Title

Estimating the Race/Ethnic-Specific Association Between Obesity and Type 2 Diabetes, and the Role of Non-Alcoholic Fatty Liver Disease

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Estimating the Race/Ethnic-Specific Association Between Obesity and Type 2 Diabetes, and the Role of Non-Alcoholic Fatty Liver Disease

by

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DISSERTATION

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Dedication

To my beloved late uncle P. Alejandro Ríos Flores, a talented artist and true friend.

To my loving and joyful daughters Samantha and Paula Rodríguez;

may God grant you wisdom and courage to make our world a better place, especially for the weak among us.

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Contributions

A version of Chapter 1 in this dissertation was accepted in *Diabetic Medicine* on 23 June 2020 in collaboration with Patrick T. Bradshaw, PhD, Stephen C. Shiboski, PhD, Alicia Fernandez, MD, Eric Vittinghoff, PhD, David Herrington, MD, Jingzhong Ding, PhD, and Alka M. Kanaya, MD. The Dissertation Committee members supervised the research that forms the basis of this dissertation chapter, the published material is substantially the product of Luis A. Rodríguez's period of study at the University of California, San Francisco and was primarily conducted and written by him. The work he completed for this published manuscript is comparable to a standard dissertation chapter.

Approved:

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Estimating the Race/Ethnic-Specific Association Between Obesity and Type 2 Diabetes, and the Role of Non-Alcoholic Fatty Liver Disease

Luis Alberto-Ríos Rodríguez

Abstract

Type 2 diabetes is one of the top-10 causes of morbidity and mortality in the US, and disproportionally affects racial/ethnic minorities. Obesity is a well-known cause of type 2 diabetes, and in the past three decades, the obesity epidemic has contributed significantly to the drastic increase in type 2 diabetes in the US. Despite this clear relationship, it is unclear if the association between obesity measures (e.g. body mass index [BMI]) and type 2 diabetes risk varies by race/ethnicity. Identifying if race/ethnic-specific BMI thresholds should be used for risk-stratification can have important clinical implications as these can be incorporated into screening guidelines. Further, the precise mechanisms that connect obesity and type 2 diabetes remain unclear. It is hypothesized that one of these mechanisms is via the development of non-alcoholic fatty liver disease (NAFLD), which exacerbates hepatic insulin resistance and promotes the onset of type 2 diabetes. Understanding the possible mediating role that NAFLD has on the obesity-type 2 diabetes relationship may be of great interest as NAFLD prevention or management could be a promising target to reduce the obesity-related burden on type 2 diabetes.

This dissertation applies advanced epidemiologic methods to answer three distinct questions utilizing data from the well-characterized Multi-Ethnic Study of Atherosclerosis cohort (2000-2011) of 6,814 White, African American, Hispanic and Chinese American adults ages 45-84 years of age. The first chapter evaluated the usefulness of the BMI as a race/ethnic-specific predictor of type 2 diabetes risk to predict the 10-year risk of type 2

diabetes according to race/ethnicity at different BMI points. We found that African American, Hispanic and Chinese American adults had similar type 2 diabetes risk at lower BMI values compared to White adults, suggesting that racial/ethnic minorities should be screened for type 2 diabetes at lower BMI cut-points. In the second chapter I applied a causal mediation analysis using marginal structural models to estimate the overall association between obesity on risk of type 2 diabetes, decomposing this into the portion of the relationship mediated, and not mediated, by the degree of liver fat accumulation (i.e. indirect and direct effects, respectively). We found that NAFLD accounted for approximately 30% of the association between obesity and type 2 diabetes risk, underscoring the importance of this mechanism as a possible target for prevention of type 2 diabetes. And the third chapter developed a practical scoring tool for predicting NAFLD using participant demographic, medical history, anthropometry and laboratory data. We found that our prediction tool was simple but highly predictive and can aid clinicians identify adults at high NAFLD risk.

Together, these projects highlight the usefulness of generalized and ectopic obesity measures that can be used to identify high-risk adults in whom screening and possible interventions can help reduce the risk of or delay the onset of type 2 diabetes.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ADA: American Diabetes Association

AHEI-2010: Alternative Healthy Eating Index-2010

ATP III: Adult Treatment Panel III

AUC: area under the curve

BMI: body mass index

CI: confidence interval

CT: computed tomography

CVD: cardiovascular disease

EASD: European Associations for the Study of Diabetes

EASL: European Associations for the Study of the Liver

EASO: European Associations for the Study of Obesity

FFA: free fatty acid

FFQ: food frequency questionnaire

FGF-21: fibroblast growth factor-21

FLI: Fatty Liver Index

GGT: gamma-glutamyltransferase

HDL: high-density lipoprotein

HR: hazard ratio

HU: Hounsfield units

IDF: International Diabetes Federation

IL-6: interleukin-6

IRB: institutional review board

MESA: Multi-Ethnic Study of Atherosclerosis

MET-min: metabolic equivalent minutes

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

NCEP: National Cholesterol Education Program

NHANES: National Health and Nutrition Examination Survey

OR: odds ratio

RBP-4: retinol-binding protein-4

SD: standard deviation

T2D: type 2 diabetes

TG: triglycerides

TNF-alpha: tumor necrosis factor-alpha

US: United States

USPSTF: United States Preventive Services Task Force

WC: waist circumference

Chapter 1 Abstract

Aims Disparities persist on the prevalence of undiagnosed type 2 diabetes in racial/ethnic minorities in the US. This study evaluated the association between BMI and incident type 2 diabetes risk by racial/ethnic group, to determine if BMI and presence of type 2 diabetes risk factors may help clinicians better target type 2 diabetes screening.

Methods This prospective cohort analysis included 5,659 adults free of type 2 diabetes at baseline from the Multi-Ethnic Study of Atherosclerosis (MESA), a population-based cohort (2000-2011). BMI was measured at baseline and time-updated at subsequent visits. Incident type 2 diabetes was defined as fasting glucose ≥7.0 mmol/L, or use of any diabetes medications.

Results The mean (SD) age was 62 (10) years and 42% of participants were White, 26% African American, 20% Hispanic, and 12% Chinese American. During follow-up, 696 (12%) new type 2 diabetes cases were observed. In age and sex-adjusted models, in the presence of one or more type 2 diabetes risk factors (the most common scenario), a 10% risk of incident type 2 diabetes was observed at a BMI of 21.7 kg/m^2 (95% CI: 20.1-22.8) in Chinese Americans, 23.8 kg/m^2 (22.7-24.9) in Hispanics, 24.7 kg/m^2 (23.7-25.6) in African Americans, and 26.2 kg/m^2 (25.1-26.9) in White participants.

Conclusions This study supports including BMI and presence of type 2 diabetes risk factors as action points for clinicians to prioritize which adults ≥45 years should be

screened. The application of race/ethnicity-specific BMI thresholds may reduce the disparity of undiagnosed type 2 diabetes observed in minority groups.

Chapter 1 Main Body

INTRODUCTION

In recent decades, the prevalence of type 2 diabetes has increased significantly [1]. Currently, 15% of US adults have type 2 diabetes [2], and if these trends continue, it is projected that the prevalence could rise to 33% by the year 2050 [3]. The burden also disproportionately affects racial/ethnic minorities; while the current prevalence is 12% among White populations, it is nearly double that among Asian Americans, African Americans and Hispanics [2]. Likewise, the prevalence of undiagnosed type 2 diabetes disproportionally affects minority groups; it is 6.4% in African Americans, 8.6% in Asian Americans, and 8.9% in Hispanics, compared to 4.2% in White populations [2].

Screening high-risk asymptomatic persons is recommended because reliable tests are available, and may lead to earlier identification and treatment that can potentially reduce progression and improve health outcomes [4]. Several societies recommend screening including the US Preventive Services Task Force (USPSTF), and the American Diabetes Association (ADA). The USPSTF recommends screening adults 40 to 70 years who are overweight/obese (body mass index [BMI] ≥25 kg/m^2) [5]. They also recommend screening persons at a lower BMI if they have risk factors such as family history of diabetes, history of gestational diabetes or polycystic ovarian syndrome, or are members of racial/ethnic minority groups. By comparison, the ADA recommends universal screening for adults 45 years or older [6]. Despite these recommendations, recent data from the National Health and Nutrition Examination Survey (NHANES) found that only half of adults 45 and older reported having been screened for diabetes [7], likely

explaining part of why a high prevalence of undiagnosed type 2 diabetes remains a problem in the US.

Under screening of minority populations may occur for many reasons. A recent study of primary care physicians found the least commonly identified risk factors were patient race/ethnicity, and lower BMI among Asian Americans [8]. Clinicians may associate diabetes with overt obesity and hence target screening to adults with BMIs over 30 kg/m^2. In this study, we used longitudinal data from a multi-ethnic cohort of adults ages 45 and older to evaluate how BMI predicted incident type 2 diabetes risk by racial/ethnic group and presence of risk factors. The overarching objective was to determine how BMI could be used to help clinicians better target screening among adults 45 and older, who continue to be screened for type 2 diabetes at suboptimal rates.

METHODS

Participant Population

We analyzed follow-up data from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, a well-characterized cohort of 6,814 participants aged 45-84 years, free of known cardiovascular disease (CVD). MESA objectives and design have been described in detail elsewhere [9]. Briefly, MESA recruited adults from six university clinics in the United States (Columbia, New York; Johns Hopkins, Baltimore; Northwestern, Chicago; University of California, Los Angeles; University of Minnesota, Twin Cities; and Wake Forest, Winston Salem). Recruitment into MESA began in the year 2000 and participants are still being followed. Approximately 38% of the cohort was White participants, 28%

African American, 22% Hispanic, and 12% Chinese American. For our analysis, we excluded participants with prevalent type 2 diabetes at baseline (N=829), or with type 1 diabetes (N=10) and participants with missing covariates (N=316). Our final sample size was 5,659 who had complete data on at least one visit.

Ethics Approval

Informed consent was obtained from all study participants and institutional review board (IRB) approval at the sites conducting MESA was obtained. Ethics approval for the use of anonymized data was obtained from the University of California San Francisco IRB on 2 January 2018 (16-21085). This study adhered to the principles detailed in the US Federal Policy for the Protection of Human Subjects.

Exposure Assessment

Anthropometric measures were taken in light clothing and no shoes and were measured twice and averaged at all study visits using standardized procedures [9]. BMI (kg/m^2) was calculated from weight (kg) divided by squared height (m^2).

Outcome Assessment

Individuals were followed for incident type 2 diabetes through Exam 5. Diabetes status was defined by fasting glucose ≥7.0 mmol/L and/or diabetes treatment, ascertained at each of the five clinic examinations (years 2000-2002, 2002-2004, 2004-2005, 2005-2007 and 2010-2012). Hemoglobin A1c (HbA1c) was only measured in exams 2 and 5,

consequently we were unable to use this diagnostic criterion to identify and exclude prevalent cases at baseline or consistently capture incident cases over time.

Diabetes Risk Factors

We considered the following risk factors: first-degree relative with diabetes, high-risk race/ethnic group (i.e. non-White participants), hypertension (≥140/90 mmHg or on therapy), high-density lipoprotein (HDL) cholesterol level <0.90 mmol/L, fasting triglyceride levels >2.82 mmol/L, and physical inactivity [5,10]. Family history of diabetes was ascertained at the second exam visit on 5,382 participants; this information was back-dated to the baseline visit and included as a risk factor in sensitivity analysis. Race/ethnicity was self-reported. Resting blood pressure was measured three times in a seated position, and the average of the last two measurements were used. Blood was drawn in the fasted state from which serum HDL-cholesterol and triglycerides were measured [9]. Duration and frequency of various physical activities during a typical week in the past month were assessed using a detailed, semi-quantitative questionnaire adapted from the Cross-cultural Activity Participation Study [9,11]. Metabolic equivalent minutes of physical activity was calculated from the duration and intensity of total intentional exercises. Sex was self-reported.

Statistical Analysis

Baseline characteristics between the four racial/ethnic groups were presented as means for continuous variables or percentages for categorical variables. Because observations of time to diagnosis of type 2 diabetes may be interval-censored in MESA, we initially fit

appropriate Weibull proportional hazards models with robust standard errors [12]. However, treating the time of incident cases of type 2 diabetes as the mid-point between two exam visits using either Weibull or Cox models resulted in essentially the same point estimates and similar standard errors as the interval censored Weibull model; accordingly, we present our results based on the Weibull model with the midpoint time. In addition, race/ethnic-specific differences in the association between BMI and type 2 diabetes risk were evaluated using interaction terms, but because these were not significant at the 10% significance level, and inclusion of these did not meaningfully alter our results, we did not include interaction in our final models.

Using the Weibull model described above we estimated the 10-year probability of developing diabetes as a function of BMI using a 3-knot restricted cubic spline, and adjusting for race/ethnicity, age (continuous), sex, and included an indicator variable for the presence of one or more risk factors (low HDL-cholesterol, hypertriglyceridemia, hypertension, or physical inactivity) [5,10]. BMI and risk factors were time-updated at each exam visit, with missing values imputed by carrying forward the last complete value; less than 3% of data was missing and carried forward. As a sensitivity analysis, we included family history of diabetes as a risk factor. Marginal estimates of the expected 10-year probability of developing type 2 diabetes as a function of BMI for each of the four racial/ethnic groups in the presence or absence of diabetes risk factors, or not accounting for risk factors were then calculated by regression standardization, averaging over the remaining covariates included in the model, as evaluated at baseline. Lastly, we used a simple line search, with step size of 0.1 kg/m^2, to find the critical BMI values for each of

the four groups corresponding to an estimated 10-year risk of type 2 diabetes of approximately 10%. Confidence intervals for the estimated BMI critical values were obtained by using bootstrap resampling with 1,000 repetitions. All statistical analyses were done in SAS V.9.4 (SAS Institute, Cary, NC) using the *PARM_ICE* macro to fit the interval-censored Weibull models, and in Stata v.15 (StataCorp, College Station, TX) using the *streg* command to fit the Weibull models, the *margins* command for regression standardization, and the *bootstrap* command to obtain confidence intervals.

RESULTS

Among the 5,659 study participants free of diabetes at baseline, 42% were White participants, 26% African American, 20% Hispanic, and 12% were Chinese American (**Table 1.1**). Mean age was 62 years. White participants had higher family incomes and education levels, particularly compared to Hispanics. Chinese Americans had a lower mean BMI compared to the other groups, while African Americans and Hispanics had a higher mean BMI than White participants. Racial/ethnic minority groups were more likely to have at least 1 diabetes risk factor compared to White participants. In particular, African Americans were more likely to have hypertension, less likely to have hypertriglyceridemia, and Hispanics and Chinese Americans were more likely to be physically inactive, compared to White participants.

Over 42,686 person-years of follow-up, 696 (12%) new cases of type 2 diabetes were observed. Crude type 2 diabetes incidence rate was 1.1 cases (95% CI 1.0 to 1.3), 2.0 cases (1.8 to 2.3), 2.2 cases (1.9 to 2.5) and 1.6 cases (1.3 to 2.0) per 100 person-years

among White participants, African Americans, Hispanics and Chinese Americans, respectively. Compared to White participants, Chinese Americans had more than twice the risk of type 2 diabetes (hazard ratio [HR]=2.6; 95% CI 2.0-3.4), while African Americans had a 30% higher risk (HR: 1.3; 95% CI: 1.1-1.6), and Hispanics had a 60% higher risk (HR: 1.6; 95% CI: 1.3-2.0), upon accounting for age, sex, and the presence of one or more diabetes risk factors. Greater BMI was associated with a higher 10-year type 2 diabetes risk across all four racial/ethnic groups, though a similar probability of type 2 diabetes risk was observed at lower BMI levels for non-White participants compared to White participants (**Figs. 1.1-1.3**).

Diabetes risk in the absence of diabetes risk factors

In a low-risk scenario (assuming no one had additional risk factors), in age and sex-adjusted models, among White participants a 10% risk of type 2 diabetes over 10 years was observed in the obese category at a BMI of 30.5 kg/m^2 (95% CI: 28.9-33.0). By comparison, this same level of risk was observed in the overweight category at BMI's of 24.3 kg/m^2 (23.2 to 25.7) in Chinese Americans, 26.8 kg/m^2 (25.8 to 28.3) in Hispanics, and 27.9 kg/m^2 (26.5 to 29.9) in African Americans (**Fig. 1.1**).

Diabetes risk in the presence of one or more diabetes risk factors

In a clinical screening scenario, assuming that everyone had at least one risk factor, in age and sex-adjusted models, a 10% risk of developing diabetes over 10 years occurred at a BMI of 21.7 kg/m^2 (95% CI: 20.1-22.8) in Chinese Americans, 23.8 kg/m^2 (22.7-24.9)

in Hispanics, 24.7 kg/m^2 (23.7-25.6) in African Americans, and 26.2 kg/m^2 (25.1-26.9) in White participants (**Fig. 1.2**).

Diabetes risk not considering other diabetes risk factors

To represent a public health screening scenario in which other diabetes risk factors are not considered, analogous estimates were made adjusting for age and sex but not accounting for any other diabetes risk factors. This resulted in estimated BMI levels associated with a 10% risk of developing type 2 diabetes over 10 years of 22.5 kg/m^2 (95% CI: 21.3 to 23.3) in Chinese Americans, 24.7 kg/m^2 (23.9-25.6) in Hispanics, 25.6 kg/m^2 (24.8-26.6) in African Americans and 27.3 kg/m^2 (26.5-28.3) in White participants (**Fig. 1.3**).

Sensitivity Analysis

In a sensitivity analysis, we included information about family history of type 2 diabetes as a risk factor, captured in exam visit 2 and back-dated to the baseline exam visit and our results were essentially unchanged from our primary findings (results not shown).

DISCUSSION

In this large multi-ethnic population-based prospective study of adults ages 45 and older free of type 2 diabetes at baseline, we found that BMI was a practical and useful predictor of type 2 diabetes risk. The BMI levels associated with a 10% risk over 10 years varied according to race/ethnicity and presence or absence of other traditional risk factors. Our primary finding showed that in high-risk individuals, who were a majority, with at least one

risk factor, screening should be considered at a BMI ≥22 kg/m^2 for Chinese Americans, ≥24 kg/m^2 for Hispanics, ≥25 kg/m^2 for African Americans, and ≥26 kg/m^2 for White participants.

This study adds to the body of literature that underscores the value of using different BMI cut-off points for different racial/ethnic groups, and presents new evidence that the cut-off points may also differ between these groups based on the presence of risk factors. Although current ADA guidelines recommend screening everyone ≥45 years, findings from NHANES (2005-2012) showed that only half of adults ≥45 reported having been screened for diabetes [7]. Our study therefore adds evidence to support including BMI and traditional risk factors as action points to prioritize and identify whom among adults ≥45 should be screened for type 2 diabetes. Further, our study confirms previous findings from cross-sectional studies [13,14], which were later used by ADA to modify its recommendations, that lower BMI cut-off points should be used to screen undiagnosed type 2 diabetes among Asian Americans, including Chinese Americans. Lastly, although we selected a 10% risk of developing type 2 diabetes over 10-years as the threshold at which screening should be considered, our findings show robust results across different risk thresholds. Regardless of the 10-year risk selected, we found that across the BMI distribution, Chinese Americans have a similar type 2 diabetes risk at about 5 BMI points lower compared to White participants, and Hispanics and African Americans about 2-2.5 BMI points lower compared to White participants.

Our findings are consistent with cross-sectional and follow-up studies in Canada [15] and the US [16–19]. In a large multi-ethnic cohort in Canada, Chiu et al. found that equivalent incidence rates of type 2 diabetes occurred at about 4 BMI points lower in black adults, 5 BMI points lower in Chinese adults and 6 BMI points lower in South Asian adults, compared to White adults with a BMI of 30 kg/m^2 [15]. In the US, using MESA follow-up data, Lutsey et al. found that similar type 2 diabetes risk occurred at lower waist circumference points among Chinese American, African Americans and Hispanics compared to White participants [17]. In another study using data from the Women's Health Initiative, Luo et al found higher rates of incident type 2 diabetes among Hispanic and Asian women compared to White women in the same BMI categories [18]. In the Nurses' Health Study, in which four percent of nurses belonged to minority groups, Shai et al found the risk of diabetes higher among Asian, Hispanic and African Americans compared to White participants [19]. In the Multi-Ethnic Cohort, the prevalence of self-reported type 2 diabetes at baseline by traditional BMI categories were two to three-fold greater for African Americans, Latinos, Japanese and Hawaiians compared to White participants [16]. And lastly, in a consortium of three integrated healthcare systems in the US, which included nearly 5 million adults, Asians, Hawaiians/Pacific Islanders, Hispanics, African Americans and American Indians/Alaskan Natives had a higher burden of type 2 diabetes and prediabetes at lower BMIs compared to White participants [20].

The relationship between increased adiposity and type 2 diabetes is more strongly linked with the distribution of body fat than overall obesity as measured by BMI. In general, greater amounts of visceral adiposity or hepatic steatosis is associated with higher risk

for insulin resistance, metabolic abnormalities and type 2 diabetes than higher levels of subcutaneous fat [21,22]. The distribution of these fat depots as well as the observed association with cardiometabolic disease vary significantly by racial/ethnic background [23–27], which may explain part of the reason why the observed association between BMI and incident type 2 diabetes is modified by race/ethnicity.

Strengths and Limitations

Our study had the following strengths. First, MESA included data from a follow-up of 11 years among a large multi-ethnic population, which allowed us to compare findings between four large US racial/ethnic groups, overcoming limitations of prior studies that used cross-sectional data [13,14]. Second, BMI was calculated from repeated measures of standing height and weight, improving upon prior studies that used self-report measures [15].

Although our study has notable strengths, there are a few limitations. First, HbA1c was only available at exams 2 and 5 and we were therefore unable to use this diagnostic criterion to identify and exclude prevalent cases at baseline or consistently capture incident cases over time. Our diagnostic method, fasting glucose and/or diabetes treatment, may have led to measurement error, as this test is less sensitive than the gold-standard two-hour glucose tolerance test, particularly for African Americans and Chinese Americans [2,28]. Future studies should augment the diagnostic method by including HbA1c and two-hour glucose tolerance tests. Second, MESA did not examine participants for the presence of acanthosis nigricans, nor collected data on polycystic ovarian

syndrome, which may have resulted in misclassification of exposure. Nevertheless, given the overlap between these conditions and the other risk factors that were included in our analyses, it is likely that misclassification of exposure was minimal, thus we would not expect our primary conclusions to be qualitatively different. Third, MESA excluded participants with known cardiovascular disease, thus our sample represents a healthier subpopulation, nevertheless since this exclusion was consistent across racial/ethnic groups, we would not expect this selection bias to qualitatively shape our inferences. Fourth, because of important between-group differences in the pathophysiological backgrounds of diabetes onset among different Asian ethnic groups [29,30], our inferences are limited to Chinese Americans. Future research should include additional Asian subgroups, including Pacific Islanders and South Asians.

CONCLUSIONS

Given that adults ages 45 and older are currently under-screened for type 2 diabetes, this study adds evidence to support including BMI and presence of traditional type 2 diabetes risk factors as action points for clinicians to prioritize and identify whom among older adults ≥45 should be screened for type 2 diabetes. Future studies should evaluate if the application of race/ethnicity-specific BMI thresholds may help reduce the high prevalence of undiagnosed type 2 diabetes, as well as the disparity observed in minority groups.

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Table 1.1 Baseline characteristics of study population by race/ethnicity group, the Multi-Ethnic Study of Atherosclerosis, 2000-2002

Characteristics	All (N=5,659)	White (*n*=2,383, 42%)	African American (*n*=1,462, 26%)	Hispanic (*n*=1,161, 20%)	Chinese American (*n*=653, 12%)
Sociodemographic					
Age, mean (SD), y	62 (10)	62 (10)	62 (10)	61 (10)	61 (10)
Women, *n* (%)	3,017 (53)	1,250 (52)	818 (57)	611 (53)	338 (52)
Family Income ≥ 75,000/y, *n* (%)	1,349 (24)	871 (37)	260 (18)	98 (8)	120 (18)
Education ≥ Bachelor's degree, *n* (%)	2,137 (38)	1,213 (51)	528 (36)	129 (11)	267 (41)
Diabetes risk factors					
BMI, mean (SD), kg/m^2	28.0 (5.3)	27.5 (4.9)	29.8 (5.8)	29.1 (4.9)	23.9 (3.3)
Family history of diabetes, *n* (%)[1]	1,882 (35)	673 (29)	588 (43)	458 (42)	163 (26)
Hypertension ≥140/90 mmHg, or on therapy, No. (%)	2,677 (47)	1,034 (42)	896 (57)	483 (39)	264 (38)
HDL cholesterol <0.90 mmol/L, No. (%)	494 (8.7)	206 (8.6)	112 (7.7)	132 (11)	44 (6.7)
Triglyceride level >2.82 mmol/L, No. (%)	355 (6.3)	157 (6.6)	24 (1.6)	125 (11)	49 (7.5)
Physical Inactivity, No. (%)[2]	1,323 (23)	414 (17)	360 (25)	370 (32)	179 (27)
One or more diabetes risk factors[3]	3,677 (65)	1,377 (58)	1,082 (74)	793 (68)	425 (65)
One or more diabetes risk factors[4]	3,983 (74)	1,554 (68)	1,124 (82)	872 (79)	433 (70)

[1] Family history of diabetes at Exam visit 2. N=5,382; White participants=2,289, African American=1,367, Latino=1,104, Chinese American=622.

[2] Physical inactivity was defined as engaging in no intentional exercises during a typical week in the past month.

[3] Risk factors include hypertension, low HDL cholesterol, high triglyceride, or physical inactivity.

[4] Risk factors include family history of diabetes, hypertension, low HDL cholesterol, high triglyceride, or physical inactivity. Including family history of diabetes, total n=5,382

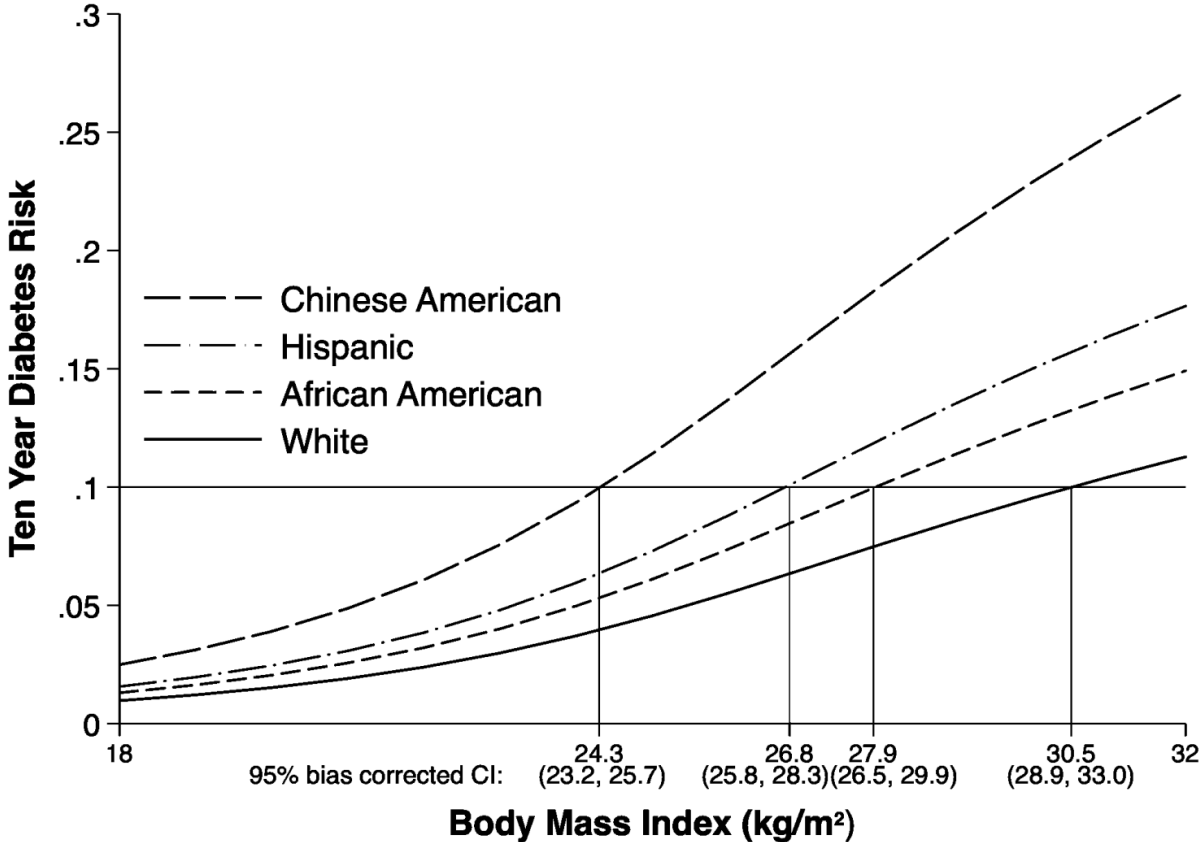

Figure 1.1 Ten-year risk of developing type 2 diabetes as a function of BMI, by race/ethnicity, in the absence of traditional risk factors. The Multi-Ethnic Study of Atherosclerosis 2000-2012

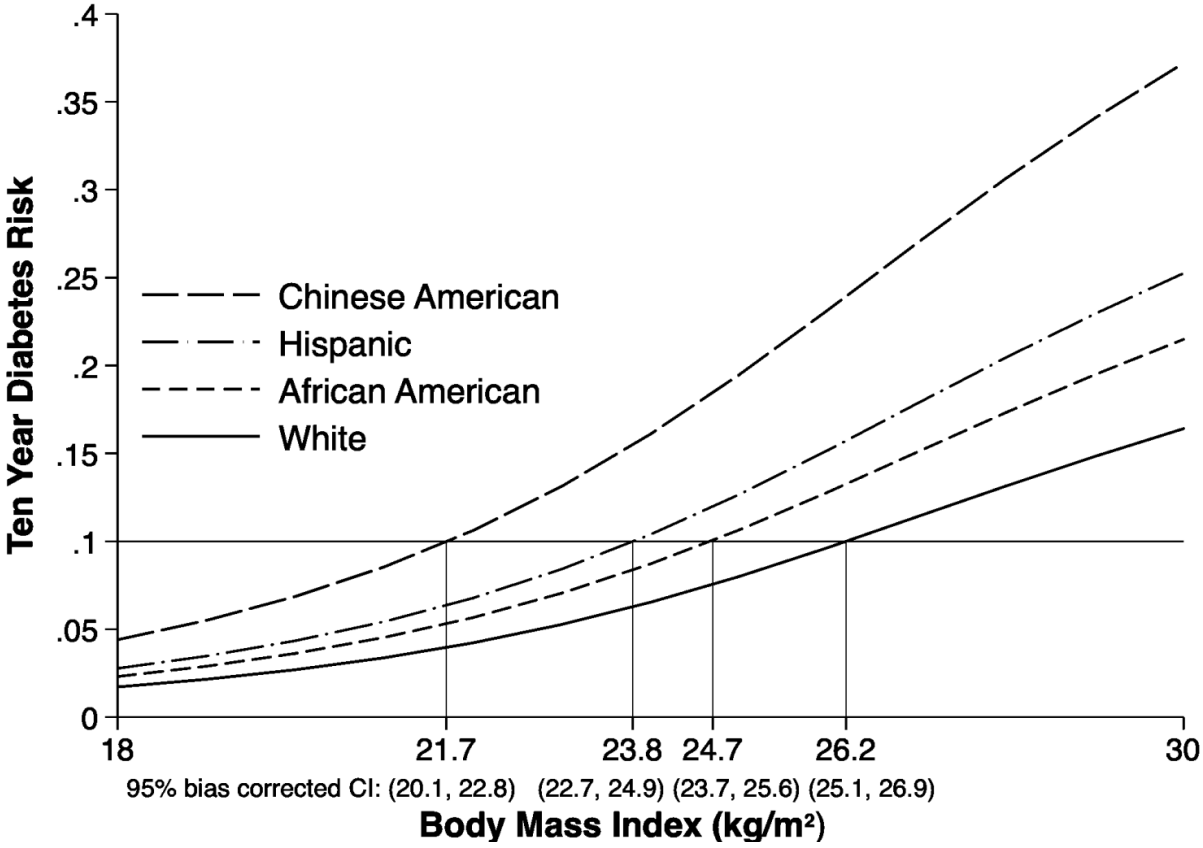

Figure 1.2 Ten-year risk of developing type 2 diabetes as a function of BMI, by race/ethnicity, in the presence of one or more traditional risk factors. The Multi-Ethnic Study of Atherosclerosis 2000-2012

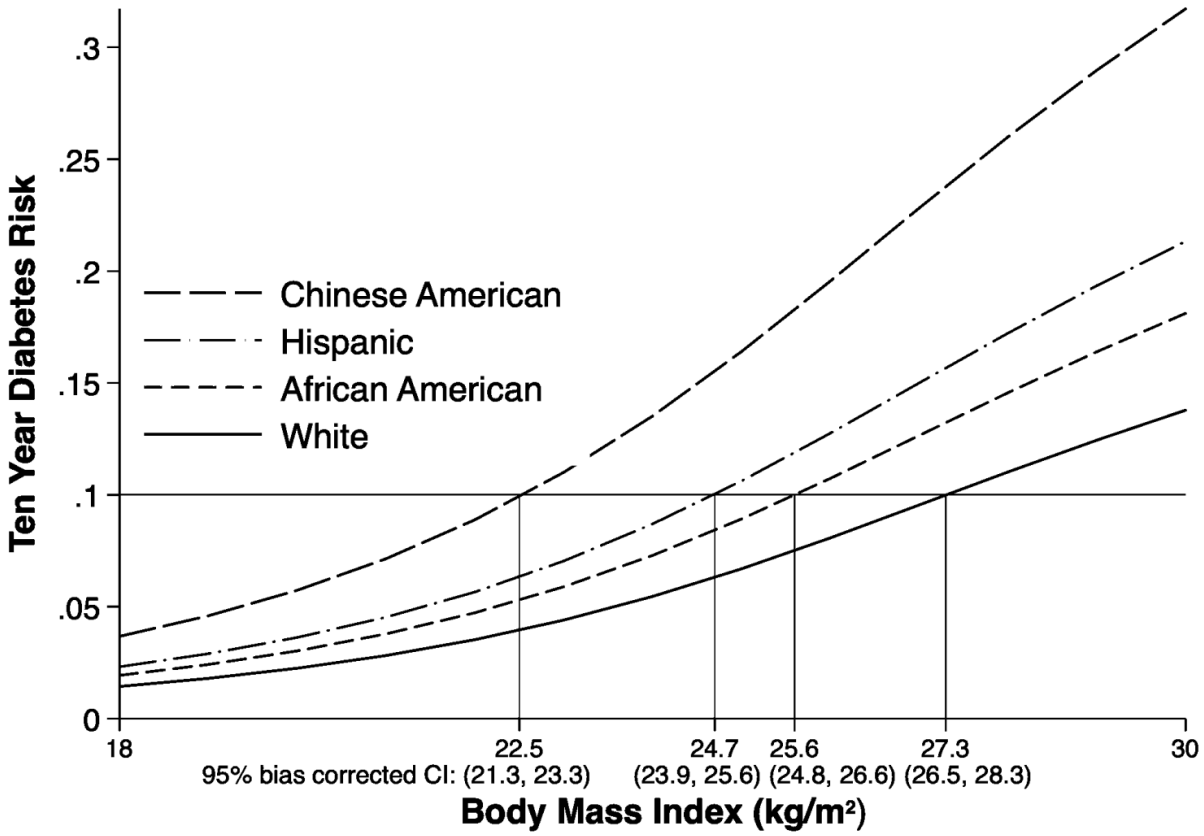

Figure 1.3 Ten-year risk of developing type 2 diabetes as a function of BMI, by race/ethnicity, not considering other traditional diabetes risk factors. The Multi-Ethnic Study of Atherosclerosis 2000-2012

Chapter 2 Abstract

Aims Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD), and NAFLD in turn is hypothesized to mediate part of the well-known effect of obesity on the risk of type 2 diabetes (T2D). We assessed the estimated effect of obesity on T2D risk and evaluated to what extent NAFLD mediates this association.

Methods This prospective analysis included data from 4,522 adults free of T2D at baseline exam from the Multi-Ethnic Study of Atherosclerosis cohort (2000-2011). Obesity at baseline was defined using BMI (<25 kg/m^2 [normal], 25-<30 [overweight], or ≥30 [obese] or <23, 23-<27.5 and ≥27.5 kg/m^2 for Chinese Americans) and waist circumference (elevated if >88 cm among women or >102 cm among men). NAFLD was determined by liver attenuation measured by CT scans at baseline and categorized into quartiles of fat density. Incident T2D cases were defined as fasting glucose ≥126 mg/dL, or use of any diabetes medications. We specified marginal structural proportional hazards models to decompose the total effect of obesity on T2D risk into its natural direct and indirect (through NAFLD) effect components.

Results Over 34,150 person-years of follow-up, unadjusted T2D incidence rates were 5.5 (95% CI 4.2 to 7.2), 14.3 (12.5 to 16.4), and 29.8 (26.6 to 33.3) cases of T2DM per 1,000 person-years among the normal, overweight and obese BMI categories, respectively. After adjusting for age, sex, race/ethnicity, education, diet and exercise, those with BMI-defined obesity were at 4.5 times the risk of T2D compared to those with normal weight (total effect hazard ratio [HR]=4.5 [95% CI=3.0-5.9]). The mediation

analysis suggested that NAFLD accounts for ~36% (95% CI=27-44%) of the association between obesity and T2D risk (direct effect HR_{BMI}=3.2 [2.3-4.6]; indirect effect through NAFLD, HR_{NAFLD}=1.4 [1.3-1.5]). A similar proportion of the total effect was explained by fatty liver when obesity was defined using waist circumference.

Conclusions These data suggest that the effect of obesity on T2D risk is partially explained by the presence of NAFLD. Future studies should evaluate if NAFLD could be an effective target to reduce the effect of obesity on T2D.

Chapter 2 Main Body

INTRODUCTION

Type 2 diabetes (T2D) is a serious chronic disease currently affecting one in seven adults in the United States (US) [1]. If trends continue, it is projected that T2D will affect as many as one in three US adults by 2050 [2]. Obesity is a well-known risk factor for T2D [3–8] and in the past three decades, the obesity epidemic has contributed significantly to the drastic increase in T2D in the US [1,9,10]. Despite the clear relationship between obesity and T2D, the precise mechanisms that connect these conditions remain unclear. It is hypothesized that at least three separate mechanisms link obesity and insulin resistance and predispose to T2D [5]: 1) increased production of adipokines/cytokines (e.g. tumor necrosis factor-alpha), promoting insulin resistance; 2) mitochondrial dysfunction, resulting in insulin resistance and B-cell dysfunction; and 3) increased ectopic fat deposition, leading to dysmetabolic sequelae. The third mechanism is of particular interest given the recent rise in non-alcoholic fatty liver disease (NAFLD) [11]. It is hypothesized that the NAFLD-specific role, on the obesity-T2D link could be due to an exacerbation of hepatic insulin resistance and alteration in the secretion of hepatokines and inflammatory biomarkers, that may promote the development of T2D [11–14].

Prior studies have found obesity to also be an established risk factor for NAFLD [11,15,16], including data from several longitudinal studies [17–23]. NAFLD in turn has been shown in multiple observational studies [24–37], and two Mendelian randomization studies [38,39], to be associated with an increased risk of T2D. All of these observational studies [24–37] have adjusted for obesity (body mass index [BMI]; weight in kg/height in

m^2) or waist circumference in an attempt to estimate independent effects of NAFLD on T2D risk, however none have used principled analytical techniques to quantify how much of the obesity-related risk for T2D is mediated through NAFLD. Understanding the possible mediating role that fatty liver, particularly (NAFLD), has on the obesity-T2D relationship may be of great interest as NAFLD prevention or management could be a promising target to reduce the obesity-related burden of T2D [40].

We hypothesize that the effect of obesity on T2D risk is explained at least in part by the degree of fat in the liver. To test this hypothesis, we used longitudinally collected data from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort to estimate the overall association between obesity on risk of T2D, and decompose this into the portion of the relationship mediated, and not mediated, by the degree of liver fat accumulation (i.e. indirect and direct effects, respectively).

METHODS

Participant Population

Our observational data comes from the well-characterized Multi-Ethnic Study of Atherosclerosis (MESA) cohort of subclinical cardiovascular disease (CVD) and risk factors of CVD progression. MESA objectives and design have been described in detail elsewhere [41]. Briefly, 6,814 participants aged 45-85 years and free of known CVD were recruited in the year 2000 from six communities in the United States and followed until present time. Participants were seen at Columbia University in New York, Johns Hopkins University in Baltimore, Northwestern University in Chicago, UCLA in Los Angeles,

University of Minnesota at Twin Cities and Wake Forest University in Winston Salem. The cohort includes those of White, African American, Hispanic, and Chinese American descent. For this study we used data from exam visits 1 through 5, conducted every 2-3 years between July 2000 and December 2011.

Exclusions

MESA excluded participants with known cardiovascular disease, active cancer treatment, pregnancy, weight > 300 pounds, cognitive inability, and anyone living in a nursing home or on a waiting list for a nursing home [41]. For this study, we further excluded participants with prevalent diabetes at baseline (n=859), participants whose computed tomography (CT) imaging did not extend inferiorly sufficiently to measure attenuation of the liver (n=75), participants with a reported history of moderately heavy alcohol use (average of >1 serving/day in women and >2 servings/day in men; n=322), history of liver cirrhosis (n=6) and use of oral steroids and class 3 antiarrhythmic medications due to their association with macrovesicular steatosis (n=84),[38] those who failed to return to at least 1 follow-up visit (n=294) or those with missing covariates of interest (n=652). Our final sample size was 4,522 who had complete data at baseline and at least one follow-up visit.

Ethics Approval

Informed consent was obtained from all study participants and institutional review board (IRB) approvals were obtained at all MESA sites. Ethical approval for the use of

anonymized data was obtained from the University of California San Francisco Committee on Human Research on 2 January 2018 (16-21085).

Exposure: Obesity

The exposure (obesity) was defined using the BMI as a proxy for generalized obesity and, in separate models, using waist circumference as a proxy for abdominal obesity. Weight and height measures were taken using standardized procedures.[51] BMI was calculated by dividing weight (in kg) by squared height (in m) and categorized according to established criteria [4,44]: normal (<25 for most race/ethnic groups and <23 kg/m^2 for Chinese Americans), overweight (25-<30 or 23-<27.5 kg/m^2 for Chinese Americans), or obese (≥30, or ≥27.5 kg/m^2 for Chinese Americans). Waist circumference (cm) was measured horizontally across the umbilicus and categorized as a binary variable using sex-specific cut-points >88 cm for women and >102 cm for men, according to the Third Report of the National Cholesterol Education Program [45]. In sensitivity analysis, we re-classified the waist circumference category using sex- and race/ethnicity-specific cut-points >80 cm for women and >94 cm for White and African American men and >90 cm for Hispanic and Chinese American men, according to the International Diabetes Federation metabolic syndrome classification [46].

Mediator: Liver fat

We considered liver fat as a potential mediator of the effect of obesity on T2D. At the baseline visit, participants received two consecutive CT scans. The scan window included the tracheal carina to below the apex of the heart, which in most subjects, included liver

images [42,47]. Liver attenuation by CT scan has been shown to be inversely correlated with liver fat deposition by liver biopsy (correlation coefficient: -0.9; p-value <0.001) [48], likewise in another study, unenhanced CT scans showed a R^2 value of 0.649 against histologic fat content in linear regressions [49], showing that CT scanning provides a useful non-invasive method for identifying fatty liver. Degree of liver attenuation was measured in three consistent regions in the parenchyma of the right hepatic lobe (each measuring about 1 cm^2) and calculated as the average density.[50] Liver fat was categorized into quartiles of Hounsfield units (HU), and inverted so that the highest quartile represented the lowest liver fat content as the referent group.

Outcome: Type 2 diabetes

Individuals were considered as having T2D if they had a fasting glucose ≥126 mg/dL and/or reported using any diabetes medications at any point during follow-up. The outcome, time to T2D, was specified as time of first observation of T2D at any time during follow-up. This time was treated as censored for those who did not develop the event by the end of follow-up.

Confounders

Informed by our directed acyclic graph [51], we assumed that the same set of covariates potentially confound the relationship between obesity and T2D, obesity and fatty liver, and fatty liver and T2D as shown in **Figure 2.1**. Measured confounders included baseline age, sex, race/ethnicity (self-reported White, African American, Hispanic or Chinese American), education (less than high school, completed high school, some college, or bachelor's degree or higher), exercise (categorized into quartiles), dietary quality

(categorized into quintiles) and total caloric intake (categorized into quintiles). Information on exercise was calculated from the duration and intensity of total intentional exercises using metabolic equivalent minutes (MET-min) per week and was measured using a detailed, semi-quantitative questionnaire adapted from the Cross-cultural Activity Participation Study [41,50]. Activities included moderate walking, dancing or individual activities, and vigorous conditioning and team/dual sports. Usual diet intake over the previous 12 months was quantified using a food frequency questionnaire (FFQ) [41,52] from which dietary quality was calculated using the Alternative Healthy Eating Index-2010 (AHEI) Score, based on the evidence for its strong link with cardiovascular disease and T2D [53]. Daily energy intake (kcal/day) was also estimated from the FFQ's.

Statistical analysis

Our study had two objectives. The first was to estimate the overall (total) effect of obesity on incident T2D risk. The second objective was to understand how much of the effect of obesity on T2D was potentially mediated by fatty liver. Our focus was etiological with the goal of addressing questions that concern the manner in which obesity mechanistically increases the risk of T2D. To accomplish this objective, we conducted a causal mediation analyses to decompose the overall effect into two separate effects: (1) the direct effect (i.e. the effect of obesity on T2D that is not mediated by fatty liver) and the indirect effect (i.e. the effect of obesity on T2D that is mediated by fatty liver) [54].

We estimated these effects with inverse probability weighted marginal structural models according to the method of Lange *et al*. [55]. The steps were as follows: first, we used

multinomial logistic regression to model the categorical mediator (liver fat) as a function of obesity and assumed confounders (age, sex, race/ethnicity, education, exercise and diet). (Results of models for the mediators are presented in **Appendix Tables 2.2-2.4**). This model was then used to obtain predicted counterfactual mediator values for each level of the exposure in each individual, so that for any individual, three mediator values were predicted when BMI was the exposure: the potential mediator value *had* the individual been normal weight, the potential mediator value *had* the individual been overweight, and the potential mediator value *had* the individual been obese. This was operationalized by constructing an extended data set by repeating each observation three times and including an auxiliary exposure variable for each counterfactual level of exposure (normal, overweight, and obese BMI categories). The original exposure variable and the auxiliary exposure variable were then weighted by dividing the probabilities corresponding to the counterfactual value observed for the mediator by using the auxiliary exposure, by the probabilities corresponding to the value actually observed for the mediator. The stability of the calculated weights was evaluated by inspection of a histogram of the final weights and verifying no extreme values [56] (near zero or excessively large) (see **Appendix Figures 1-3**). A marginal structural model for the relationship between obesity and T2D outcome was then estimated by fitting a parametric proportional hazards model with a Weibull distribution with robust SEs, and incorporating weights estimated in the first stage of modeling. Instead of estimating a separate model for the exposure (obesity) conditional on confounders, we included the same set of covariates from the liver fat model, which results in weights that are typically much more stable as these do not involve inverse probability weighting of the exposure distribution

[55]. In our data, T2D event times were not observed exactly, but were only known to occur within the interval of time between the two surrounding exam visits. Correspondingly, we used estimation methods that accounted for the interval-censored event times [57–59]. Ninety five percent confidence intervals (CIs) for the total, indirect, and direct effects were estimated using 1,000 bootstrapped samples. The proportion mediated by the mediator was calculated as the ratio of the natural indirect effect to the total effect. Separate models were repeated using waist circumference as a proxy for central obesity following the same steps as described above.

We also assessed whether indirect and direct effects differed between racial/ethnic groups using covariate-by-exposure interactions as described by Lange *et al.*,[59] and tested their significance using a Wald test. None of the interactions were statistically significant ($p>0.05$) (see **Appendix Table 2.1**) thus we removed interactions from final models. Baseline characteristics between the three BMI categories were presented as proportion for categorical variables, and mean (standard deviation) or median [interquartile range] for continuous variables depending on their distribution. All statistical analyses were done in Stata v.15 (StataCorp, College Station, TX).

RESULTS

Descriptive characteristics by BMI category among 4,522 adults in MESA are presented in **Table 2.1**. Over a median 9.1 years of follow-up, 557 new cases of T2D occurred (12%). Incidence rates were 5.5 (95% CI: 4.2, 7.2), 14.3 (12.5, 16.4), and 29.8 (26.6, 33.3) per 1,000 person-years among those in normal, overweight, and obese BMI

categories, respectively. There was a clear association between liver fat quartile and BMI category; among those in the normal BMI category, fewer participants were in the highest quartile of liver fat, conversely, among those in the obese category, more participants were in the highest quartile of liver fat. The mean age (SD) of the population was 62 ± 10 years, and roughly half were female. Adults in the normal BMI category were more likely to be Chinese American or White, whereas adults in the obese BMI category were more likely to be African American or Hispanic. There was also an educational gradient with a greater proportion of those with at least a bachelor's degree having a normal BMI, compared to those with lower educational attainment. Lastly, those in the obese BMI category had lower dietary quality, consumed more calories, and exercised less than their counterparts in the normal or overweight BMI categories.

Estimates of the total, natural direct and natural indirect effects of obesity on T2D risk are shown in **Tables 2.2 and 2.3**. After covariate adjustment, those with BMI-defined obesity were at 4.5 times the risk of T2D compared to those with normal weight (total effect hazard ratio [HR]=4.5 [95% CI=3.0-5.9]). The mediation analysis suggested that NAFLD was responsible for ~36% (95% CI=27-44%) of the relationship between obesity and T2D risk (indirect effect of BMI through NAFLD, HR_{NAFLD}=1.4 [1.3-1.5]; direct effect, $HR_{BMI\text{-}obese}$=3.2 [2.3-4.6])). Those with overweight had more than twice the risk of T2D compared to those with normal weight (HR=2.2 [1.5-3.0]), with NAFLD responsible for ~27% (18-41) of this relationship (indirect effect, HR_{NAFLD}=1.2 [1.1-1.3]); direct effect, $HR_{BMI\text{-}overweight}$=1.9 [1.3-2.7]). Similarly, when using waist circumference as a proxy for central obesity, those with elevated waist circumference were at 2.7 times the risk of T2D

compared to those with normal waist circumference (total effect HR=2.7 [2.2-3.2]), with NAFLD responsible for ~32% (24-40) of this relationship (indirect effects (HR_{NAFLD}=1.2 [1.1-1.3]); direct effect, $HR_{WC\text{-}high}$=2.2 [1.8-2.6]).

Sensitivity analysis

In a sensitivity analysis, when using race/ethnic- and sex-specific categories for waist circumference according to the International Diabetes Federation metabolic syndrome classification [46], the estimated total, and natural direct and indirect effects of central obesity on T2D risk were marginally higher compared to when using high waist circumference according to the Third Report of the National Cholesterol Education Program, but the proportion explained by fatty liver was similar (~27% [95%CI 21-33] of the total effect) (**Table 2.4**).

DISCUSSION

In this multi-ethnic population-based cohort study of 4,522 adults in the US, our mediation analysis suggests that obesity increases the risk of incident T2D through NAFLD. To our knowledge, this is the first analysis to decompose the complex links between obesity, NAFLD and incident T2D.

There is much prior knowledge demonstrating the prospective associations between each one of these links (obesity-T2D, obesity-NAFLD, and NAFLD-T2D). First, consistent with multiple prior studies that have evaluated the obesity-T2D link, we found strong evidence of a total effect of obesity on incident T2D [3–8]. Second, longitudinal studies of Israeli,

Korean, Chinese, and Italian populations have shown the importance of generalized or central obesity, or weight gain in the development of NAFLD [17–23]. Furthermore, two of these studies demonstrated an increased rate of NAFLD remission among those who had weight loss during the observation periods [17,22]. And third, the presence of NAFLD has been shown in multiple observational studies to be associated with an increased risk of incident T2D across different racial/ethnic groups [24–37]. Likewise, a Mendelian randomization study found that liver fat was causally associated with insulin resistance, a precursor of T2D, as well as with a small but significant increase in T2D risk [38]. However, in this last study, these associations were observed only among individuals with liver disease (e.g. fibrosis) [38]. In our analysis, due to lack of histology, we were unable to distinguish between simple steatosis from more advanced liver disease (e.g. fibrosis). Future studies should distinguish between these conditions and assess their mediating role separately.

Accumulating evidence implicates free fatty acids (FFAs) as the primary culprit of liver injury [60]. Accumulation of fat in the liver can be caused by obesity-related factors including an influx of FFAs into the liver, and an imbalance of adipokines (increased proinflammatory cytokines or decreased adiponectin) as well as increased *de novo* lipogenesis from excessive carbohydrates, certain amino acids, and dietary fat [60,61]. It is hypothesized that NAFLD-specific role, (especially non-alcoholic steatohepatitis [NASH] with varying levels of fibrosis) on the obesity-T2D link could be due to an exacerbation of hepatic insulin resistance and alteration in the secretion of hepatokines, such as retinol-binding protein (RBP)-4, fetuin-A, fibroblast growth factor (FGF)-21, or of

inflammatory biomarkers such as C-reactive protein, tumor necrosis factor (TNF)-alpha and interleukin-6 (IL)-6 [11–14]. These hepatokines and inflammatory cytokines negatively affect hepatic gluconeogenesis, glycogen synthesis and insulin signaling, which in turn directly affect the risk of incident T2D [11].

Our results should be interpreted in light of several assumptions required in the analytic approach. First, as with all analyses with an etiological objective, mediation analyses assume all relevant confounders of the obesity-T2D, obesity-NAFLD, and NAFLD-T2D relationships were identified and accounted for. Second, valid estimation of natural effects also assumes that there are no mediator-outcome confounders that are caused by exposure. We believe these assumptions are unlikely to have been significantly violated, as we used principled methods (e.g. causal diagrams) to identify relevant confounders of these relationships, however there may be others that we did not consider or were unmeasured, including genetics, as well as individual, interpersonal and neighborhood-level social determinants of health. Third, interpretation of these associations as etiological relationships requires the assumption of consistency, or well-defined exposures. Although BMI has been criticized in this regard [62], in populations with elevated BMIs, studies that target weight reduction have consistently found similar health benefits, including reduced T2D risk, or improved T2D management, regardless of the intervention [5–7]. Fourth, positivity, or the positive probability of the mediator observed at all levels of exposure and confounders, is required. Positivity was evaluated empirically and we found overall good overlap of all included confounders by BMI category. Fifth, temporality is necessary to establish causality [63] and as both exposure and mediator

were measured at baseline, it is possible that the mediator could have preceded the exposure. However we believe this is highly unlikely as prior studies have shown that in most adults, liver fat arises due to central obesity and insulin resistance [11,64], and many epidemiological studies have consistently found obesity to precede NAFLD [17–23]. Future studies can improve on this possible limitation by modeling this association longitudinally. Lastly, marginal structural models via inverse probability weighting requires correct specification of models for the estimation of these weights (see **Appendix Figs. 2.1-2.3** showing that no observation was given unreasonably large weights).

Our study also has a few additional limitations. First, to be enrolled in MESA, participants must be at least 45 years old and free of CVD, and to be included in these analyses we excluded participants with T2D at baseline. Because persons with high BMIs tend to develop CVD and T2D at higher rates and at younger ages, and because we restricted our analyses to those free of these conditions, we may have underestimated the total estimated effect of obesity on T2D risk. Second, MESA did not include oral glucose tolerance tests, considered gold standard for diagnosing diabetes, or repeat measures of glycated hemoglobin (HbA1c), so we may have had some outcome misclassification. And lastly, we only evaluated the mediating role of NAFLD, future studies can expand on this initial work and evaluate other mechanisms that mediate the obesity-T2D relationship (e.g. visceral and intramuscular fat). Third, because presently there are no known effective NAFLD treatments that do not also include obesity reduction, we did not estimate controlled effects, which are useful in prescriptive settings [54], for instance in estimating the effects of an exposure on an outcome, holding a mediator value at a particular level

(e.g. no liver fat). And lastly, understanding the mechanisms linking obesity and T2D, and the role that targeting NAFLD may play in T2D prevention has been an area of active but inconclusive research [38,39,65,66], and interventional studies may be necessary to more conclusively understand these links.

In conclusion, these data suggest that the effect of obesity on T2D risk is partially explained by the presence of NAFLD. Understanding the relative impact of the NAFLD pathway provides valuable knowledge that can be incorporated into strategies to reduce the negative effect of obesity on T2D at the population level. Consistent with prior studies [40], these results support that more evidence is needed to evaluate if NAFLD could be an effective target to reduce the effect of obesity on T2D.

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Table 2.1 Baseline characteristics overall, and by BMI category, among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011)

	BMI Category[a]			
Characteristic	**Total (*n* = 4,522; 100%)**	**Normal (*n* = 1,221; 27%)**	**Overweight (*n* = 1,913; 42%)**	**Obese (*n* = 1,388; 31%)**
Incident diabetes cases, n (%)	557 (12)	54 (4.4)	207 (11)	296 (21)
Diabetes incidence rate per 1,000 person-years (95% CI)	16.3 (15, 17.7)	5.5 (4.2, 7.2)	14.3 (12.5, 16.4)	29.8 (26.6, 33.3)
Liver fat HU units, n (%)				
Quartile 1 (70 to 110) (lowest fat)	1,138 (25)	395 (32)	491 (26)	252 (18)
Quartile 2 (64 to <70)	1,136 (25)	392 (32)	484 (25)	260 (19)
Quartile 3 (57 to <64)	1,144 (25)	323 (26)	487 (25)	334 (24)
Quartile 4 (-27 to <57) (highest fat)	1,104 (25)	111 (9.1)	451 (24)	542 (39)
Age, mean ± SD	62 ± 10	62 ± 11	62 ± 10	61 ± 10
Sex, n (% female)	2,417 (54)	692 (57)	898 (47)	827 (60)
Race/Ethnicity, n (%)				
White	1,853 (41)	612 (50)	753 (39)	488 (35)
African American	1,159 (26)	219 (18)	451 (24)	489 (35)
Hispanic	973 (22)	178 (15)	456 (24)	339 (24)
Chinese American	537 (12)	212 (17)	253 (13)	72 (5.2)
Education, n (%)				
Less than high school	734 (16)	168 (14)	340 (18)	226 (16)
Completed high school	797 (18)	203 (17)	335 (18)	259 (19)
Some college	1,277 (28)	331 (27)	509 (27)	437 (31)
≥Bachelor's degree	1,714 (38)	519 (43)	729 (38)	466 (34)
Diet Quality, AHEI, mean ± SD	55 ± 10	57 ± 10	55 ± 10	52 ± 10
Kcal/day, median [interquartile range]	1522 [1116-2067]	1427 [1044-1926]	1518 [1116-2029]	1627 [1175-2201]
Intentional Exercise, MET-min/week, median [interquartile range]	840 [165-2100]	1043 [315-2363]	975 [210-2130]	630 [0-1713]

Abbreviations: BMI, body mass index; HU, Hounsfield units

[a]BMI categories: Chinese American normal <23, overweight 23-27.4, obese ≥27.5; other: normal <25, overweight 25-29.9, obese ≥30

Table 2.2 Direct and Indirect Effects of generalized obesity (BMI category[a]) on incident type 2 diabetes with liver fat attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis

Exposure		HR (95% CI)
Overweight vs. normal BMI[a]	Direct Effect	1.9 (1.4, 2.7)
	Indirect Effect	1.2 (1.1, 1.3)
	Total Effect	2.2 (1.5, 3.0)
Obese vs. normal BMI[a]	Direct Effect	3.2 (2.3, 4.6)
	Indirect Effect	1.4 (1.3, 1.5)
	Total Effect	4.5 (3.0, 5.9)

Abbreviations: HR, hazard ratio; BMI, body mass index
[a]BMI categories: Chinese American normal <23, overweight 23-27.4, obese ≥27.5; other: normal <25, overweight 25-29.9, obese ≥30

Table 2.3 Direct and Indirect Effects of central obesity (elevated waist circumference[a]) on incident type 2 diabetes with liver fat attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis

Exposure		HR (95% CI)
Elevated vs. normal waist circumference[a]	Direct Effect	2.2 (1.8, 2.6)
	Indirect Effect	1.2 (1.1, 1.3)
	Total Effect	2.7 (2.2, 3.2)

Abbreviations: HR, hazard ratio
[a]Elevated waist circumference according to the Third Report of the National Cholesterol Education Program[7]: >102cm for men and >88cm for women

Table 2.4 Direct and Indirect Effects of central obesity (elevated waist circumference[a]) on incident type 2 diabetes with liver fat attenuation as a Mediator, Multi-Ethnic Study of Atherosclerosis

Exposure		HR (95% CI)
Elevated vs. normal waist circumference[a]	Direct Effect	2.6 (1.9, 3.5)
	Indirect Effect	1.2 (1.1, 1.3)
	Total Effect	3.1 (2.2, 4.0)

Abbreviations: HR, hazard ratio
[a]Elevated waist circumference according to the International Diabetes Federation metabolic syndrome classification[8]: >94cm for White and African American men, >90cm for Chinese American and Hispanic men and >80cm for all women

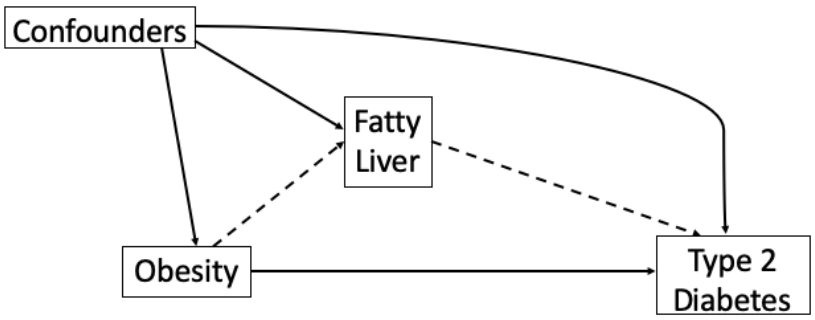


Figure 2.1 Hypothesized causal diagram of the effects of obesity on type 2 diabetes where “confounders” denote the same set of socio-demographic and lifestyle confounders (i.e. age, sex, race/ethnicity, education, diet and exercise) of the associations between obesity and fatty liver, obesity and type 2 diabetes, and fatty liver and type 2 diabetes. The solid arrow between obesity and type 2 diabetes represents the direct effect and dashed arrows represent the indirect effect via fatty liver.

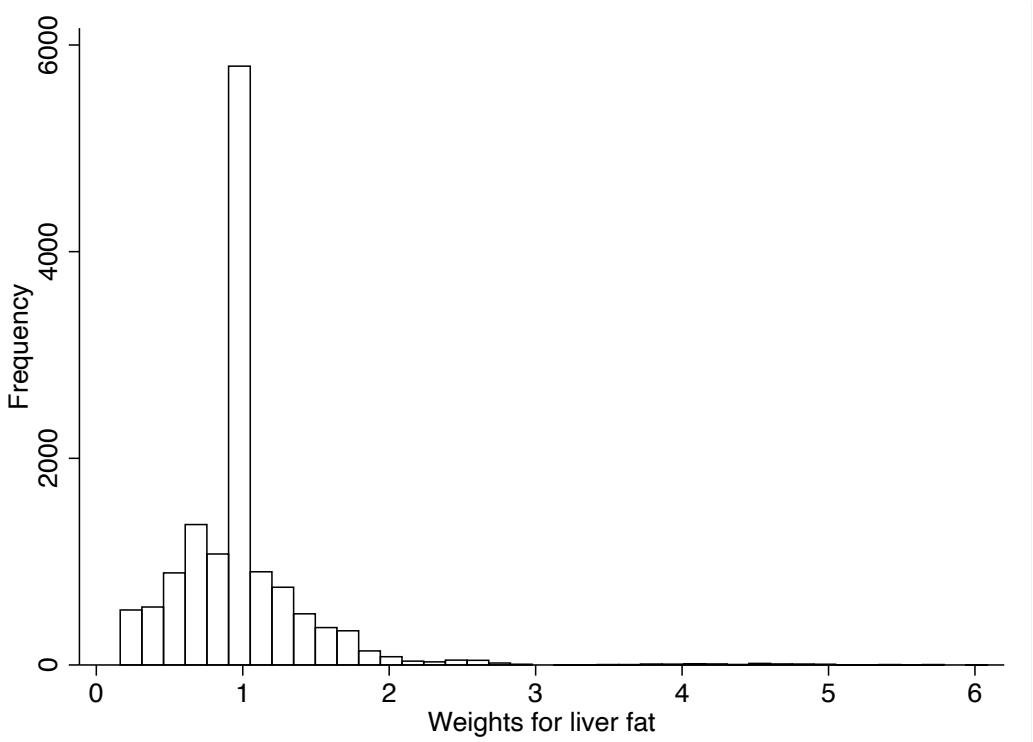
Appendix Material

Appendix Table 2.1 Direct and Indirect Effects of BMI on Incident Type 2 Diabetes with Liver Fat Attenuation as a Mediator with exposure-race/ethnicity interaction, Multi-Ethnic Study of Atherosclerosis, by race/ethnicity

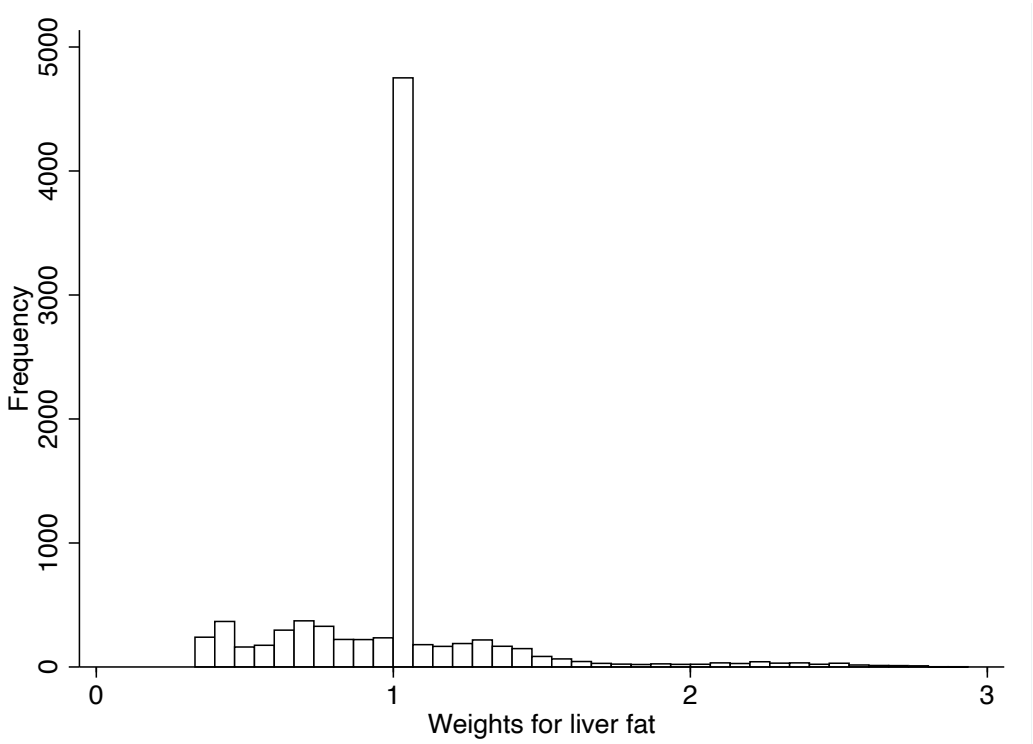
Exposure		White HR (95% CI)	African American HR (95% CI)	Hispanic HR (95% CI)	Chinese American HR (95% CI)
Overweight vs. normal BMI[a]	Direct Effect	2.9 (1.6, 5.3)	1.8 (0.9, 3.7)	1.4 (0.7, 2.6)	1.6 (0.8, 3.4)
	Indirect Effect	1.2 (1.1, 1.3)	1.1 (1.0, 1.2)	1.2 (1.1, 1.3)	1.2 (1.0, 1.4)
	Total Effect	3.5 (1.4, 5.5)	2.0 (0.7, 3.4)	1.6 (0.5, 2.7)	1.9 (0.6, 3.2)
Obese vs. normal BMI[a]	Direct Effect	4.0 (2.2, 7.2)	3.4 (1.7, 6.5)	2.5 (1.3, 4.9)	3.5 (1.5, 8.2)
	Indirect Effect	1.5 (1.3, 1.7)	1.2 (1.1, 1.3)	1.5 (1.3, 1.7)	1.3 (1.0, 1.8)
	Total Effect	5.8 (2.4, 9.2)	4.1 (1.5, 6.7)	3.6 (1.3, 6.0)	4.6 (1.3, 7.9)

Abbreviations: HR, hazard ratio; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

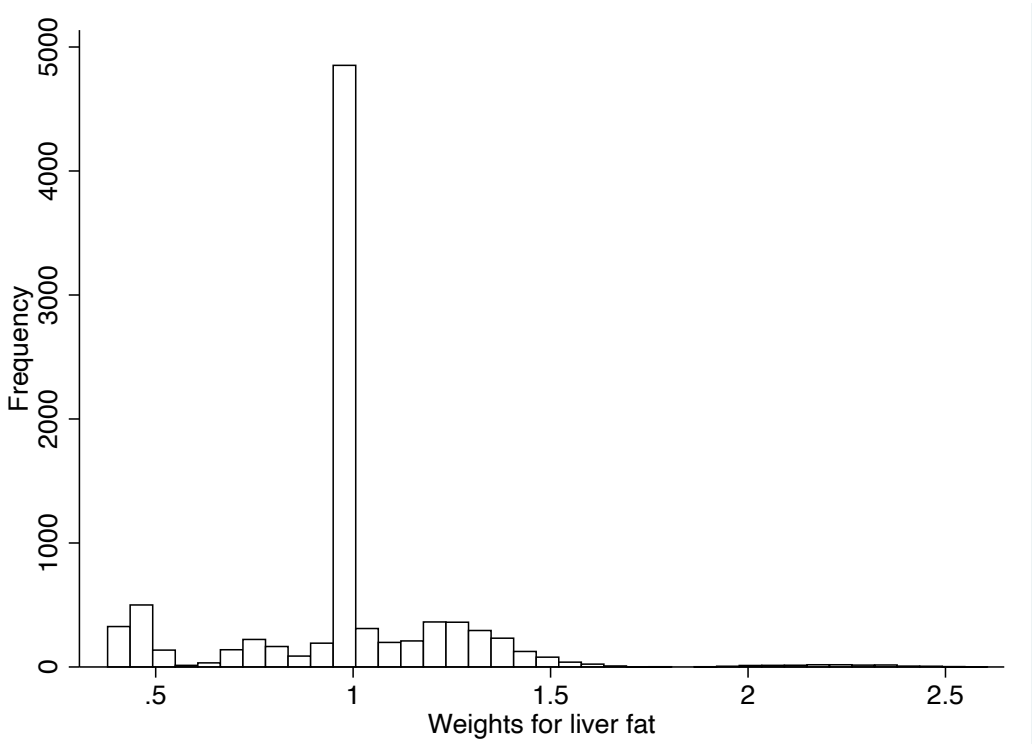
[a]BMI categories: Chinese American normal <23, overweight 23-27.4, obese ≥27.5; other: normal <25, overweight 25-29.9, obese ≥30



Appendix Figure 2.1 Histogram of weights for liver fat, using BMI as obesity measure, among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011). BMI categories: Chinese American normal <23, overweight 23-27.4, obese ≥27.5; other: normal <25, overweight 25-29.9, obese ≥30



Appendix Figure 2.2 Histogram of weights for liver fat, using waist circumference as obesity measure, among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011). Elevated waist circumference according to the Third Report of the National Cholesterol Education Program[7]: >102cm for men and >88cm for women



Appendix Figure 2.3 Histogram of weights for liver fat, using waist circumference as obesity measure, among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011). Elevated waist circumference according to the International Diabetes Federation metabolic syndrome classification[8]: >94cm for White and African American men, >90cm for Chinese American and Hispanic men and >80cm for all women.

Appendix Table 2.2 Fitted multinomial logistic regression model for the mediator (liver fat attenuation quartile), conditioning on exposure (obesity as measured by BMI categories), age, sex, race/ethnicity, education, diet and exercise among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011)

	Liver Fat Attenuation (HU units)							
	Quartile 1 (70 to 110) (lowest fat) reference		**Quartile 2 (64 to <70)**		**Quartile 3 (57 to <64)**		**Quartile 4 (-27 to <57) (highest fat)**	
Exposure	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Overweight vs. normal BMI[a]	1	--	1.1 (0.9, 1.3)	0.36	1.3 (1.0,1.6)	0.02	3.4 (2.7, 4.4)	<0.01
Obese vs. normal BMI[a]	1	--	1.2 (0.9, 1.5)	0.19	1.9 (1.5, 2.3)	<0.01	9.0 (6.8, 11.8)	<0.01

Abbreviations: HU, Hounsfield units; OR, odds ratio; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

[a]BMI categories: Chinese American normal <23, overweight 23-27.4, obese ≥27.5; other: normal <25, overweight 25-29.9, obese ≥30

Appendix Table 2.3 Fitted multinomial logistic regression model for the mediator (liver fat attenuation quartile), conditioning on exposure (obesity as measured by elevated waist circumference[a]), age, sex, race/ethnicity, education, diet and exercise among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011)

	Liver Fat Attenuation (HU units)							
	Quartile 1 (70 to 110) (lowest fat) reference		**Quartile 2 (64 to <70)**		**Quartile 3 (57 to <64)**		**Quartile 4 (-27 to <57) (highest fat)**	
Exposure	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Elevated vs. normal waist circumference[a]	1	--	1.1 (0.9, 1.4)	0.18	1.4 (1.2, 1.7)	<0.01	3.7 (3.0, 4.5)	<0.01

Abbreviations: OR, odds ratio; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

[a]Elevated waist circumference according to the Third Report of the National Cholesterol Education Program[7]: >102cm for men and >88cm for women

Appendix Table 2.4 Fitted multinomial logistic regression model for the mediator (liver fat attenuation quartile), conditioning on exposure (obesity as measured by elevated waist circumference[a]), age, sex, race/ethnicity, education, diet and exercise among 4,522 men and women in the Multi-Ethnic Study of Atherosclerosis (2000-2011)

	Liver Fat Attenuation (HU units)							
	Quartile 1 (70 to 110) (lowest fat) reference		**Quartile 2 (64 to <70)**		**Quartile 3 (57 to <64)**		**Quartile 4 (-27 to <57) (highest fat)**	
Exposure	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Elevated vs. normal waist circumference[a]	1	--	1.1 (0.9, 1.3)	0.49	1.3 (1.1, 1.6)	<0.01	3.1 (2.4, 3.9)	<0.01

Abbreviations: OR, odds ratio; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared)

[a]Elevated waist circumference according to the International Diabetes Federation metabolic syndrome classification[8]: >94cm for White and African American men, >90cm for Chinese American and Hispanic men and >80cm for all women.

Chapter 3 Abstract

Background Many adults have risk factors for non-alcoholic fatty liver disease (NAFLD). Screening all adults with risk factors for NAFLD using imaging is not feasible.

Objective To develop a practical scoring tool for predicting NAFLD using participant demographics, medical history, anthropometrics and lab values.

Methods We used cross-sectional data from 6,194 White, African American, Hispanic, and Chinese American participants from the Multi-Ethnic Study of Atherosclerosis cohort, ages 45-85 years. NAFLD was identified by liver computed tomography (≤40 Hounsfield units indicating >30% hepatic steatosis) and data on 14 predictors was assessed for predicting NAFLD. Random forest variable importance was used to identify the minimum subset of variables required to achieve the highest predictive power. This subset was used to derive (*n*=4,132) and validate (*n*=2,063) a logistic regression-based score (NAFLD-MESA Index). A second NAFLD-Clinical Index excluding laboratory predictors was also developed.

Results NAFLD prevalence was 6.2%. The model included eight predictors: age, sex, race/ethnicity, type 2 diabetes, smoking history, body mass index, gamma-glutamyltransferase (GGT) and triglycerides (TG). The NAFLD-Clinical Index model excluded GGT and TG. In the NAFLD-MESA model, the derivation set achieved an $AUC_{NAFLD\text{-}MESA}$ =0.83 (95% CI, 0.81 to 0.86), and the validation set an $AUC_{NAFLD\text{-}MESA}$ =0.80 (0.77 to 0.84). The NAFLD-Clinical Index model was ($AUC_{Clinical}$ =0.78 [0.75 to 0.81]

in the derivation set, and $AUC_{Clinical}$ =0.76 [0.72 to 0.80] in the validation set; $p_{Bonferroni\text{-}adjusted}$ <0.01).

Conclusions The two models are simple but highly predictive tools that can aid clinicians identify individuals at high NAFLD risk who could benefit from imaging.

Chapter 3 Main Body

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease in westernized societies [1], with a global prevalence of around 25% [2]. NAFLD represents a spectrum of disease from fat accumulation in the liver, to inflammation and progressive fibrosis, and eventual progression to cirrhosis and hepatocellular carcinoma [3–5]. In addition recent evidence showed that NAFLD complications were not only confined to advanced liver disease but also may contribute to major extrahepatic conditions [6,7], including a nearly two-fold increase in the risk of incident type 2 diabetes [8], and among patients with NAFLD, cardiovascular disease (CVD) and extrahepatic malignancies currently account for a greater proportion of mortality than liver disease [9].

To diagnose NAFLD there must be evidence of hepatic steatosis by imaging (e.g. ultrasound) or histology, and absence of secondary causes of hepatic fat accumulation, such as excessive alcohol consumption or chronic use of steatogenic medications [4]. Because of the high prevalence of NAFLD risk factors, such as overweight or the metabolic syndrome, assessing all patients at risk for NAFLD using imaging is not feasible [10]. A simplified algorithm to screen patients at high risk of NAFLD is therefore desirable. In clinical settings, especially in those with limited resources, clinicians could prioritize who should receive an imaging study or a more invasive liver biopsy for diagnosis. Likewise, in research settings, investigators could identify high-risk participants.

Previous non-invasive risk scores have been developed for detecting hepatic steatosis. These include the Fatty Liver Index (FLI) [11], the Lipid Accumulation Product [12], the Hepatic Steatosis Index [13], the NAFLD Liver Fat Score [14], the SteatoTest [15], and the NAFLD Ridge Score [16]. These tests have a moderate performance in identifying NAFLD, (area under the curve [AUC] range 0.79-0.87) and some have been externally validated [17–21]. All were developed in racially/ethnically homogeneous populations so it is unclear how they would perform in a heterogeneous adult population in the US. Furthermore, all risk scores primarily included clinical laboratory markers, which are not always readily available in clinical practice. The aim of this study was therefore to develop, in a large multi-ethnic cohort in the US, a practical scoring tool for predicting NAFLD risk based on participant demographics, medical history, anthropometrics and routine lab values, referred to as the NAFLD-MESA Index. Since laboratory measures are often not readily available, a secondary aim was to develop a second NAFLD-NAFLD-Clinical Index that does not require any laboratory variables. And lastly, we compare the performance of our two models against the FLI, which we additionally validate in our sample, to quantify any observed difference in classification performance.

MATERIALS AND METHODS

Data source

The Multi-Ethnic Study of Atherosclerosis (MESA) [22] is a well-characterized cohort of 6,814 participants aged 45-85 and free of known CVD. Established in 2000, participants were recruited from six communities of the US from the following University clinics: Columbia University in New York, Johns Hopkins University in Baltimore, Northwestern

University in Chicago, UCLA in Los Angeles, University of Minnesota at Twin Cities and Wake Forest University in Winston Salem. Racial/ethnic distribution was: 38% White, 28% African American, 22% Hispanic, and 12% Chinese American. Informed consent was obtained from all study participants and institutional review board (IRB) approval was obtained by the MESA sites. Ethics approval for the use of anonymized data was obtained from the University of California San Francisco IRB on 2 January 2018 (16-21085).

Sample population

MESA excluded participants with known CVD, active cancer treatment, pregnancy, weight >300 pounds, cognitive inability, and anyone living in a nursing home or on a waiting list for a nursing home [22]. We further excluded participants whose computed tomography (CT) imaging did not extend inferiorly sufficiently to measure liver fat attenuation (n = 56) [23]; participants with a reported history of moderately heavy alcohol use (average >1 serving/day in women and >2 servings/day in men) (n = 261), history of liver cirrhosis (n = 5) and use of oral steroids or class 3 antiarrhythmic medications due to their association with macrovesicular steatosis (n = 72) [24]. Our final sample size was 6,194 from the baseline visit between 2000-2002.

Outcome Measure: Non-Alcoholic Fatty Liver Disease

At the baseline visit, participants received two consecutive CT scans. Scan window included the tracheal carina to below the apex of the heart, which included liver images [24,25]. Liver attenuation by CT scan has been shown to be inversely correlated with liver fat deposition by liver biopsy (correlation coefficient: -0.9; p-value < 0.001), showing that

CT scanning provides a useful non-invasive method for identifying moderate to severe fatty liver [26]. Degree of liver attenuation was measured in three consistent regions in the parenchyma of the right hepatic lobe (each measuring about 1 cm^2) and calculated as the average density [23]. We used a previously validated threshold of ≤40 Hounsfield units (HU) for the identification of a binary classification of moderate to severe hepatic steatosis (>30% liver fat; NAFLD) [27,28].

Potential Predictors

Fourteen candidate predictors were identified *a-priori* based on their known association with NAFLD [29,30] or components of the metabolic syndrome [31] and their availability in routine clinical practice. These included: body mass index (BMI), waist circumference (WC), waist-to-hip ratio, age, sex, race/ethnicity, education, smoking history, recent weight change, gamma-glutamyltransferase (GGT) (u/L), triglycerides (mg/dL), type 2 diabetes, high density lipoprotein (HDL)-cholesterol (mg/dL), and hypertension.

Predictor Measurements

Anthropometric measures were taken using standardized procedures [32]. BMI was categorized according to established criteria [33–35]: normal weight (<25 kg/m^2), overweight (25-<30 kg/m^2), and obesity (30-<35 kg/m^2 grade 1, ≥35 kg/m^2 grade 2) for White, African American and Hispanic participants, and normal weight (<23 kg/m^2), overweight (23-<27.5 kg/m^2), obesity (≥27.5 kg/m^2) for Chinese Americans. WC (cm) was measured horizontally across the umbilicus and hip circumference (cm) at the greatest circumference around the buttocks[50]. An average of two waist and hip measurements

were used. WC was categorized into three groups according to guidelines [36]: <88, 88-102, >102cm. Age was initially categorized into decade groups, but then further modified into three categories to maximize discrimination ability: 45-<65, 65-<75 and 75-85. Sex was self-reported (male/female). Highest achieved education was classified into four categories: less than high school, high school, some college, bachelor's degree or higher. Race/ethnicity was self-reported (White, African American, Hispanic or Chinese American). Smoking history was categorized as never, former, or current. Recent weight change was calculated comparing measured weight at study baseline to self-reported highest weight over the prior three years, and calculated as % of weight loss/gain. GGT was categorized into quartiles according to units per liter (<5, 5-<8, 8-<14 and ≥14). Triglycerides were measured in the fasted state and categorized into three categories: <75, 75-<150, ≥150mg/dL to maximize model performance. Type 2 diabetes was defined as fasting glucose ≥126 mg/dL and/or on any diabetes treatment. HDL cholesterol was classified using the ATP III criteria of <40 mg/dL (Low), and ≥60 (high) [36], and we added intermediate categories 40-49, and 50-59 mg/dL to improve discrimination. Lastly, resting blood pressure was measured three times in the seated position and the average of the last two measurements were used.

Statistical Analysis of Participant Characteristics

Baseline characteristics, anthropometric data and clinical parameters are reported as means and standard deviation (SD) or median and interquartile range depending on their distribution, or as counts and proportions.

Risk-score Derivation

To select the optimal subset of predictor variables that minimize error in NAFLD prediction, we used a conditional random forest classification algorithm that accounts for variable correlation in the importance calculation. Estimation was based on the R *party* package [37] using the full sample. Random forest classification is a nonparametric, ensemble classification tree method that incorporates bootstrap aggregation in assessment of variable importance [38]. The use of the full sample for variable selection is acceptable since the algorithm incorporates bootstrapping and accounts for any over-optimistic identification of important variables as a result. From the original 14 variables, the random forest identified nine predictor variables that were most influential in minimizing prediction error. WC was identified as an important variable, but was subsequently removed from the final set of predictor variables because it is not regularly or accurately measured in current routine clinical settings, furthermore including it did not significantly improve the model performance, thus leaving eight variables for the final model.

To develop and validate our final model, called the NAFLD-MESA index, we selected a random 2/3 of the sample (*n*=4,151) for model training, and the remaining (*n*=2,063) for model validation. A risk score for the final multivariate model was derived using a modified version of the Framingham Heart Study approach described by Sullivan et al [39]. Briefly, a logistic regression model was fitted to the NAFLD outcome using the eight predictor variables. Model coefficients were then converted to points, with 1 point indicating the risk equivalent to the smallest coefficient (type 2 diabetes). A total risk score was then

calculated for each study participant by adding all points from each of the eight variables included in the final model. A detailed algorithm describing risk score point derivation is included in the Appendix. We assessed presence of two-way multiplicative interaction in separate models using likelihood ratio tests, including between race and BMI, sex and BMI, sex and age category, and sex and smoking. None of these were statistically significant at the 5% level, so the final model included only main effects.

We used a similar approach to construct a second model excluding laboratory variables (GGT and TG). Because TG requires an overnight fast, and both TG and GGT require a blood draw, these measures may not be readily available in routine clinical care setting. The corresponding risk score was based on the training and validation sets described above. The smallest coefficient in this case (equivalent to 1 point) was being a former smoker. A Chi-squared test comparing the estimated AUC was used to compare the two models (i.e. with and without TG and GGT); we present Bonferroni-adjusted p-values given that multiple pairwise differences were tested, including stratified models by race/ethnicity.

Internal discrimination and calibration

To assess the ability of our models to discriminate between study participants with and without NAFLD, we constructed ROC curves, and calculated sensitivity, specificity, interval likelihood ratios and estimated post-test probability of NAFLD at various intervals. The intervals were selected from visually inspecting the ROC curves to identify slope changes. Interval likelihood ratios were obtained by dividing the proportion of participants

with NAFLD over the proportion of participants without NAFLD in each interval. Calibration performance was assessed on the validation sample using goodness of fit measures, Brier scores, and graphically using a calibration plot. The Hosmer-Lemeshow goodness of fit test evaluates the discrepancy between observed and expected outcomes using a Chi-squared statistic based on quantiles of the model-derived outcome probabilities [40]. Brier scores are the mean square error between outcomes and predictions and are useful in assessing predictive accuracy of binary predictor models [41]; a Brier score closer to zero points to a relatively superior model. Lastly, study participants were grouped into quintiles of NAFLD risk and the average predicted risk of each quintile was plotted against the average observed risk. If a model is well calibrated, the observed percentages should be close to the predicted percentages near the 'line of equality', which represents perfect calibration.

Model performance compared to the Fatty Liver Index

We compared the performance of our NAFLD-MESA and NAFLD-Clinical Index models against the FLI to quantify any observed difference in classification performance. The FLI includes BMI (kg/m^2) and WC (cm) and log-transformed serum TG (mg/dl) and GGT (U/l) concentrations according to Bedogni et al [11] to obtain a score between 0 and 100 using the following formula based on the corresponding logistic model:

$$FLI = \frac{e^{(0.953\times\ln(TG) + 0.139\times BMI + 0.718\times\ln(GGT) + 0.053\times WC - 15.745)}}{1+ e^{(0.953\times\ln(TG) + 0.139\times BMI + 0.718\times\ln(GGT) + 0.053\times WC - 15.745)}} \times 100$$

We compared the AUC of our NAFLD-MESA and NAFLD-Clinical Index models to the AUC of the FLI using a Chi-squared test. In sensitivity analysis, for a fairer comparison we modified the FLI predictors (e.g. made them categorical, or linear) to potentially better fit our data and improve its discrimination performance. Analyses were conducted using R version 3.6.1 (Vienna, Austria) and Stata 15.0 (College Station, Texas, USA).

RESULTS

Characteristics of Participants

A total of 6,194 participants were included in the study. Participants included in the derivation and validation sets had similar distributions of important covariates (**Table 3.1**). Participant characteristics were presented by NAFLD status. Participants with NAFLD were younger and had more components of the metabolic syndrome including a higher BMI, WC, TG, systolic blood pressure, and GGT. In addition, participants with NAFLD were more likely to be Hispanic, have a lower educational background, have type 2 diabetes, and be never smokers.

Predictors of NAFLD

The final logistic regression model for the NAFLD-MESA point-based system included the following predictors: BMI, GGT, TG, sex, smoking history, age, type 2 diabetes, and race/ethnicity. Our second NAFLD-Clinical Index model included all of these variables except GGT and TG (**Table 3.2**). When coefficients were converted to risk-score points, high levels of TG or BMI had the greatest risk contribution, followed by younger age category, higher GGT, then race/ethnicity (with African Americans having the lowest risk),

sex, smoking history and lastly type 2 diabetes. In the second NAFLD-Clinical Index model, high BMI and younger age category had the greatest risk contributions, followed by race/ethnicity, type 2 diabetes, sex and lastly smoking history.

Discrimination performance

ROC curves were constructed using the point-based system and AUC estimated with NAFLD. In our full NAFLD-MESA model, the derivation set achieved an $AUC_{NAFLD\text{-}MESA}$ =0.83 (95% CI, 0.81 to 0.86), and the validation set an $AUC_{NAFLD\text{-}MESA}$ =0.80 (0.77 to 0.84) (**Figure 3.1**). Our NAFLD-Clinical Index model without GGT and TG performed marginally lower than our full model ($AUC_{Clinical}$ =0.78 [0.75 to 0.81] in the derivation set, and $AUC_{Clinical}$ =0.76 [0.72 to 0.80] in the validation set; $p_{Bonferroni\text{-}adjusted}$ <0.01) (**Figure 3.2**).

We provided the interval likelihood ratio and post-test probability at each two-unit interval for both models (**Table 3.3)**. We considered a post-test probability of NAFLD greater than the average pre-test probability (prevalence) as suitable cut-offs for higher suspicion of NAFLD. In the NAFLD-MESA index, this corresponded to a binary cut-off ≥22 points which had a sensitivity of 75%, a specificity of 72%, and a post-test probability >8%. Similarly, in our NAFLD-Clinical Index, the corresponding binary cut-off was ≥20 points, which had a sensitivity of 80%, specificity of 60% and post-test probability >8%.

Internal calibration

In our NAFLD-MESA model, the Hosmer-Lemeshow goodness-of-fit test had a p=0.24, mean bias was 0.002, mean absolute error was 0.106, and the Brier score was 0.053 for

the validation set, indicating that our validation model had acceptable calibration and prediction performance. In our second NAFLD-Clinical Index model, the Hosmer-Lemeshow goodness-of-fit test had a p=0.39, mean bias was 0.002, mean absolute error was 0.11, and the Brier score was 0.05. Graphically, when comparing the predicted NAFLD risk against the observed NAFLD prevalence by NAFLD risk quintiles, we found that both the NAFLD-MESA and NAFLD-Clinical Index models slightly overestimated risk overall, but the estimates by quintiles were close to the line of equality (**Appendix Figures 3.1-3.2**).

Comparison with the Fatty Liver Index

Compared to the FLI, when applied to our full cohort (*n*=6,194) our NAFLD-MESA index outperformed the FLI ($AUC_{NAFLD\text{-}MESA}$ =0.83 [95% CI: 0.81, 0.85] vs. AUC_{FLI} =0.78 [0.76, 0.80]); $p_{Bonferroni\text{-}adjusted}$ <0.01). On the other hand, our NAFLD-Clinical Index model performed similar to the FLI ($AUC_{Clinical}$ =0.78 [0.75 0.80]; $p_{Bonferroni\text{-}adjusted}$ 1.00) (**Table 3.4**). In race/ethnicity stratified analyses, we found that our NAFLD-MESA index also performed better than the FLI among African Americans ($AUC_{NAFLD\text{-}MESA,\ African\ Americans}$ =0.83 [0.78, 0.88] vs. $AUC_{FLI,\ African\ American}$ =0.79 [0.73, 0.84]); $p_{Bonferroni\text{-}adjusted}$ 0.01) and Hispanics ($AUC_{NAFLD\text{-}MESA,\ Hispanics}$ =0.79 [0.76, 0.83] vs. $AUC_{FLI,\ Hispanics}$ =0.74 [0.70, 0.78]); $p_{Bonferroni\text{-}adjusted}$ <0.01), though similar in White participants and Chinese Americans (**Appendix Table 3.1**). Lastly, in sensitivity analysis, modifying the FLI predictors to improve its performance in our data resulted in minimal AUC changes of less than one percent (data not shown).

DISCUSSION

In this large population-based cross-sectional study of White, African American, Hispanic and Chinese American adults over the age of 45 years, we developed two practical indices that use a point-based system to discriminate between individuals with and without NAFLD with good precision. The index showed adequate discrimination, supporting its use in clinical settings to prioritize who should be referred for an imaging study for NAFLD diagnosis. Likewise, in research settings, researchers can use the index to identify high or low NAFLD risk individuals. The NAFLD-MESA index was built using one common anthropometric measure, five health and demographic characteristics, and two biomarkers available from routine clinical visits. We also developed a NAFLD-Clinical Index excluding biomarkers (GGT and TG), and found it to perform only marginally lower than the full NAFLD-MESA index, indicating its use appropriate when laboratory tests are not readily available.

Machine learning can allow the identification of potentially highly predictive variables, that otherwise may have gone unexplored using traditional methods such as stepwise logistic regression [42]. The algorithm identified some of the same variables included in prior risk models, but also an additional three variables not previously included (sex, age and smoking). Consistent with prior studies [29,30], BMI, GGT, TG, type 2 diabetes and race/ethnicity in our index were independent predictors of NAFLD. Age and sex also have been associated with NAFLD, but their association vary across the life-course. NAFLD prevalence increases with age until about 50 years, particularly among men [43,44]. In populations <50, men generally have a higher NAFLD risk compared to women, whereas

among post-menopausal women, the risk of NAFLD has been found to be similar to men their age [43,44]. NAFLD prevalence decreases after about the age of 50 in men and around the age of 70 in women [44]. These findings are consistent in MESA with adults >75 years having the lowest risk of NAFLD. In our model, women had a slightly higher risk than men. On the other hand, the association between cigarette smoking history and NAFLD is less clear. In MESA, current smokers had the lowest NAFLD prevalence, and this was consistent across different strata of BMI (data not shown). As we did not control for other ectopic fat stores in our models, it is possible for residual confounding to explain at least part of this inverse association.

Our models share some of the same predictors used in a number of indices for NAFLD that have been previously developed [11–16]. These have shown to have a moderate predictive ability (AUC range 0.79-0.87), yet all were developed among homogeneous populations outside of the US. Thus, it is unclear whether these indices would work just as well for people in the US who are more racially/ethnically diverse or exposed to a more obesogenic environment. To test this hypothesis, we compared our NAFLD-MESA and Clinical indices to the FLI, originally developed among a cohort of Italian adults and has been externally validated in other ethnically-homogeneous populations with moderate discrimination performance (AUC range 0.79-0.83) [17–21]. We found that in our population, our NAFLD-MESA index outperformed the FLI by five percent of AUC, and our NAFLD-Clinical Index had a comparable performance compared to the FLI. In particular, the NAFLD-MESA performed marginally better than the FLI among African Americans and Hispanics, but similar among White participants and Chinese Americans.

Because of unavailability of one or more variables, we were unable to validate any of the other previously developed NAFLD indices.

Our study has limitations. First, NAFLD diagnosis in our study was based on CT scans which are insensitive to mild hepatic steatosis [27,28]. This resulted in outcome misclassification of mild NAFLD cases who may be at-risk for liver disease progression or extrahepatic conditions. Additionally, we could not evaluate non-alcoholic steatohepatitis due to the lack of histologic data. Second, other than the FLI, we were unable to compare the performance of our models against other previously published fatty liver indices as we did not have necessary variables (e.g. alanine transaminase). And lastly, we were unable to externally validate our index in contemporary clinical populations.

The current American Association for the Study of Liver Diseases NAFLD Practice Guidance do not recommend routine screening for NAFLD in high-risk groups attending primary care, diabetes, or obesity clinics [4]. They advise against routine screening in part because of lack of knowledge related to long-term benefits and cost-effectiveness of screening and due to the unproven utility of ultrasound or transient elastography as screening tools. On the other hand, the 2016 European Associations for the Study of the Liver (EASL), of Diabetes (EASD) and of Obesity (EASO) recommend screening high-risk individuals (with obesity or metabolic syndrome) for NAFLD by liver enzymes and/or ultrasound as part of routing work-up [45]. Likewise, the American Diabetes Association recommends routine screening of nonalcoholic steatohepatitis and liver fibrosis in

patients with type 2 diabetes and fatty liver on ultrasound [46]. Due to the high prevalence of NAFLD risk factors, such as obesity or the metabolic syndrome, routine screening for NAFLD would likely overwhelm imaging services. And importantly, the sensitivity for NAFLD using a BMI >30 kg/m^2 is likely too low, especially for those of Asian origin, who have a lower BMI distribution. The NAFLD-MESA index addresses this important limitation, making it easier to identify high-risk individuals, and by reducing the proportion of the population referred to imaging studies. For instance, by applying the NAFLD-MESA index cut-off to MESA, only about 1/3 of the study population would be referred for an imaging study, compared to about 75% of individuals with BMI in overweight or obese categories and/or with type 2 diabetes. Nevertheless, we agree with prior authors [47] that further research should evaluate if targeted NAFLD screening using a tool such as this one is cost-effective.

CONCLUSION

In conclusion, the NAFLD-MESA and NAFLD-Clinical indices adequately discriminate between individuals with and without moderate to severe NAFLD (>30% hepatic fat) and perform better or similar to the previously validated FLI. These indices can aid clinical decision making by risk stratifying and referring those at high risk for imaging studies. Likewise, in research settings, this index may aid in identifying high-risk individuals for inclusion or identification in interventional or observational studies.

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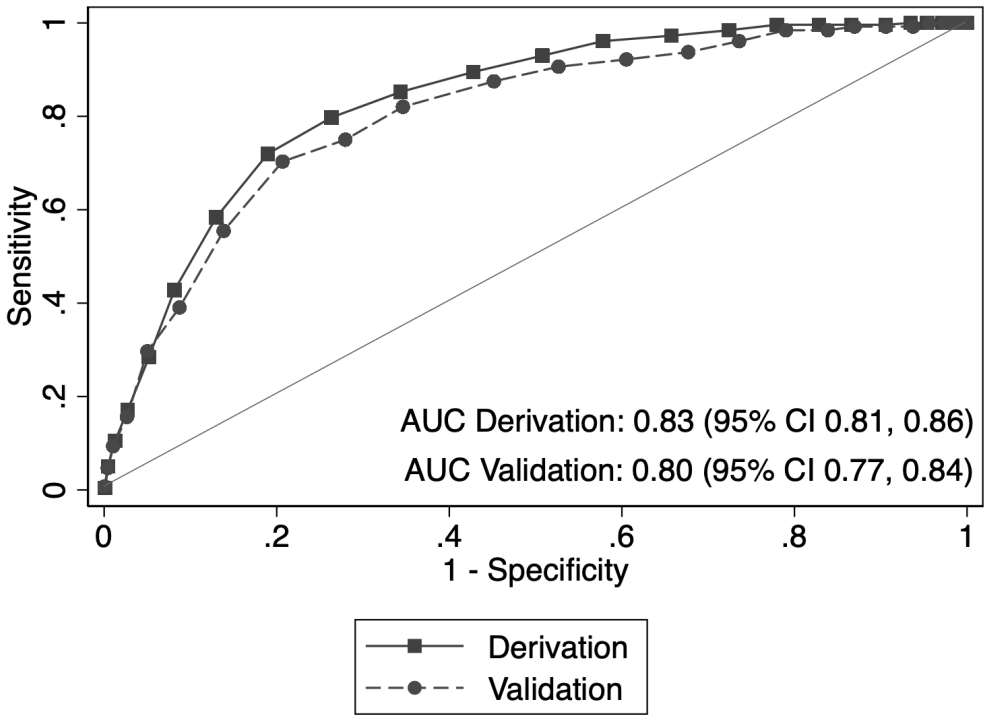


Figure 3.1 Area under the receiver operating characteristic curve using the point system on the derivation (*n*=4,151) and validation (*n*=2,063) model 1: NAFLD-MESA. AUC, area under the curve

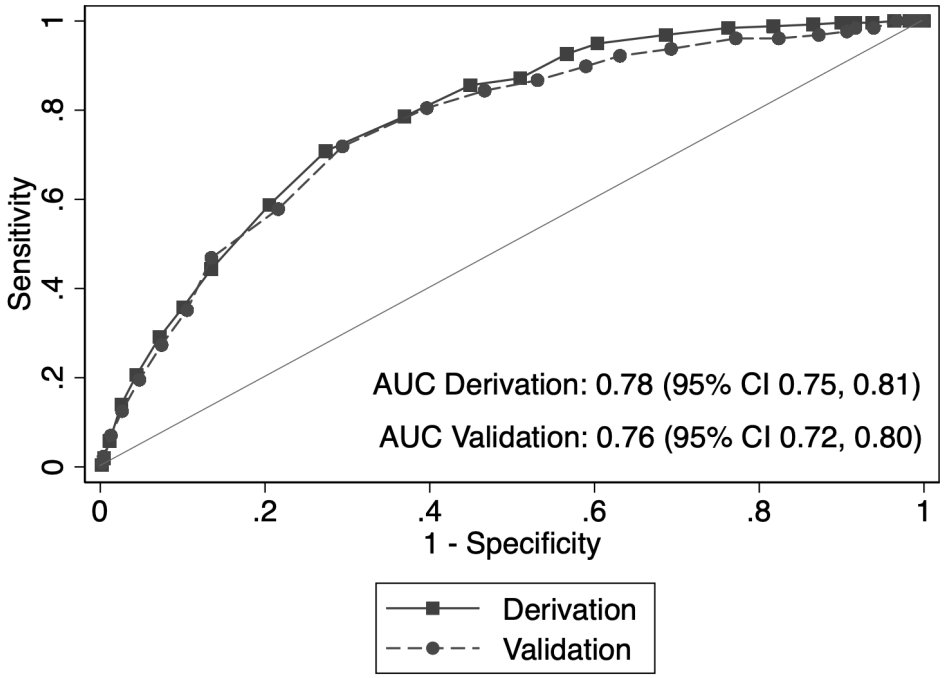


Figure 3.2 Area under the receiver operating characteristic curve using the point system on the derivation (*n*=4,151) and validation (*n*=2,063) model 2: NAFLD-Clinical Index.
AUC, area under the curve

Table 3.1 Characteristics of study participants with and without NAFLD in the development and validation samples

	Development sample (*n* = 4,132)		**Validation sample (*n* = 2,063)**	
Characteristic	NAFLD (*n* = 257) 6.2%	Non-NAFLD (*n* = 3,874) 93.8%	NAFLD (*n* = 128) 6.2%	Non-NAFLD (*n* = 1,935) 93.8%
Age, years	59 (8)	62 (10)	59 (9)	63 (10)
Sex				
Female	160 (7.3)	2,040 (92.7)	66 (6.1)	1,024 (93.9)
Male	97 (5.0)	1,834 (95.0)	62 (6.4)	911 (93.6)
Race/Ethnicity[c]				
White	93 (6.2)	1,420 (93.9)	39 (5.3)	702 (94.7)
African American	40 (3.4)	1,137 (96.6)	29 (5.0)	553 (95.0)
Hispanic	94 (10.0)	836 (90.0)	45 (9.3)	437 (90.7)
Chinese American	31 (6.1)	481 (93.9)	15 (5.8)	243 (94.2)
BMI, kg/m^2	32 (6)	28 (5)	33 (6)	28 (5)
Waist circumference, cm	107 [97 – 118]	97 [88 – 106]	108 [98 – 118]	97 [88 – 106]
Type 2 diabetes[b]	57 (22.2)	481 (12.4)	31 (24.2)	248 (12.8)
Triglycerides[a], mg/dL	154 [118 – 221]	108 [77 – 158]	163 [116 – 221]	110 [77 – 157]
GGT, u/L	13 [9 – 22]	8 [5 – 13]	13 [9 – 21]	8 [5 – 13]
HDL cholesterol[a], mg/dL	43 [37 – 50]	48 [40 – 58]	41 [36 – 48]	48 [40 – 59]
Systolic blood pressure, mm Hg	127 [116 – 142]	123 [111 – 140]	130 [116 – 143]	124 [111 – 140]
Diastolic blood pressure, mm Hg	73 [66 – 81]	72 [65 –78]	74 [67 – 82]	72 [67 – 79]
Education ≥ bachelor's degree	73 (28)	1,346 (35)	35 (27)	680 (35)
Cigarette smoking history				
Never	153 (59.5)	1,990 (51.4)	69 (53.9)	997 (51.5)
Former	82 (31.9)	1,396 (36.0)	41 (32.0)	710 (36.7)
Current	22 (8.6)	488 (12.6)	18 (14.1)	228 (11.8)
Exercise, MET-min	525 [0 – 1377]	840 [158 – 2100]	600 [0 – 1755]	818 [105 – 1943]

Results are presented as mean (SD) or median [interquartile range] for continuous variables, or n (%) for categorical variables. Abbreviations: BMI, body mass index; GGT, Gamma-Glutamyltransferase; HDL, high density lipoprotein; MET-min, metabolic equivalent minutes

[a]Fasting sample

[b]Defined as having a fasting blood glucose ≥126mg/dL, or on anti-diabetic medications

[c]Self-reported

Table 3.2 NAFLD-MESA index and NAFLD-Clinical Index predictors (derivation set, *n*=4,131)

Predictor	NAFLD-MESA Index		NAFLD-Clinical Index	
	Coefficient (95% CI)	Points	Coefficient (95% CI)	Points
BMI category (kg/m^2)[a]				
Normal	0	0	0	0
Overweight	0.9 (0.3, 1.5)	3	1.3 (0.7, 1.9)	6
Obese, grade 1	1.6 (1.0, 2.1)	5	2.1 (1.6, 2.7)	9
Obese, grade 2	2.1 (1.5, 2.7)	6	2.7 (2.1, 3.3)	11
GGT quartile (u/L)				
First (<5)	0	0	–	–
Second (5-7.9)	0.6 (0, 1.1)	2	–	–
Third (8-13.9)	1.3 (0.8, 1.9)	4	–	–
Fourth (≥14)	1.8 (1.3, 2.4)	5	–	–
Triglycerides[b] (mg/dL)				
<75	0	0	–	–
75-149	1.8 (1.0, 2.5)	5	–	–
≥150	2.0 (1.2, 2.8)	6	–	–
Female sex	0.6 (0.3, 0.9)	2	0.3 (0.0, 0.5)	1
Cigarette smoking history				
Never	0.8 (0.3, 1.3)	2	0.5 (0.0, 1.0)	2
Former	0.5 (0.0, 1.0)	1	0.2 (-0.3, 0.7)	1
Current	0	0	0	0
Age category (years)				
44-64	1.6 (0.9, 2.4)	5	1.8 (1.0, 2.5)	8
65-74	1.3 (0.5, 2.1)	4	1.3 (0.6, 2.1)	6
75-84	0	0	1	0
Type 2 diabetes[c]	0.3 (0, 0.7)	1	0.5 (0.2, 0.8)	2
Race/Ethnicity[d]				
White	0.9 (0.5, 1.3)	3	1.0 (0.6, 1.4)	4
African American	0	0	0	0
Hispanic	1.0 (0.6, 1.4)	3	1.3 (0.9, 1.6)	5
Chinese American	1.0 (0.5, 1.6)	3	1.3 (0.8, 1.8)	6

[a] BMI cut-points are <25 normal, 25-<30 overweight, 30-<35 obese grade 1 for White adults, African Americans and Hispanics and ≥35 obese, grade 2 for White adults, African Americans and Hispanics. <23 normal, 23-<27.5 overweight and ≥27.5 obese for Chinese Americans

[b] Fasting sample

[c] Defined as having a fasting blood glucose ≥126mg/dL, or on anti-diabetic medications

[d] Self-reported

Table 3.3 NAFLD-MESA index and NAFLD-Clinical Index interval table

Interval	% of population in interval	% D+ in interval	% D- in interval	Interval LR	Post-test probability in interval
NAFLD-MESA Index					
26 to 30	7	30%	5%	5.9	28%
24 to <26	10	26%	9%	2.9	16%
22 to <24	**14**	**20%**	**14%**	**1.4**	**8%**
20 to <22	17	13%	17%	0.7	5%
18 to <20	15	5%	15%	0.3	2%
16 to <18	13	4%	13%	0.3	2%
NAFLD-Clinical Index					
24 to 30	12	35%	11%	3.3	18%
22 to <24	12	23%	11%	2.0	12%
20 to <22	**18**	**23%**	**18%**	**1.3**	**8%**
18 to <20	13	6%	13%	0.5	3%
16 to <18	10	5%	10%	0.5	3%
14 to <16	13	4%	14%	0.3	2%

Abbreviations: D+, disease positive (NAFLD ≤40 Hounsfield units in CT scan); D-, disease negative (>40 Hounsfield units in CT scan); LR, likelihood ratio

Table 3.4 Comparing the AUC of the NAFLD-MESA and NAFLD-Clinical Index models using the point system to the AUC of the Fatty Liver Index using the regression equation according to Bedogni et al.

	Anthropometry and characteristics							Clinical parameters		AUC	
Model	**BMI**	**WC**	**Sex**	**Smoke**	**Age**	**Race/ Ethnicity**	**T2D**	**GGT**	**TG**	**Full Cohort**	***p*-value[a]**
NAFLD-MESA	✓		✓	✓	✓	✓	✓	✓	✓	0.83 (0.81, 0.85)	--
NAFLD-Clinical Index	✓		✓	✓	✓	✓	✓			0.78 (0.75, 0.80)	<0.01
Fatty Liver Index	✓	✓						✓	✓	0.78[b] (0.76, 0.80)	<0.01

AUC, area under the curve; BMI, body mass index; WC, waist circumference; T2D, type 2 diabetes; GGT, Gamma-Glutamyltransferase; TG, triglycerides

[a]Chi^2 test Bonferroni-adjusted *p*-value comparing the AUC of each model to the AUC of the NAFLD-MESA Index model

[b]Chi^2 test Bonferroni-adjusted *p*-value =1.00 comparing the AUC of the FLI to the AUC of the NAFLD-Clinical Index model

Appendix

The risk score algorithms for the Steato-MESA and non-clinical models were derived using a modified version of the Framingham Heart Study approach described by Sullivan et al. *Statistics in Medicine,* 2004 (38). What follows is an outline of the development of the Steato-MESA Index.

1. Estimate the parameters of the multiple logistic regression

Consider the model:

$$\text{logit}(P[Y|X]) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p$$

Y = outcome variable (*Y*=1 indicates presence of NAFLD; *Y*=0 absence of NAFLD)
X_p= candidate variables reflecting dichotomous or categories of predictors
β_p= estimates of the regression coefficients based on the multiple logistic regression

2. Organize the predictors into categories and determine reference values

Our final model included the following eight predictors: body mass index (BMI), GGT, triglycerides, sex, smoking history, age, type 2 diabetes, and race/ethnicity. Each variable was categorized into *jth* biologically relevant groups or into quartiles and modified as necessary to maximize model performance. The reference value for each dichotomous or categorical dummy variable was set=0.

3. Determine the referent predictor profile and set the constant *B*

Determine the category for each predictor associated with the lowest risk, which serves as the base category, and is assigned 0 points in the scoring system. Categories reflecting higher risk are assigned positive points. The constant, *B*, is the number of regression units that reflect 1 point in the final points system. In the Steato-MESA points system, we based the constant on the smallest *B* coefficient, $B_{T2D,}$ *e*quivalent to having type 2 diabetes.

4. Determine the number of points for each category of each predictor

Predictor	Predictor categories	Coefficient	Calculated Points = Coefficient/ B_{T2D}	Allocated Points (Rounded)
BMI category (kg/m^2)[a]	Normal	0	0.0000	0
	Overweight	0.9101	2.6984	3
	Obese, grade 1	1.5527	4.6035	5
	Obese, grade 2	2.1400	6.3450	6
GGT quartile (u/L)	First (<5)	0	0.0000	0
	Second (5-7.9)	0.5553	1.6465	2
	Third (8-13.9)	1.3205	3.9153	4
	Fourth (≥14)	1.8209	5.3988	5
Triglycerides[b] (mg/dL)	<75	0	0.0000	0
	75-149	1.7678	5.2414	5
	≥150	1.9897	5.8994	6
Sex	Male	0	0.0000	0
	Female	0.6063	1.7977	2
Cigarette smoking history	Never	0.7697	2.2822	2
	Former	0.5036	1.4931	1
	Current	0	0.0000	0
Age category (years)	44-64	1.6247	4.8171	5
	65-74	1.2941	3.8369	4
	75-84	0	0.0000	0
Type 2 diabetes[c]	No	0	0.0000	0
	Yes	0.3373	1.0000	1
Race/ Ethnicity[d]	White	0.9173	2.7198	3
	African American	0	0.0000	0
	Hispanic	0.9751	2.8910	3
	Chinese American	1.0490	3.1101	3
Intercept	–	-9.8943	–	–

[a] BMI cut-points are <25 normal, 25-29.9 overweight, 30-34.9 obese grade 1 for White adults, African Americans and Hispanics and ≥35 obese, grade 2 for White adults, African Americans and Hispanics. <23 normal, 23-27.4 overweight and ≥27.5 obese for Chinese Americans

[b] Fasting sample

[c] Defined as having a fasting blood glucose ≥126mg/dL, or on anti-diabetic medications

[d] Self-reported

5. Derive estimated risk ($\hat{p}$, probability) for each participant, based on the Points Score model:
Point totals range from 0-30.

$$\hat{p} = \frac{1}{1 + \exp(-\sum_{i=0}^{p} \beta_0 + \beta_{T2D} * Points)}$$

Possible Points	Estimated risk	Possible Points	Estimated risk	Possible Points	Estimated risk
0	0.0%	11	0.2%	22	7.8%
1	0.0%	12	0.3%	23	10.6%
2	0.0%	13	0.4%	24	14.2%
3	0.0%	14	0.6%	25	18.8%
4	0.0%	15	0.8%	26	24.5%
5	0.0%	16	1.1%	27	31.3%
6	0.0%	17	1.5%	28	38.9%
7	0.1%	18	2.1%	29	47.2%
8	0.1%	19	3.0%	30	55.6%
9	0.1%	20	4.1%		
10	0.1%	21	5.7%		

6. For comparison, derive estimated risk (*P,* probability) for each participant, based on the Logistic model:

$$\hat{p} = \frac{1}{1 + \exp(-\sum_{i=0}^{p} \beta_i X_i)}$$

Where $\beta's$ are the final Logistic regression coefficients, *X*'s are the participant's values on the *p* predictors.

-9.8943	(intercept)		+
0	*	BMI category Normal	+
0.9101	*	BMI category Overweight	+
1.5527	*	BMI category Obese, grade 1	+
2.1400	*	BMI category Obese, grade 2	+
0	*	GGT quartile (u/L) First (<5)	+
0.5553	*	GGT quartile (u/L) Second (5-7.9)	+
1.3205	*	GGT quartile (u/L) Third (8-13.9)	+
1.8209	*	GGT quartile (u/L) Fourth (≥14)	+
0	*	Triglycerides <75	+
1.7678	*	Triglycerides 75-149	+

1.9897	*	Triglycerides ≥150	+
0	*	Male	+
0.6063	*	Female	+
0.7697	*	Cigarette smoking history Never	+
0.5036	*	Cigarette smoking history Former	+
0	*	Cigarette smoking history Current	+
1.6247	*	Age category 44-64	+
1.2941	*	Age category 65-74	+
0	*	Age category 75-84	+
0	*	Type 2 diabetes No	+
0.3373	*	Type 2 diabetes Yes	+
0.9173	*	Race/Ethnicity White	+
0	*	Race/Ethnicity African American	+
0.9751	*	Race/Ethnicity Hispanic	+
1.0490	*	Race/Ethnicity Chinese American	+

A comparison of the Logistic regression model and the Risk Score, using two examples:
NAFD risk (points given in parentheses)

1. A 62-year-old (5) Chinese American (3) woman (2) with GGT 8.9 (4) and fasting TG 129 (5) with a BMI of 24 Kg/m² (3), never smoker (2), without a history of type 2 Diabetes (0), has a cumulative Point Score of 24, equivalent to an estimated NAFLD risk of **14.2%.**

   For comparison, her estimated NAFLD risk using the Logistic regression:

$$\hat{p} = \frac{1}{1 + \exp(-\sum_{i=0}^{p} \beta_i X_i)}$$

β_0 = -9.8943

$\sum_{i=0}^{p} \beta_i X_i$ = 1.6247 + 1.0490 + 0.6063 + 1.3205 + 1.7678 + 0.9101 + 0.7697 + 0

= -9.8943 + 8.0481 = -1.8462

$\hat{p}$ = $\frac{1}{1+\exp(-(-1.8462))}$

$\hat{p}$ = 0.136 = **13.6%**

2. An 80-year-old (0) African American (0) man (0) with GGT 11 (4) and fasting TG 126 (5) with a BMI of 29 Kg/m² (3), former smoker (1), and history of type 2 Diabetes (1), has a cumulative Point Score of 14, equivalent to an estimated NAFLD risk of **0.6%.**

   For comparison, his estimated NAFLD risk using the Logistic regression:

$$\hat{p} = \frac{1}{1 + \exp(-\sum_{i=0}^{p} \beta_i X_i)}$$

β_0 = -9.8943

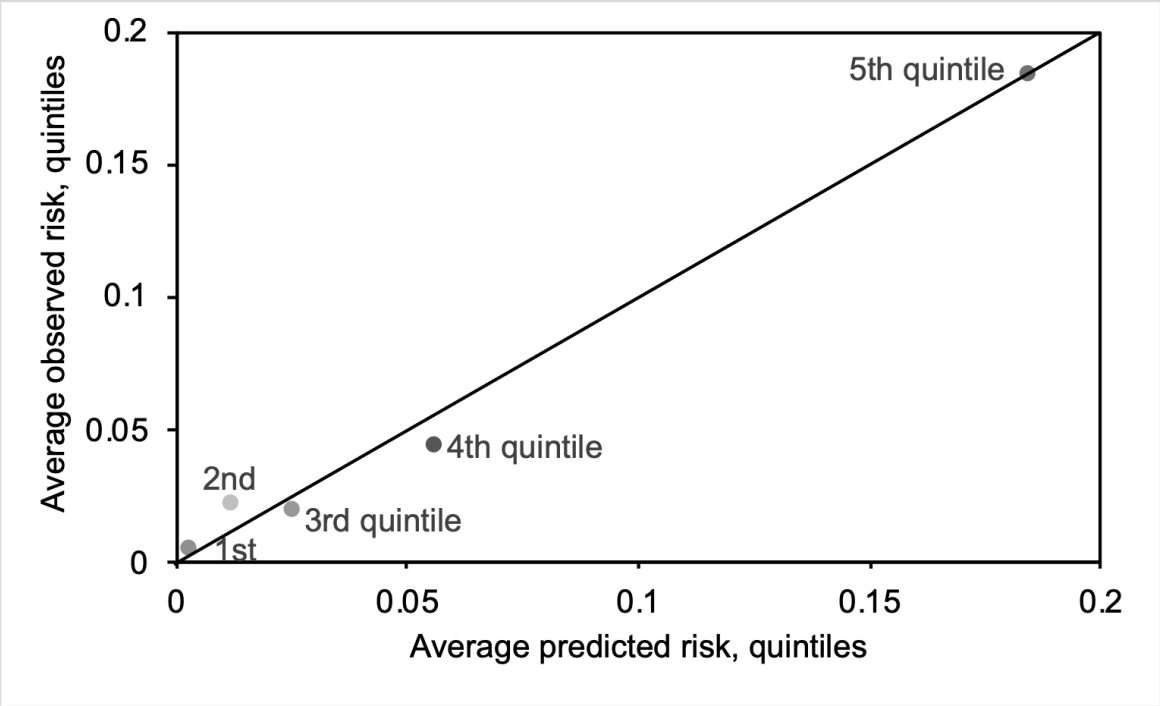
$\sum_{i=0}^{p} \beta_i X_i$ = 0 + 0 + 0 + 1.3205 + 1.7678 + 0.9101 + 0.5036 + 0.3373

= -9.8943 + 4.8393 = -5.055

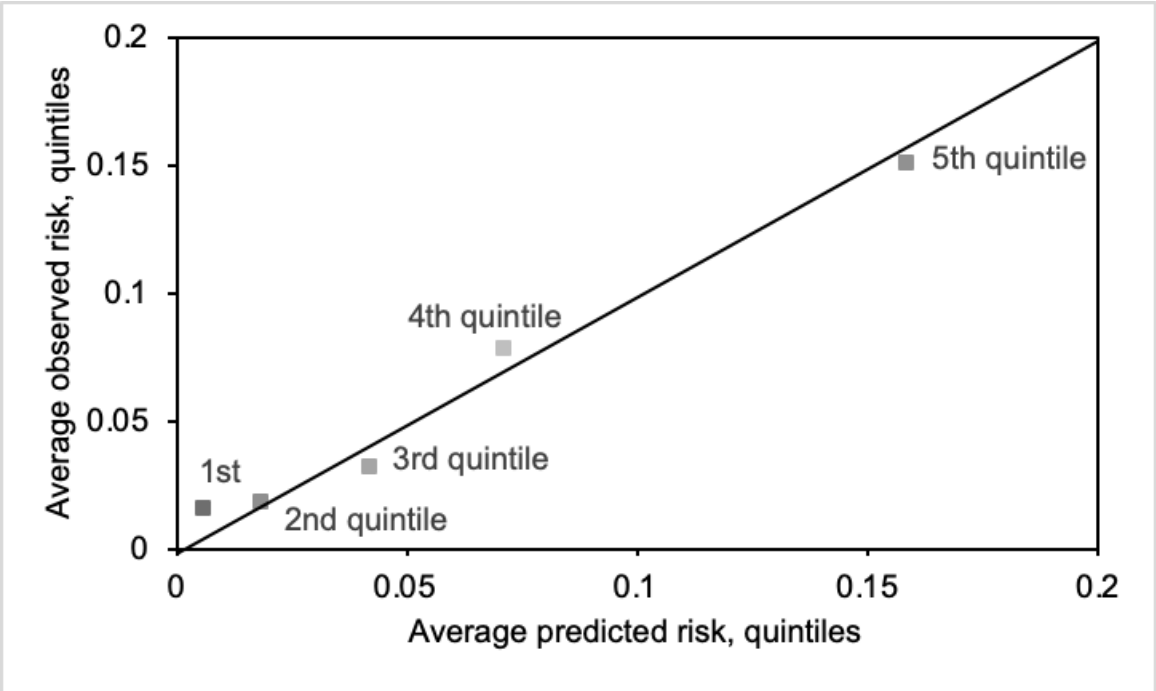
$\hat{p}$ = $\frac{1}{1+\exp(-(-5.055))}$

$\hat{p}$ = 0.006 = **0.6%**

Appendix Figures and Tables



Appendix Figure 3.1 Calibration plot showing average predicted vs. actual risk in each quintile of predicted risk in validation set (n=2,063) of model 1: NAFLD-MESA



Appendix Figure 3.2 Calibration plot showing average predicted vs. actual risk in each quintile of predicted risk in validation set (n=2,063) of model 2: NAFLD-Clinical Index

Appendix Table 3.1 Comparing the AUC of the NAFLD-MESA and NAFLD-Clinical Index models using the point system to the AUC of the Fatty Liver Index using the regression equation according to Bedogni et al., by race/ethnicity. The Multi-Ethnic Study of Atherosclerosis (2000-2002)

Model		All Set	*p*-value[a]	*p*-value[b]
NAFLD-MESA	All	0.83 (0.81, 0.85)	--	--
	White	0.84 (0.80, 0.87)	--	--
	African American	0.83 (0.78, 0.88)	--	--
	Hispanic	0.79 (0.76, 0.83)	--	--
	Chinese American	0.79 (0.73, 0.85)	--	--
NAFLD-Clinical Index	All	0.78 (0.75, 0.80)	<0.01	--
	White	0.79 (0.75, 0.82)	<0.01	--
	African American	0.76 (0.71, 0.82)	<0.01	--
	Hispanic	0.73 (0.69, 0.77)	<0.01	--
	Chinese American	0.73 (0.66, 0.80)	0.05	--
Fatty Liver Index	All	0.78 (0.76, 0.80)	<0.01	1.0
	White	0.83 (0.79, 0.86)	0.64	<0.01
	African American	0.79 (0.73, 0.84)	0.01	0.56
	Hispanic	0.74 (0.70, 0.78)	<0.01	1.00
	Chinese American	0.78 (0.71, 0.84)	1.00	0.22

[a] Test comparing NAFLD-Clinical Index and FLI to NAFLD-MESA, by race/ethnicity strata, Bonferroni-adjusted, in combined set

[b] Test comparing FLI to NAFLD-Clinical Index, by race/ethnicity strata, Bonferroni-adjusted, in combined set

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