Obesity and type 2 diabetes : cardiovascular and cerebral aspects

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Chapter 3

Is hepatic triglyceride content associated with aortic pulse wave velocity and carotid intima-media thickness? The Netherlands Epidemiology of Obesity study

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ABSTRACT

Background

Nonalcoholic fatty liver disease is associated with an increased risk of cardiovascular disease. The purpose of this study was to test the hypothesis that hepatic triglyceride content is associated with subclinical vascular impairment and is not confounded by various cardiometabolic risk factors.

Methods

This study was approved by the institutional review board, and all participants gave written informed consent. In this cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity study, a population-based cohort study, 1899 participants (52% men; mean age, 55 years ± 6 [standard deviation]) underwent magnetic resonance (MR) spectroscopy and MR imaging to assess hepatic triglyceride content, aortic pulse wave velocity (PWV), and visceral fat. Carotid intima-media thickness (IMT) was acquired and measured by trained research nurses according to standard procedures. Multivariate regression analyses were used to study associations of hepatic triglyceride content with total and regional aortic PWV and carotid IMT while adjusting for several possible confounding factors, including the metabolic syndrome.

Results

Total aortic PWV (mean difference, 0.5 m/s; 95% confidence interval [CI]: 0.3, 0.7) and carotid IMT (mean difference, 37 μm; 95% CI: 25, 49) were higher in participants with hepatic steatosis. After adjusting for various covariates, a 10-fold increase in hepatic triglyceride content was associated with an increased mean aortic PWV of 0.19 m/s (95% CI: 0.03, 0.36) in total and an increased mean aortic PWV of 0.42 m/s (95% CI: 0.03, 0.81) in the abdominal segment. A 10-fold increase in hepatic triglyceride content was also associated with an increased mean carotid IMT of 15 μm (95% CI: 0, 29) but not after additional adjustments for visceral and total body fat.

Conclusions

In this relatively large population-based cohort study, hepatic triglyceride content was associated with aortic pulse wave velocity and carotid IMT. These associations were only partly explained by the metabolic syndrome and visceral adiposity, suggesting a possible specific contribution of hepatic steatosis to subclinical vascular impairment.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is being diagnosed with increasing frequency and is considered to be the most common chronic liver disease in Western countries, with a prevalence of 20%-30% in the general population[1, 2] and a prevalence of 90% in patients with severe obesity[3]. The recent literature shows an ongoing worldwide trend of increasing body mass index (BMI), with a global prevalence of severe obesity (BMI ≥ 35 kg/m^2) of 2.3% in men and 5.0% in women in 2014[4].

NAFLD has been associated with increased brachial-ankle pulse wave velocity (PWV)[5, 6] and increased carotid intima-media thickness (IMT)[7]. PWV of the aorta is a marker for central arterial stiffness, and it is a prognostic marker for cardiovascular disease[8-10]. Aortic PWV assessed with magnetic resonance (MR) imaging has an advantage over brachial-ankle PWV, in that the true path length of the pulse wave along the aorta can be directly assessed, even in the presence of a tortuous course of the aorta, and regional elastic properties of the aorta can be studied depending on the number of aortic segments examined[11]. Increased carotid IMT is an early indicator of generalized atherosclerosis and is a risk factor for myocardial infarction and stroke[12].

Obesity is recognized as an important contributor to NAFLD[13], increased arterial stiffness[14, 15], and subclinical atherosclerosis[16]. Additionally, NAFLD and cardiovascular disease share common risk factors, which are clustered in the metabolic syndrome[17]. These risk factors may confound observed associations between NAFLD and surrogate markers of cardiovascular disease. We have previously shown that hepatic triglyceride content is associated with decreased diastolic function after adjustment for the individual components of the metabolic syndrome and visceral adiposity[18]. However, it is unclear whether hepatic triglyceride content is truly associated with aortic PWV and carotid IMT or if hepatic steatosis and subclinical vascular impairment are merely common consequences of the obese state. We hypothesized that hepatic triglyceride content is associated with subclinical vascular changes. Thus, the purpose of our study was to investigate the relationships between hepatic triglyceride content and aortic PWV and carotid IMT and to what extent these relationships were explained by the metabolic syndrome and measurements of overall and abdominal obesity in a relatively large number of individuals.

METHODS

Study design and study population

This is a cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study. The NEO study is a population-based prospective cohort study, with an oversampling of individuals with a BMI of 27 kg/m^2 or higher. A detailed description

of the study design and data collection has been published elsewhere[19]. In short, men and women aged 45-65 years with a self-reported BMI of 27 kg/m^2 or higher from Leiden, the Netherlands, and the surrounding area were included between September 2008 and October 2012. In addition, all inhabitants aged 45-65 years in one municipality (Leiderdorp, the Netherlands) were invited irrespective of their BMI, allowing for a reference distribution of BMI. For pragmatic reasons (time), only three participants could undergo MR imaging and MR spectroscopy each day. Thus, we randomly selected the first three participants without contraindications for MR imaging and MR spectroscopy. This resulted in a subset of approximately 30% of all NEO study participants who underwent MR imaging of the aorta and abdomen in addition to proton (^{1}H) MR spectroscopy of the liver. In our current study, we included participants with MR imaging of the aorta and abdomen, ^{1}H MR spectroscopy of the liver, and a carotid IMT measurement. Exclusion criteria for this analysis were missing data of any of the aforementioned measurements, missing data of any parameter used in the multivariate regression analyses, a history of liver disease, alcohol consumption of more than 10 units per day, or any combination thereof. The relationship between hepatic steatosis and left ventricular diastolic function was previously described in a subset ($n = 714$) of the current study population[18]. Our study was approved by the medical ethics committee of Leiden University Medical Center, and all participants provided written informed consent.

Data collection

A history of cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. History of liver disease included cirrhosis and hepatitis. Total body fat was estimated with a bioimpedance device (TBF-310; Tanita International Division, Yiewsley, England). Blood was sampled after an overnight fast of at least 10 hours. Metabolic syndrome was defined by the updated National Cholesterol Education Program Adult Treatment Panel III report[20] (**Appendix**).

MR studies

All MR studies were performed with a 1.5 Tesla whole-body MR imager (Philips Medical Systems, Best, the Netherlands). Detailed information, including imaging parameters, can be found in the **Appendix**.

Hepatic triglyceride content

Hepatic spectral data were acquired with a point-resolved spectroscopy sequence with free breathing. All spectral data were analyzed with the reader blinded to all study parameters and patient information. Spectra were initially included when automatic fitting was successful. When line shapes were distorted by eddy currents or as a result of poor shimming, spectral data were rejected. In addition, mean line widths of the spectra were calculated. Hepatic triglyceride content relative to water was calculated as the sum of signal amplitudes

of methyl and methylene divided by the signal amplitude of water and then multiplied by 100.

Aortic PWV

Through-plane flow measurements of the ascending, proximal descending, mid-descending, and distal descending aorta were acquired. Aortic PWV was calculated by dividing the aortic path length between the measurement sites by the transit time between the arrival of the systolic wave front at these sites, and it is expressed in meters per second.

Visceral adipose tissue

Visceral adipose tissue was assessed with a fast spin-echo MR imaging protocol. At the level of the fifth lumbar vertebra, three transverse images were acquired during one breath hold. Visceral adipose tissue was quantified by converting the number of pixels to square centimeters and adding the areas of the individual sections by using in-house software (MASS; Leiden University Medical Center, Leiden, the Netherlands)[21].

Carotid IMT

Ultrasonography (US) was used to measure carotid IMT in the left and right common carotid arteries. Additional information is provided in the **Appendix**.

Statistical analyses

In the NEO study, individuals with a BMI of 27 kg/m^2 or higher were oversampled. To correctly represent associations in the general population[22], adjustments for the oversampling of individuals with a BMI of 27 kg/m^2 or higher were made by weighting individuals toward the BMI distribution of participants from the Leiderdorp municipality[23], whose BMI distribution was similar to the BMI distribution of the Dutch general population[24]. All results were based on weighted analyses; consequently, the results apply to a population-based study without oversampling of participants with a BMI of 27 kg/m^2 or higher. Participants were stratified into a group with hepatic steatosis and a group without hepatic steatosis according to a hepatic triglyceride content cut-off value for hepatic steatosis of 5.56%[2]. Baseline characteristics of participants were expressed as mean ± standard deviation, as median and interquartile range, or as a percentage. Comparisons between groups were tested with the two-tailed independent samples *t* test. Hepatic triglyceride content showed a right skewed distribution at visual inspection of the data; therefore, log transformation was applied. Linear regression analyses were performed to study the association of the log of hepatic triglyceride content with aortic PWV and carotid IMT: First, we estimated the crude associations of hepatic triglyceride content with aortic PWV and carotid IMT (model 1). Second, we adjusted for age, sex, heart rate, systolic and diastolic blood pressure, alcohol consumption, pack years of smoking, and use of antihypertensive and lipid-lowering drugs

(model 2). In model 3, the remaining components of the metabolic syndrome besides blood pressure (waist circumference, serum triglycerides, high-density lipoprotein cholesterol level, and fasting plasma glucose level) were added. To adjust more precisely for the effects of abdominal and total body fat, we additionally adjusted for these parameters in model 4. In additional analyses, we excluded participants who used lipid-lowering drugs because of their known hepatic lipid-lowering effects[25]. Because serum triglyceride, high-density lipoprotein cholesterol, and fasting plasma glucose levels could be possible mediators in the relationship between hepatic triglyceride content and vascular stiffness, we repeated analyses without correction for these factors. We calculated variance inflation factors to check for multicollinearity in our regression models. Variance inflation factor values were less than 10 in all models and were considered appropriate. Regression coefficients, 95% confidence intervals (CIs), and *P* values were reported. Regression coefficients can be interpreted as the difference in outcome associated with a 10-fold increase in hepatic triglyceride content. $P < 0.05$ was considered indicative of a significant difference. Statistical analysis was performed with IBM SPSS Statistics for Windows (version 20.0; IBM, Armonk, NY) and Stata (version 12; Stata, College Station, Tex) software.

RESULTS

From the total of 6673 participants in the NEO study, 2581 underwent MR imaging and spectroscopy. Because of our high-throughput study protocol, only a limited time slot was available per subject, and this did not allow time for a repeat examination when technical failures were recognized. For this reason, the technical failure rate of MR spectroscopy in the liver was approximately 20%. There was no difference in BMI ($P = 0.186$) or waist circumference ($P = 0.130$) between participants with successful MR spectroscopy and those with unsuccessful MR spectroscopy. After consecutive exclusion of participants with missing data of hepatic triglyceride content ($n = 498$), aortic PWV ($n = 60$), carotid IMT ($n = 15$), pack years of smoking ($n = 37$), plasma glucose level ($n = 12$), liver disease ($n = 8$), visceral fat ($n = 6$), use of alcohol ($n = 5$), total body fat ($n = 3$), heart rate ($n = 1$), and diastolic blood pressure ($n = 1$) and after exclusion of participants with a history of liver disease ($n = 23$) and alcohol consumption of more than 10 units per day ($n = 13$), 1899 participants (52% men, 96% white) were included in the present analysis (**Figure 1**).

PWV of the descending aorta could not be assessed in 177 participants (9%) due to technical errors. Separate analyses of the thoracic and abdominal segments of the descending aorta were performed in a subgroup of 1490 participants (78%).

Maximum detection limits were exceeded in 77 participants (4%) for the aortic arch PWV, in 81 participants (5%) for the thoracic descending aortic PWV, and in 25 participants (2%)

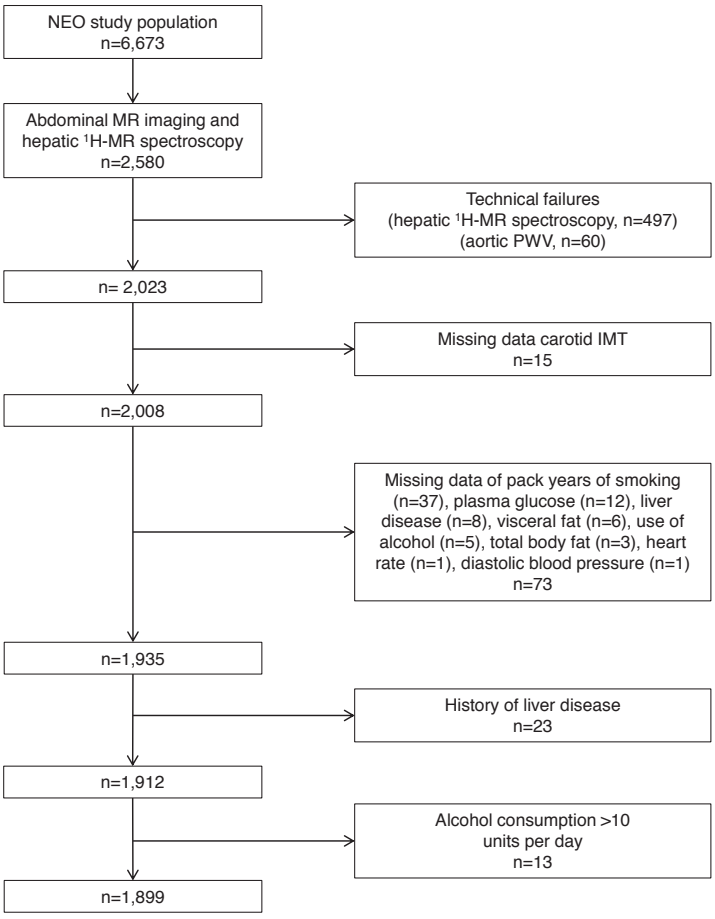

Figure 1. Flowchart shows details of the study design.

for the abdominal descending aortic PWV. The maximum PWV values, as calculated for each individual, were assigned to these participants.

Weighted participant characteristics of the total study population (*n* = 1899) are described in **Table 1**. Participants had a mean age of 55 years ± 6, and a median BMI of 25.5 kg/m2 (interquartile range, 23.1-27.9 kg/m^2). Median hepatic triglyceride content was 2.64% (interquartile range, 1.33%-6.18%), and 29% of the study population had hepatic steatosis. Mean line widths of the spectra were 45.7 Hz ± 15.5 (water, 4.7 ppm), 46.8 Hz ± 19.1 (methylene, 1.3 ppm), and 43.2 Hz ± 17.9 (methyl, 0.9 ppm). Mean PWV values were 6.6 m/s ± 1.3 (total aorta), 6.6 m/s ± 1.8 (aortic arch), 6.7 m/s ± 1.9 (total descending aorta), 7.3 m/s ± 2.4 (thoracic segment of descending aorta), and 6.4 m/s ± 2.1 (abdominal segment of descending aorta). Mean carotid IMT was 615 μm ± 92. All aortic PWV and carotid IMT values were higher in participants with hepatic steatosis. Examples of hepatic triglyceride content quantification and aortic PWV measurements are shown in **Figure 2**.

Table 1. Characteristics of participants aged 45-65 years in the Netherlands Epidemiology of Obesity study stratified by hepatic triglyceride content (n = 1899)

Characteristic	Without hepatic steatosis (hepatic triglyceride content ≤ 5.56%)	With hepatic steatosis (hepatic triglyceride content > 5.56%)	Mean difference*	*P* value
Percentage of study population (%)	71	29	NA	NA
Male sex (%)	40	61	NA	< 0.001
Age (y)	55 ± 5	57 ± 7	1.7 (0.9, 2.4)	< 0.001
BMI (kg/m^2)	24.7 (22.2-26.7)	27.7 (25.4-30.6)	3.5 (3.0, 4.0)	< 0.001
Total body fat (%)				
Men	23 ± 4	27 ± 7	4.5 (3.6, 5.4)	< 0.001
Women	35 ± 5	41 ± 7	6.4 (5.3, 7.5)	< 0.001
VAT (cm^2)				
Men	282 ± 127	426 ± 222	143.4 (117.2, 169.7)	< 0.001
Women	164 ± 81	324 ± 184	160.6 (138.6, 182.6)	< 0.001
Hepatic triglyceride content (%)	1.78 (1.08-2.95)	10.89 (7.06-17.72)	12.16 (11.21, 13.11)	< 0.001
Total aortic PWV (m/s)	6.4 ± 1.1	6.9 ± 1.7	0.5 (0.3, 0.7)	< 0.001
Aortic arch PWV (m/s)	6.5 ± 1.5	6.7 ± 2.3	0.2 (0.0, 0.4)	0.053
Descending aortic PWV (m/s)	6.6 ± 1.5	7.1 ± 2.7	0.6 (0.3, 0.9)	< 0.001
Thoracic descending aortic PWV (m/s)	7.1 ± 2.0	7.8 ± 3.2	0.7 (0.4, 1.1)	< 0.001
Abdominal descending aortic PWV (m/s)	6.3 ± 1.7	6.9 ± 3.1	0.6 (0.2, 1.0)	< 0.001
Carotid IMT (μm)	604 ± 80	641 ± 11	37 (25, 49)	< 0.001
Heart rate (beats/min)	67 ± 8	70 ± 14	2.9 (1.5, 4.3)	< 0.001
Smoking history (no. of pack years)	6.9 ± 10.2	10.6 ± 19.2	3.8 (2.3, 5.3)	< 0.001
Systolic blood pressure (mmHg)	128 ± 15	135 ± 21	7.3 (5.0, 9.6)	< 0.001
Diastolic blood pressure (mmHg)	82 ± 9	87 ± 12	4.9 (3.6, 6.2)	< 0.001
Metabolic syndrome (%)	15	55	NA	< 0.001
Waist circumference (cm)†				
Men	94.3 ± 8.1	102.1 ± 12.8	7.9 (6.2, 9.6)	< 0.001
Women	82.7 ± 8.9	95.5 ± 14.7	12.8 (10.7, 14.9)	< 0.001
Fasting serum triglyceride level (mmol/L)†	0.9 (0.7-1.3)	1.5 (1.0-2.1)	0.7 (0.6, 0.8)	< 0.001
Fasting serum HDL cholesterol level (mmol/L)†	1.7 ± 0.4	1.4 ± 0.5	-0.3 (-0.4, -0.2)	< 0.001
Hypertension (%)†	54	75	NA	< 0.001
Fasting plasma glucose level (mmol/L)†	5.2 ± 0.6	5.9 ± 1.8	0.6 (0.4, 0.8)	< 0.001
Diabetes (%)	2	11	NA	< 0.001
History of cardiovascular disease (%)	5	6	NA	0.286
AST level (units/L)	23.6 ± 5.4	26.9 ± 10.1	3.3 (2.5, 4.1)	< 0.001
ALT level (units/L)	22.1 ± 6.8	31.6 ± 18.2	9.5 (8.2, 10.8)	< 0.001
Use of antihypertensive drugs (%)	13	30	NA	< 0.001
Use of RAS antagonists	8	18	NA	< 0.001

Table 1. Characteristics of participants aged 45-65 years in the Netherlands Epidemiology of Obesity study stratified by hepatic triglyceride content (n = 1899) (continued)

Characteristic	Without hepatic steatosis (hepatic triglyceride content ≤ 5.56%)	With hepatic steatosis (hepatic triglyceride content > 5.56%)	Mean difference*	*P* value
Use of lipid-lowering drugs	7	15	NA	< 0.001

Unless otherwise indicated, data are mean ± SD, median and interquartile range, or percentage. Results are based on analyses weighted toward the BMI distribution in the general population (n = 1899). The number of participants with a measurement of aortic PWV in the descending aorta was 1722, and the number of participants with aortic PWV in the thoracic and abdominal segments of the descending aorta was 1490. ALT = alanine aminotransferase, AST = aspartate aminotransferase, HDL = high-density lipoprotein, NA = not applicable, RAS = renin-angiotensin system, VAT = visceral adipose tissue.

* Data in parentheses are the 95% CI.

† Component of the metabolic syndrome (National Cholesterol Education Program Adult Treatment Panel III).

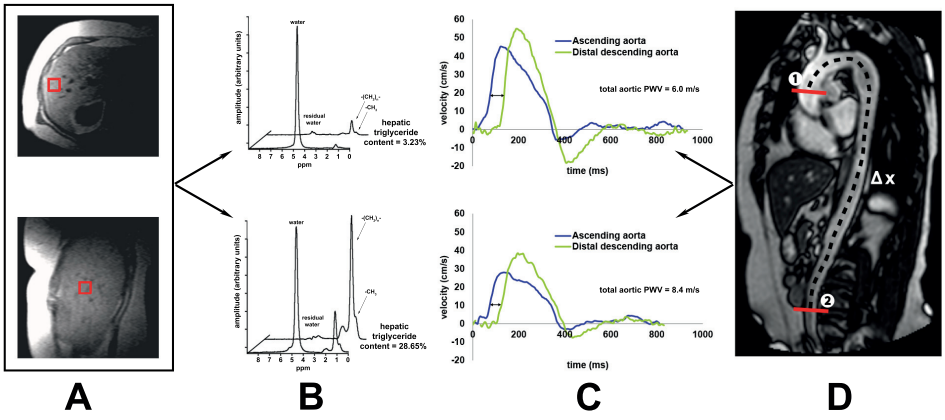

Figure 2. Assessment of hepatic triglyceride content and aortic PWV in a participant without (BMI, 26.0 kg/m^2) and in a participant with (BMI, 30.6 kg/m^2) hepatic steatosis. A, ^{1}H MR spectroscopy of the liver was used to measure hepatic triglyceride content. An 8-mL voxel was positioned in the right liver lobe, avoiding major vascular structures and extrahepatic visceral fat. Transverse (upper) and sagittal (lower) scout images are shown. B, Representative examples of MR spectral data in a participant without (upper graph; hepatic triglyceride content, 3.23%) and in a participant with (lower graph; hepatic triglyceride content, 28.65%) hepatic steatosis. Spectra were obtained without (front) and with (back) water suppression. Resonances from protons of methylene (peak at 1.3 ppm, $[CH_2]_n$) and methyl (peak at 0.9 ppm, CH_3) are highlighted. C, Systolic wave propagation of aortic flow was evaluated from maximal velocity-time curves that were obtained at each measurement site. Upper: PWV of the total aorta of the participant without hepatic steatosis was 6.0 m/s. Lower: PWV of the total aorta of the participant with hepatic steatosis was 8.4 m/s. D, PWV assessment with through-plane velocity-encoded MR imaging. The two measurement sites used to calculate total aortic PWV are shown. Aortic PWV (in meters per second) was calculated by dividing the aortic path length between the ascending aorta (1) and the distal descending aorta (2) by the transit time between the arrival of the systolic wave front at these sites.

Table 2. Differences in aortic PWV associated with a 10-fold increase in hepatic triglyceride content

Model	Difference	95% CI	*P* value
Total aorta (*n* = 1899)			
Model 1	0.56	0.40, 0.73	< 0.001
Model 2	0.15	0.00, 0.29	0.046
Model 3	0.15	-0.01, 0.31	0.072
Model 4	0.19	0.03, 0.36	0.022
Aortic arch (*n* = 1899)			
Model 1	0.36	0.14, 0.58	< 0.001
Model 2	0.02	-0.22, 0.25	0.882
Model 3	-0.02	-0.30, 0.26	0.886
Model 4	0.00	-0.28, 0.28	0.999
Descending aorta (*n* = 1722)			
Model 1	0.60	0.34, 0.87	< 0.001
Model 2	0.19	-0.05, 0.44	0.126
Model 3	0.23	-0.03, 0.49	0.078
Model 4	0.28	0.02, 0.54	0.038
Thoracic descending aorta (*n* = 1490)			
Model 1	0.93	0.60, 1.26	< 0.001
Model 2	0.29	-0.07, 0.65	0.111
Model 3	0.21	-0.20, 0.62	0.309
Model 4	0.28	-0.13, 0.70	0.179
Abdominal descending aorta (*n* = 1490)			
Model 1	0.58	0.25, 0.90	< 0.001
Model 2	0.17	-0.17, 0.51	0.327
Model 3	0.33	-0.06, 0.72	0.100
Model 4	0.42	0.03, 0.81	0.037

Reported differences represent differences in aortic PWV associated with a 10-fold increase in hepatic triglyceride content. Corresponding 95% CIs and *P* values are shown. Model 1 = crude association. Model 2 = model 1 + age, sex, heart rate, systolic and diastolic blood pressure, alcohol use, smoking history (in number of pack years), and use of antihypertensive and lipid-lowering drugs. Model 3 = model 2 + all components of metabolic syndrome. Model 4 = model 3 + visceral adipose tissue + total body fat. Results are based on analyses weighted toward the BMI distribution of the general population.

Table 2 shows the associations between hepatic triglyceride content and total and regional aortic PWV in the whole study population. The association with total aortic PWV attenuated after adjustments for several covariates. After adjustments for all covariates, the regression coefficient was 0.19 m/s (95% CI: 0.03, 0.36), representing an increase in mean total aortic PWV of 0.19 m/s per 10-fold increase in hepatic triglyceride content. After adjustments for all covariates, a 10-fold increase in hepatic triglyceride content was associated with a PWV increase of 0.28 m/s (95% CI: 0.02, 0.54) in the total descending aorta and a PWV

increase of 0.42 m/s (95% CI: 0.03, 0.81) in the abdominal segment of the descending aorta. No associations were observed between hepatic triglyceride content and PWV of the aortic arch (0.00 m/s; 95% CI: −0.28, 0.28 per 10-fold increase in hepatic triglyceride content) or the thoracic segment of the descending aorta (0.28 m/s; 95% CI: −0.13, 0.70 per 10-fold increase in hepatic triglyceride content).

Table 3 shows the association between hepatic triglyceride content and mean carotid IMT. In model 3, the regression coefficient was 15 µm (95% CI: 0, 29), representing an increase in mean carotid IMT of 15 µm for every 10-fold increase in hepatic triglyceride content. After additional adjustments for visceral and total body fat, this attenuated to 14 µm (95% CI: −1, 29).

The additional analyses in which we excluded participants who used lipid-lowering drugs showed similar results. For example, in model 4, the increase in mean total aortic PWV was 0.21 m/s (95% CI: 0.03, 0.38; $P = 0.020$), and the increase in mean carotid IMT was 16 µm (95% CI: 0, 32; $P = 0.049$) per 10-fold increase in hepatic triglyceride content. The additional analyses in which we did not adjust for triglycerides, high-density lipoprotein cholesterol level, and fasting plasma glucose level yielded slightly higher regression coefficients. For example, in model 4, the increase in mean total aortic PWV was 0.24 m/s (95% CI: 0.08, 0.40; $P = 0.004$), and the increase in mean carotid IMT was 18 µm (95% CI: 3, 32; $P = 0.015$) per 10-fold increase in hepatic triglyceride content.

Table 3. Differences in carotid IMT associated with a 10-fold increase in hepatic triglyceride content

Model	Difference	95% CI	*P* value
1	50	39, 61	< 0.001
2	32	19, 44	< 0.001
3	15	0, 29	0.044
4	14	-1, 29	0.060

Reported differences indicate differences in carotid IMT for every 10-fold increase in hepatic triglyceride content. Corresponding 95% CIs and *P* values are shown. Model 1 = crude association. Model 2 = model 1 + age, sex, heart rate, systolic and diastolic blood pressure, alcohol use, smoking history (in number of pack years), and use of antihypertensive and lipid-lowering drugs. Model 3 = model 2 + all components of metabolic syndrome. Model 4 = model 3 + visceral adipose tissue + total body fat. Results are based on analyses weighted toward the BMI distribution of the general population.

DISCUSSION

The main findings in our cohort study are that hepatic triglyceride content was associated with aortic pulse wave velocity (in particular, with regional abdominal aortic pulse wave velocity) and carotid IMT. These associations were only partly confounded by the individual components of the metabolic syndrome, and they remained after adjustments for these confounding factors. The association with aortic pulse wave velocity also remained after adjustment for visceral adipose tissue.

Aortic PWV is a measure of aortic stiffness and is recognized as an important surrogate marker of cardiovascular disease. Aortic stiffness can be assessed with different noninvasive methods. Most studies investigating the relationship between hepatic steatosis and arterial stiffness used oscillometry or tonometry devices to assess brachial-ankle or carotid-femoral PWV[5, 26-28]. These techniques are relatively inexpensive, but they are limited by the absence of vascular imaging; thus, the vascular length traveled by the pulse wave must be estimated[8]. To our knowledge, studies on whether the relationship between hepatic steatosis and vascular stiffness is attributed to concomitant risk factors have yielded conflicting results. In a large community-based cohort of mostly white subjects studied by Long et al[29], several vascular function measures, including carotid-femoral PWV, were associated with NAFLD, and these associations were largely attributed to coexisting cardiometabolic risk factors. In contrast, a large Korean epidemiologic study by Kim et al[6] and a large population-based Chinese study by Huang et al[30] reported that NAFLD was associated with brachial-ankle PWV, independent of the metabolic syndrome. Both studies also investigated carotid IMT, but only Huang et al[30] found a relationship between NAFLD and carotid IMT independent of conventional cardiovascular risk factors, including components of the metabolic syndrome. Our results in a relatively large predominantly white population enable us to confirm that an association exists between hepatic steatosis and vascular stiffness and subclinical atherosclerosis, which is not confounded by various cardiometabolic risk factors. Moreover, our study adds to the current knowledge that there are regional differences in the association of hepatic steatosis with aortic PWV.

Strengths of our cohort study were its relatively large size, the combined use of MR techniques to directly assess hepatic steatosis and aortic PWV in addition to the carotid IMT measurements, and detailed phenotyping of all participants that enables us to control for important confounding factors. Hepatic MR spectroscopy has shown better diagnostic accuracy in the identification and quantification of hepatic steatosis than has CT or US[31]. Furthermore, by using MR imaging, we were able to assess the true aortic path length, thereby enabling us to estimate central arterial PWV more precisely than we could with brachial-ankle PWV. Moreover, this technique enabled us to investigate the PWV of multiple aortic regions. Further strengths of our study were the availability of detailed information about the components of the metabolic syndrome and other potential confounding variables, including visceral adipose tissue and total body fat.

Currently, alternative noninvasive methods other than MR spectroscopy are available to measure hepatic steatosis. Quantitative imaging approaches to fat quantification are generally preferable to MR spectroscopy, as they provide spatial coverage of the liver[32]. Proton density fat fraction measurement with MR imaging showed good diagnostic accuracy in the quantification of steatosis[33]. Because our study started in 2008, this technique was unfortunately not yet validated at the time. Future trials monitoring change in hepatic steatosis are

needed to evaluate the extent to which hepatic fat measurements with MR techniques may serve as a biomarker for treatment response in patients with vascular disease.

To our knowledge, we are the first to observe a specific association of hepatic steatosis with stiffness of the abdominal aorta. It is known that the high elastin-to-collagen ratio in the aortic wall progressively declines toward the periphery[34, 35]. Elastin degradation of the media layer is a major reason for vascular stiffening in the large arteries during aging. Vascular inflammation and proliferation of smooth muscle cells in the subendothelial space cause an accumulation of collagen, resulting in an increased PWV[36]. Furthermore, cytokines are involved in low-grade inflammation and are associated with greater arterial stiffness in healthy adults[37] and men with type 1 diabetes[38]. Hence, circulating inflammatory cytokines in the state of hepatic steatosis could have a greater effect on distal regions of the aorta. This pattern of a predominantly distal decrease in aortic elastic function is supported by previous studies on obesity[39, 40].

We also observed an association between hepatic triglyceride content and subclinical atherosclerosis after taking individual components of the metabolic syndrome into account, whereas visceral fat abolished this relationship. This is consistent with findings described by Targher et al[41]. In conditions of insulin resistance, increased lipolysis in visceral adipose tissue generates an increased flux of free fatty acids. This increased flux promotes hepatic triglyceride accumulation, which is associated with enhanced oxidative stress, abnormal lipoprotein metabolism, and secretion of proinflammatory markers, such as interleukin-6, tumor necrosis factor α, and C-reactive protein[17, 42, 43]. These abnormalities may play an important role in the proatherogenic effect of NAFLD[7, 44-47]. Our data show that visceral fat is a confounder in the relationship between hepatic steatosis and subclinical atherosclerosis, but not in the relationship with aortic stiffness. It is conceivable that the pathophysiologic pathways of atherosclerosis and central arterial stiffness are not identical and that the role of visceral fat in the development of atherosclerosis is possibly more significant. Nonetheless, weight loss not only has been shown to decrease visceral adipose tissue[48] and carotid IMT and to improve inflammatory profile[49], but has also been shown to decrease arterial stiffness in overweight and obese people[39, 50].

There were several limitations to our study. First, hepatic steatosis was not proven by biopsy. Liver biopsy is the reference standard used to assess NAFLD and its necroinflammatory form, nonalcoholic steatohepatitis[51]. However, for ethical reasons, it was not possible to perform liver biopsies in such a relatively large study population. Alternatively, localized ^{1}H MR spectroscopy provides a sensitive quantitative noninvasive method with which to estimate hepatic triglyceride content[2]. Second, we aimed to study the relationship between hepatic steatosis and subclinical vascular impairment in the general population. Some participants who were more likely to have hepatic steatosis were excluded from MR imaging because their body circumference was larger than 170 cm. As a result, our findings only pertain to people with a body circumference less than 170 cm. Third, by adjusting for the components

of the metabolic syndrome, we may have overadjusted the associations being studied because from certain components, such as glucose, cholesterol, and triglyceride concentrations, it is not clear to what extent they are truly confounding factors or may be part of the causal pathway between NAFLD and cardiovascular disease. Such overadjustment for these components may have led to an underestimation of the regression coefficients. A fourth limitation of our study was the relatively high rate of technical failure for MR spectroscopy of the liver and, to a lesser extent, for MR imaging of the aorta. We were challenged by a high-throughput study protocol with only a limited time slot available per study participant. Nevertheless, technical failure of MR spectroscopy occurred randomly throughout the MR subgroup, and we do not believe it influenced our results. In addition, hepatic triglyceride content did not differ between participants with and those without PWV measurement. Furthermore, the same high-throughput study protocol with detailed phenotyping of the NEO study participants enabled us to answer our research question and to perform the present analyses with adjustment for many confounding factors. A fifth limitation is the use of a point-resolved spectroscopy sequence, which may systematically overestimate hepatic steatosis[52]. We chose to use point-resolved spectroscopy as the ^{1}H MR spectroscopy sequence because of the double signal-to-noise ratio when compared with stimulated echo acquisition mode and to keep our methods in line with those used in the population study by Szczepaniak et al[2]. Because our primary analyses were based on hepatic triglyceride content as a continuous variable, a systematic overestimation of hepatic triglyceride content would not affect the strength (i.e., the regression coefficient calculated from the multivariate regression analysis) or precision (95% CIs) of the associations between hepatic triglyceride content and aortic PWV and carotid IMT, nor would it affect our conclusions. A sixth limitation of our study is its observational cross-sectional design, which precludes a causal interpretation. Prospective studies are required to investigate the effect of hepatic steatosis on incident cardiovascular events. Finally, our study population primarily consists of white people, and our findings need to be confirmed in other ethnic groups.

In conclusion, our study showed that hepatic triglyceride content is associated with aortic stiffness, particularly stiffness of the abdominal aorta, and subclinical atherosclerosis. These associations were only partly explained by the metabolic syndrome and visceral adiposity, suggesting a possible specific contribution of hepatic steatosis to subclinical vascular impairment.

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APPENDIX

METABOLIC SYNDROME

According to the updated National Cholesterol Education Program Adult Treatment Panel III report[20], patients with the metabolic syndrome have any three of the following five characteristics: (a) waist circumference of at least 102 cm in men or at least 88 cm in women, (b) a serum triglyceride level of at least 1.7 mmol/L or currently taking medication for an elevated triglyceride level, (c) a high-density lipoprotein cholesterol level less than 1.03 mmol/L in men or less than 1.3 mmol/L in women or currently taking medication to reduce high-density lipoprotein cholesterol level, (d) systolic blood pressure of 130 mm Hg or higher and/or diastolic blood pressure of 85 mm Hg or higher or currently taking antihypertensive drugs; and (e) elevated fasting glucose level of 5.6 mmol/L or higher or currently taking medication for an elevated glucose level.

HEPATIC TRIGLYCERIDE CONTENT

Hepatic ^{1}H magnetic resonance (MR) spectra were acquired, as previously described[18, 53]. A body coil for radiofrequency transmission and a surface coil (diameter of 17 cm) for signal receiving were used. Furthermore, automated three-dimensional volume first-order iterative shimming was performed. In short, an 8-mL voxel was positioned in the right liver lobe while avoiding major vascular structures and abdominal subcutaneous and visceral adipose tissue depots. Sixty-four signals were acquired with water suppression (repetition time ms/echo time ms, 2900/23). Data points (n = 1024) were collected by using a 1000-Hz spectral line. Without changing any parameters, four signal averages without water suppression (repetition time, 10 s) were obtained as an internal reference. Areas of resonances from protons of water (4.7 ppm), methyl (0.9 ppm), and methylene (1.3 ppm) groups in fatty acid chains of the hepatic triglyceride were evaluated by using Java-based MR-user interface software (jMRUI, version 3.0; A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)[54].

AORTIC PULSE WAVE VELOCITY

Aortic pulse wave velocity (PWV) was determined by using a previously described protocol[55]. In short, a scout view of the aorta was obtained. Next, retrospectively electrocardiographically gated gradient-echo sequences with velocity encoding were performed during free breathing to assess flow at the following levels: perpendicular to the ascending aorta at the level of the pulmonary trunk, perpendicular to the descending aorta just below the diaphragm, and just above the aortic bifurcation. This resulted in through-plane flow measurements of the ascending, proximal descending, mid-descending, and distal descending aorta. A maximum number of phases was reconstructed to ensure high temporal resolution. Maximum velocity encoding was set to 200 m/s. Wave propagation was evaluated from maximal velocity-time curves that were obtained at each sampling site. Aortic PWV was calculated by dividing the aortic path length between the measurement sites by the transit time between the arrival of the systolic wave front at these sites. The foot of the systolic velocity wave front was detected by assessing the intersection point of the horizontal line modeling the constant diastolic flow and a line along the upslope of the systolic wave front, modeled by linear regression along 20%-80% of the range of the flow velocity values along this upslope. Aortic path length measurements and manual contour drawing in the aortic velocity maps was performed by using in-house software packages (MASS and FLOW; Leiden University Medical Center, Leiden, the Netherlands). The maximum detectable PWV was calculated by multiplying the repetition time (5 ms) by two and then multiplying by the distance between each measurement site.

CAROTID INTIMA-MEDIA THICKNESS

Ultrasonography was used to measure carotid intima-media thickness (IMT) in the far wall of the left and right common carotid arteries along a 15-mm long section, 10-mm proximal to the bifurcation, and in the supine position. A 7.5-10.0-MHz linear array transducer (Art Laboratory, version 2.1; Esaote, Maastricht, the Netherlands) in the B-mode setting was used to depict the distal common carotid artery, and an online wall track system was used to detect the lumen-intima and media-adventitia boundaries. Carotid IMT was measured in three predefined angles per side (180˚, 135˚, and 90˚ for the right common carotid artery; 180˚, 225˚, and 270˚ for the left common carotid artery) during six heartbeats. We calculated the mean carotid IMT for each participant by averaging all 36 carotid IMT measurements within each individual.

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