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Non-alcoholic fatty liver disease presence and severity are associated with aortic stiffness beyond abdominal obesity: The ELSA-Brasil

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HIGHLIGHTS

- There is a cardiovascular risk (CV) gap beyond traditional risk factors.
- Non-alcoholic fatty liver disease (NAFLD) is independently related to CV risk.
- NAFLD association with pulse wave velocity (PWV) is controversial.
- NAFLD is associated with PWV across race/ethnic diverse ELSA-Brasil population.
- The worldwide CVD burden of disease related to NAFLD is potentially relevant.

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ABSTRACT

Background and aims: It is uncertain whether non-alcoholic fatty liver disease (NAFLD) is associated with aortic stiffness in a racial/ethnically diverse and admixed society. We addressed whether NAFLD presence and severity were associated with carotid-femoral pulse wave velocity (cf-PWV) in individuals free of cardiovascular disease.
Methods: In 7196 individuals free of cardiovascular disease at the baseline Brazilian longitudinal study of adult health, we classified NAFLD presence and severity (mild, moderate and severe) by ultrasound hepatic attenuation. We measured cf-PWV using a non-invasive validated device (Complior SP, Artech Medicale France). We compared cf-PWV of NAFLD individuals to those without by analysis of covariance adjusted for demographics, life style, waist circumference, and arterial pressure. We also analyzed the cf-PWV trend from no-NAFLD to severe NAFLD.
Results: In unadjusted analysis, from no-NAFLD to severe NAFLD, there were slightly older individuals, lower frequency of smokers, more prevalent diabetes and hypertension. In adjusted analysis, there was slightly higher cf-PWV (m/s) (95%CI) in NAFLD *vs.* no-NAFLD, respectively 9.32 (9.22, 9.41) and 9.24 (9.15, 9.33) ($p = 0.037$). Across NAFLD severity spectrum, adjusted cf-PWV increased from 9.24 (9.15, 9.33) in no-NAFLD to 9.69 (9.46, 9.93) in severe NAFLD (p for trend association = 0.001). In sensitivity analysis, diabetes adjustment nullified the association of binary NAFLD with cf-PWV, but not that of increasingly severe NAFLD.
Conclusions: In racial/ethnically diverse individuals free of cardiovascular disease, NAFLD is associated with aortic stiffness beyond abdominal obesity. The specific NAFLD impact on CVD worldwide is potentially relevant.

1. Introduction

Cardiovascular disease (CVD) is the leading cause for death and disability around the world [1]. Despite the major contribution of traditional risk factors to CVD burden, 10–15% of individuals with manifest CVD have no risk traditional factors [2]. It is of great interest to unveil subclinical and underappreciated pathways that may underlie this CVD risk gap.

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent hepatic disease worldwide, which affects approximately 15–30% of the

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general adult population [3]. Given CVD represents its most frequent related morbid-mortality [4], CVD burden related to NAFLD is potentially enormous. However, NAFLD is highly correlated with insulin resistance, incident diabetes, traditional and novel cardiovascular risk factors, and metabolic syndrome [5–7]. NAFLD has been associated with incident CVD apart from dysglycemia, atherogenic dyslipidemia, high blood pressure and obesity [8–10]. Furthermore, severe NAFLD has been associated with even higher risk of incident fatal and non-fatal CVD events [11]. Given that, it is of great interest to address specific pathophysiological mechanisms underlying NAFLD association with CVD.

Augmented aortic stiffness derives from a structural modification in large arteries, in which disrupted turnover favors collagen accumulation over elastin. Carotid femoral pulse wave velocity (cf-PWV), its gold standard measurement, is associated with incident CVD events above and beyond traditional risk factors [12–15]. However, too little is known about cf-PWV determinants beyond age ad blood pressure [16–18]. Previous studies have found that NAFLD is associated with cf-PWV in relatively small or highly selected Western populations from specialty hospital services [7,19–21]. In the Framigham population, a community-based population, there was no association between NAFLD and cf-PWV [22]. In China and Korea population-based studies, there have been more consistent findings on the association of NAFLD with brachial ankle pulse wave velocity (ba-PWV) [23–26], another valid measure of aortic stiffness. Therefore, the association of NAFLD with cf-PWV seems especially controversial in Western populations.

NAFLD is highly prevalent worldwide and it is paramount to unveil whether it is associated with aortic stiffness across diverse race/ethnicities. The ensuing CVD burden on the population might be substantial. We elected to address whether NAFLD presence and severity is associated with cf-PWV beyond obesity in the racial/ethnically diverse and admixed ELSA-Brasil population.

2. Materials and methods

2.1. Study population

This is a cross-sectional study addressing the NAFLD association with cf-PWV in the baseline ELSA-Brasil (The Brazilian Longitudinal Study of Adult Health) population, a prospective cohort of 15,105 civil servants aged 35 to 74 living in 6 cities in Brazil [27]. All employees and retired individuals of the participating institutions were initially eligible, except for those pregnant at enrollment or in the last 4 months, with severe cognitive or communication impairment; or residing outside study's center corresponding metropolitan area. Further details of this cohort were described elsewhere [27–30]. All the Institutional Review Boards of the participating institutions approved the protocol, which conforms to the ethical guidelines of the 1975 Declaration of Helsink, and all participants provided informed consent.

We excluded those with: self-referred hepatitis, alcohol consumption above 210 g/week for men or 140 g/week for women, steroids usage, body mass index below 18.5 kg/m^2, previous myocardial infarction, angina pectoris, stroke or cardiac surgery and those with extreme cf-PWV values (less than 4 m/s or over 20 m/s) to avoid potential measurement errors (Fig. 1).

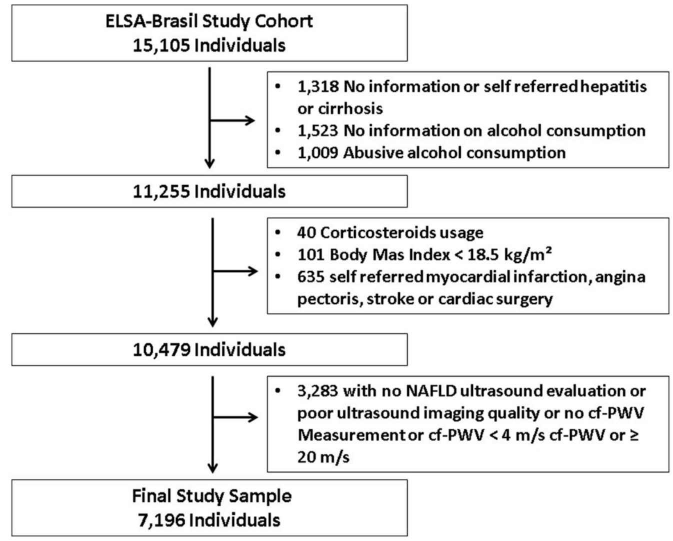

Fig. 1. Study population flow chart.

2.2. Data collection

Demographic, anthropometric, life-style and self-described race/ethnicity data were collected on site by questionnaires and clinical exam between August 2008 and December 2010 [28]. Height, weight and waist circumference (WC) (cm) were measured according to standard techniques, and BMI was calculated by dividing body weight by squared height in meters (kg/m^2). Leisure-time physical activity was classified according to the frequency and intensity by the International Physical Activity Questionnaire (IPAQ) [31], in which physically active were those with at least 150 min of moderate-intensity, or 75 min of high-intensity leisure-time aerobic physical activity, or proportionally equivalent of both intensity regimens each week. Any weekly activity below that threshold was classified as partly active, and those with no activity as sedentary.

We asked patients to avoid consuming alcohol and caffeinated beverages, and abstain from exercising within 12 h before the exams. Immediately before acquiring cf-PWV and after 5 min rest, blood pressure was measured three times with 1 min interval in the sitting position. The mean of the 2 last measurements defined blood pressure value. Mean arterial pressure was defined by the formula (systolic arterial pressure + 2*diastolic arterial pressure)/3. Hypertension was defined by blood pressure above 140/90 mmHg or pharmacologic antihypertensive treatment. Diabetes was defined by self-referred disease or routine use of diabetes medication, fasting plasma glucose ≥126 mg/dL, glycated hemoglobin (HbA1c) ≥ 6.5%, or 2-h plasma glucose during the oral glucose tolerance test ≥ 200 mg/dL.

Blood samples were drawn from participants in fasting state. Glomerular filtration rate was estimated according to CKD EPI calculation. Gamma-glutamyltransferase (GGT) was measured by kinetic colorimetric assay; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by enzymatic assay (ADVIA Chemistry); total cholesterol, high-density cholesterol and triglycerides by the enzymatic colorimetric method. Low-density cholesterol was calculated by the Friedewald equation if triglycerides ≤400 mg/dL or directly measured by enzymatic colorimetric method otherwise; glucose was measured by hexokinase method (ADVIA Chemistry), glycated hemoglobin (HbA1c) by high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, California); and high sensitivity C reactive protein (hs-CRP) by immunochemistry nephelometry (BN II Siemens® nephelometer).

2.3. Hepatic imaging and NAFLD classification

Ultrasound imaging is a non-invasive, relatively accessible and reliable method for classifying and grading NAFLD severity [32,33]. Despite better diagnostic accuracy of computed tomography and magnetic resonance for NAFLD, they are not as suitable for epidemiological studies compared with ultrasound due to their costs, limited access and adverse events. The most common ultrasonography parameters for assessing NAFLD are parenchymal brightness, deep-beam attenuation, vascular blurring of portal veins, and compared echogenicity between liver and kidney (hepatorenal index). Among them, we have chosen hepatic deep-beam attenuation due to its better diagnostic performance as previously described [34].

Images were acquired by board-certified radiologists or trained radiology technicians in a high-resolution B-mode scanner (SSA-790A, Aplio XG, Toshiba Medical System) and a convex array transducer (model PVT-375BT), using a central frequency of 1.9–5.0 MHz [34]. Image acquisition was standardized and obtained on the antero-posterior axis of the right hepatic lobe including the gallbladder, inferior vena cava and hepato-diafragmatic angle. The images were all read at the ELSA-São Paulo site by board-certified radiologists in a workstation using the IMAGE ARENA –TOM TEC software®, and additionally crosschecked by a senior ultrasound radiologist according to the quality control protocol previously described [34]. From the 10,479 eligible individuals according to the inclusion and exclusion criteria, 7196 had acceptable, good or excellent imaging quality and cf-PWV values within 4.1 and 20.0 m/s (Fig. 1).

We defined NAFLD presence and severity according to deep-beam hepatic attenuation [34]. Visualization of the diaphragm posterior to the right hepatic lobe: normal (complete viewing), mild NAFLD (> 50% viewing of the diaphragm), moderate NAFLD (< 50% viewing of the diaphragm), and severe NAFLD (no viewing of the diaphragm).

Finally, for comparing the effect of NAFLD classification method on the associations, we did the same analysis based on fatty liver index (FLI) [35,36]. As described by Bedogni et al., it infers NAFDL based on body mass index, waist circumference, triglycerides and gama glutamil transferase [36]. For binary classification, we used the score 60 as the threshold. For assessing NAFLD severity there is no FLI based classification equivalent to the ultrasound method described herein. Due to that we chose the rule in and rule out NAFLD thresholds (respectively ≥60 and < 30 score) for classifying it in three probability categories (low, intermediate and high). In the one hand, the aforementioned classification is not comparable to the ultrasound severity classification described. Even though, we preferred to use these standard FLI parameters on the literature instead of arbitrarily defining an equivalent to the ultrasound severity classification.

2.4. Carotid femoral pulse wave velocity

The cf-PWV was measured by using a validated automated device (Complior, Arthech Medicale, France) after resting in a supine position in a temperature-controlled room between 20° and 24° Celsius according to ELSA-Brasil protocols [17,31]. For cf-PWV measurement sensors were located at the right carotid and femoral arteries for pulse wave detection by the computer software. An inelastic tape was used to measure the distance of the sternum furcula to the right femoral site where the pulse was recorded. The cf-PWV was calculated by dividing the distance from the suprasternal furcula to the femoral pulse measurement site over the time delay between carotid and femoral pulse waves, quantified in meters per second (m/s). The final cf-PWV value was obtained by the arithmetic average of 10 consecutive cardiac cycles in regular heart rhythm. Data were acquired locally in each of the 6 ELSA-Brasil sites and validated at a centralized reading center for all participants [31].

2.5. Statistical analysis

We grouped participants according to the presence and severity of NAFLD: no-NAFLD, mild, moderate, and severe NAFLD. Their demographics, clinical and laboratorial characteristics were described by median (interquartile range) for continuous variables and by frequency (percentage) for categorical ones. Given the ordinal nature of NAFLD severity groups, we run trend analysis by using Jonckheere-Terpstra for continuous variables and Cox-Mantel-Haenszel for categorical ones. As race/ethnicity is not an ordinal variable, we tested it by χ square test.

In order to address the independent association of NAFLD presence and severity with cf-PWV, we run adjusted linear regression model by means of PROC GLM command by using SAS 9.4. We run analysis for trend along the NAFLD severity gradient. Models were progressively adjusted for; model 1: age, sex, race/ethnicity. Model 2, model 1 variables plus mean arterial pressure and anti-hypertensive use. Model 3, model 2 variables plus smoking habit, current alcohol consumption, physical activity, familial history of premature coronary heart disease, and waist circumference. In order to address the potential mediators in the association, we further adjusted model 3 by adding the following variables each at a time: diabetes, HDL-cholesterol and triglycerides (natural log), high sensitivity C reactive protein (natural log), and uric acid. We further adjusted the sensitivity models by exchanging HbA1c for diabetes, and BMI for waist circumference in fully adjusted models. We tested for effect modification according to demographic, anthropometric and cardiovascular risk factors strata as described by Harada et al. [35]. All analyses were run by SAS version 9.4 (SAS Institute, Cary, NC) and statistical significance set at two-tailed p value < 0.05.

3. Results

Among the 7196 individuals from the overall sample, unadjusted median (interquartile) and frequency (%) characteristics were: age, 50 years (45–57); women, 3995 (55.5%); White, 4058 (57.0%); Brown, 1778 (25.0%); Black, 1043 (14.6%); Asian, 181 (2.5%), Indigenous, 66 (0.9%). NAFLD was present in 2625 (36.5%), being 1634 (22.7%) with mild NAFLD, 845 (11.7%) with moderate and 146 (2.0%) with severe NAFLD. The median (interquartile) cf−PWV value was 8.90 m/s (8.10–10.00).

From no-NAFLD to severe NAFLD, individuals were slightly older, but presented no race/ethnicity differences, had higher BMI and waist circumference, more prevalent sedentary behavior, but less active smokers (Table 1). Still on the same direction, there was increasing prevalence of hypertension, diabetes, lower HDL-c and higher triglycerides, namely worsening insulin resistant dyslipidemia. Increasingly severe NAFLD presented higher ALT, GGT, uric acid, and hs-CRP. In the binary NAFLD ultrasound classification, 3681 (80.5%) of no-NAFLD had the same status based on FLI. Besides that, 1464 (55.8%) of ultrasound based NAFLD had congruent classification by FLI.

In unadjusted analysis, NAFLD presented higher cf-PWV in meters per second (m/s) (95%CI) compared with no-NAFLD ($p < 0.001$), respectively 9.61 m/s (9.54, 9.68) and 9.03 m/s (8.98, 9.08) (Table 2). In age, sex and race/ethnicity adjusted comparison the difference persisted; NAFLD cf-PWV, 9.59 m/s (9.49, 9.69) versus no-NAFLD 9.21 m/s (9.12, 9.30) ($p < 0.001$). Further adjusting for arterial pressure, anti-hypertensives, life style, familial history of coronary heart disease, and waist circumference (model 3), cf-PWV difference was attenuated, nut not nullified, 9.32 (9.22, 9.41) for NAFLD *versus* 9.24 m/s (9.15, 9.33) for no-NAFLD ($p = 0.037$).

In sensitivity analyzes for mediators, the association was nullified by diabetes ($p = 0.224$) and insulin resistant dyslipidemia, namely HDL cholesterol and triglycerides ($p = 0.073$) (Table 2). In sensitivity analysis, by exchanging HbA1c for diabetes or BMI for WC results were similar (data not shown).

From no-NAFLD to increasingly severe NAFLD there was higher unadjusted cf-PWV; no-NAFLD cf-PWV, 8.70 m/s (7.90–9.80) and severe NAFLD was 10.00 m/s (9.10–11.10), p for trend < 0.001 (Table 3). In age, sex and race/ethnicity adjusted models cf-PWV ranged from 9.21 m/s (9.12, 9.3) for no-NAFLD to 10.26 m/s (10, 10.51) for severe NAFLD (Model 1, p for trend < 0.001). Further adjusting for arterial pressure, anti-hypertensives, life style, familial history of coronary heart disease, and waist circumference (model 3), attenuated but did not nullify the aforementioned trend ($p < 0.001$).

In sensitivity analyzes further adjusting one each a time for; diabetes, HDL-c/triglycerides, hsCRP and uric acid the association between NAFLD severity and cf-PWV was not nullified (Table 3). By exchanging BMI for waist circumference or HbA1c for diabetes did not change the results (data not shown).

In stratified analyzes for binary NAFLD association with cf-PWV (see Table 1 in Ref. [35]), there was significant effect modification for waist circumference startum (p for interaction = 0.001) (Fig.2A). Within larger waist circumference individuals, NAFLD had higher cf-PWV, 9.65 (9.50, 9.79), as compared to no-NAFLD, 9.50 (9.36, 9.65), ($p = 0.004$). By contrast, within smaller waist circumference individuals there was no contrast between them ($p = 0.199$). However, in age, gender, race/

Table 1
Characteristics according to non-alcoholic fatty liver disease presence and severity.[a],[b]

NAFLD presence and severity N (%)	No 4571 (63.5%)	Mild 1634 (22.7%)	Moderate 845 (11.7%)	Severe 146 (2.0%)	*p value* for trend
Age (years)	50 (44–57)	51 (45–58)	53 (46–58)	53 (46–59)	< 0.001
Male gender N (%)	1622 (46.0%)	824 (38.9%)	497 (49.7%)	258 (46.4%)	0.649
Race					0.062
White N (%)	2582 (57%)	883 (54.8%)	504 (60.2%)	89 (61%)	
Brown N (%)	1128 (24.9%)	417 (25.9%)	192 (22.9%)	41 (28.1%)	
Black N (%)	658 (14.5%)	255 (15.8%)	119 (14.2%)	11 (7.5%)	
Asian N (%)	125 (2.8%)	36 (2.2%)	18 (2.2%)	2 (1.4%)	
Indigenous N (%)	40 (0.9%)	19 (1.2%)	4 (0.5%)	3 (2.1%)	
Waist circumference (cms)	85.7 (78.7–93.2)	92.9 (86.3–100.3)	100.3 (93.4–107.4)	103.6 (97.7–111)	< 0.001
Body Mass Index (kg/m^2)	25.0 (22.9–27.7)	27.5 (25.0–30.4)	30.0 (27.1–33.1)	31.2 (28.7–34.8)	< 0.001
Current alcohol user N (%)	3515 (76.9%)	1234 (75.5%)	625 (74.0%)	116 (79.5%)	0.174
Tobacco use					0.027
Never	2677 (58.6%)	882 (54.0%)	451 (53.4%)	72 (49.3%)	
Former	1311 (28.7%)	542 (33.2%)	295 (34.9%)	63 (43.2%)	
Current	583 (12.8%)	210 (12.9%)	99 (11.7%)	11 (7.5%)	
Physical activity					< 0.001
Sedentary	2668 (59.4%)	1023 (63.5%)	587 (70.3%)	101 (69.7%)	
Insufficiently active	583 (13%)	208 (12.9%)	89 (10.7%)	20 (13.8%)	
Active	1239 (27.6%)	381 (23.6%)	159 (19%)	24 (16.6%)	
Family history CHD	667 (14.8%)	246 (15.2%)	159 (19.2%)	23 (15.9%)	0.008
Hypertension N (%)	1146 (25.1%)	620 (38.0%)	407 (48.2%)	77 (52.7)	< 0.001
Hypertension treatment N (%)	936 (20.5%)	488 (29.9%)	324 (38.3%)	52 (35.9%)	< 0.001
Systolic arterial pressure (mmHg)	121 (112–132)	126 (116–138)	128 (120–140)	130 (122–145)	< 0.001
Diastolic arterial pressure (mmHg)	74 (68–80)	77 (70–83)	79 (73–86)	82 (76–89)	< 0.001
Mean arterial pressure (mmHg)	90 (83–97)	93 (86–101)	96 (89–103)	99 (92–107)	< 0.001
Diabetes N (%)	508 (11.1%)	351 (21.5%)	272 (32.2%)	67 (45.9%)	< 0.001
Glucose (mg/dL)	102 (97–109)	106 (100–115)	110 (103–122)	115 (106–131)	< 0.001
HbA1c (%)	5.2 (4.9–5.7)	5.4 (5–5.8)	5.5 (5.1–6.0)	5.5 (5.1–6.3)	< 0.001
Dyslipidemia N (%)	2453 (53.7%)	9998 (61.1%)	519 (61.4%)	82 (56.2%)	< 0.001
Total cholesterol (mg/dL)	208 (184–236)	216 (190–243)	215 (193–242)	211 (189–243)	< 0.001
HDL cholesterol (mg/dL)	57 (48–67)	53 (45–63)	50 (43–58)	47 (41–54)	< 0.001
LDL cholesterol (mg/dL)	127 (107–150)	132 (110–154)	130 (111–154)	128.5 (108–152)	< 0.001
Triglycerides (mg/dL)	100 (74–139)	126 (89–180)	153 (108–221)	163.5 (120–226)	< 0.001
Lipoprotein(a) (mg/dL)	12.3 (8.6–19.0)	12.2 (8.9–19.9)	12.4 (8.7–18.0)	12.4 (10.7–19.9)	0.746
GFR (mL/min/1.73m^2)	83 (74–95)	82 (72–94)	82 (72–94)	82 (70–91.1)	0.001
ALT (U/L)	22 (17–29)	25 (19–34)	31 (23–42)	34 (25–49)	< 0.001
AST (U/L)	23 (19–27)	24 (20–28)	25 (21–31)	26 (21–33)	< 0.001
GGT (U/L)	23 (16–35)	28 (20–43)	34 (24–53)	34 (25–48)	< 0.001
Serum uric acid (mg/dL)	5.0 (4.2–6.0)	5.7 (4.7–6.7)	6.2 (5.3–7.3)	6.6 (5.6–7.6)	< 0.001
hs-CRP (mg/L)	1.18 (0.61–2.62)	1.61 (0.83–3.66)	2.10 (1.08–4.41)	2.27 (1.26–4.77)	< 0.001
Fatty Liver Index	27.1 (12.2–52.7)	54.6 (30.0–75.8)	77.3 (57.4–89.4)	85.9 (70.8–92.6)	< 0.001

[a] Continuous variables displayed by median (interquartile range) and categorical by number (percentage). *P value* for trend by Cox Mantel Haenszel for categorical variables and Jonckheere-Terpstra for continuous ones. For race/ethnicity, χ^2 was used as it is not an ordinal variable.
[b] Glomerular filtration rate according to CKD EPI.
ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT (gamma-glutamyl transferase), hs-CRP (high sensitivity C reactive protein), and cf-PWV (carotid femoral pulse wave velocity).

Table 2
Carotid femoral pulse wave velocity according to NAFLD presence.[a],[b]

	No-NAFLD	NAFLD	*p value*
Unadjusted	9.03 (8,98, 9.08)	9.61 (9.54, 9.68)	< 0.001
Model 1	9.21 (9.12, 9.30)	9.59 (9.49, 9.69)	< 0.001
Model 2	9.25 (9.17, 9.33)	9.39 (9.29, 9.48)	< 0.001
Model 3	9.24 (9.15, 9.33)	9.32 (9.22, 9.41)	0.037
Sensitivity analysis for mediating mechanisms			
Model 3 + Diabetes	9.24 (9.15, 9.33)	9.28 (9.18, 9.38)	0.224
Model 3 + HDL-c/TG	9.24 (9.15, 9.33)	9.31 (9.21, 9.40)	0.073
Model 3 + hsCRP	9.24 (9.15, 9.33)	9.32 (9.22, 9.41)	0.035
Model 3 + Uric Acid	9.24 (9.15, 9.33)	9.32 (9.22, 9.41)	0.032

[a] Adjustment covariates for Model 1: age, sex, race/ethnicity. Model 2: M1 + mean arterial pressure and anti-hypertensive treatment. Model 3: M2 + alcohol consumption, smoking habit, physical activity, familial history of premature coronary heart disease and waist circumference.
[b] HDL-cholesterol (HDL-c), triglycerides (TG), and high sensitivity C reactive protein (hsCRP).

ethnicity, BMI, hypertension, diabetes or alcohol consumption strata there was no effect modification for binary NAFLD association with cf-PWV. In NAFLD severity stratified analyzes there were no effect modification for any of the aforementioned strata (see Table 2 in Ref. [35]) (Fig.2B).

For NAFLD classification based on the FLI, there was no association between NAFLD presence with cf-PWV (see respectively Tables 3 and 4 in Ref. [35]). In sensitivity analysis adjusting for BMI instead of waist circumference, however, binary NAFLD based on FLI was associated with cf-PWV (NAFLD, 9.36 [9.26–9.47]; no-NAFLD, 9.21 [9.12–9.30], $p = 0.001$). The NAFLD probability based on FLI was not associated with cf-PWV (see Table 4 in Ref. [35]).

4. Discussion

In a racially/ethnically diverse and admixed population free of CVD, NAFLD is associated with aortic stiffness measured by cf-PWV independently from abdominal obesity. There is also increasing cf-PWV

Table 3
Adjusted carotid femoral pulse wave velocity according to NAFLD severity.[a],[b]

NAFLD Severity	No	Mild	Moderate	Severe	*p value* for trend
Unadjusted	8.70 (7.90–9.80)	9.10 (8.30–10.30)	9.50 (8.70–10.60)	10.00 (9.10–11.10)	< 0.001
Model 1	9.21 (9.12, 9.30)	9.47 (9.37, 9.58)	9.70 (9.57, 9.84)	10.26 (10.00, 10.51)	< 0.001
Model 2	9.25 (9.17, 9.33)	9.33 (9.23, 9.42)	9.44 (9.32, 9.56)	9.78 (9.55, 10.01)	< 0.001
Model 3	9.24 (9.15, 9.33)	9.27 (9.17, 9.38)	9.35 (9.23, 9.48)	9.69 (9.46, 9.93)	0.001
Sensitivity analysis for mediating mechanisms					
Model 3 + Diabetes	9.24 (9.15, 9.32)	9.25 (9.15, 9.36)	9.30 (9.18, 9.43)	9.59 (9.36, 9.83)	0.022
Model 3 + HDL-c/TG	9.24 (9.15, 9.33)	9.27 (9.16, 9.37)	9.34 (9.21, 9.47)	9.68 (9.44, 9.92)	0.002
Model 3 + Inflammation	9.24 (9.15, 9.33)	9.27 (9.17, 9.38)	9.36 (9.23, 9.49)	9.69 (9.46, 9.93)	0.001
Model 3 + Uric Acid	9.23 (9.15, 9.32)	9.27 (9.17, 9.38)	9.36 (9.23, 9.48)	9.70 (9.46, 9.94)	0.001

[a] Adjustment covariates for Model 1: age, sex, race/ethnicity. Model 2: M1 + mean arterial pressure and anti-hypertensive treatment. Model 3: M2 + alcohol consumption, smoking habit, physical activity, familial history of premature coronary heart disease and waist circumference.
[b] HDL-cholesterol (HDL-c), triglycerides (TG), and high sensitivity C reactive protein (hsCRP).

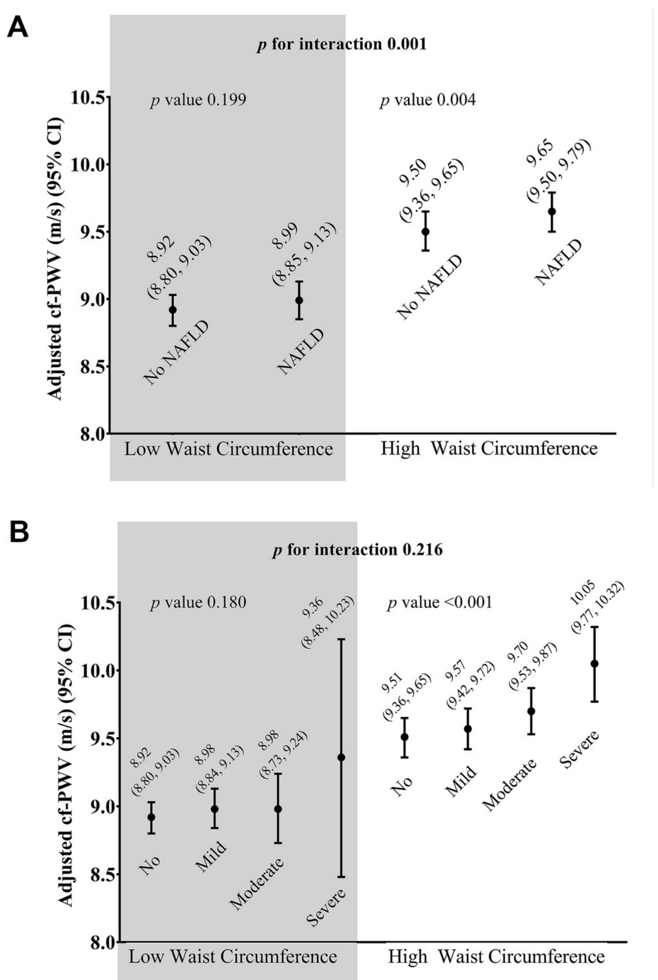

Fig. 2. NAFLD and adjusted carotid femoral pulse wave velocity.
Fully adjusted analysis for the association of NAFLD presence (A) or its graded severity (B) with cf-PWV (*p* for trend). Adjustment covariates age, sex, race/ethnicity, mean arterial pressure, and anti-hypertensive medication, alcohol consumption, smoking habit, physical activity, familial history of premature coronary heart disease, and waist circumference.

along more severe NAFLD, which may better capture the association with aortic stiffness and the ensuing CVD risk. Importantly, our results are widely generalizable and do not differ across diverse and admixed races/ethnicities. This association is independent from hypertension, obesity and low grade chronic inflammation. Future investigations should clarify whether preventing or reversing NAFLD will have a favorable impact on arterial stiffness.

Previous studies have addressed the association of NAFLD with aortic stiffness. In two European populations, NAFLD presence (binary) was associated with higher cf-PWV [19,20]. However, there were some limitations in their design as the relatively small sample and adjustment for limited set of confounders. In the Framigham Heart Study for its side, NAFLD was associated with peripheral arterial tonometry response but not with cf-PWV [22]. One potential reason for the discrepancy with our data is their adjustment model for glycaemia and insulin resistant dyslipidemia. Even though, the cross sectional design of both studies does not distinguish whether insulin resistance is a confounder or mediator on the association. Other one is that the Framingham sample is not as diverse as our population from the race/ethnic standpoint, but our stratified analyses do not lend evidence for effect modification from White, to Brown, to Black individuals. In population-based cohorts in Korean adults, NAFLD presence and severity have been associated with ba-PWV, which aligns with our results [23,26]. In a large sample of Chinese individuals as well, NAFLD presence (binary) was associated with ba-PWV [24]. Therefore, our study expands the validity of NAFLD presence and severity association with cf-PWV to racial/ethnically diverse Western populations, independently of confounders.

The NAFLD association with incident CVD, apart from traditional cardiovascular risk factors, has been shown in diverse studies [8–11]. Furthermore, there is even higher risk for fatal and non-fatal cardiovascular with severe NAFLD [11], which parallels the associations studied herein. Addressing the specific mechanisms by which NAFLD is associated with CVD may open new frontiers for preventive strategies. Besides that, NAFLD seems to be a unique dimension of ectopic fat as it does not mirror overall and abdominal obesity. In our study population, 19.4% of lean people (BMI < 25 kg/m^2) had NAFLD, meanwhile 34.8% of obese (BMI > 30 kg/m^2) did not. Given the high population prevalence of NAFLD and ultrasound availability for its measurement, it is plausible to consider it as a complimentary method for CVD risk prevention strategies. In one meta-analysis of 15,877 individuals followed for 7.7 years, for each 1 m/s cf-PWV increase there is 15% higher risk for CVD events, CVD or overall mortality [14]. The small cf-PWV differences across NAFLD studied herein have limited clinical and individual impact. However, NAFLD is highly prevalent worldwide, which may imply in a relevant CVD burden of disease from the population standpoint.

Along increasing NAFLD severity there is concomitant worsening cardiometabolic profile, namely hypertension, diabetes, dyslipidemia and inflammation. Despite some confounding, or potentially mediating effect, on the NAFLD-cf-PWV association, CVD risk markers are just partly associated with NAFLD. We hypothesize there may be specific and direct mechanisms linking fatty liver to aortic stiffness. NAFLD is associated with hepatic-specific insulin resistance, which unleashes the hepatic production of glucose and triglyceride rich lipoproteins even in apparently healthy individuals [37,38]. Advanced end glycation products may ensue, which have been associated with oxidative and inflammatory processes in the arterial wall [39], and metalloproteinases local activation [40]. Excessive stimulation of metalloproteinases favors abnormal/uncoiled collagen accumulation over normal elastin and is the main underlying mechanism for increased aortic stiffness. The contribution of these mechanisms is supported by the partial attenuation of diabetes and insulin resistant dyslipidemia on our binary NAFLD analysis. Given previous data on the prospective association of NAFLD with incident diabetes [5], assigning diabetes as a mediator rather than a confounder is reasonable.

NAFLD is associated with inflammatory cytokines independently from obesity and other metabolic risk factors [41,42]. As inflammation has been linked to increased aortic stiffness [40,43,44], this is another potential mechanism for our findings. In our study, the interaction between larger waist circumference and binary NAFLD towards higher cf-PWV may derive from incremental inflammation between inter-visceral and intra-hepatic fat. Lack of such interaction between waist circumference and graded NAFLD does not invalidate our hypothesis due to the low statistical power related to low frequencies of moderate and severe NAFLD. Given larger waist circumference and NAFLD are considerably dissimilar (see Table 1 in Ref. [35]), inter-visceral and intrahepatic fat may have specific clinical implications. In this regard, it has was previously shown that inter-visceral fat cell volume is independently associated to aortic stiffness in obese submitted to bariatric surgery [45]. One aspect that may contradict the inflammatory hypothesis in our study is that hsCRP did not nullified the results. However, hsCRP is a downstream marker that does not fully reflect the complex inflammatory milleau [46]. By contrast, polymorphisms in interleukin-6, an upstream regulator of the inflammatory chain, are associated with life span cardiovascular risk by genome wide association [47,48]. It is plausible, therefore, that NAFDL may affect cf-PWV by inflammatory pathways apart from hsCRP. However, a common pathophysiological determinant for both NAFLD and cf-PWV as aging, adipose tissue dysfunction and still unknown factors cannot be rule out.

Our study should be considered in the context of its population and methods. It includes a large sample of men and women, not selected from specialized medical centers, and with very diverse and admixed race/ethnicities. Therefore, our findings are widely generalizable. However, our cross sectional design demands cautious consideration and is limited for inferring causality. We also do not account for other medications potentially related to NAFLD apart from steroids. However, we judge that would have minor interference in our results as our sample is massively composed of apparently healthy individuals. Regarding non-invasive identification of NAFLD, ultrasound is insensitive to hepatic fat accumulation lower than 30%. It is, however, a universal limitation across large population studies not involving specialized tertiary centers with histopathological diagnosis for NAFLD. It is unknown whether our findings extends to the milder spectrum of NAFLD exclusively detected by liver histopathology. Despite being a non-standard way of addressing that, supposedly milder NAFLD based on FLI probability score did not show any association with cf-PWV [35]. Besides that, there was no specific identification of steatohepatitis, which is associated with advanced subclinical atherosclerosis [49] and higher cardiovascular and overall mortality [50,51]. Therefore, NAFLD histopathology may have implications on cf-PWV not considered here. We did not measure inter-visceral abdominal fat directly, which could elucidate its individual role versus NAFLD. Even though, WC and ultrasound imaging are valid measurements for respectively measuring abdominal obesity and NAFLD in population studies [32,33].

4.1. Conclusion

In racial/ethnically diverse individuals free of CVD, NAFLD presence and severity is associated with aortic stiffness beyond abdominal obesity. Future investigations should clarify whether preventing or reversing NAFLD severity will have a favorable impact on aortic stiffness.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Paulo H. Harada (conception, design, data analysis, interpretation and drafting of the manuscript), Isabela M. Benseñor (critical review), Luciano Drager (critical review), Alessandra C Goulart (critical review), José G Mill (critical review), and Paulo A. Lotufo (conception and critical review).

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