.4 (1.5)	<0.001
Fasting Serum Insulin* (μU/mL), Mean (SE)	6.4 (0.76)	11.1 (0.43)	14.9 (0.56)	23.4 (1.4)	<0.001
HOMA-IR*, Mean (SE)	1.8 (0.20)	2.9 (0.13)	3.9 (0.16)	7.4 (0.43)	<0.001
HbA1c, Mean (SE)	5.4 (0.05)	5.5 (0.03)	5.7 (0.04)	6.6 (0.07)	<0.001
Diabetes, % (SE)	8.1 (1.1)	8.9 (0.81)	10.5 (1.4)	38.7 (1.8)	<0.001
Number of Metabolic Abnormalities†, Mean (SE)	0.6 (0.06)	1.8 (0.04)	3.0 (0.05)	3.9 (0.04)	<0.001
Metabolic Syndrome‡, % (SE)	6.2 (1.9)	22.0 (2.0)	67.4 (2.3)	91.2 (1.6)	<0.001
Lipid Profile					
Total Cholesterol (mg/dL), Mean (SE)	188.7 (2.4)	210.8 (2.2)	216.4 (1.9)	222.1 (2.4)	<0.001
High-Density Lipoprotein Cholesterol (mg/dL), Mean (SE)	62.1 (1.1)	50.9 (0.57)	44.2 (0.53)	38.2 (0.57)	<0.001
Hyperlipidemia, % (SE)	7.5 (2.3)	29.0 (2.3)	58.4 (2.7)	81.6 (1.8)	<0.001
low-density lipoprotein cholesterol (mg/dL), Mean (SE)	112.1 (3.7)	133.2 (2.9)	134.2 (1.78)	133.8 (2.9)	<0.001
Triglycerides (mg/dL), Mean (SE)	76.6 (3.9)	119.9 (3.2)	176.9 (4.2)	294.7 (8.9)	<0.001
Liver Function					
Alkaline Phosphatase (U/L), Mean (SE)	78.9 (1.6)	91.4 (1.6)	91.6 (1.7)	97.8 (1.4)	<0.001
Serum Albumin (g/dL), Mean (SE)	4.2 (0.03)	4.1 (0.02)	4.1 (0.03)	4.1 (0.02)	0.003
Bilirubin (mg/dL), Mean (SE)	0.63 (0.02)	0.55 (0.01)	0.57 (0.02)	0.63 (0.02)	0.781
Ferritin (ng/mL), Mean (SE)	112.5 (6.5)	127.4 (5.3)	159.0 (6.7)	197.8 (8.0)	<0.001
Gamma-Glutamyltransferase* (U/L), Mean (SE)	24.0 (2.2)	33.2 (2.7)	42.8 (3.9)	47.9 (3.3)	<0.001
Aspartate Aminotransferase (AST) (U/L), Mean (SE)	22.5 (1.0)	22.2 (0.57)	24.4 (0.88)	25.3 (0.65)	0.012
Alanine Aminotransferase (ALT) (U/L), Mean (SE)	14.2 (0.94)	19.1 (0.63)	24.5 (1.4)	25.6 (0.88)	<0.001
AST/ALT Ratio, Mean (SE)	1.7 (0.05)	1.4 (0.04)	1.2 (0.03)	1.2 (0.03)	<0.001
Kidney Function					
Urea Nitrogen (mg/dL), Mean (SE)	14.5 (0.30)	14.0 (0.27)	14.2 (0.22)	14.6 (0.21)	0.620
Creatinine (mg/dL), Mean (SE)	1.1 (0.01)	1.1 (0.01)	1.1 (0.01)	1.09 (0.01)	0.188
Uric Acid (mg/dL), Mean (SE)	4.7 (0.08)	5.5 (0.06)	6.1 (0.06)	6.2 (0.08)	<0.001

* Among a sub-sample of adults who reported at least 8 hours of fasting before examination

† (1) hyperglycemia (i.e., fasting blood glucose over 100 mg/dl, or pharmacological treatment), (2) dyslipidemia (i.e., fasting HDL cholesterol level less than 40 mg/dl, men, or 50 mg/dl, women, or pharmacological treatment) (3) hypertriglyceridemia (i.e., fasting triglyceride (TG) level over 150 mg/dl, or pharmacological treatment), (4) central obesity (i.e., waist circumference over 40 inches ,men, or 35 inches, women), or (5) hypertension (i.e., systolic blood pressure (SBP) over 130 mmHg, or pharmacological treatment)

‡ At least three metabolic abnormalities

% = Weighted Proportion; SE= Standard Error

4.3.3 Metabolic Syndrome Severity and Mortality in NAFLD

The mean and median years of follow-up for NAFLD patients were 18.2 and 19.2, respectively. During the 23 years of follow-up, the overall cumulative mortality incidence from all-causes was 29.1% (741 deaths) in NAFLD. During the same period, the cause-specific cumulative mortality incidence was 7.3% (174 deaths) with heart disease, 5.7% (126 deaths) from diabetes, and 6.2% (115 deaths) related to hypertension. The unadjusted cumulative mortality over the 23 years of follow-up increased with rises in the MetS severity score for deaths related to all-causes (Figure 4-2). As such, the all-cause unadjusted cumulative mortality for NAFLD patients in the first, second, third, and fourth MetS severity score quartile were 10.2%, 24.8%, 30.6%, and 48.0%, respectively. Similarly, the cause-specific cumulative mortality associated with heart disease, diabetes, and hypertension increased with a higher MetS severity score quartile (Table 4-3).

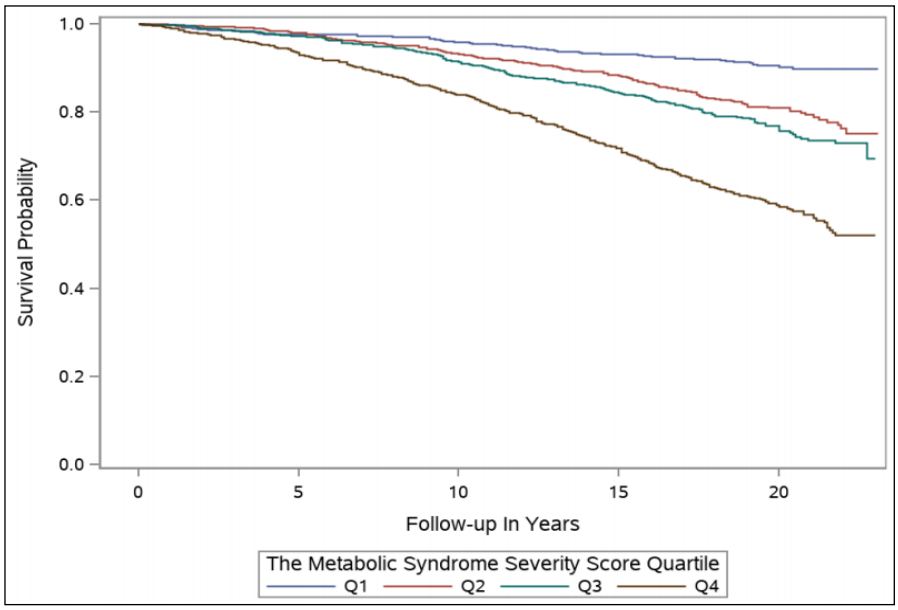

Figure 4-2 Cumulative All-Cause Mortality Incidence from by the Metabolic Syndrome Severity Score Quartile among Adults with NAFLD, (NAHNES III), (n=3,088)

Table 4-3 Number of Deaths and Cumulative Incidence of Mortality over 23 Years by Metabolic Syndrome Severity Score Quartiles, Adults with Non-Alcoholic Fatty Liver Disease (NAFLD), (NHANES III), (n=3,088)

Metabolic Syndrome Severity Quartile	All Cause		Heart Disease		Diabetes		Hypertension	
	Number of Deaths	% (95% CI)	Number of Deaths	% (95% CI)	Number of Deaths	% (95% CI)	Number of Deaths	% (95% CI)
Q1	64	10.2 (8.0 – 13.0)	12	2.1 (1.1 – 4.0)	3	0.70 (0.20 – 2.5)	6	1.0 (0.42 – 2.1)
Q2	153	24.8 (20.6 – 29.6)	32	5.2 (3.5 – 7.6)	9	1.3 (0.67 – 2.4)	24	4.5 (2.8 – 7.2)
Q3	182	30.6 (23.7 – 38.8)	39	6.9 (4.8 – 10.0)	18	3.2 (2.0 – 5.3)	26	9.3 (3.5 – 23.4)
Q4	342	48.0 (43.5 – 42.7)	91	14.9 (11.8 – 18.7)	96	17.7 (13.9 – 22.4)	59	10.8 (7.9 – 14.7)

All cause excluding adults who died in accidents [i.e., unintentional injuries (V01-X59, Y85-Y86)], heart disease [i.e., ICD-10 codes I00-I09 (Acute rheumatic fever and chronic rheumatic heart diseases), I11 (Hypertensive heart disease), I13 (Hypertensive heart and renal disease), I20-I25 (Ischemic heart diseases), and, I26-I51 (Other heart diseases)], diabetes [i.e., ICD-10 codes (E10-E14)], and hypertension [i.e., ICD-10 codes (I10-I12)]

The mortality incidence rate for adults with NAFLD followed similar trends to the unadjusted cumulative mortality incidence trajectories (Figure 4-3). In NAFLD, the all-cause mortality incidence rate was 13.5 per 1,000 person-years, while the cause-specific mortality incidence rates associated with heart disease, diabetes, and hypertension were 3.2 per 1,000 person-years, 2.3 per 1,000 person-years, and 2.1 per 1,000 person-years, respectively. An estimated 46.2% of all deaths occurred amongst NAFLD patients in the fourth MetS severity score quartile, compared to 8.6% for those in the first quartile. In turn, the all-cause mortality incidence rates were 4.9 per 1,000 person-years and 25.3 per 1,000 person-years, for the first and fourth MetS severity score quartiles, respectively. The cause-specific mortality incidence rates for heart disease, diabetes, and hypertension also increased with rises in the MetS severity score quartiles.

The MetS severity score was a significant predictor for all-cause and cause-specific adjusted mortalities in NAFLD. A quartile increase in MetS severity score was associated with increased in the risk of all-cause mortality adjusted Hazard Ratio (aHR) 1.36 (95% CI; 1.17 – 1.57), heart disease-related mortality aHR 1.70 (95% CI; 1.17 – 2.47), diabetes-related mortality aHR 3.64 (95% CI; 2.27 – 5.83), and hypertension-related mortality aHR 1.87 (95% CI; 1.14 – 3.04) (Data not shown). In the adjusted models with the severity score included as a categorical variable, those in the fourth quartile had aHR 2.12 (95% CI; 1.17 – 3.84) times the risk of all-cause mortality, aHR 8.26 (95% CI; 1.68 – 40.58) higher risk of diabetes-related mortalities and aHR 8.68 (95% CI; 1.47 – 51.08) increase risk of hypertension-related mortality, relative to NAFLD patients in the first MetS severity score quartile (Table 4-4).

Table 4-4 Adjusted Mortality Hazard Ratios (HR) by Metabolic Syndrome Severity Score Quartiles in Adults with Non-Alcoholic Fatty Liver Disease (NAFLD), the National Health and Nutrition Examination Survey (NHANES III) 1988-2011 (n=3,088)

Metabolic Syndrome Severity Quartile	All Cause	Heart Disease	Diabetes	Hypertension
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Q1	Reference	Reference	Reference	Reference
Q2	1.21 (0.71 – 2.06)	1.38 (0.30 – 6.38)	0.65 (0.10 – 4.15)	3.58 (0.44 – 29.38)
Q3	1.07 (0.56 – 2.03)	1.27 (0.26 – 6.18)	1.50 (0.25 – 9.03)	2.54 (0.40 – 16.32)
Q4	**2.12 (1.17 – 3.84)**	3.53 (0.74 – 16.86)	**8.26 (1.68 – 40.58)**	**8.68 (1.47 – 51.08)**

CI; Confidence Interval, Q; Quartile

All cause excluding adults who died in accidents [i.e., unintentional injuries (V01-X59, Y85-Y86)], heart disease [i.e., ICD-10 codes I00-I09 (Acute rheumatic fever and chronic rheumatic heart diseases), I11 (Hypertensive heart disease), I13 (Hypertensive heart and renal disease), I20-I25 (Ischemic heart diseases), and, I26-I51 (Other heart diseases)], diabetes [i.e., ICD-10 codes (E10-E14)], and hypertension [i.e., ICD-10 codes (I10-I12)]

Adjusted for attained age, gender, race/ethnicity, education level, marital Status, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index percentile, chronic kidney disease, family history of diabetes, family history of myocardial infarction, and history of cancer

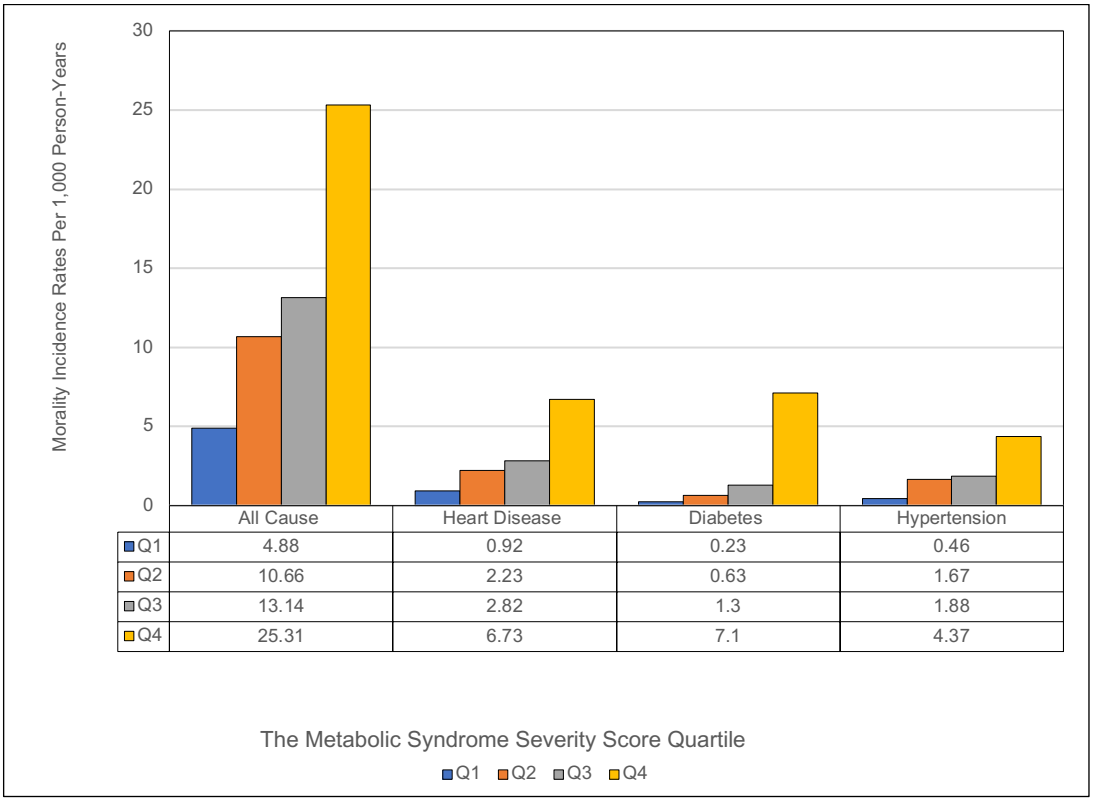

Figure 4-3 Mortality Incidence Rates of Per 1000 Person-Years by the Metabolic Syndrome Severity Score Quartile (Q) amongst Adults with NAFLD, (NHANES III), (n=3,088)

In the RCS analysis, significant non-linear dose-response trends were observed in the relationship between increased risk of mortality and higher MetS severity score in all adjusted models (Figure 4-4). Generally, the risk of mortality in NAFLD increased with higher MetS severity scores relative to the median severity value. In contrast, the risk of mortality was lower for NAFLD patients with MetS severity scores below the median value up to the 20th severity percentile. A two-tail dose-response was observed in the relationship between an increase in MetS severity score and mortality risks from diabetes and hypertension. Table 4-5 outlines the aHR estimates for mortality risk in reference to the median MetS severity score value. Notably, significant increased adjusted mortality risks were observed for all severity estimates above the median MetS severity value. The

risk of mortality from all causes, heart disease, diabetes, and hypertension was aHR 2.16 (1.61 – 2.90), aHR 3.41 (1.70 – 6.81), aHR 18.92 (7.59 – 47.13), and aHR 3.98 (1.30 – 12.18), respectively, times the mortality risk for NAFLD patients in the 99th MetS severity percentile compared to those with median severity.

Table 4-5 Adjusted Hazard Ratios of Mortality Related to (A) All-Cause (B) Heart Disease (C) Diabetes (D) Hypertension for Metabolic Syndrome Score Percentiles In Reference to the Median Severity Percentile, (NAHNES III), (n=3,088)

Metabolic Syndrome Severity Percentile	All Cause	Heart Disease	Diabetes	Hypertension
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
50th	Reference	Reference	Reference	Reference
60th	1.16 (1.10 – 1.23)	1.27 (1.11 – 1.45)	1.74 (1.46 – 2.05)	1.29 (1.05 – 1.59)
70th	1.36 (1.21 – 1.53)	1.64 (1.24 – 2.16)	3.21 (2.24 – 4.60)	1.72 (1.11 – 2.67)
80th	1.61 (1.34 – 1.93)	2.14 (1.40 – 3.27)	6.10 (3.49 – 10.66)	2.33 (1.18 – 4.61)
90th	1.94 (1.51 – 2.50)	2.88 (1.59 – 5.21)	12.53 (5.72 – 27.43)	3.28 (1.25 – 8.55)
99th	2.16 (1.61 – 2.90)	3.41 (1.70 – 6.81)	18.92 (7.59 – 47.13)	3.98 (1.30 – 12.18)

All cause excluding adults who died in accidents [i.e., unintentional injuries (V01-X59, Y85-Y86)], heart disease [i.e., ICD-10 codes I00-I09 (Acute rheumatic fever and chronic rheumatic heart diseases), I11 (Hypertensive heart disease), I13 (Hypertensive heart and renal disease), I20-I25 (Ischemic heart diseases), and, I26-I51 (Other heart diseases)], diabetes [i.e., ICD-10 codes (E10-E14)], and hypertension [i.e., ICD-10 codes (I10-I12)]
Adjusted for attained age, gender, race/ethnicity, education level, marital Status, access to health insurance, alcohol intake, smoking status, body mass index, abdominal obesity, physical activity, healthy eating index percentile, chronic kidney disease, family history of diabetes, family history of myocardial infarction, and history of cancer
CI; Confidence Interval

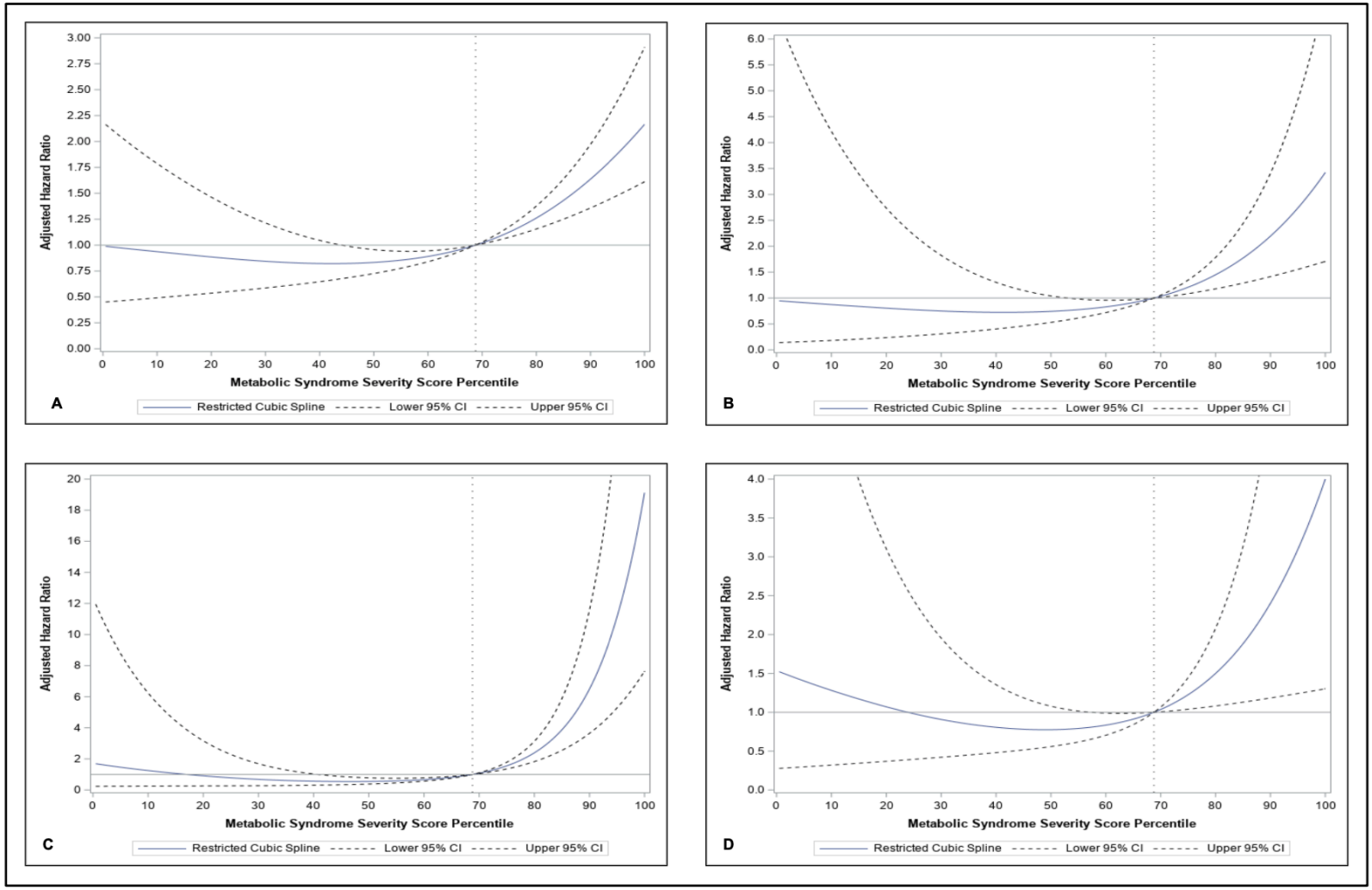

Figure 4-4 Adjusted Hazard Ratios of Mortality Related to (A) All-Cause (B) Heart Disease (C) Diabetes (D) Hypertension for Different Metabolic Syndrome Severity Score Percentiles in Reference to the Median Severity Percentile (69th), (NAHNES III), (n=3,088)

4.4 Discussion

Previously conducted studies on the association between MetS and the risk of mortality in NAFLD have utilized the harmonization definition of this syndrome. While this definition factors in disease occurrence, it treats, equally, the effects of any of the possible ten combinations of metabolic abnormalities on survival in NAFLD. Furthermore, the traditional definition of MetS does not account for the racial and gender disparities in MetS severity and the corresponding effects of those disparities on the risk of mortality in NAFLD. To address this knowledge gap, we used the MetS severity score, a validated gender-race specific Z-score, to assess the association between MetS severity and the risk of mortality in NAFLD.

In this population-based cohort study with 23 years of follow-up, the prevalence of NAFLD differed by race/ethnicity and was highest in Mexican Americans. NAFLD patients in our study were older, obese with low levels of physical activities compared to the general US adult population. The MetS severity was significantly higher in NAFLD patients relative to participants without NAFLD. In NAFLD, increases in the MetS severity were associated with linear dose-response in biomarkers for cardiovascular disease, insulin resistance, and lipid abnormalities. The MetS severity score was a significant predictor for all-cause and cause-specific adjusted mortalities in NAFLD. We also found non-linear dose-response relationships between increased adjusted mortality risks and higher MetS severity score.

The prevalence of NAFLD in our study was 26.7% (95% CI; 24.3% – 29.1%). NAFLD prevalence was highest in Mexican Americans (33.7%) and lowest amongst Black

non-Hispanics (23.2%). Those estimates are consistent with NAFLD prevalence reported by other studies. In the US, the prevalence of NAFLD was estimated by other studies to be 26.36% (95% CI; 23.82-29.07).[16] A study of racial and ethnic disparities in NAFLD reported the age-adjusted NAFLD prevalence to be 21.2%, 12.5%, and 11.6% in Mexican Americans, non-Hispanic Whites, and non-Hispanic Blacks, respectively.[58] Such race/ethnic dipartites in NAFLD prevalence was confirmed by a meta-analysis of 343,393 individuals in which the relative risk (RR) of NAFLD was 1.36 (95% CI, 1.08–1.73) for Hispanics and 0.68 (95% CI, 0.54–0.84) for Blacks when compared respectively to Whites.[23]

We detected a significant association between age and NAFLD occurrence. Age has been found in multiple studies to be associated with increased risk of NALFD, NASH, and advanced fibrosis.[2] The relationship between age and increased risks of advanced hepatic outcomes is attributed to a longer duration of disease amongst older patients. Namely, a study on age and the risk of liver outcomes found the prevalence of NAFLD to be less than 20% in those younger than age 20 and 40% amongst those ages 60 or older.[59] A study of NHANES 2011-2014 data reported a 4% increase in the adjusted odds of NAFLD with one year increase in age.[60] In this study, the prevalence of advanced fibrosis in NAFLD was significantly associated with an increase in age.

Obesity is a well-established risk for NAFLD development.[61] Obesity is marked by elevated accumulations of TG throughout the body. In the hepatocytes, increased uptake of TG results in cell-specific lipotoxicity, which raises the risk of comorbidities such as NAFLD.[62] Individuals with high visceral adiposity may suffer from increased plasma free fatty acids, which is due to impaired insulin function related to peripheral Insulin

resistance.[63] Studies have reported the prevalence of steatosis and NASH to be 65% and 20%, respectively in individuals with BMI 30.0–39.9 kg/m^2, and 85% and 40%, respectively in morbidly obese person (BMI ≥40 kg/m^2).[64] Similarly, the prevalence of NASH and NAFLD amongst obese patients undergoing bariatric surgery is 37% and 91%, respectively.[65] Our study findings highlight similar results of the increased obesity burden in NAFLD patients.

Currently, there are no approved pharmacologic or other modalities of treatment for NAFLD. Lifestyle modifications that are suggested as treatments for NAFLD follow those recommended for MetS and include increasing physical activity and weight loss.[66] Such lifestyle changes have been shown to reduce the risk of NAFLD significantly. In Meta-analysis of six cohort studies (32,657 participants) the highest level of physical activity was associated with 21% reduction in the risk of NAFLD development relative to the lowest physical activity levels.[67] A meta-analysis of randomized trials showed that weight loss, meeting or exceeding 7%, can improve histological markers of disease. However, fewer than 50% of subjects across several trials were able to achieve this level of weight loss.[68] The prevalence of physically active adults in our study was significantly lower compared to the general adult population. This finding is comparable to results from a population study where NAFLD patients spent less time than controls participating in any level of physical activity.[69]

Our study shows that both the prevalence and severity of MetS were significantly higher in NAFLD patients. A recent US-based study revealed significant associations between individual components of MetS and NAFLD. In individuals with increased waist circumference, the prevalence of NAFLD (31%, 8.7% with advanced fibrosis) greatly

exceeded controls.[20] NAFLD patients with increased waist circumference were predominantly female, older, and less educated. NAFLD prevalence in subjects with diabetes (41%, 18% with advanced fibrosis), also greatly exceeded control prevalence. The prevalence of NAFLD in subjects with high triglyceride levels was 35% (8% with advanced fibrosis).

An increase in the severity of MetS was associated with clinical characteristics related to cardiovascular factors, metabolic control, lipid profile, liver function, and kidney function. A meta-analysis of 27 cross-sectional studies reported an association between NAFLD and markers of atherosclerosis, including increased carotid intima-media thickness, coronary calcification, endothelial dysfunction, and arterial stiffness, independent of traditional cardiometabolic risk factors and metabolic syndrome.[70] In turn, the pooled odds ratio of CVD in NAFLD relative to NAFLD free adults is 2.02 (95% CI; 1.81–2.31).[71] According to a meta-analysis of nineteen observational studies, the risk of diabetes is HR 2.22 (95% CI; 1.84–2.60) in NAFLD compared to those with no NAFLD.[72] The risk of incident MetS in NAFLD compared to no-NAFLD ranges between Risk Ratio (RR) 1.80 and 3.22.[73] NAFLD patients have 2.12- and 1.79- folds increase in odds and risks of chronic kidney disease, respectively.[74] Our finding of higher MetS severity could help to explain the increased risks of diabetes, MetS, cardiovascular disease and chronic kidney disease in NAFLD.[35,68,72,73,75]

NAFLD has been shown to increase the risk of mortality from all-cause, liver-related, and CVD-related. In NAFLD, the most common causes of deaths are CVD, malignancies, and liver disease.[2] The incidence rate of all-cause mortality was higher in NAFLD vs. no NAFLD (13.52 deaths per 1,000 person-years vs. 11.75 deaths per 1,000

person-years). Those estimates are similar to findings for a global meta-analysis where the incidence rate of all-cause mortality was 11.77 deaths per 1,000 person-years.[10] In a meta-analysis of forty cohort studies, NAFLD patients had higher all-cause mortality compared to the general population pooled odds ratio 1.57 (95% CI; 1.18–2.10).[71] Similarity, compared to the adults without NAFLD, NAFLD patients have HR 9.32 (95% CI; 9.11–9.33) for liver-related [76], and pooled odds ratio 1.59 (95% CI; 1.42–1.78) for CVD-related mortality.[71]

The association between MetS and the risk of mortality in NAFLD has been assessed in multiple studies.[77-79] In a population-based study using NHAES III data, the risk of overall mortality and CVD-related mortality was HR 2.22 (95% CI; 1.26–3.91) and HR 4.58 (95% CI; 1.53–13.76), respectively for NAFLD patients with versus without MetS. Our study adds to those findings by accounting for the effects of the aggerate MetS severity on survival in NAFLD. In our study, significant non-linear dose-response trends were observed in the relationship between increased risk of mortality and higher MetS severity score in all adjusted models. Specifically, the risk of mortality in NAFLD increased with higher MetS severity scores relative to the median severity value. In contrast, the risk of mortality was lower for NAFLD patients with MetS severity scores below the median value up to the 20th severity percentile.

The relationship between MetS severity and survival in NAFLD is a step towards tackling the current public health burden of this disease. Results from the underlined research could aid both clinicians and public health practitioners in planning and executing secondary and tertiary prevention efforts related to long-term mortality in NAFLD. Secondary prevention efforts could take place by using the MetS severity score

as a screening tool to identify patients at the highest risk of mortality in NAFLD. The MetS severity score could also be used as a tertiary prevention tool whereby the progression of severity is monitored with the aim of designing interventions to mitigate the chances of more advanced hepatic manifestations in NAFLD.

Our findings are not without limitations. Liver biopsies are the gold standard for NAFLD diagnosis. NAFLD assessment was done using ultrasonography, which could result in misclassifications. Furthermore, we could not evaluate the role of NASH in survival due to the lack of liver biopsy data. Ascertainments of exposure and baseline characteristics were conducted cross-sectionally. Alcohol intake was assessed based on self-reporting, which might result in underestimation. ICD-9 codes were used to identify cause-specific mortalities for deaths that took place before 1998. This could have potential minor misclassification when those ICD-9 codes are transformed into ICD-10 causes. The analysis was limited to noninstitutionalized adults who were able to participated; hence, those with severe disease were less likely to participate.

4.5 Conclusions

Previously research on the natural history of NAFLD has solely focused on identifying the presence of risk factors for disease progression without accounting for the effects of MetS severity on survival in NAFLD. To tackle this knowledge gap, we utilized the MetS severity score to examine the association increased MetS severity and the risk of long-term mortality in adults with NAFLD. NAFLD is marked by significantly higher MetS severity compared to US adults. In NAFLD, an increase in the MetS severity corresponded to a linear rise in biomarkers for cardiovascular disease, insulin resistance, lipid abnormalities,

and decreases in both liver and kidney functions. Almost half of all deaths in NAFLD patients take place amongst adults in the highest MetS severity quartile. A quartile increase in the MetS severity score was associated with an increased risk of all-cause mortality, heart disease-related), diabetes-related mortality, and hypertension-related mortality. Significant non-linear dose-response trends were observed in the relationship between increased risk of mortality and higher MetS severity score in all adjusted models. Significant increases in adjusted mortality risks amongst adults with NAFLD were observed for all severity estimates above the median MetS severity values. Those findings show the utility of MetS severity as a driving force of increased risk of mortality in NAFLD. While current treatment options for patients with NAFLD are limited and indirect, the MetS severity score could be used as screening tool that could aid tertiary prevention measures in NAFLD patients.

4.6 References

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