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Noninvasive tests for nonalcoholic fatty liver disease in a multi-ethnic population: The HELIUS study

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is increasing in prevalence and severity globally, prompting noninvasive testing, yet limited data exist on noninvasive liver tests (NITs) including transient elastography (TE) in ethnically diverse populations. Therefore, we studied prevalence and ethnic differences in NAFLD with NITs in the multi-ethnic HEalthy Life In an Urban Setting (HELIUS) cohort. NITs of liver steatosis (Fatty Liver Index [FLI]) and fibrosis (Fibrosis-4 index [FIB-4], and aspartate aminotransferase–to–platelet ratio [APRI]) were assessed in 10,007 participants. A subpopulation of 399 participants, selected on high-risk criteria for NAFLD (obesity, type 2 diabetes mellitus [T2DM], and/or elevated NITs), was examined with TE. FLI was ≥ 60 in 27.3% of 10,007 participants, indicating steatosis. Most participants (71.8%) had FIB-4 < 1.30, excluding advanced liver fibrosis, and 1.1% (n = 113) had high FIB-4 (FIB-4 ≥ 2.67), indicating likely advanced liver fibrosis. In the TE subpopulation, 37.8% and 17.3% had steatosis and fibrosis (continuation attenuation parameter [CAP] ≥ 280 dB/m, liver stiffness measurement [LSM] ≥ 7.0 kPa, respectively). Turkish participants had highest adjusted odds ratio (OR) for elevated LSM (1.72, 95% confidence interval [CI] 0.59–5.01) and Ghanaians the lowest (0.24, 95% CI 0.09–0.65). Ghanaians had lowest adjusted OR for elevated CAP: 0.18 (95% CI 0.09–0.37). In diabetics, CAP and LSM were 17.6% and 14.6% higher than in nondiabetics, respectively. Correlations of FIB-4 and APRI with LSM were absent and weak. *Conclusion*: Liver steatosis proxy FLI was elevated in 27.3% of this multi-ethnic population. In Turkish background and in those with T2DM, proxies for steatosis and fibrosis were high, whereas in Ghanaian background, NITs were generally low. Together, this warrants awareness for NAFLD among high-risk populations, taking ethnic

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background into account. The absence of clear correlation between FIB-4 and APRI with LSM questions the accuracy of these fibrosis NITs to detect advanced fibrosis in the general population.

INTRODUCTION

In concurrence with the increasing prevalence of obesity and type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD) has become the most prevalent liver disease worldwide.[1] The pathophysiology of NAFLD is complex, with visceral obesity and insulin resistance as root causes. The NAFLD spectrum ranges from isolated steatosis to nonalcoholic steatohepatitis (NASH) to liver fibrosis and ultimately cirrhosis and hepatocellular carcinoma.[2] Most patients with NAFLD, up to 80%, have isolated steatosis.[3] Although the group of individuals who progress to fibrosis is smaller than those who remain having isolated steatosis, it is important to diagnose and stage NAFLD. NAFLD fibrosis strongly correlates with liver-related complications and liver-related all-cause mortality. Unfortunately, NAFLD including its advanced fibrotic stages often still remains underdiagnosed.[4] Currently, the gold standard for identifying and staging NAFLD is liver biopsy, yet its invasive nature renders it unsuitable in routine clinical work-up or in most epidemiological studies. The increasing prevalence of NAFLD, including severe fibrotic stages, has prompted international guidelines to recommend early identification of NAFLD fibrosis with noninvasive liver tests (NITs).[5]

Multiple studies have evaluated the performance of Fibrosis-4 index (FIB-4) and aspartate aminotransferase (AST)–to–platelet ratio index (APRI) to identify NAFLD fibrosis.[6,7] In addition to NITs for fibrosis, NITs to assess steatosis have also been developed. Because of wide availability and acceptable sensitivity of ultrasound, these steatosis NITs have not reached clinical care studies, but have shown value in epidemiological research. One of the best characterized and validated steatosis NITs is the Fatty Liver Index (FLI), an algorithm that combines body mass index, waist circumference, and plasma levels of gamma-glutamyltransferase, and triglycerides.[8]

These steatosis and fibrosis NITs were derived and validated in populations of Western descent, but limited data are present from other ethnic backgrounds, constituting a clear knowledge gap, as NAFLD is a global problem affecting all ethnic backgrounds.[9] Two studies from the United States using liver magnetic resonance spectroscopy or liver histology have reported on differences in the prevalence and severity of NAFLD between groups with different ethnic backgrounds. In these, American Hispanics were the most affected ethnic group, whereas African Americans were the least affected with NAFLD.[10]

To increase our understanding of noninvasive assessment of NAFLD in diverse ethnic backgrounds, we conducted the NAFLD In the healthy Life in an urban sEtting (NILE) study, nested in the multi-ethnic HEalthy Life In an Urban Setting (HELIUS) cohort study. Here we report on the differences in NAFLD prevalence and severity between ethnicities and between participants with different risk factors. Finally, we evaluated correlations between different NAFLD NITs.

METHODS

Design of the HELIUS cohort

The HELIUS study is a prospective observational cohort study into the impact of ethnicity on health in adults from different ethnic groups living in Amsterdam, the Netherlands. The detailed design has been published previously.[11] Briefly, adults between 18–70 years of age from multiple ethnic groups were randomly selected from the municipal register, stratified by ethnicity. Included were people from Surinamese (both self-reported African or South-Asian Surinamese), Turkish, Moroccan, Ghanaian, and Dutch descent. Data for HELIUS baseline and follow-up visits were collected by physical examination, blood sampling, and a self-reported questionnaire (including alcohol use, T2DM, and cardiovascular history). All participants provided written informed consent. See the Supporting Information for more details.

Design of the NILE study

The NILE study consisted of two phases and therefore two different populations (see Figure 1 for the design). Phase 1 consisted of the measurement of FLI, FIB-4, and APRI[6–8] in all HELIUS follow-up participants (n = 10,585). More information about these NITs is found in the Supporting Information and Table S1. FLI was categorized into three groups: FLI < 30, FLI 30–59, and FLI ≥ 60 as a proxy for no, potential, and likely liver steatosis, respectively. FIB-4 was categorized in three groups: FIB-4 < 1.30, FIB-4 1.30–2.66, and FIB-4 ≥ 2.67 as a proxy for no, potential, and likely advanced liver fibrosis, respectively. APRI was categorized into two groups: APRI < 0.42 and APRI ≥ 0.42 as a proxy for no and potential advanced liver fibrosis, respectively.

Phase 2, the transient elastography (TE) substudy, consisted of a nested substudy including TE measurement. This was conducted in a selected subpopulation of HELIUS follow-up participants (n = 409). We invited two groups: one at risk of NAFLD (metabolic risk group, n = 346) and one control group (n = 53). Participants were included in the

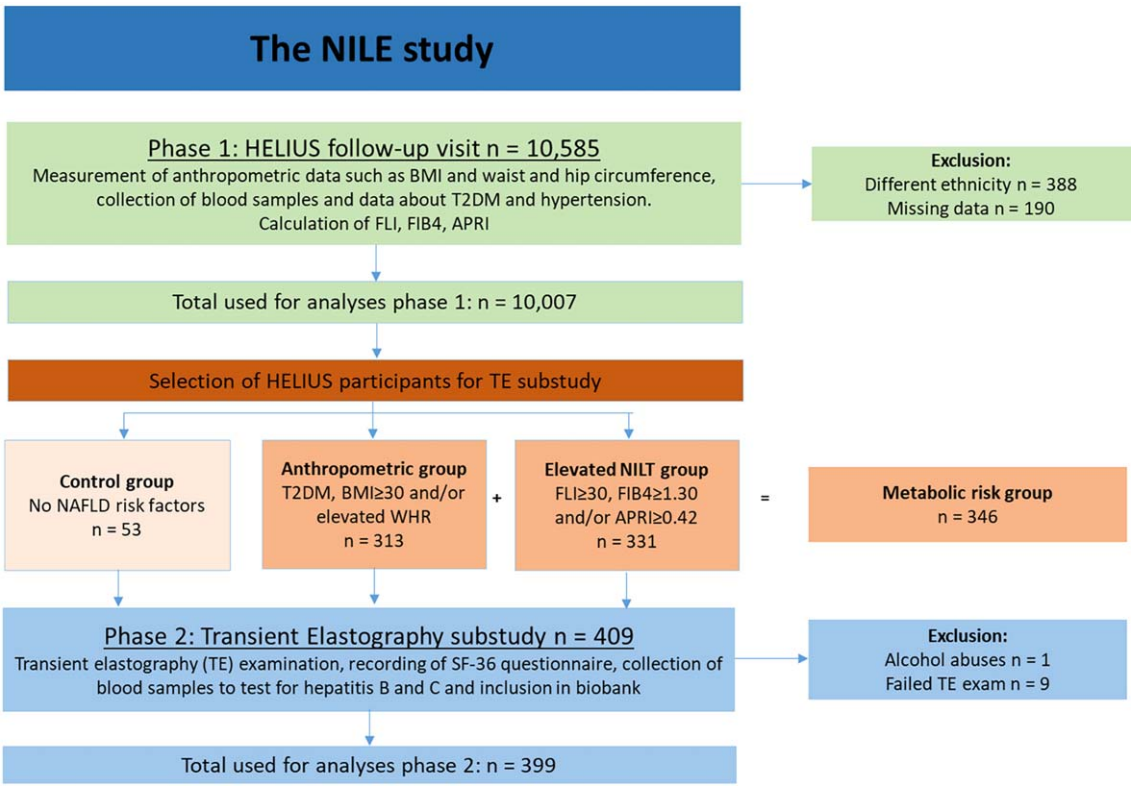

FIGURE 1 Flowchart of the NAFLD In the healthy Life in an urban sEtting (NILE) study. APRI, aspartate aminotransferase–to–platelet ratio index; BMI, body mass index; FIB-4, Fibrosis-4 index; FLI, Fatty Liver Index; HELIUS, HEalthy Life In an Urban Setting; NAFLD, nonalcoholic fatty liver disease; SF-36, 36-Item Short Form Health Survey; T2DM, type 2 diabetes mellitus; TE, transient elastography; WHR, waist–hip ratio

metabolic risk group if one or more of the following criteria, measured during Phase 1, were met: (a) T2DM (self-reported and/or T2DM medication use), (b) obesity (body mass index [BMI] $\geq$ 30 kg/m^2), (c) elevated waist–hip ratio (WHR), (i.e., $\geq$0.90 for men and $\geq$0.85 for women),[12] (d) elevated FLI ($\geq$30), (e) elevated FIB-4 ($\geq$1.30), and/or (f) elevated APRI ($\geq$0.42). The control group included participants who met none of these criteria. Exclusion criteria for the TE substudy were excessive alcohol use, defined as >21 units/week for men and > 14 units/week for women, known history of hepatitis B or C, and use of systemic corticosteroids. HELIUS follow-up participants were checked for the inclusion and exclusion criteria and invited for an additional study visit. Selection was randomized by a computer, stratified for age groups and ethnicity.

TE substudy visits

During the TE visits, a 36-Item Short Form Health Survey (SF-36)[13] was taken; anthropometric data and blood samples were collected; and a TE was performed by an experienced physician (AvD). TE has been validated against liver biopsy for identifying NAFLD steatosis and fibrosis in patients with NAFLD.[14] In addition, blood pressure and heart rate were recorded following standard procedure. Blood samples were collected to be tested for hepatitis B and C and stored in the biobank. Medical history was recorded including hypertension, T2DM, and medication use. TE included controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), recorded on a FibroScan 530 Compact (Echosens) with M or XL probe by guidance of the device's algorithm, in fasted condition ($\geq$2 h), with a minimum of 10 measurements.[14]

Data analyses

Participant characteristics are expressed per ethnicity as percentages, means with SD or medians with interquartile range (IQR), depending on the data distribution. BMI (kg/m^2) was categorized into three categories: normal (BMI 18–25 kg/m^2), overweight (BMI 26–29 kg/m^2), and obesity (BMI $\geq$ 30 kg/m^2). Hypertension was defined as systolic blood pressure $\geq$ 140 mm Hg, diastolic blood pressure $\geq$ 90 mm Hg, or the use of anti-hypertensive medication.

In Phase 1, ethnic differences in NITs were analyzed against the total group (n = 10,007), and in Phase 2 against the total metabolic risk group (n = 346); the control group was not included in the comparisons of Phase 2. Unpaired Student's *t*-test or Mann–Whitney *U*-test was used depending on the data distribution. To adjust for confounding, three logistic regression models (Models A, B, and C) were used, with Dutch ethnicity as reference. All models were adjusted for age, sex, BMI, and T2DM.

These logistic regression models had three different outcome measures: Model A, dichotomous CAP measurement (≥280 dB/m); and Model B, dichotomous LSM (≥7.0 kPa) calculated in the total TE substudy (n = 399). The outcome measure of Model C was dichotomous LSM (≥7.0 kPa) calculated in all TE substudy participants with elevated CAP (≥280 dB/m, n = 151). Outcome measures were presented as adjusted odds ratios (ORs).

Pearson correlation coefficients were used for normally distributed data to calculate the correlation between TE and NITs, BMI, T2DM, and WHR. Where appropriate, skewed data were log-transformed before correlation analysis.

To examine whether ethnic differences could be explained by common risk factors for NAFLD, a stepwise logistic regression model was performed. The first model corrected for age and gender (Model 1); the second model consisted of Model 1 and BMI, T2M, and WHR (Model 2); and the third model consisted of all of these variables and hypertension and CVD 10 years risk according to Framingham (Model 3). These models and comprehensive methods are shown in the Supporting Information. In addition, the Supporting Information provides (1) an analysis comparing NITs between participants with T2DM and elevated BMI, (2) methods to assess relations between SF-36 data and NITs, and (3) a matching approach correcting for age, sex at birth, BMI, and T2DM.

RESULTS

Participant characteristics of the HELIUS follow-up population

We included 10,007 participants in the analysis. Mean age was 52.4 years (SD = 13); 56.7% were female; and 72.2% had another background than Dutch. Of the ethnic minority groups, 81.3% were first-generation migrants. We excluded Javanese Surinamese descent or unknown ethnic background (n = 388) and individuals with insufficient data to calculate NITs (n = 190). Table 1 gives characteristics of the HELIUS follow-up population.

Average BMI in the HELIUS population was 27.3 kg/m^2. A total of 1255 participants had T2DM (12.5%), with highest percentage in South-Asian Surinamese (22.2%). Liver enzymes and triglycerides were on average comparable between the different ethnic groups.

Prevalence and severity of NAFLD as assessed by NITs in the HELIUS follow-up population

In the total population, median FLI was 34.0, whereas 27.3% (n = 2727) had a FLI ≥ 60. In the Turkish subgroup, we observed the highest percentage of FLI ≥ 60: 36.3% (n = 397).

Based on FIB-4, 71.8% (n = 7189) of the participants had no advanced liver fibrosis (FIB-4 < 1.30), whereas 1.1% (n = 113) had advanced fibrosis (FIB-4 ≥ 2.67). In the Ghanaian subgroup, we observed the highest percentage of FIB-4 ≥ 2.67: 2.5% (n = 22). In all ethnic groups, more than 90% of participants were categorized in the low APRI category (APRI < 0.42), except for the Ghanaian subgroup. Mean FIB-4 and APRI were highest in Ghanaian participants (FIB-4 = 1.30 [SD = 0.55]; APRI = 0.31 [SD = 0.15]) compared with the total population. See Figure 2 and Table 1 for more data.

Participant characteristics of the TE substudy

An invitation for the NILE study was sent to 655 HELIUS follow-up participants, of whom 409 (62.4%) individuals responded and went to the TE substudy visit. Of these 409 participants, 9 were excluded due to failed TE measurements and 1 due to excessive alcohol use (> 30 units/week), rendering 399 participants for analysis across all six ethnic groups (median age = 58.0 and 48.6% female). Of these, 53 participants were controls, encompassing all six different ethnicities. The median of liver enzymes (AST, alanine aminotransferase, and GGT were highest in Ghanaian and lowest in Turkish participants). The opposite trend is seen for triglycerides, with the lowest median in Ghanaian (0.77 mmoL/L; IQR 0.54–1.10) and highest median in Turkish participants (1.21 mmoL/L; IQR 0.66–1.54). For an overview of characteristics of the TE substudy (see Table 2).

Participants with T2DM were older than nondiabetic participants (66.0 vs. 56.0 years; $p < 0.001$). FLI was significantly higher in participants with T2DM compared with nondiabetics (70.4 vs. 51.1; $p < 0.001$), whereas FIB-4 and APRI were not significantly different between these groups ($p = 0.473$ and $p = 0.157$, respectively). See Table S2 for more differences between participants with and without T2DM.

The median age of the metabolic risk group (n = 346) was higher compared with control (59.0 vs. 47.0 years; $p < 0.001$). The median FLI was 64 (IQR 35–84) in the metabolic risk group and 12 (IQR 6–20) in the control group ($p < 0.001$). A comparably large difference was seen for FIB-4 as fibrosis proxy with 1.40 (IQR 0.96–1.78) in the metabolic risk group and 0.77 (IQR 0.60–1.11) in the control group ($p < 0.001$). See Table S2 for more differences between participants with and without T2DM, Table 3 for more details about the metabolic risk group, and Table S4 for all differences between metabolic risk and control groups.

TABLE 1 Characteristics of total HELIUS follow-up population

	Dutch	African Surinamese	Ghanaian	Moroccan	South-Asian Surinamese	Turkish	Total
N (%)	2784 (27.8)	2087 (20.9)	896 (9.0)	1518 (15.2)	1627 (16.3)	1095 (10.9)	10,007 (100.0)
Sex, female, n (%)	1458 (52.4)	1320 (63.2)	545 (60.8)	847 (55.8)	935 (57.5)	571 (52.1)	5676 (56.7)
Age, mean (SD)	53.70 (13.26)	55.85 (11.57)	52.32 (10.25)	47.89 (12.28)	52.96 (12.70)	47.53 (11.40)	52.35 (12.58)
Migration generation, NA, n (%)	2784 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2784 (27.8)
First generation	0 (0.0)	1786 (85.6)	864 (96.4)	1118 (73.6)	1301 (80.0)	801 (73.2)	5870 (58.7)
Second generation	0 (0.0)	301 (14.4)	32 (3.6)	400 (26.4)	326 (20.0)	294 (26.8)	1353 (13.5)
BMI, mean (SD)	24.97 (4.06)	28.37 (5.29)	29.48 (4.70)	28.24 (4.78)	26.85 (4.66)	28.84 (5.16)	27.31 (4.99)
T2DM, n (%)	125 (4.5)	295 (14.1)	135 (15.1)	207 (13.6)	361 (22.2)	132 (12.1)	1255 (12.5)
WHR, mean (SD)	0.89 (0.09)	0.91 (0.08)	0.92 (0.08)	0.91 (0.09)	0.94 (0.09)	0.91 (0.09)	0.91 (0.09)
AST, median (IQR)	24 (21, 28)	24 (21, 29)	27 (23, 32)	23 (20, 27)	24 (20, 29)	23 (19, 27)	24 (20, 28)
ALT, median (IQR)	22 (17, 28)	20 (16, 27)	23 (18, 29)	21 (16, 28)	23 (18, 31)	23 (17, 32)	22 (17, 29)
GGT, median (IQR)	19 (14, 28)	25 (18, 35)	28 (21, 38)	20 (14, 29)	21 (15, 32)	21 (14, 32)	22 (15, 32)
Thrombocytes, mean (SD)	262 (60)	273 (68)	248 (67)	266 (64)	281 (66)	271 (63)	268 (64)
Triglycerides, median (IQR)	1.10 (0.73)	0.95 (0.56)	0.78 (0.41)	1.08 (0.59)	1.26 (0.82)	1.33 (0.90)	0.91 (0.64, 1.32)
FLI, mean (SD)	21 (8, 49)	38 (17, 66)	42 (21, 69)	41(17, 65)	36 (16, 63)	44(18, 74)	34 (14, 63)
FLI $<$ 30, n (%)	1697 (61.0)	844 (40.4)	335 (37.4)	604 (39.8)	695 (42.7)	411 (37.5)	4586 (45.8)
FLI $\geq$ 60, n (%)	527 (18.9)	623 (29.9)	297 (33.1)	438 (28.9)	445 (27.4)	397 (36.3)	2727 (27.3)
FIB-4, mean (SD)	1.05 (0.78, 1.38)	1.13 (0.84, 1.47)	1.21 (0.92, 1.57)	0.92 (0.68, 1.22)	0.95 (0.70, 1.29)	0.85 (0.63, 1.09)	1.02 (0.75, 1.35)
FIB-4 1.30–2.66, n, (%)	809 (29.1)	735 (35.2)	351 (39.2)	294 (19.4)	382 (23.5)	134 (12.2)	2705 (27.0)
FIB-4 $\geq$ 2.67, n (%)	25 (0.9)	29 (1.4)	22 (2.5)	14 (0.9)	20 (1.2)	3 (0.3)	113 (1.1)
APRI, mean (SD)	0.23 (0.19, 0.29)	0.23 (0.18, 0.30)	0.28 (0.21, 0.37)	0.23 (0.18, 0.29)	0.22 (0.17, 0.29)	0.22 (0.17, 0.28)	0.23 (0.18, 0.30)
APRI $\geq$ 0.42, n (%)	132 (4.7)	148 (7.1)	139 (15.5)	82 (5.4)	97 (6.0)	50 (4.6)	648 (6.5)

Note: BMI shown in kg/m^2, AST, ALT, and GGT in U/L, thrombocytes in* 10^{e}9/L, triglycerides in mmol/L, CAP in dB/m, and LSM in kPa.
Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; BMI, body mass index; FIB4, fibrosis-4 index; FLI, fatty liver index; GGT, gamma-glutamyltransferase; IQR, interquartile range; NA, not applicable; T2DM, type 2 diabetes mellitus; WHR, waist-to-hip ratio.

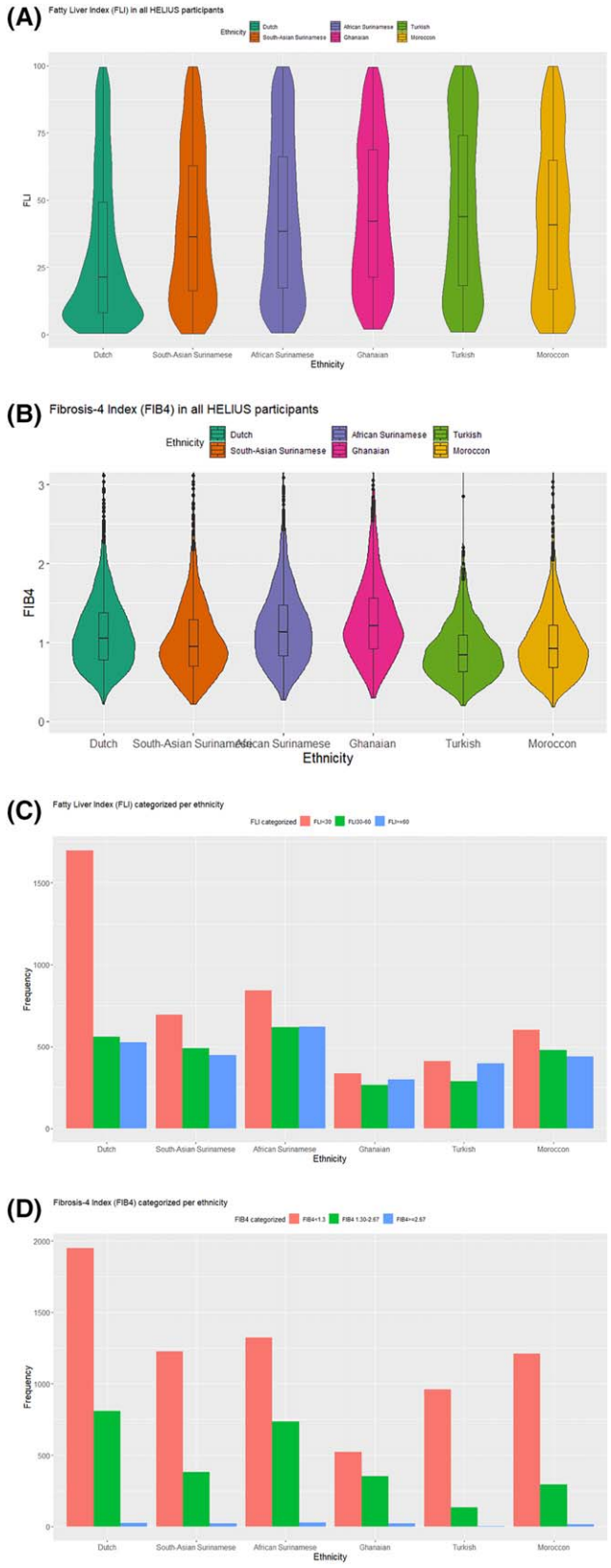

FIGURE 2 Violin plots and bar plots of FLI (A) FIB-4 (B), categorized FLI (C), and categorized FIB-4 (D), respectively, in HELIUS follow-up population stratified by ethnicity; every ethnicity was significantly different from the total group

NAFLD as assessed with NITs in the TE substudy population adjusted for confounders

Dichotomized CAP and LSM (cutoff 280 dB/m and 7.0 kPa, respectively) were corrected for age, gender, BMI, and WHR using logistic regression models. South-Asian Surinamese participants had the highest adjusted OR for elevated CAP (1.30, 95% confidence interval [CI] 0.73–2.32; Table 3, Model A). Turkish participants had the highest adjusted OR for elevated LSM (1.72, 95% CI 0.59–5.01, Model B), followed by South-Asian Surinamese participants (adjusted OR 1.04, 95% CI 0.53–2.01). Ghanaian participants showed the lowest adjusted OR (0.18, 95% CI 0.09–0.37 and 0.24, 95% CI 0.09–0.65) for elevated CAP and LSM, respectively.

Dichotomized LSM adjusted ORs were also calculated for all participants with elevated CAP (n = 151, Model C). In this population, the highest adjusted OR for elevated LSM was found in Turkish participants (adjusted OR 3.19, 95% CI 0.56–18.16) and the lowest in Moroccan participants (adjusted OR 0.38, 95% CI 0.27–3.30).

Severity of NAFLD as assessed by NITs in the metabolic risk group

In the metabolic risk group, 41.3% (n = 143) of the participants had steatosis according to an elevated CAP compared with 15.1% (n = 8) in the control group. In South-Asian Surinamese participants, the median CAP was highest at 288 dB/m (IQR 251–338), whereas in Ghanaian participants, both median FLI and CAP were lowest (50.0, IQR 27.2–69.2 and 245 dB/m, IQR 222–276, respectively). Median FLI was highest in Turkish participants (87.9, IQR 51.9–89.4).

According to high LSM, 17.3% (n = 69) of the participants in the metabolic risk group had indications for liver fibrosis. Among Turkish participants, this percentage was highest at 38.5% (n = 5) and among Ghanaian participants lowest at 6.7% (n = 4). A FIB-4 $\geq$ 2.67 was most common in South-Asian Surinamese participants with 11.8% (n = 9).

Figure 3 shows violin plots of CAP and LSM stratified per ethnicity, including the control group. Clinical characteristics of the metabolic risk group stratified per ethnicity can be found in Table S3.

Stratification for BMI and T2DM of NITs in the metabolic risk group

Compared with the normal weight group, the obesity group had higher median CAP values in all ethnicities. The obese Dutch, South-Asian Surinamese, and Turkish participants had median CAP levels above the cutoff for liver steatosis (312 dB/m, IQR 281–361;

TABLE 2 Characteristics of the participants in the NILE TE substudy (n = 399)

	Dutch	African Surinamese	Ghanaian	Moroccan	South-Asian Surinamese	Turkish	Total group
N (%)	102 (25.6)	90 (22.6)	63 (15.8)	34 (8.5)	89 (22.3)	21 (5.3)	399 (100)
Sex, female, n (%)	50 (49.0)	44 (48.9)	29 (46.0)	16 (47.1)	44 (49.4)	11 (52.4)	194 (48.6)
Age, median (IQR)	58.5 (49.2, 69.8)	59.0 (48.2, 69.8)	58.0 (51.0, 63.0)	53.0 (44.2, 67.2)	60.0 (48.0, 67.0)	46.0 (38.0, 56.0)	58.0 (48.0, 67.0)
BMI, median (IQR)	27.4 (23.8, 32.4)	30.6 (26.9, 34.0)	29.1 (26.6, 32.4)	28.7 (25.7, 32.5)	27.8 (24.3, 31.1)	26.7 (23.6, 34.4)	28.8 (25.0, 32.5)
T2DM, n, (%)	10 (9.8)	16 (17.8)	16 (25.4)	7 (20.6)	22 (24.7)	1 (4.8)	72 (18.0)
WHR, mean (SD)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.9 (0.1)	1.0 (0.1)
AST, median (IQR)[a]	28 (23, 32)	27 (22, 35)	29 (24, 35)	25 (20, 28)	27 (22, 36)	22 (20, 25)	27 (22, 33)
ALT, median (IQR)[a]	27 (19, 36)	25 (18, 36)	27 (20, 35)	22 (17, 28)	26 (19, 38)	19 (15, 34)	25 (19, 35)
GGT, median (IQR)[a]	22 (16, 35)	29 (22, 50)	31 (23, 40)	23 (17, 33)	22 (16, 35)	19 (16, 31)	22 (16, 35)
Thrombocytes, median (IQR)[a]	232 (190, 276)	251 (202, 293)	239 (180, 277)	272 (222, 312)	246 (207, 303)	266 (229, 305)	232 (190, 276)
Triglycerides, median (IQR)[a]	1.06 (0.75, 1.49)	0.96 (0.62, 1.31)	0.77 (0.54, 1.10)	0.89 (0.80, 1.30)	1.07 (0.85, 1.56)	1.21 (0.66, 1.54)	1.06 (0.75, 1.49)
FLI, median (IQR)[a]	49 (21, 87)	68 (40, 82)	50 (25, 69)	54 (28, 83)	45 (23, 81)	27 (14, 89)	56 (24, 82)
FLI < 30, n, (%)	40 (39.2)	17 (19.1)	21 (33.3)	9 (26.5)	30 (33.7)	11 (52.4)	128 (32.2)
FLI ≥ 60, n, (%)	47 (46.1)	53 (59.6)	27 (42.9)	15 (44.1)	38 (42.7)	8 (38.1)	188 (47.2)
FIB-4, median (IQR)[a]	1.39 (0.93, 1.69)	1.35 (0.95, 1.70)	1.40 (1.03, 1.83)	0.94 (0.76, 1.24)	1.41 (0.87, 1.76)	0.70 (0.61, 1.03)	1.39 (0.93, 1.69)
FIB-4 1.30–2.66, n, (%)	49 (48.5)	43 (48.3)	29 (46.0)	7 (21.9)	38 (42.7)	2 (9.5)	168 (42.5)
FIB-4 ≥ 2.67, n, (%)	8 (7.8)	4 (4.4)	7 (10.8)	0 (0.0)	9 (10.1)	0 (0.0)	28 (7.0)
APRI, median (IQR)[a]	0.30 (0.21, 0.45)	0.30 (0.21, 0.43)	0.33 (0.23, 0.46)	0.22 (0.18, 0.31)	0.28 (0.20, 0.47)	0.23 (0.16, 0.29)	0.30 (0.21, 0.45)
APRI ≥ 0.42, n, (%)	30 (29.4)	24 (26.7)	23 (36.5)	2 (5.9)	30 (33.7)	1 (4.8)	110 (27.6)
CAP, median (IQR)	266 (219, 319)	258 (219, 298)	243 (218, 276)	258 (213, 302)	280 (245, 320)	244 (219, 281)	261 (222, 306)
CAP ≥ 280, n, (%)	44 (43.1)	30 (33.3)	14 (22.2)	13 (38.2)	44 (49.4)	6 (28.6)	151 (37.8)
LSM, median (IQR)	4.9 (4.0, 6.4)	5.0 (4.1, 6.0)	4.5 (3.9, 5.2)	5.3 (4.2, 6.5)	4.9 (4.0, 6.6)	5.0 (4.0, 6.9)	4.90 (4.00, 6.20)
LSM ≥ 7.0, n, (%)	21 (20.6)	16 (17.8)	4 (6.3)	6 (17.6)	17 (19.1)	5 (23.8)	69 (17.3)

Note: BMI is shown in kg/m2, AST/ALT/GGT in U/L, thrombocytes in* 10e9/L, triglycerides in mmol/L, CAP in dB/m, and LSM in kPa.
Abbreviations: ALT, alanine aminotransferase; APRI, AST to platelet ratio; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; FIB4, fibrosis-4 index; FLI, fatty liver index; GGT, gamma-glutamyltransferase; LSM, liver stiffness measurement; T2DM, type 2 diabetes mellitus; TE, transient elastography; WHR, waist-to-hip ratio.
[a]Blood values measured during the HELIUS follow-up visit.

TABLE 3 Logistic regression to assess prevalence and severity of NAFLD as assessed by NITs, with Dutch ethnicity as reference (all models are adjusted for age, gender, BMI, and T2DM)

	Adjusted odds ratio Model A	CI 95%	Adjusted odds ratio Model B	CI 95%	Adjusted odds ratio Model C	CI 95%
Intercept	0.00	0.00–0.00	0.00	0.00–0.00	0.00	0.00–0.00
South-Asian Surinamese	1.30	1.30–0.73	1.03	0.53–2.01	1.24	0.50–3.12
African Surinamese	0.29	0.16–0.53	0.62	0.32–1.20	1.94	0.75–5.01
Ghanaian	0.18	0.09–0.37	0.24	0.09–0.65	0.69	0.03–1.11
Moroccan	0.46	0.20–1.05	0.74	0.30–1.86	0.38	0.27–3.29
Turkish	0.35	0.12–1.05	1.72	0.59–5.01	3.19	0.56–18.16
Age	1.00	1.00–1.01	1.03	1.01–1.05	1.03	1.00–1.06
Sex	1.95	1.27–2.99	2.02	1.23–3.33	5.11	2.32–11.25
BMI	1.28	1.22–1.35	1.16	1.10–1.21	1.20	1.11–1.31
T2DM	3.44	2.03–5.85	1.38	0.77–2.45	1.79	0.83–3.85

Note: Model A: logistic regression for CAP (cutoff ≥ 280 dB/m) in the TE substudy (n = 399); Model B: logistic regression for LSM (cutoff ≥ 7 kPa) in the TE substudy (n = 399); Model C: logistic regression for LSM (cutoff ≥ 7 kPa) in participants with elevated CAP (n = 151).
Abbreviation: NIT, noninvasive liver test.

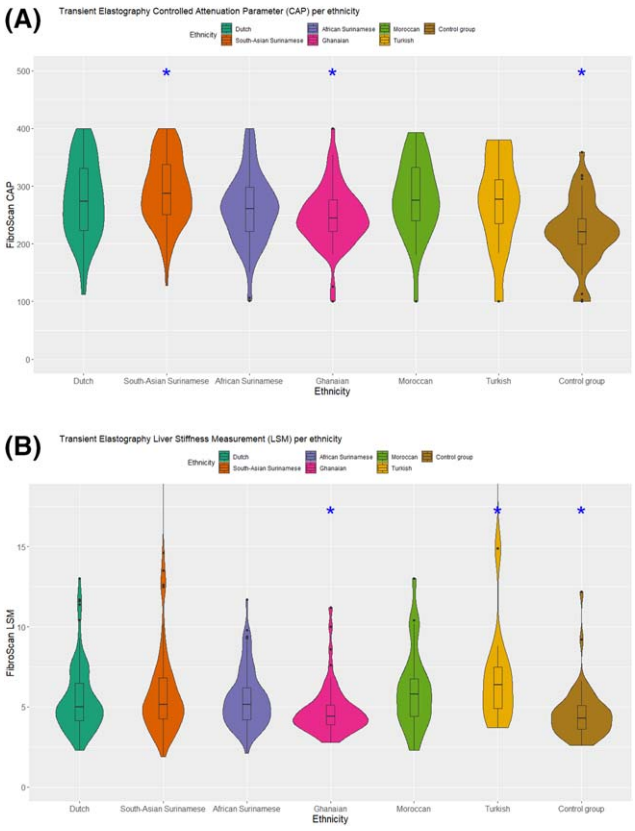

FIGURE 3 Violin plot of controlled attenuation parameter (CAP) (A) and liver stiffness measurement (LSM) (B) in the TE substudy participants stratified per ethnicity, including the control group. Significance was calculated per ethnicity in comparison with the metabolic risk group (n = 346). In addition, the median CAP and LSM in the control group were both significantly different from the metabolic risk group. *Indicates significant difference compared with metabolic risk group (n = 346)

296 dB/m, IQR 276–355; and 281 dB/m, IQR 258–337; respectively).

Median CAP and LSM scores were higher in participants with T2DM compared with nondiabetics (CAP = 300 dB/m, IQR 259–362 vs. 260 dB/m, IQR 227–302, respectively; and LSM = 5.6 kPa, IQR 4.5–7.0 vs. 4.9 kPa, IQR 4.1–6.2, respectively). Within the group of participants with T2DM, median CAP (367 dB/m, IQR 323–391) and LSM (7.40 kPa, IQR 5.95–7.80) were highest in the Dutch participants. The median LSM in the Dutch participants was already above the cutoff for liver fibrosis.

The Ghanaian participants without T2DM had the lowest median CAP and LSM (245 dB/m, IQR 224–270 and 4.35 kPa, IQR 3.85–5.10, respectively). See more details of stratification for T2DM in Figures S1 and S2.

Interrelations between NITs and biometric data

FIB-4 and LSM were not correlated, whereas APRI and LSM were significantly, but weakly correlated ($R = 0.15$, $p = 0.002$). CAP and LSM both correlated with BMI ($R = 0.45$, $p < 0.001$ and $R = 0.26$, $p < 0.001$, respectively), with waist circumference ($R = 0.52$, $p < 0.001$ and $R = 0.32$, $p < 0.001$, respectively), with WHR ($R = 0.31$, $p < 0.001$ and $R = 0.28$, $p < 0.001$, respectively), and, remarkably, with plasma triglycerides ($R = 0.41$, $p < 0.001$ and $R = 0.20$, $p < 0.001$, respectively).

DISCUSSION

This study investigated the prevalence and severity of NAFLD in a multi-ethnic population by studying NITs for

liver steatosis and fibrosis. Three notions stand out. First, liver steatosis in this multi-ethnic adult population was highly prevalent. This was highest in the metabolic risk group of the TE substudy: 41.3%, with 17.3% concomitant prevalence of liver fibrosis.

Second, we found noticeable differences in NITs across the different ethnic groups. Despite a higher-than-average prevalence of obesity and T2DM, Ghanaian participants had lowest steatosis scores, based on both FLI in the Ghanaian subgroup of the entire HELIUS cohort and CAP within the TE substudy population. After adjustment for age, sex, BMI and T2DM, we observed a trend of Ghanaian participants having the lowest ORs for both elevated CAP and LSM. In contrast, Turkish participants had the highest FLI scores in both the entire HELIUS population and in the metabolic risk group. Although the 95% CIs were large for all of the adjusted ORs, a trend was observed for Turkish participants having the highest adjusted OR for LSM, compared with the Dutch participants.

The third main finding was the absence of correlation between LSM and FIB-4 within the total TE substudy group and within the different ethnic subgroups. For example, while Turkish participants had the highest LSM, they had the lowest FIB-4 scores in both the total HELIUS and the TE substudy group. This may imply that FIB-4 has variable diagnostic value across different ethnicities.

High prevalence of NAFLD steatosis and fibrosis

In the HELIUS population, 27.3% of the participants had steatosis with FLI $\geq$ 60, which was comparable to the Dutch population–based Lifelines Cohort Study with 167,729 participants, reporting a prevalence of 22.0%. However, in the Lifelines Cohort Study, no participants with migration background were included.[15] Fortunately, most participants diagnosed with NAFLD have isolated steatosis. However, the detection of NAFLD remains important for early detection of NASH and/or fibrosis.[3] According to a FIB-4 $\geq$ 2.67, 1.1% of the HELIUS participants were likely to have advanced liver fibrosis. The National Health and Nutrition Examination Survey performed in the United States (1999–2016) had similar results with prevalence of FIB-4 $\geq$ 2.67 of 1.4%.[3]

HELIUS participants with T2DM had higher median FLI compared with nondiabetics in all six ethnicities. This fits with the notion of insulin resistance of visceral and peripheral adipose tissue as a consequence of calorie overload. Indeed, several studies show that insulin resistance and compensatory hyperinsulinemia result in increased hepatopetal flux of free fatty acids and increased *de novo* lipogenesis, respectively.[16] This relation and our findings warrant awareness among diabetes health care professionals to keep in mind silent NAFLD progression toward advanced fibrotic stages.

Differences in NAFLD between ethnicities

Interestingly, despite highest BMI and high prevalence of T2DM, Ghanaian participants tended to have lowest liver steatosis and fibrosis. However, it should be noted that the 95% CI of these adjusted ORs are large. In line, prevalence of NAFLD, as assessed with ultrasound, was 24% in African Americans compared with a higher prevalence in European Americans and Hispanics of 33% and 45%, respectively, in an American population study.[17] The mechanisms underlying these ethnic prevalence differences are incompletely understood due to lack of well-characterized multi-ethnic cohort studies. Ancestry can play a role in ethnic differences in the occurrence of steatosis. Romeo et al. previously found that the phospholipase domain-containing 3 (PNPLA3) gene was strongly associated with increased liver fat content. The PNPLA3 variant S453I—protective against liver fat accumulation—was more prevalent in African Americans (Minor allele frequency = 0.104) when compared with European Americans.[18]

Interestingly, our study had two different groups with African background: African Surinamese and Ghanaians. In contrast to Ghanaians, African Surinamese participants in our study did not have less NAFLD steatosis and fibrosis. We have no explanation for this difference, yet it raises two hypotheses. First, these groups may differ in average dietary pattern, in particular Western diet, in turn affecting risk for obesity and NAFLD. Indeed, a dietary study in HELIUS showed that African Surinamese participants scored high in the consumption of sugar-sweetened beverages, enriched in fructose, a known inducer of hepatic steatosis.[19] In contrast, a study among the Ghanaian population in European cities including Amsterdam showed a highly diverse diet that may protect from NAFLD.[20] When following this notion that African background may offer relative protection against severe stages of NAFLD, another explanation for the disparity in NAFLD prevalence in African Surinamese versus Ghanaian participants might pertain to a potentially more mixed background in African-Surinamese participants. Dutch, South-Asian, and native American admixture is known to be present, and these backgrounds may offer relatively less protection against NAFLD. Further studies including genetics may shed light on these apparent differences and could point to new protective genetic factors for NAFLD and even leads for therapy.

No correlation between FIB-4 and LSM, yet positive findings for WHR

Another notable finding was the absence of correlation between LSM and FIB-4, both in the total TE substudy population and within the ethnic subgroups. In participants from African descent, the levels of LSM and FIB-4 showed almost an opposite trend. Although Ghanaian participants scored lowest on fibrosis according to LSM, they had highest FIB-4 and APRI values. In addition, African-Surinamese participants of the TE substudy scored second highest on median FIB-4 and APRI. We have no explanation for the discrepancy between fibrosis measured with LSM and with FIB-4 and APRI, yet in concordance a recent study also reported a poor correlation between LSM and FIB-4 in populations at risk for NAFLD. This study suggested waist circumference to be a better first screening tool to identify high liver stiffness and fibrosis in at-risk populations.[21] This is in line with visceral adipose tissue as the root cause of NAFLD.[22] Our TE substudy supports this notion, with significant correlations between waist circumference as well as WHR and both CAP and LSM. Interestingly, WHR also had the highest precision in predicting T2DM in the HELIUS population.[23] Together, this warrants caution in relying on FIB-4 as a first-line test for NAFLD in clinical care paths, especially in ethnically diverse populations. Second, it may suggest that waist circumference and WHR can be potential tools to screen for liver steatosis and even fibrosis.

Strengths and limitations

Studies of NAFLD in population-based studies with different ethnicities from across the globe are scarce. Our large and diverse study makes a robust contribution to this clinically relevant topic. We assessed the general population for NAFLD using NITs. In addition, we studied an at-risk subpopulation using TE. In the control group of this substudy, we included all six ethnicities to provide a representative comparator to our metabolic risk group.

Limitations include absence of histological assessment of NAFLD, yet this is ethically and technically implausible in such large population studies. All used NITs have been derived in original studies against NAFLD liver histology, the current gold standard, and have acceptable diagnostic accuracy. However, they are not equally accurate as this gold standard, which necessitates some caution when interpreting our findings. Indeed, we do not provide histological validation of diagnostic accuracy of NITs in different ethnic backgrounds, which remains a clear knowledge gap in the field. Our manuscript does provide prevalence of elevated NITs in different ethnic populations, another clear knowledge gap in the field, under the reasonable assumption that NITs perform comparably well in different ethnic populations.[6,7,14] Another limitation was the relatively high mean age of the metabolic risk and diabetics groups compared with controls, in the TE substudy, which might have resulted in overestimation of severe cases of NAFLD. In the TE substudy, Moroccan and Turkish participants were relatively underrepresented, which could have caused insufficient power to show differences between these ethnic groups. For example, the 95% CIs were large in the logistic regression models in which we adjusted for confounders. Furthermore, we recommend future research into the differences in NAFLD prevalence and severity in multi-ethnic populations with histological validation.

CONCLUSIONS

The high prevalence of liver steatosis and fibrosis, especially in the presence of cardiometabolic risk factors, warrants clinical awareness for NAFLD among high-risk populations in general practice and endocrinology practices. The absence of clear correlation between FIB-4 and APRI with LSM questions the accuracy of these fibrosis NITs to detect advanced fibrosis in a multi-ethnic general population. Differences in NAFLD between ethnic backgrounds, especially the indications for less NAFLD in Ghanaian participants and potentially more NAFLD in Turkish participants, have consequences for clinical management of NAFLD. Guidelines and screening strategies for NAFLD should be mindful of potential differences in ethnic risk profiles.

AUTHOR CONTRIBUTIONS

Study design: AMD and AGH. *Protocol draft preparation*: AMD, AGH, and HG. *Manuscript draft*: AMD. *Statistical analyses and interpretation*: AMD and HG. *Design of the HELIUS study*: HG and BJB. All authors reviewed and critically revised the manuscript.

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CONFLICT OF INTEREST

Nothing to report.

DATA AVAILABILITY STATEMENT

The HELIUS data are owned by the Amsterdam University Medical Centers. location AMC in Amsterdam, the Netherlands. Any researcher can request the data by submitting a proposal to the HELIUS Executive Board as outlined at http://www.heliusstudy.nl/en/researchers/collaboration (email: heliuscoordinator@amsterdamumc.nl). The HELIUS Executive Board checks proposals for compatibility with the general objectives, ethical approvals, and informed consent forms of the HELIUS study. There are no other restrictions to obtaining the data, and all data requests will be processed in the same manner.

ETHICS APPROVAL STATEMENT

The HELIUS study has been approved by the institutional review board of the AMC at the University of Amsterdam.

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