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Type 2 diabetes mellitus increases the risk of hepatic fibrosis in individuals with obesity and nonalcoholic fatty liver disease

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Abstract

Objective: This study assessed the impact of diabetes mellitus (DM) on nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) with advanced fibrosis prevalence in adults with overweight or obesity in the United States.

Methods: Participants (National Health and Nutrition Examination Survey [NHANES] 2015-2016 database) included 834 middle-aged patients with DM (21.7%) and 3,007 without DM (78.3%). NAFLD was defined by Fatty Liver Index (FLI) ≥ 60 or United States FLI (USFLI) ≥ 30. Moderate-to-high and high risk of advanced fibrosis was defined by fibrosis-4 index (FIB-4) ≥ 1.67 and ≥ 2.67, respectively, and NAFLD fibrosis scores > 0.676 also indicated a high risk.

Results: NAFLD prevalence increased with BMI. Steatosis was higher in individuals with overweight with DM versus without DM (USFLI ≥ 30: 48.3% vs. 17.4%; $p < 0.01$) and in individuals with obesity with DM versus without DM (USFLI ≥ 30: 79.9% vs. 57.6%; $p < 0.01$). DM significantly increased the proportion of individuals at moderate-to-high risk of fibrosis (FIB-4 ≥ 1.67: 31.8% vs. 20.1%; $p < 0.05$). In the high risk of advanced fibrosis group (FIB-4 ≥ 2.67), the risk almost doubled (3.8% vs. 7.1%). Among individuals with obesity, DM increased the proportion of adults with moderate and high risk of fibrosis by 1.8- and 2.5-fold, respectively ($p < 0.01$ and $p = 0.39$, respectively, vs. without DM).

Conclusions: In this US cohort, DM modestly impacted steatosis, which was primarily obesity-driven. DM added a significant risk of fibrosis to individuals with overweight or obesity, suggesting that screening is imperative in adults with DM.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States. Individuals with obesity or diabetes mellitus (DM) have the highest risk of developing its more severe form, nonalcoholic steatohepatitis (NASH), with inflammation,

hepatocyte injury, and severe fibrosis. There are ~18.2 million people in the United States with DM and NAFLD, with about one-third having NASH (1-3). The American Diabetes Association (ADA) recommended screening for advanced fibrosis in all patients with prediabetes or DM with elevated plasma alanine aminotransferase (ALT) and/or hepatic steatosis (4). NAFLD not only predisposes individuals to

advanced liver diseases (cirrhosis and hepatocellular carcinoma), but it also is often associated with worse insulin resistance (5), dyslipidemia (6), DM (7), and cardiovascular disease (CVD) (8). Over the next 20 years, NASH in patients with DM will be responsible for ~812,000 liver-related deaths and ~1.37 million cardiovascular-related deaths, at an estimated cost of $55.8 billion (1,3).

The diagnosis of NAFLD is usually based on history and serum diagnostic panels that combine clinical parameters with routine laboratory tests, followed by imaging and liver biopsy (2,9,10). The Fatty Liver Index (FLI) (11) and United States FLI (USFLI) (12) are the most suitable validated diagnostic panels (12). A recent meta-analysis based largely on liver ultrasonography studies reported a worldwide prevalence of NAFLD in ~55% of patients with DM (13). However, ultrasonography may underestimate steatosis (14). Studies using controlled attenuation parameter (15-19) or magnetic resonance-based techniques (the gold standard) (20-22) have suggested an even higher prevalence of steatosis in patients with DM, but access to these imaging techniques is more costly and often limited. However, the true target of screening is fibrosis, not steatosis per se, because fibrosis (not steatosis) is associated with an increased risk of mortality from end-stage liver disease and CVD (8-10,23). Individuals with DM and elevated ALT or steatosis (4) are especially at risk for steatohepatitis with advanced liver fibrosis, defined as having either fibrosis stage F3 (advanced fibrosis) or F4 (cirrhosis) (9). An earlier diagnosis would facilitate treatment according to current guidelines with lifestyle management, vitamin E, or pioglitazone (9,10,24,25). Additionally, many new drugs are under development (26). However, the true prevalence of advanced fibrosis associated with NASH in the general US population, or in those with DM, remains unclear. The most widely accepted blood diagnostic panels are the fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS) (9,10,27-30), as they can predict liver-related mortality in patients with NASH (31). However, their use in patients with DM has suffered from small sample size, study design heterogeneity, and populations in which patients with and without DM were analyzed together (15,16,20-22,30,32). Additionally, few analyses have been performed in the United States (21,30) or have examined the contributing role of obesity to fibrosis in patients with DM.

We aimed to address the prevalence of steatosis and of NASH-associated advanced liver fibrosis in a US population, taking advantage of the large, mixed ethnicity population–based data from the National Health and Nutrition Examination Survey (NHANES) 2015-2016, which was conducted by the US National Center for Health Statistics (NCHS) (33-35).

METHODS

Data source

Data from the NHANES 2015-2016 cycle collected by the NCHS were obtained for this study (33,34). The survey combined interviews with physical examinations designed to provide a cross-sectional view of

Study Importance

What is already known?

► Individuals with obesity or diabetes mellitus (DM) with elevated alanine aminotransferase or steatosis are believed to be at a high risk for steatohepatitis with advanced liver fibrosis (nonalcoholic steatohepatitis fibrosis) and cirrhosis.

► However, this knowledge derives largely from selected populations and has not yet been demonstrated in a large cohort in the general US population, in which the interaction and/or added risk of having both overweight or obesity and DM for the development of fibrosis remains unclear.

What does this study add?

► In this large US cohort, steatosis was primarily driven by obesity and was only modestly impacted by DM.

► In contrast, the presence of DM added a significant risk of advanced fibrosis and about a twofold risk of cirrhosis to individuals with overweight or obesity.

► Owing to the fact that advanced fibrosis is an established risk factor for cirrhosis and increased mortality, screening is imperative in adults with DM.

How might these results change the direction of research or the focus of clinical practice?

► This study highlights the additive impact of DM on the development of steatosis and fibrosis for individuals with overweight or obesity, as well as the need for improved diagnostic approaches in the future.

► Based on the current findings, the American Diabetes Association guidelines that recommend screening for nonalcoholic fatty liver disease in individuals with elevated alanine aminotransferase or steatosis are a first step in the right direction.

the health status of adults in the United States. The NHANES interviews collected data on demographic, socioeconomic, and medical conditions, whereas the physical examinations consisted of physiological measurements and laboratory testing.

Participants

Participants were selected if they were ≥18 years old and had undergone a medical examination (Figure 1). Of the 9,971 participants surveyed during the NHANES 2015-2016 cycle, a total of 3,841 were ≥18 years old, had available medical examination
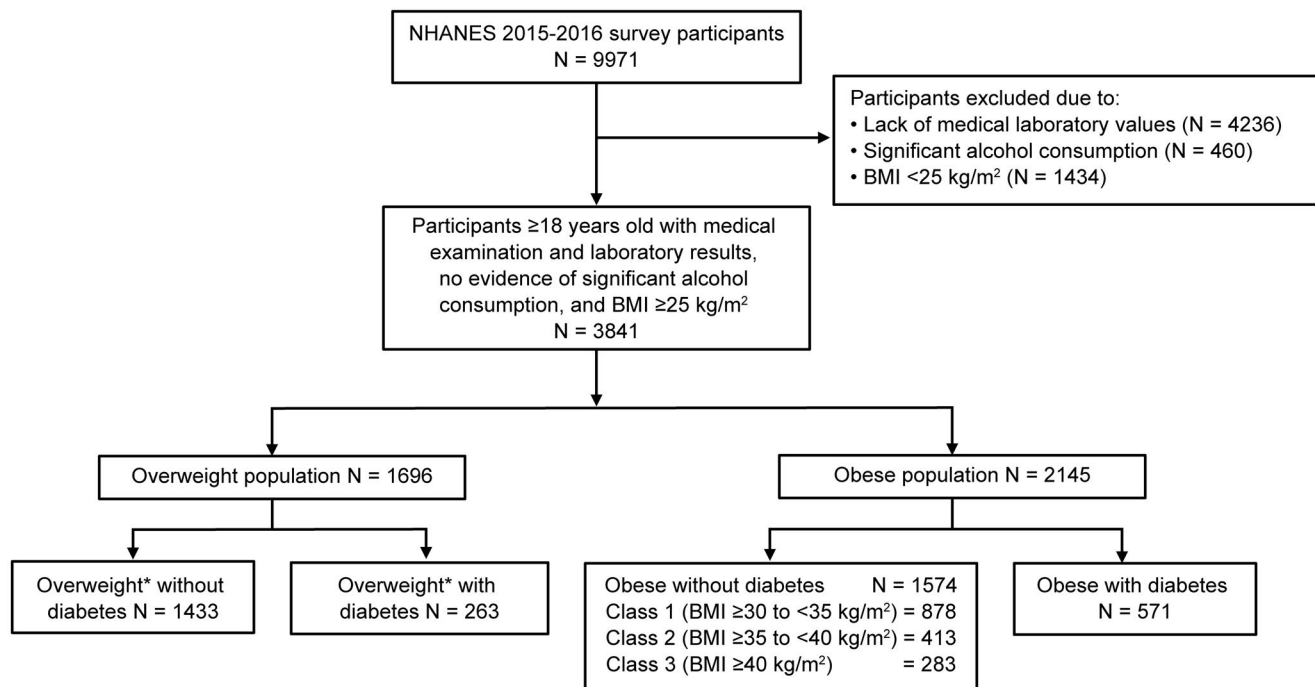
FIGURE 1 Flow diagram of NHANES participants and study subgroups. NHANES = National Health and Nutrition Examination Survey

and laboratory results, no evidence of significant alcohol consumption (≤2 drinks/d for men and ≤1 drink/d for women), and a BMI ≥ 25 kg/m^2. There were 1,696 participants with overweight (BMI ≥ 25-<30) and 2,145 participants with obesity (BMI ≥ 30) included in the analysis. The population with overweight was composed of 263 participants with DM, defined as having a self-reported physician diagnosis of DM, receipt of DM medications, or glycated hemoglobin (HbA1c) ≥ 6.5%, and there were 1,433 participants with no evidence of DM. The population with obesity was composed of 571 participants with evidence of DM and 1,574 participants "without DM." Hypertension was defined from the physical exam data as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg. CVD was a patient-reported diagnosis of coronary heart disease, angina, heart attack, or stroke.

Study measures

The presence of NAFLD was predicted using two indices: the FLI, comprising triglycerides, BMI, gamma-glutamyl transferase (GGT), and waist circumference (11), and the more recently developed USFLI (12), comprising age, ethnicity, GGT, waist circumference, fasting glucose, and fasting insulin (4). FLI was calculated as follows:

$$FLI = (e^{0.953 \times \log_e [\text{Triglycerides}] + 0.139 \times BMI + 0.718 \times \log_e [\text{GGT}] + 0.053 \times \text{Waist Circumference} - 15.745)} / (1 + e^{0.953 \times \log_e [\text{Triglycerides}] + 0.139 \times BMI + 0.718 \times \log_e [\text{GGT}] + 0.053 \times \text{Waist Circumference} - 15.745}) \times 100$$

An FLI cutoff value of ≥ 60 was used to denote NAFLD (11). For FLI values ≥ 60, the reported sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are 44%, 90%, 67%, and 76%, respectively (36).

Owing to the fact that NHANES is a US database, we also established USFLI scores, believed to be more precise in assessing steatosis (12), at least as validated in US populations and considering relevant variables such as insulin resistance (i.e., fasting plasma glucose and insulin) and ethnicity. USFLI (12) was calculated using the following formula, where Non-Hispanic Black and Mexican American have a value of 1 if the participant is of that ethnicity and 0 if not of that ethnicity:

$$USFLI = (e^{-0.8073 \times \text{Non-Hispanic Black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{Age} + 0.6151 \times \log_e [\text{GGT}] + 0.0249 \times \text{Waist Circumference} + 1.1792 \times \log_e [\text{Insulin}] + 0.8242 \times \log_e [\text{Glucose}] - 14.7812)} / (1 + e^{-0.8073 \times \text{Non-Hispanic Black} + 0.3458 \times \text{Mexican American} + 0.0093 \times \text{Age} + 0.6151 \times \log_e [\text{GGT}] + 0.0249 \times \text{Waist Circumference} + 1.1792 \times \log_e [\text{Insulin}] + 0.8242 \times \log_e [\text{Glucose}] - 14.7812}) \times 100$$

A USFLI cutoff value of ≥30 was used to diagnose fatty liver, with a reported sensitivity, specificity, likelihood ratio positive, and likelihood ratio negative values of 62%, 88%, 5.2, and 0.43, respectively (12).

The USFLI could be used only in a subset of NHANES participants, as serum fasting insulin was unavailable in 58% of study

participants. Therefore, we focused on FLI for the overall population analysis.

The FIB-4, NFS, and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) were chosen as the diagnostic panels to identify participants with advanced fibrosis, as these are well validated, supported by the literature (9,10,27-29,31,37,38), and most widely used in clinical practice. NFS, FIB-4, and APRI were calculated as follows:

$$FIB-4 = Age\,(years) \times AST\,(U/L)/Platelet\,\left(\times 10^9/L\right) \times \sqrt{ALT}\,(U/L)$$

$$NFS = -1.675 + 0.037 \times Age\,(years) + 0.094 \times BMI\,(kg/m^2) + 1.13 \times$$
$$(Impaired\,Fasting\,Glucose\,or\,DM) + 0.99 \times (AST/ALT) - 0.013$$
$$\times Platelet\,(\times 10^9/L) - 0.66 \times Albumin\,(g/dL),\,in\,which\,Impaired$$
Fasting Glucose or DM had a value of 1 if the participants had impaired fasting glucose and 0 if they did not

$$APRI = \left[(AST/Upper\,Limit\,of\,Normal\,AST\,Range) \times 100\right]/Platelet\,\left(10^9/L\right)$$

We chose the traditionally accepted cutoffs to define the relative risk of having clinically significant fibrosis for each panel. Participants with FIB-4 < 1.3 were considered as being at the lowest risk of advanced liver fibrosis, followed by those with FIB-4 ≥ 1.3 and <1.67. The cutoff of ≥1.67 was chosen based on a prior cohort we studied that indicated the cutoff of ≥1.67 as a good discriminatory value to identify those with clinically relevant fibrosis among patients with DM (29). Participants with FIB-4 ≥ 1.67 to < 2.67 were classified as having a moderate risk, whereas those with FIB-4 ≥ 2.67 were classified as having a high risk of advanced liver fibrosis (F3 or higher; ≥F3) based on the literature (9,10,27-29,31). For NFS, we also used accepted cutoffs for the risk of liver fibrosis (9,10,27-29,31). An NFS value of <−1.455 was considered as low risk, −1.455 to 0.676 as intermediate risk, and >0.676 as high risk for advanced liver fibrosis (≥F3). The reported areas under the receiver operating characteristic curve for the diagnosis of advanced fibrosis have been reported to be 0.78 to 0.80 for FIB-4 and 0.72 to 0.75 for NFS (28-31). For instance, based on a recent analysis by Anstee et al. (27), for a threshold of <1.3 for FIB-4, the sensitivity, specificity, PPV, and NPV were 82%, 57%, 83%, and 56%, respectively. In contrast, FIB-4 with a cutoff value of ≥2.67 has a sensitivity of 36%, specificity of 93%, PPV of 93%, and NPV of 37%. A cutoff of <−1.455 for NFS is able to detect advanced fibrosis with a sensitivity of 89%, specificity of 37%, PPV of 85%, and NPV of 46%. NFS with a threshold value > 0.676 had a sensitivity, specificity, PPV, and NPV of 88%, 89%, 93%, and 26%, respectively.

At upper and lower cutoff values (<0.50, >1.50), APRI has been reported to have a sensitivity of 31%, specificity of 99%, PPV of 67%, and NPV of 94% (29).

Statistical analyses

The data were analyzed using descriptive statistics. Health indices were used to approximate the prevalence of NAFLD and NASH with fibrosis among populations of participants with overweight and participants with obesity. Group comparisons were made using the χ^2 test for categorical measures and the Student t test for continuous measures. Comparisons involving steatosis and fibrosis indices were adjusted for multiple comparisons using the Holm–Bonferroni method. Categorical measures were reported as percentages, and continuous measures were reported as mean (SD) values. Logistic regression models examining the impact of DM on fibrosis while controlling for age were performed. Corresponding odds ratios (ORs) and 95% confidence intervals (CIs) are reported.

Analyses were conducted using the survey weights provided with the NHANES data. The weights were used to account for the complex survey design, including nonresponse and oversampling of certain age and ethnic groups. Weighting of the NHANES data produced results that were representative of the US noninstitutionalized civilian resident population. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Data were collected from 3,841 participants ≥ 18 years old without evidence of significant alcohol consumption and BMI ≥ 25. Of these, a total of 2,145 participants (56%) had a diagnosis of obesity, and 1,696 (44%) had overweight. Among included participants, 834 (21.7%) had a self-reported physician diagnosis of DM, were taking DM medications, or had HbA1c ≥ 6.5%, a prevalence consistent with previous Centers for Disease Control and Prevention reports in patients with higher BMI and older age (https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf).

Table 1 shows the baseline characteristics and risk factors for the development of NAFLD, categorized into participants with either overweight or obesity with or without DM. CVD and hypertension were significantly more prevalent in participants with overweight or obesity with DM versus without DM ($p < 0.05$-0.001). Patients with DM were older versus those without DM. In the group of individuals with overweight, there were fewer female participants with DM versus without DM ($p < 0.05$). There were no significant differences in ethnicity between the groups; this is representative of a typical US population. Metabolic parameters (HbA1c, glucose, and lipids) were worse, as expected, in those with DM versus without DM.

Prevalence of hepatic steatosis (NAFLD)

Table 2 summarizes the NAFLD and liver fibrosis indices across the different cohorts. A diagnosis of hepatic steatosis by FLI ≥ 60 was observed more frequently among individuals with obesity with DM versus without DM (94.4% vs. 90.1%; $p = 0.396$). This was higher than that in the participants with overweight with DM (52.4%) or without DM (32.5%; $p = 0.074$ vs. with DM).

When hepatic steatosis was calculated using USFLI ≥ 30, a similar trend was observed but with an overall lower prevalence of

TABLE 1 Baseline characteristics of the NHANES populations with overweight and obesity

	Population with overweight[†]		Population with obesity[‡]	
	Without DM (n = 1,433)	With DM[§] (n = 263)	Without DM (n = 1,574)	With DM[§] (n = 571)
Age (y), mean (SD)	48 (9.2)	63 (4.4)***	46 (9.0)	57 (5.9)***
Sex, female (%)	47	32.2**	55.3	55.3
Ethnicity (%)				
Non-Hispanic White	64.7	58.2	61.6	58.2
Non-Hispanic Black	10.1	11.3	12.5	16.6
Hispanic[¶]	16.4	21.3	19.3	17.8
Other[¶¶]	8.8	9.2	6.6	7.4
BMI (kg/m^{2}), mean (SD)	27.4 (0.7)	27.7 (0.2)	35.9 (5.0)	37.0 (3.0)*
History of CVD[¶¶¶] (%)	6.3	21.5**	7.2	23.1***
Hypertension[¶¶¶¶] (%)	25.4	42.8**	29.5	43.7**
DM (%)	NA	100.0	NA	100.0
Glucose (mg/dL), mean (SD)	93 (7.3)	144 (12.0)***	96 (7.4)	149 (20.5)***
HbA1c (%), mean (SD)	5.4 (0.2)	7.3 (0.4)***	5.5 (0.2)	7.3 (0.6)***
Total cholesterol (mg/dL), mean (SD)	199 (21)	178 (7)**	195 (19)	185 (12)**
HDL (U/L), mean (SD)	56 (9)	50 (3)**	50 (7)	46 (4)**
LDL (U/L), mean (SD)	119 (11)	96 (4)***	118 (11)	106 (6)***
Triglycerides (mg/dL), mean (SD)	158 (83)	185 (22)	171 (58)	208 (43)*
ALT (U/L), mean (SD)	25 (6)	28 (3)*	29 (10)	29 (5)
AST (U/L), mean (SD)	25 (5)	27 (2)	26 (5)	29 (8)*
Platelet count (10[9]/L), mean (SD)	233 (26)	225 (10)	247 (28)	239 (18)*

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVD, cardiovascular disease; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable; NHANES, National Health and Nutrition Examination Survey.

[†]BMI ≥ 25 to <30 kg/m^{2}.

[‡]BMI ≥ 30 kg/m^{2}.

[§]Self-reported based on physician diagnosis and/or taking DM medications.

[¶]Hispanic includes "Mexican American" and "other Hispanic."

[¶¶]Other includes "non-Hispanic Asian" and "other race, including multi-racial" categories.

[¶¶¶]CVD was a patient-reported diagnosis of coronary heart disease, angina, heart attack, or stroke.

[¶¶¶¶]Hypertension was defined from the physical exam data as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥90 mm Hg.

*$p < 0.05$ vs. without DM; **$p < 0.01$ vs. without DM; ***$p < 0.001$ vs. without DM. The without DM group was used as the reference group within the population with overweight and obesity.

NAFLD (Table 2). In participants with overweight, the prevalence of steatosis was higher in participants with DM (48.3%) versus without DM (17.4%; $p < 0.01$), with a similar trend in patients with obesity (79.9% vs. 57.6%, respectively; $p < 0.01$).

In order to further explore the role of obesity, we examined proportions of steatosis by different BMI categories of individuals with DM versus without DM. Figure 2 illustrates that the proportion of individuals with steatosis increased with BMI, either measured by FLI ≥ 60 (not shown) or USFLI ≥ 30, increasing proportionally with obesity class and only marginally impacted by DM status at higher BMI levels, with a plateau when BMI reached ~35 and not significantly different in BMI > 30 with USFLI or with FLI (data not shown).

However, there was a discordance in prevalence between patients with obesity with steatosis using FLI versus USFLI. With USFLI ≥ 30, the proportion of individuals nearly doubled from class 1 to class 3

obesity, the highest percentage being recorded among those with class 3 obesity (BMI ≥ 40; 84.8% and 90.9%, without DM and with DM, respectively; Figure 2). In contrast, FLI ≥ 60 appeared to indicate that nearly all participants with class 2 obesity (99.3%) and all participants with class 3 obesity (100%) had steatosis (nonsignificant vs. class 2; $p < 0.001$ vs. class 1 obesity). When only patients with DM were considered with either index, a similar pattern evolved when stratified by BMI category, with the highest increase in steatosis among patients with overweight with DM (with ~50% having steatosis; Figure 2).

When the role of glycemic control was examined (Figure 3 and Supporting Information Table S1), there was no further impact of worse glycemic control on the prevalence of steatosis by FLI when comparing patients with HbA1c ≤ 7.0% versus uncontrolled DM (data not shown). When examining steatosis by USFLI ≥ 30, we observed an increase in steatosis with uncontrolled DM (80.6% in patients with HbA1c > 8.0% vs. 64.6% in controlled DM with a HbA1c ≤ 7.0%; $p = 0.41$ vs. ≤7.0%).

TABLE 2 Hepatic steatosis and fibrosis indices among participants

	Overweight group[†]		Obesity group[‡]		Overall	
	Without DM (n = 1,433)	With DM[§] (n = 263)	Without DM (n = 1,574)	With DM[§] (n = 571)	Without DM (n = 3,007)	With DM[§] (n = 834)
Steatosis indices						
FLI > 60 (%)	32.5	52.4	90.1	94.4	62.6	82.6[***]
USFLI[¶] ≥ 30 (%)	17.4	48.3[**]	57.6	79.9[**]	38.4	70.8[***]
Advanced fibrosis risk stratification						
FIB-4 < 1.3 (%; lowest risk)	56.1	38.3	66.7	49.7[*]	61.5	46.4[*]
FIB-4 ≥ 1.3 to < 1.67 (%[¶¶]; low risk)	19.5	27.8	17.3	19.3	18.4	21.8
FIB-4 ≥ 1.67 to < 2.67 (%)[¶¶]; moderate risk)	19.0	24.1	13.7	24.9[*]	16.3	24.7
FIB-4 ≥ 2.67 (%; high risk of advanced fibrosis)	5.4	9.7	2.4	6.1	3.8	7.1
FIB-4 ≥ 1.67 (%; moderate-to-high risk)	24.3	33.9	16.1	31.0[**]	20.1	31.8[*]
NFS < −1.455 (%)	70.8	12.6[***]	53.7	9.7[***]	61.9	10.5[***]
NFS −1.455 to 0.676 (%)	27.0	68.4[***]	39.8	56.2[**]	33.7	59.7[***]
NFS > 0.676 (%)	2.2	18.9[**]	6.4	34.1[***]	4.4	29.8[***]

Abbreviations: DM, diabetes mellitus; FIB-4, fibrosis-4 index; NFS, nonalcoholic fatty liver disease fibrosis score; USFLI, United States Fatty Liver Index.

[†]BMI ≥ 25 to <30 kg/m².

[‡]BMI ≥ 30 kg/m².

[§]Self-reported based on physician diagnosis and/or taking DM medications.

[¶]USFLI measurements required fasting insulin data, which were not available for all participants of the survey.

[¶¶]Cutoff based on Bril et al. (30).

*$p < 0.05$ vs. without DM; **$p < 0.01$ vs. without DM; ***$p < 0.001$ vs. without DM; p values adjusted for multiple comparisons using the Holm–Bonferroni method.
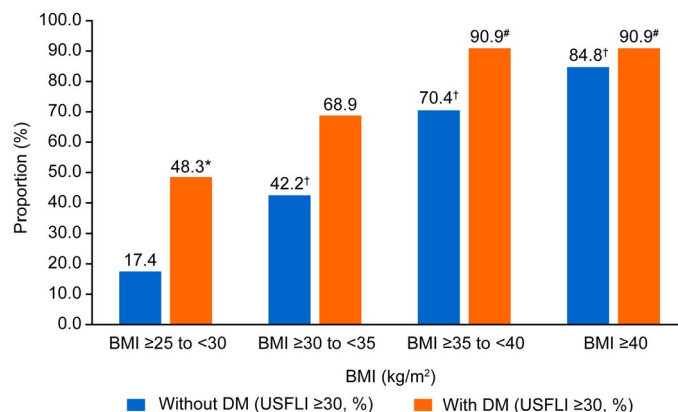

FIGURE 2 Prevalence of steatosis by BMI in participants with DM (orange bars) vs. without DM (blue bars). Values in participants with DM (orange bars) vs. without DM (blue bars) were higher only in the BMI ≥ 25 to <30 category (*$p < 0.01$ for participants with DM vs. without DM at the same BMI category). All values in participants with BMI ≥ 30 were significantly higher than in participants with BMI ≥ 25 to <30 ([†]$p < 0.01$ for participants without DM vs. BMI ≥ 25 to <30 and [#]$p < 0.01$ for participants with DM vs. BMI ≥ 25 to <30), except for participants with DM and BMI ≥ 30 and <35. P values were adjusted for multiple comparisons. DM = diabetes mellitus; USFLI = United States Fatty Liver Index

Prevalence of advanced liver fibrosis

As detailed in the "Study measures" section, we stratified patients by either FIB-4 or NFS, following validated cutoffs (9,10,27-29,31). A FIB-4 < 1.3 was considered as the lowest risk of

advanced liver fibrosis, followed by a low risk if the FIB-4 was ≥ 1.3 to <1.67.

The risk of moderate-to-advanced fibrosis by FIB-4 was much higher in individuals with DM versus those without DM (31.8% vs. 20.1%; $p < 0.05$; Table 2 and Figure 4). A moderate risk of advanced
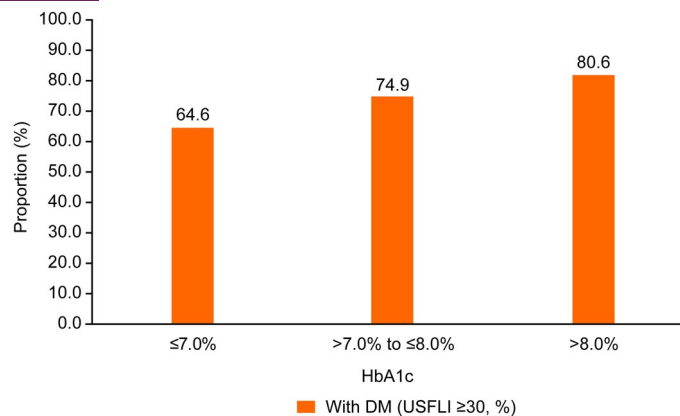
FIGURE 3 Prevalence of steatosis by HbA1c in adults with type 2 DM, measured by USFLI ≥ 30. USFLI ≥ 30 in patients with HbA1c > 8.0% or HbA1c > 7.0% to ≤8.0% was not significantly different than in the reference group ($p > 0.05$ vs. ≤7.0%). HbA1c data were missing for 18 individuals; these patients were not included in the analysis. P values were adjusted for multiple comparisons. DM = diabetes mellitus; HbA1c = glycated hemoglobin; USFLI = United States Fatty Liver Index
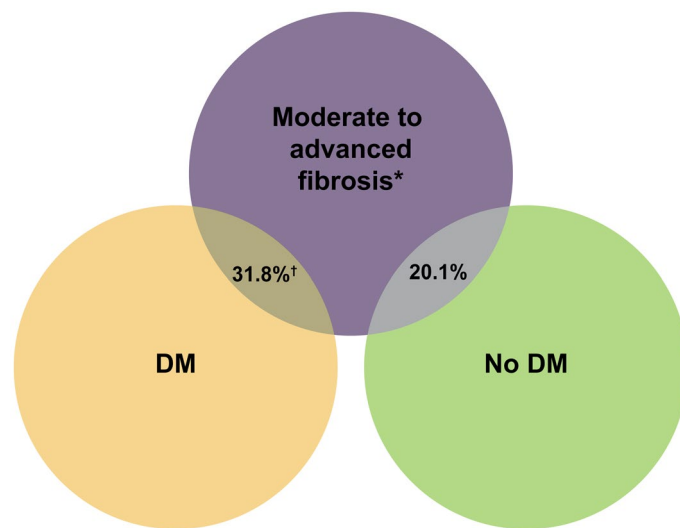

FIGURE 4 Individuals with and without DM with moderate-to-high risk of liver fibrosis. *At moderate-to-advanced risk of fibrosis (FIB-4 ≥ 1.67). [†]$p < 0.01$ vs. without DM. DM = diabetes mellitus

liver fibrosis (FIB-4 ≥ 1.67 to <2.67) was observed in 13.7% to 19% of participants with overweight or obesity without DM versus a much higher risk in those with DM (24.1%-24.9%; Table 2 and Figure 5A). Advanced fibrosis (FIB-4 ≥ 2.67 or ≥F3) was ~twofold higher in patients with overweight with DM versus without DM, with a similar increase in patients with obesity with DM versus without DM (2.6-fold increase; both nonsignificant; Table 2 and Figure 5B). Taken together, DM doubled the risk of advanced liver fibrosis; however, given the small number of patients in each group (from 51 patients with obesity to 81 patients with overweight with DM), it fell short of statistical significance.

In contrast to FIB-4, NFS appeared to classify many more patients with DM into either a moderate risk (NFS = −1.455-0.676 or "gray zone") or high risk (NFS > 0.676) of having advanced liver fibrosis (Table 2). Strikingly, the risk of advanced fibrosis increased ~sixfold in patients with obesity with DM versus without DM (34.1% vs. 6.4%; $p < 0.001$; Table 2).

When the role of glycemic control was examined, the prevalence of fibrosis by FIB-4 ≥ 1.67 or <2.67 did not increase with worse glycemic control; in fact, the proportion of patients with a FIB-4 ≥ 2.67 was unexpectedly lower at a higher HbA1c (>8.0%; see Supporting Information Table S1).

Participants with DM were significantly older compared with those without DM in both the overweight (mean age = 62.8 vs. 47.5 y; $p < 0.0001$) and obese cohorts (57.1 vs. 46.0 y; $p < 0.0001$). However, we examined the influence of DM on the prevalence of fibrosis using logistic regression models controlling for age. In these analyses, DM was still associated with a twofold increase in the proportions at moderate-to-high risk of fibrosis in patients with obesity (FIB-4 ≥ 1.67; OR = 2.06; 95% CI: 1.30-3.25) and a greater than fivefold increase in the proportions with advanced fibrosis as measured by NFS > 0.676 in both the overweight (OR = 5.66; 95% CI: 3.02-10.61) and obese groups (OR = 5.70; 95% CI: 3.74-8.68). There were too few patients with DM < 45 years of age to draw any significant
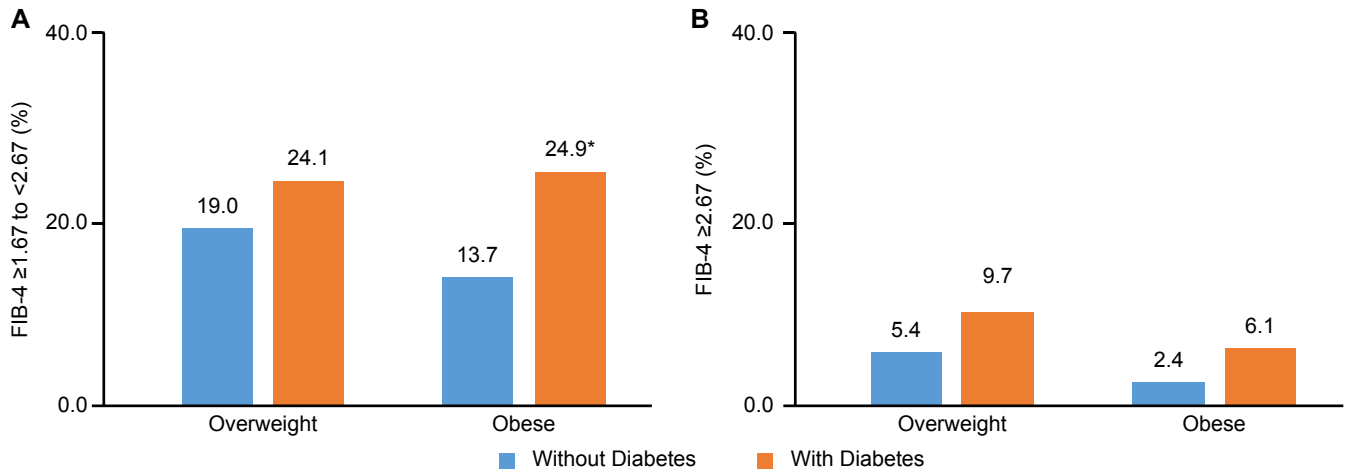
FIGURE 5 (**A**) Proportion of individuals at moderate risk (FIB-4 ≥ 1.67 to <2.67) of having NASH with liver fibrosis (risk of fibrosis stage F2), and (**B**) proportion of patients at high risk (FIB-4 ≥ 2.67) of having NASH with advanced liver fibrosis (risk of fibrosis stage F3 [advanced fibrosis] or F4 [cirrhosis]). *$p < 0.05$. P values were adjusted for multiple comparisons. FIB-4 = fibrosis-4 index; NASH = nonalcoholic steatohepatitis

conclusion (23 patients with overweight and 112 patients with obesity). However, in the age subgroup of 45 to 64 years, more patients with DM compared with those without DM had a FIB-4 ≥ 1.67 in both those with obesity (8.6% vs. 26.3%, respectively; $p < 0.01$) or with overweight (10.4% vs. 17.7%, respectively; $p = 0.303$). Advanced fibrosis (FIB-4 ≥ 2.67) in the age subgroup of 45 to 64 years was also higher when comparing individuals with overweight or obesity with DM versus without DM (5.3% vs. 1.1%), although the result was not statistically significant ($p = 0.109$). In the smaller group of individuals ≥ 65 years old ($n = 479$), the prevalence of moderate-to-advanced fibrosis was not significant, although there was a ~30% greater prevalence of advanced fibrosis in individuals with obesity with DM versus without DM (11.2% vs. 8.8%, respectively; $p = 0.469$).

DISCUSSION

This study offers a novel examination in the US population of the role of DM regarding its association with steatosis and liver fibrosis and considering the confounding role of obesity. The key findings can be summarized as follows: (1) liver steatosis is primarily associated with excessive adiposity (higher BMI) and more modestly associated with DM; (2) type 2 DM (T2DM) promotes advanced fibrosis among individuals who already have overweight or obesity; and (3) common tools used to screen for steatosis (FLI vs. USFLI) and fibrosis (FIB-4 vs. NFS) showed discordant results in individuals with obesity with DM, calling for their critical reappraisal. Collectively, these findings validate the 2021 ADA recommendations (4) regarding the need for screening adults with T2DM for advanced fibrosis in the presence of elevated ALT levels or steatosis.

Although obesity and DM may be associated with a higher risk of steatosis (5,6,15), no prior study, to our knowledge, had separated both risk factors in a large US population to establish their relative impact. A key observation was that the impact of obesity

on steatosis seemed to plateau at a BMI of ~35 kg/m^2 (Figure 2), with modest (by USFLI) or no worsening (by FLI) beyond this point. There was a discordance between USFLI and FLI, the latter apparently "overdiagnosing" steatosis (e.g., ~99% of patients with BMI ≥ 35 having steatosis by FLI). While a confirmatory liver imaging study was unavailable, the reported prevalence of NAFLD using imaging is ~60% in adults with a BMI of 30 to 40 (39) and between ~60% and ~80% in those with BMI ≥ 40 (40,41). Steatosis, assessed by liver biopsy at the time of bariatric surgery, has been reported to be present in 79% (42) to 83% (43) of individuals with a BMI between 46 and 56. Therefore, a prevalence of steatosis by FLI ≥ 90% is highly unlikely in a cohort of less individuals with less severe obesity (Table 2 and Figure 2). This high prevalence with FLI is likely because BMI is part of the FLI index calculation. Therefore, caution must be exercised when using FLI for the diagnosis of NAFLD in populations predominantly of individuals with obesity. In contrast, USFLI appeared to be in better agreement with liver imaging and biopsy prevalence rates. Finally, DM increased the risk of steatosis largely when BMI ≤ 35.0 (Figure 2), whereas only the worse glycemic control increased the proportion of patients with steatosis (HbA1c > 8.0%; Figure 3), as previously described by Portillo et al. (44).

The role of advanced fibrosis as a predictor of future cirrhosis is well established (23,27). Screening for NASH in adults with DM is aimed at identifying those with advanced fibrosis (F ≥ 3) at high risk of cirrhosis (9,24,25). However, because noninvasive diagnostic panels/imaging techniques cannot reliably identify steatohepatitis, clinicians depend on steatosis or ALT to identify patients at risk of developing NASH with fibrosis. Moreover, point-of-care imaging is not widely available in non-hepatology settings, which creates a greater need for serum diagnostic panels to guide referrals to hepatologists. FIB-4 and NFS are the most widely used, but their performance has not been carefully assessed before in a large US population categorized by obesity and DM status. A key finding is that the NFS likely "overreads" the prevalence of

advanced fibrosis (≥F3). The observed 18.9% and 34.1% prevalence of advanced fibrosis in individuals with overweight and obesity with DM, respectively, is higher than in any prior study (20-22) and unlikely to be accurate. Having BMI as well as DM (yes/no) embedded in the NFS calculation appears to be the reason. The prevalence of advanced liver fibrosis (≥F3) in adults with obesity and DM has ranged from ~6% to 20% (20-22,30). The 8.7% prevalence rate of ≥F3 in individuals with obesity with DM in this study is in agreement with reports using magnetic resonance elastography (7%) (20) or liver biopsy (~5%) (13,16) but lower than with elastography (15,16,32). Diagnostic panels may underperform in terms of sensitivity for advanced fibrosis in patients with DM (29,31), with ~40% of individuals falling in an indeterminate zone (≥1.3 and <2.67). This limits its usefulness for primary care referral to a specialist. Recently, Bril et al. (29) identified a FIB-4 cutoff (≥1.67) that could reduce the indeterminate zone to about half, along with reducing the need for referral to a specialist. However, AST ≥ 38 IU/L had a similar specificity. Future studies will need to validate its clinical value. Finally, age ≥ 65 years increases the prevalence of advanced fibrosis, and higher predictive cutoffs (e.g., FIB-4 > 2.0) have been recommended to minimize false positives in older patients. Still, when APRI was used (which does not include age in the equation), results were similar to those reported with FIB-4 (data not shown).

Finally, our results are in line with prior limited available recent data work in patients with T2DM screened in the outpatient setting by vibration-controlled transient elastography (VCTE). Lomonaco et al. (45) reported a prevalence of steatosis of 70% and advanced fibrosis of 9% (liver stiffness measure ≥ 9.7 kPa) in 561 participants with T2DM. In a cohort of patients from the NHANES III undergoing VCTE screening, Ciardullo et al. (46) reported, in 825 patients with T2DM, a prevalence of steatosis of 74% and advanced fibrosis (liver stiffness measure ≥ 9.7 kPa) of 15.4%. The prevalence of steatosis in the DM group in the current study (70.8% by USFLI) was comparable with the aforementioned results (Table 2 and Figure 3). However, that of advanced fibrosis (7.1% by FIB-4 ≥ 2.67) was somewhat lower but comparable with that in an analysis from the same NHANES database from 2005 to 2016 (18), in which the proportion of those with advanced fibrosis was 5.9% when using FIB-4 and higher but similar to ours when using NFS (26.8% compared with 29.8% in our analysis, both likely overestimating the risk of advanced fibrosis). It is likely that a proportion of patients in the subgroup with a FIB-4 ≥ 1.67 to < 2.67 would account for missed patients with fibrosis observed in the higher proportion of patients by VCTE imaging. Of note, a limitation of the study is not having a confirmatory liver biopsy. This is a challenge for any large screening study. It is known that the relatively low sensitivity and PPV of blood-based testing is a limitation of blood-based approaches that calls for complementary strategies for patients at high risk of advanced liver disease (such as individuals with obesity, metabolic syndrome, and/or T2DM).

In summary, this study highlights the additive impact of DM on the development of steatosis and fibrosis for individuals with overweight or obesity, as well as the need for improved diagnostic approaches in the future. Based on the current findings, the ADA guidelines that recommend screening for NAFLD in individuals with elevated ALT or steatosis are a first step in the right direction.●

CONFLICT OF INTEREST

DB has nothing to disclose. EMR is a former employee of AstraZeneca plc. MES is an employee of Evidera, which received consulting fees related to this work. SSS is an employee of MedImmune, LLC. KC has received research support as principal investigator for the University of Florida from Cirius Therapeutics, Echosens, Inventiva, Novartis International AG, Novo Nordisk A/S, Poxel SA, and Zydus Pharmaceuticals, Inc. KC is also a consultant for Allergan plc, Altimmune, Arrowhead Pharmaceuticals, AstraZeneca plc, Bristol Myers Squibb, Boehringer Ingelheim, Coherus BioSciences, Eli Lilly and Company, Fractyl Health, Inc., Hanmi Healthcare, Genentech, Inc., Gilead Sciences, Inc., Intercept Pharmaceuticals, Janssen Pharmaceuticals, Pfizer, Inc., Prosciento, Inc., Madrigal Pharmaceuticals, and Novo Nordisk A/S.

AUTHOR CONTRIBUTIONS

DB contributed to the analysis of the results and to the writing and critical revisions of the manuscript. EMR contributed to the analysis of the results and critical revisions of the manuscript. MES contributed to data analysis and critical revisions of the manuscript. SSS contributed to critical revisions of the manuscript. KC contributed to the study design, analysis of the results, and to the writing and critical revisions of the manuscript. KC is the guarantor of this work and, as such, takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors had access to the data and approved the final version of the manuscript for submission.

PRIOR ABSTRACT PUBLICATION

An abstract of this work has been presented as a poster at the Endocrine Society Annual Scientific Meeting (ENDO) 2019 conference, March 23 through 26, 2019, in New Orleans, Louisiana, and at the European Association for the Study of Diabetes (EASD) 2019 conference, September 16 through 20, 2019, in Barcelona, Spain.

DATA AVAILABILITY STATEMENT

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca plc's data sharing policy described in the following link: https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.