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No association between aortic stiffness and liver steatosis in morbidly obese patients

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HIGHLIGHTS

- We studied 120 unselected severely obese patients undergoing bariatric surgery.
- Aortic stiffness was not related to biopsy-confirmed liver steatosis.
- Hypertension was the major factor contributing to increased aortic stiffness.

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ABSTRACT

Background and aims: Patients with non-alcoholic fatty liver disease are characterized by increased aortic stiffness, but it is unclear whether this is related to non-alcoholic fatty liver disease itself or concomitant metabolic syndrome components, including hypertension and diabetes. Previous studies were methodologically limited by ultrasound-based assessment of liver steatosis or performing liver biopsy in patients with more severe disease. Therefore, we prospectively measured aortic pulse wave velocity (aPWV) in non-selected obese subjects admitted for bariatric surgery with liver biopsy, allowing assessment of the association between aortic stiffness and biopsy-confirmed liver steatosis.

Methods: We evaluated 120 consecutive severely obese patients (79 females; mean age 42 ± 10 years, mean body mass index 45.0 ± 5.3 kg/m^2) without cardiac disease or alcohol-induced liver disease, who were admitted for bariatric surgery. The presence or absence of liver steatosis was defined by wedge liver biopsy. aPWV was measured with the Doppler method at the time of preoperative transthoracic echocardiography.

Results: Based on liver biopsy results, 82 patients (68%) had liver steatosis and 38 (32%) had no steatosis. Univariate linear regression analysis showed that age, mean arterial pressure, liver steatosis, heart rate, female gender, and diabetes were significantly associated with aPWV. However, only age, mean arterial pressure, heart rate, and diabetes remained significant in the multivariate model ($p \leq 0.001$).

Conclusions: We found no independent association between biopsy-confirmed liver steatosis and aortic stiffness measured by Doppler aPWV in morbidly obese individuals. Aortic stiffness in these subjects is related to comorbidities and not to non-alcoholic fatty liver disease itself.

1. Introduction

Aortic stiffness measured by aortic pulse wave velocity (aPWV) has an established prognostic value in cardiovascular disease in the general population [1]. It was hypothesized that increased aortic stiffness found in patients with non-alcoholic fatty liver disease (NAFLD) may play a role in mediating an increased cardiovascular risk in this population [2]. NAFLD-associated factors such as inflammation, increased triglyceride levels, and adipokine imbalance, were proposed to explain an adverse effect of NAFLD on aortic wall properties [2–4].

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Table 1
Characteristics of the study group.

Variable	NAFLD present N = 82	NAFLD absent N = 38	*p* value
Age, years	43.00 ± 9.50	39.76 ± 10.28	0.0933
Females, n (%)	46 (56.10%)	33 (86.84%)	0.0009
BMI, kg/m^2	44.56 [42.01–47.80]	44.00 [40.63–46.60]	0.2850
Weight, kg	130 [114–146]	120 [114–138]	0.1574
Height, cm	171.02 ± 11.00	168.05 ± 6.81	0.0732
Hypertension, n (%)	55 (67.07%)	17 (44.74%)	0.0201
Diabetes, n (%)	19 (23.17%)	9 (23.68%)	0.8649
Percentage of hepatocytes with steatosis, %	25 [20–50]	2 [1–3]	< 0.0001
Use of antihypertensive medications, n (%)	50 (60.98%)	15 (39.47%)	0.0278
ACEI/ARB, n (%)	38 (46.34%)	15 (39.47%)	0.4809
Beta-blocker, n (%)	26 (31.71%)	8 (21.05%)	0.2282
Diuretic, n (%)	20 (24.39%)	8 (21.05%)	0.8649
Use of hypoglycemic agents, n (%)	24 (29.27%)	8 (21.05%)	0.3437
Fasting blood glucose, mg/dL	102 [90–122]	92 [88–100]	0.0013
HbA1c, %	5.8 [5.5–6.2]	5.4 [5.2–5.6]	< 0.0001
HbA1c > 6.5%, n (%)	17 (20.73%)	1 (2.63%)	0.0209
TC, mg/dL	177.43 ± 37.59	183.14 ± 35.62	0.4387
LDL-C, mg/dL	103.75 ± 33.84	110.78 ± 32.98	0.3180
HDL-C, mg/dL	40.89 ± 9.9	47.78 ± 11.01	0.0009
TG, mg/dL	157.5 [129–240.5]	114.5 [92.5–139]	< 0.0001
AST, U/L	29 [23–42]	22 [19–25]	0.0002
ALT, U/L	38 [27–65]	23 [19–32]	< 0.0001
GGTP, U/L	37.5 [24–62]	22.5 [18–31]	0.0001
CRP, mg/L	5.8 [3.0–9.2]	5.55 [4–12.50]	0.6773
SBP, mmHg	137 [130–145]	131.5 [120–138]	0.0056
DBP, mmHg	84 [80–90]	80 [74–83]	0.0025
MAP, mmHg	102.08 ± 8.64	96.88 ± 8.70	0.0027
HR, bpm	73.11 ± 10.69	72.11 ± 8.93	0.6157
APWV, m/s	6.73 [6.32–7.85]	6.36 [5.84–6.75]	0.0029

Data reported as mean ± standard deviation, number (percentage), or median [interquartile range] as applicable.
ACEI, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; aPWV, aortic pulse wave velocity; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; GGTP, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; MAP, mean arterial pressure; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

However, evaluation of aortic stiffness in NAFLD may be confounded by common coexistence of elevated blood pressure and increased glucose levels. This most common liver abnormality, typically associated with abdominal obesity, is considered a hepatic manifestation of metabolic syndrome. Hypertension and diabetes, both typical features of metabolic syndrome, are well established factors that increase aortic stiffness and have a negative impact on cardiovascular prognosis. Therefore, evaluation of the independent prognostic significance of each of these variables individually may be challenging.

Another potential problem in studying the association between aPWV and NAFLD is related to the diagnostic modalities used. In most studies, NAFLD was diagnosed by ultrasound that has a low accuracy for detecting mild steatosis [5–14]. This may lead to over-representation of patients with more advanced steatosis and metabolic abnormalities in NAFLD groups. To date, only few studies evaluated the association between aPWV and NAFLD confirmed by liver biopsy, considered the gold standard for the diagnosis of fatty liver disease [15–17]. However, patients in these studies were preselected by increased transaminase levels, suggesting the presence of non-alcoholic steatohepatitis (NASH) and thus more advanced metabolic abnormalities.

Therefore, we decided to prospectively measure aPWV in a non-selected population of very obese subjects admitted for bariatric surgery with liver biopsy, allowing assessment of the association between aortic stiffness measured by aPWV and biopsy-confirmed liver steatosis.

2. Patients and methods

We evaluated 120 consecutive severely obese patients (body mass index [BMI] > 35 kg/m^2) without known cardiac disease including arrhythmia or a history of excessive alcohol use who were admitted for bariatric surgery (laparoscopic sleeve gastrectomy) to a tertiary care, university surgical department. Women accounted for 66% of the study population (n = 79). The mean age was 42 ± 10 years, and the mean BMI was 45.0 ± 5.3 kg/m^2. Excessive alcohol use was defined as self-reported daily alcohol consumption ≥30 g for men and ≥20 g for women. Diabetes was defined as the diagnosis of diabetes in medical records or use of any antidiabetic drug. The demographic, clinical, and laboratory characteristics of the study group are presented in Table 1.

2.1. Liver biopsy

Wedge liver biopsy was performed during the bariatric surgery as a part of the routine surgical protocol implemented in the university surgical department. A tissue sample of approximately 10 × 5 mm was acquired from the subcapsular part of the left lobe (segment III according to the Couinaud classification). Liver biopsy specimens were fixed in formalin and embedded in paraffin. Histopathological evaluation was performed by a single experienced pathologist. Semiquantitative histopathological assessments were done according to the Clinical Research Network for Nonalcoholic Liver Disease recommendations [18]. The reported histopathological findings included the percentage of hepatocytes with steatosis, NAFL activity score (NAS), and the presence or absence of NASH. The percentage of hepatocytes with steatosis was evaluated using a visual semiquantitative method. Liver steatosis was defined as the presence of > 5% of hepatocytes with fatty infiltration. NASH was defined as the NAS score ≥5 and the presence of hepatocyte ballooning.

2.2. Aortic pulse wave velocity measurements

Aortic pulse wave velocity was measured with the Doppler method

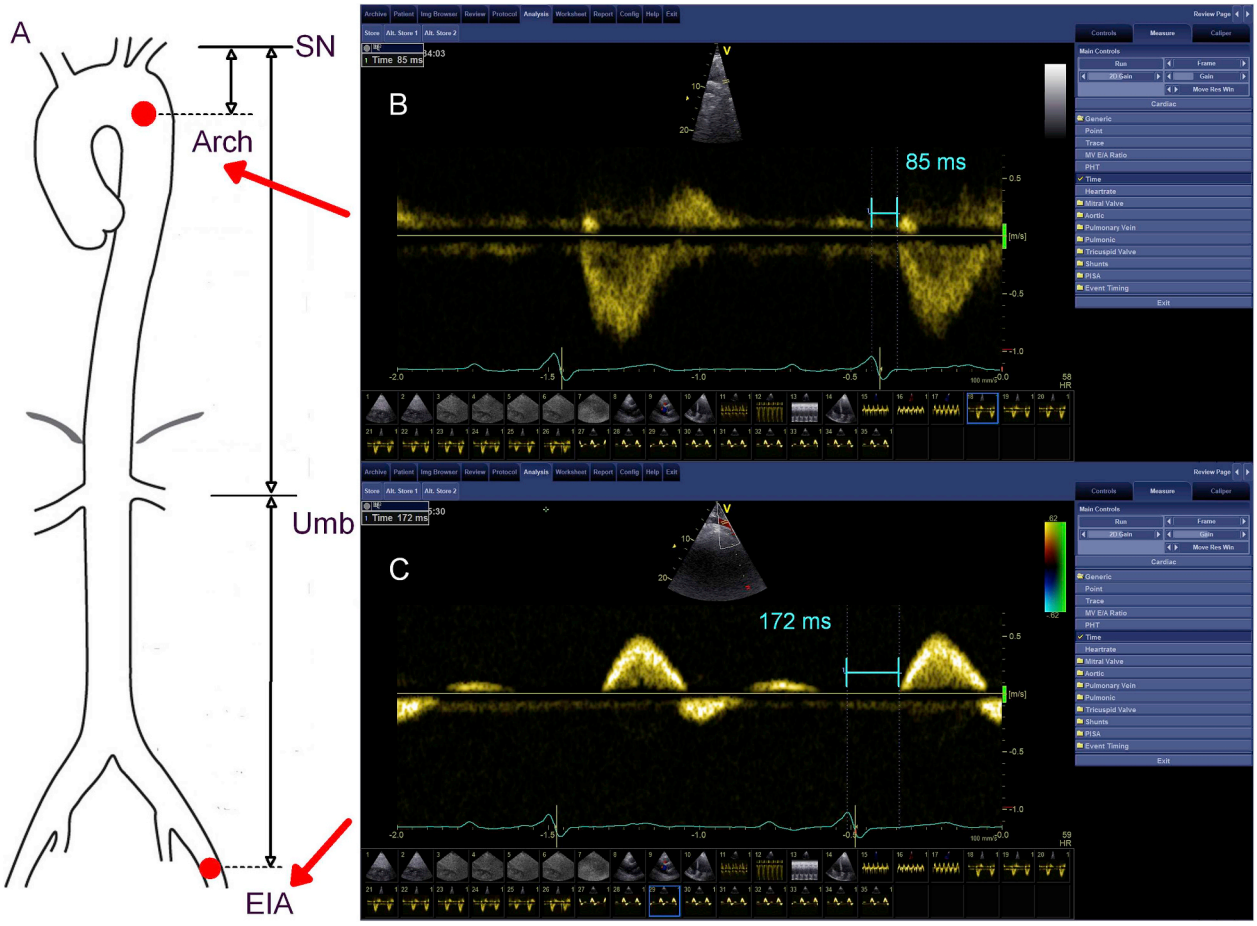

Fig. 1. Aortic pulse wave velocity measurement.
(A) Diagram showing body surface measurements for calculation of the distance between the two points of Doppler recordings. (B) Example of a Doppler recording from the distal aortic arch for transit time measurement. (C) Example of a Doppler recording from the left external iliac artery for transit time measurement. Arch, distal aortic arch; EIA, external iliac artery (in the left groin); SN, suprasternal notch; Umb, umbilicus.

at the end of transthoracic echocardiographic examination (VIVID 9, GE Healthcare, Horton, Norway; 2.5 MHz cardiac sector probe) performed for perioperative risk assessment. This approach to aPWV measurements has been recently demonstrated to show a very good concordance with invasively assessed aPWV using the intraarterial pressure wave method [19]. Patients were evaluated 1–2 days before surgery, in a fasting state, after at least 15 min of rest in a supine position. Ten consecutive Doppler waveforms were recorded with a cardiac sector probe at the sweep speed of 100 mm/s, first in the distal aortic arch assessed from a suprasternal view and then in the left distal external iliac artery (EIA) assessed in the left groin area. Acquisition of adequate quality Doppler recordings was possible in all patients. Images were stored and evaluated with a dedicated software (Echopac, GE Healthcare, United States). Transit time was calculated as the difference between the time from QRS to the beginning of EIA Doppler waveform (Fig. 1C) and the time from QRS to the beginning of distal aortic arch Doppler waveform (Fig. 1B). Times averaged over ten consecutive cardiac cycles were used for analysis. The distance between the two points of Doppler recordings was measured over the body surface with a measuring tape. The distance from the suprasternal notch to the umbilicus was added to the distance from the umbilicus to the left groin. Then, the distance between the suprasternal notch and the Doppler sample volume in the distal aortic arch was subtracted (Fig. 1A). Care was taken to measure the distances in a straight line between the measuring points, above the skin surface to avoid distance overestimation by redundant abdominal fat tissue. Doppler echocardiography, distance measurements and off-line transit time evaluations were performed by a single experienced echocardiographer blinded to the results of liver biopsies. Blood pressure was measured at the end of examination using the automated oscillometric method (Microlife, Watch BP Office, Switzerland) with the cuff size properly adjusted to the arm circumference.

2.3. Statistical analysis

Statistical analysis was performed using Statistica 13.1 package (TIBCO Software Inc., Palo Alto, USA). Continuous variables with normal distribution were presented as mean values ± standard deviation (SD), and non-normally distributed variables were reported as median and interquartile range (IQR). Differences between groups were compared using the unpaired Student *t*-test for normally distributed data and the Mann-Whitney *U* test for non-normally distributed data. Discrete variables were compared using the chi-square test with Yates correction if required. Multiple linear regression analyses were performed to evaluate the association between aPWV and other variables including the presence of NAFLD, age, gender, blood pressure, diabetes, and heart rate. NAFLD was included in the model as a categorical covariate. While aPWV showed a non-normal distribution, natural log transformed aPWV was distributed normally and thus it was used in our linear model. BMI as an indicator of the severity of obesity and the percentage of hepatocytes with steatosis as an indicator of the severity of steatosis were not distributed normally and the correlation between these variables and aPWV was assessed using the Spearman correlation coefficient.

Table 2
Multivariate linear regression analysis of aPWV as a dependent variable.

aPWV (ln) dependent variable, adjusted $R^2 = 0.57$			
Variable	Regression coefficient (CI)	Standardized regression coefficient (CI)	*p* value
Age	0.007 (0.005–0.009)	0.453 (0.321–0.585)	< 0.001
MAP	0.007 (0.004–0.009)	0.393 (0.256–0.531)	< 0.001
HR	0.003 (0.001–0.005)	0.183 (0.054–0.312)	0.006
NAFLD	0.032 (−0.010 to 0.075)	0.097 (−0.032 to 0.225)	0.138
Diabetes	0.074 (0.026–0.121)	0.201 (0.072–0.329)	0.003
Female	0.005 (−0.038 to 0.048)	0.066 (−0.116 to 0.148)	0.811

aPWV, aortic pulse wave velocity; CI, confidence interval; HR, heart rate; MAP, mean arterial pressure; NAFLD, non-alcoholic fatty liver disease.

The study was approved by the local Ethical Committee and all patients gave an informed consent for the ultrasound examination. All procedures were performed in in accordance to all the relevant national regulations, institutional guidelines and the tenets of the Helsinki Declaration.

3. Results

Eighty-two patients (68%) had liver steatosis and 38 patients (32%) had no steatosis based on liver biopsy results. Among patients with NAFLD, 31 (38%) met the criteria for NASH. Females outnumbered men in both groups, especially in the group without liver steatosis. With similar basic anthropometric measures (weight, height, BMI) in both groups, patients with NAFLD demonstrated typical features of metabolic syndrome, including increased blood pressure, blood glucose and triglyceride levels and lower HDL cholesterol levels. As expected, patients with NAFLD had significantly elevated hepatic enzyme levels compared to the no-steatosis group.

Univariate linear regression analysis showed that age, mean arterial pressure (MAP), the presence of NAFLD, heart rate, female gender, and diabetes were significantly associated with aPWV. However, only age, MAP, heart rate, and diabetes remained statistically significant in the multivariate model (Table 2). Overall, gender was not significantly associated with aPWV in multivariate model. The potential effect of gender on the relation between aPWV and NAFLD was also analyzed separately in both gender subgroups. In males (n = 41), aPWV did not differ between those with and without NAFLD ($p = 0.76$, Mann-Whitney *U* test). In females (n = 79), there was a significant association between PWV and the presence of NAFLD in the univariate linear regression analysis ($p = 0.005$) but it was no longer significant when age and MAP were included in the multivariate model ($p = 0.16$).

Regarding the effects of the severity of steatosis and obesity, we found no correlation between aPWV and the percentage of hepatocytes with steatosis in the NAFLD group (Spearman correlation coefficient r = 0.2; $p = 0.065$), and between aPWV and BMI both in the NAFLD group (r = −0.01; $p = 0.88$) and in all patients (r = 0.12; $p = 0.17$).

4. Discussion

The results of our study show that liver fat content is not independently associated with aPWV in morbidly obese patients. Although in univariate analysis aPWV was increased in patients with NAFLD compared to those without liver steatosis, this relation was no longer significant after blood pressure, heart rate, and diabetes were taken into account.

Blood pressure is an important determinant of aortic PWV, with higher distending pressure increasing wall stiffness independently of its other adverse long-term effects on the structural components of aortic walls [20]. Increased blood pressure is a major component of the metabolic syndrome and it is believed to result most likely from insulin resistance and hyperinsulinemia acting through various mechanisms [21]. Thus, even if a potential independent effect of NAFLD on aortic stiffness exists, it is small and insignificant when compared to the dominant effect of blood pressure.

In our patients, diabetes and hyperglycemia were other factors independently associated with increased aPWV, although to a lesser extent when compared to blood pressure. This association is well known and common for various populations [22,23]. It is believed that an adverse effect of advanced glycation end-products on the arterial wall and decreased nitric oxide endothelial synthesis play a major role in an increased aortic stiffness in diabetes [22].

Heart rate was also independently associated with increased aPWV, and this finding is consistent with the evidence obtained in a non-NAFLD population that heart rate has an effect on aPWV that is independent from the effect of blood pressure itself [24]. It has been suggested that viscoelastic properties of the aortic wall may explain this phenomenon [25].

In our study, aPWV was measured using Doppler echocardiography, the modality recently validated versus simultaneously performed invasive intraarterial pressure wave measurement, considered the reference method for evaluating aPWV [19]. Although the carotid-femoral approach using tonometry is most commonly used for non-invasive aPWV measurement, its results are often overestimated due to problems with distance appreciation [1]. Moreover, detection of a good quality pulse for tonometry in the carotid and femoral artery could be difficult in extremely obese subjects. In contrast, echocardiography enables detection of a good quality Doppler signal by prior artery identification using color Doppler mode, especially in the inguinal area.

Our findings refer only to patients with severe obesity and are in contrast to the results of some other studies performed in less obese populations. However, most of those studies used only ultrasound evaluation as a method to identify liver steatosis. Despite being widely accessible, non-invasive, and of good diagnostic value in at least moderate steatosis, the ultrasound diagnosis of NAFLD has significant limitations in patients with low levels of steatosis (> 5% and less than 20–30% of hepatocytes with fatty infiltration). As a result, the diagnosis of NAFLD by ultrasound may lead to overrepresentation of individuals with more advanced steatosis and thus more metabolic abnormalities. Patients with low levels of steatosis and less advanced metabolic abnormalities may be categorized by ultrasound as having no steatosis even if they may in fact have significant NAFLD, which makes interpretation of the results of those studies more problematic.

In fact, only three previous studies evaluated the association between aPWV and NAFLD diagnosed by liver biopsy [15–17]. Unfortunately, studies that evaluate NAFLD with liver biopsy may be affected by a patient selection bias, as this invasive procedure is usually reserved for patients with abnormally elevated transaminase levels that are typical for NASH. This is a more advanced form of NAFLD in terms of liver and metabolic dysfunction. In addition, the absence of NAFLD in the control groups is usually not confirmed by liver biopsy.

Thus, our study population seems to be unique in terms of no preselection of the studied patients and histological confirmation of no steatosis in the control group. However, inclusion of only morbidly obese individuals creates some other potential limitations that should be taken into account. First, the relation between aortic stiffness and BMI is a complex one, as it was demonstrated that elevated body fat was associated with reduced aPWV until middle age, and then with increased aPWV later in life [26]. Second, obesity may particularly affect the adequacy of travel distance measurements and in consequence PWV results. In particular, central obesity that often coexists with NAFLD may overestimate travel distance when routinely measured using a flexible tape on the skin surface [27]. This may lead to falsely increased PWV values. Therefore, we measured distance as a straight line several centimeters over the skin, between the points of Doppler signal acquisition (suprasternal notch, left groin area) and the umbilicus. We observed that there was no impairment of Doppler signal quality even in

extremely obese patients. On the contrary, both standard two-dimensional ultrasound visualization of the aortic arch and the proximal descending aorta and the Doppler signal quality seemed better than in many non-obese patients, mostly due to a larger suprasternal acoustic window. However, the Doppler signal acquisition in EIA was slightly more difficult due to excessive abdominal fat but the use of color Doppler for identification of the vessel allowed good quality spectral Doppler recordings in all patients.

Third, females were overrepresented in our study group, which is a typical pattern for cohorts of bariatric patients, and sex-related differences in body fat composition were reported to influence the relation between obesity and aPWV [28]. In our study population, the significant association between aPWV and NAFLD in the univariate linear regression analysis was driven mainly by the female subgroup. In males, aPWV did not differ between subjects with NAFLD and those with no steatosis. However, the low number of very obese males without NAFLD makes it difficult to offer a firm conclusion regarding a potential effect of gender on the relation between aPWV and NAFLD.

Another limitation of the study is its relatively small sample size which may generate error. However, with 120 patients and 6 covariates, the linear model does not seem over-fitted by commonly used criteria [29]. Finally, our findings may not be relevant for non-obese (lean or overweight) subjects with NAFLD, a population with somewhat different characteristics in terms of the burden of cardiovascular risk factors and subclinical atherosclerosis, metabolic risk profile, genetic factors, liver disease, and mortality risk [30,31].

In our cohort, age, blood pressure, heart rate, and diabetes were independently associated with aPWV, with the strongest associations observed for age and blood pressure. Although patients with morbid obesity have unique characteristics and may be perceived as a particularly difficult group for aPWV measurements, our results are in line with most studies that reported correlation coefficients for aPWV [23], thus corroborating the validity of our methodology and results.

4.1. Summary and conclusions

In the present study, we found no independent association between liver steatosis evaluated by liver biopsy and aortic stiffness measured by aPWV using the Doppler method in a population of morbidly obese individuals. Our results contribute to the ongoing debate whether NAFLD itself has an independent adverse effect on the cardiovascular system [32,33], including an adverse effect on aortic stiffness, or whether increased aortic stiffness and cardiovascular risk observed in patients with NAFLD are only related to concomitant components of the metabolic syndrome, which are established cardiovascular risk factors. Our findings favor the latter explanation, suggesting that there is no evident independent effect of NAFLD on aortic stiffness. Some previous studies, showing an independent association between NAFLD and aortic stiffness, might have been limited by several methodological issues, including ultrasound-based diagnosis of liver steatosis and patient selection bias towards performing liver biopsy in those with an already more severe disease (NASH). Based on our findings, one cannot expect a direct significant effect on aortic stiffness when treating and/or preventing NAFLD, and the therapeutic efforts to reduce aortic stiffness in these subjects should be targeted at the metabolic syndrome components, in particular blood pressure and glucose control, rather than NAFLD itself.

Conflicts of interest

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

Author contributions

Grzegorz Styczyński: study design, data collection, study supervision, literature review, data analysis, drafting the manuscript.

Piotr Kalinowski: data collection, reviewing the manuscript.

Łukasz Michałowski: data collection, reviewing the manuscript.

Rafał Paluszkiewicz: study design, data collection, study supervision.

Bogna Ziarkiewicz-Wróblewska: study design, data collection, study supervision.

Krzysztof Zieniewicz: study design, data collection, study supervision.

Emanuel Tataj: data analysis, reviewing the manuscript.

Cezary Szmigielski: study design, data collection, study supervision, literature review, reviewing the manuscript.

Piotr Jędrusik: study supervision, writing the final manuscript.

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