Obesity and type 2 diabetes : cardiovascular and cerebral aspects

Widya, R.L.; Widya R.L.

Chapter 2

Association between hepatic triglyceride content and left ventricular diastolic function in a population-based cohort: the Netherlands Epidemiology of Obesity study

Ralph L. Widya
Renée de Mutsert
Martin den Heijer
Saskia le Cessie
Frits R. Rosendaal
J. Wouter Jukema
Johannes W.A. Smit
Albert de Roos
Hildo J. Lamb

for the NEO Study Group

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ABSTRACT

Background

Nonalcoholic fatty liver disease is associated with increased prevalence and incidence of cardiovascular disease. The purpose of the study was to investigate the association between hepatic triglyceride content and left ventricular (LV) diastolic function while taking potential confounding factors into account, including the components of the metabolic syndrome.

Methods

The study was approved by the institutional review board, and all participants gave informed consent. In this cross-sectional analysis of baseline data from the Netherlands Epidemiology of Obesity study, a population-based, prospective cohort study, participants (45% men; mean age ± standard deviation, 55.3 years ± 6.2) underwent magnetic resonance (MR) spectroscopy and MR imaging to assess hepatic triglyceride content and LV diastolic heart function (ratio of peak filling rates of the early filling phase and atrial contraction [E/A ratio]). Multivariate linear regression analysis was performed while adjusting for confounding factors, and results were additionally stratified according to body mass index.

Results

Adjustment for age, sex, heart rate, alcohol consumption, pack-years of smoking, all components of the metabolic syndrome, and visceral adiposity attenuated crude observed associations. A 10-fold increase in hepatic triglyceride content was associated with a change in mean E/A ratio of −0.004 (95% confidence interval [CI]: −0.134, 0.125) in the total study population, −0.194 (95% CI: −0.430, 0.042) in the normal-weight subgroup, 0.079 (95% CI: −0.090, 0.248) in the overweight subgroup, and −0.109 (95% CI: −0.186, −0.032) in the obese subgroup.

Conclusions

Fatty liver itself could, at least in obesity, pose a risk of myocardial dysfunction above and beyond known cardiovascular risk factors that are clustered within the metabolic syndrome. The association in the obese subgroup was small, and future studies with larger samples sizes are required to investigate to what extent the association exists and differs in normal-weight, overweight, and obese persons to unravel its clinical relevance.

INTRODUCTION

Obesity has reached epidemic proportions during the past decades and is a well-established risk factor for various diseases, including nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease[1]. NAFLD covers a spectrum of simple steatosis to nonalcoholic steatohepatitis and cirrhosis[2]. It is the most common liver disease, with a prevalence of 20%-30% in the general population, increasing to 70%-90% among persons who are obese or have type 2 diabetes[3]. NAFLD is therefore considered as a hepatic manifestation of the metabolic syndrome. NAFLD, and in particular nonalcoholic steatohepatitis, has been associated with increased prevalence[4, 5] and incidence[3] of cardiovascular disease and with decreased myocardial phosphate metabolism[6]. Cardiac disease is increasingly observed in persons with obesity, including subclinical impairment of left ventricular (LV) diastolic function, a precursor to overt heart failure[7]. Abnormalities of diastolic function have a major role in exercise intolerance in patients with heart failure[8]. Nevertheless, limited data are available concerning the relationship between NAFLD and myocardial function. Individuals with metabolic syndrome have a twofold risk of developing cardiovascular disease[9]. Potential underlying mechanisms, however, remain unclear. Cardiac myocyte contractile function is decreased by macrophages[10]. Although speculative, it can be hypothesized that macrophage recruitment in individuals with NAFLD could be present not only in the liver, but also in the heart, causing diastolic dysfunction. Alternatively, it has been suggested that increased intrahepatic cytokine expression plays a key role in the progression of cardiovascular disease[3].

Furthermore, the individual components that define the metabolic syndrome (three of the following five: high waist circumference, high serum triglyceride level, decreased serum high-density lipoprotein cholesterol level, high blood pressure, high fasting plasma glucose level) are also considered risk factors of both NAFLD and cardiovascular disease. This means that these components, and possibly the metabolic syndrome itself, may be responsible for an observed association between hepatic triglyceride content and diastolic function[3]. In other words, it is yet unclear whether the association between NAFLD and cardiovascular disease is a consequence of shared risk factors within the metabolic syndrome or exists independently of these risk factors.

Localized hydrogen 1 (^{1}H) magnetic resonance (MR) spectroscopy is a sensitive, quantitative, noninvasive method for measuring hepatic triglyceride content[11]. Cardiac MR imaging is a highly accurate and reproducible technique for assessing LV diastolic function[12, 13]. We hypothesized that hepatic triglyceride content is associated with LV diastolic function in a population-based cohort independent of the possible confounding effect of the metabolic syndrome. Therefore, the purpose of this study was to investigate the association between hepatic triglyceride content and LV diastolic function while taking potential confounding factors into account, including the components of the metabolic syndrome.

METHODS

Study population

Men and women aged 45-65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher from Leiden and the surrounding area (Midwest of the Netherlands) were eligible to participate in the Netherlands Epidemiology of Obesity (NEO) study. In addition, all inhabitants aged 45-65 years of one municipality (Leiderdorp) were invited irrespective of their BMI, which allowed for a reference distribution of BMI. The study population of the present analysis consists of participants who had undergone MR imaging and ^{1}H MR spectroscopy. Exclusion criteria for this analysis were a history of cardiovascular disease, history of liver disease, alcohol consumption of more than 10 units per day, and use of statins and/or other lipid-lowering drugs. The study was approved by the medical ethics committee of the Leiden University Medical Center, and all participants provided written informed consent.

Study design

The present study is a cross-sectional analysis of baseline data from the NEO study. The NEO study is a population-based, prospective cohort study. Detailed information about the study design and data collection is available in the **Appendix**.

Metabolic syndrome

Characteristics of the metabolic syndrome were based on the updated National Cholesterol Education Program Adult Treatment Panel III definition[14] (see **Appendix**).

MR studies

MR imaging and spectroscopy were performed with a 1.5 Tesla whole-body MR unit (Philips Medical Systems, Best, the Netherlands). More detailed information, including imaging parameters, can be found in the **Appendix**.

MR spectroscopy

Hepatic ^{1}H MR spectra were obtained as described previously[15]. In short, an 8-mL voxel was positioned in the right lobe of the liver. A point-resolved spectroscopy sequence was used to acquire spectroscopic data during continuous breathing with automated shimming. Spectra were obtained with and without water suppression. Spectral data were fitted by using Java-based MR user interface software (jMRUI, version 3.0; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)[16]. Mean line widths of the spectra were calculated. The resonances that were fitted and used for calculation of the triglycerides were methylene (peak at 1.3 ppm, $[CH_2]_n$) and methyl (peak at 0.9 ppm, CH_3). The hepatic triglyceride content relative to water was calculated with the following formula: (signal amplitude of methylene + methyl) / (signal amplitude of water) × 100.

MR imaging

Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue areas were quantified with a turbo spin-echo MR imaging protocol. At the level of the fifth lumbar vertebra, three transverse images were acquired during one breath hold. The entire heart was imaged in the short-axis orientation by using electrocardiographically gated breath-hold balanced steady-state free precession imaging to assess LV dimensions and mass. To determine diastolic function, an electrocardiographically gated gradient-echo sequence was performed with velocity encoding to measure blood flow across the mitral valve. Diastolic parameters included peak filling rates of the early filling phase (E) and atrial contraction (A) and their ratio (E/A ratio). Image postprocessing was performed with in-house-developed software packages (MASS and FLOW; Leiden University Medical Center, Leiden, the Netherlands), and decisions were made by consensus between two experienced observers (R.L.W. and H.J.L., with 5 and > 15 years of experience in cardiovascular MR imaging, respectively).

Statistical analyses

Additional information about the statistical analyses is provided in the **Appendix**. Baseline characteristics of participants are summarized as means ± standard deviations, medians and 25th and 75th percentiles, or as percentages according to categories of BMI. To this extent, participants were stratified into subgroups according to World Health Organization criteria (BMI of < 25 kg/m^2, 25-30 kg/m^2, and ≥ 30 kg/m^2). Comparisons among groups were tested with the two-tailed independent samples *t* test or the χ^2 test where appropriate. Multivariate linear regression analyses were used to study the association between hepatic triglyceride content and E/A ratio while adjusting for potential confounders in different models (**Appendix**). Hepatic triglyceride content showed a right-skewed distribution; to use this variable in the regression analysis, a log-transformation was applied (log hepatic triglyceride content). We examined the presence of interaction between hepatic triglyceride content and BMI in their association with E/A ratio by including product terms to the final model. Regression (β) coefficients, 95% confidence intervals (CIs), and *P* and R^2 values were reported. $P < 0.05$ was considered indicative of a statistically significant difference. Statistical analysis was performed with software (SPSS for Windows, version 17.0 [SPSS, Chicago, Ill] and Stata, version 12 [Stata, College Station, Tex]).

RESULTS

Between September 3, 2008, and September 28, 2012, 6673 participants were included in the NEO study, of whom 2580 underwent MR imaging and MR spectroscopy. Of those 2580 subjects, 1207 underwent cardiovascular MR imaging. Cardiovascular MR imaging failed because of technical errors in 35 participants. In another 246 participants, ^{1}H MR

spectroscopy of the liver could not be completed owing to technical errors (*n* = 241) or because the participant felt unwell (*n* = 5), for example because of claustrophobia. In our high-throughput study protocol, only a limited time slot was available per subject, and this

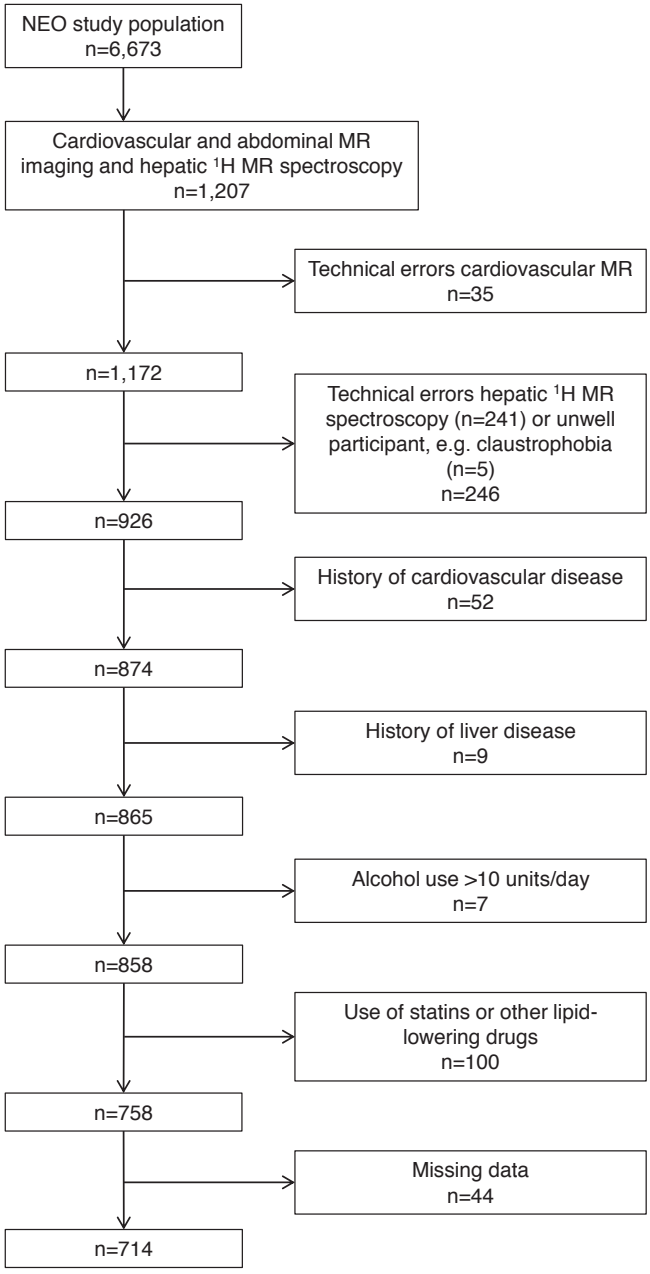

Figure 1. Study flowchart.

did not allow for repeat imaging when technical failures were recognized. The failure rate of MR spectroscopy was not related to age, sex, BMI, waist circumference, VAT, total body fat, or LV E/A ratio. Participants in whom MR spectroscopy was unsuccessful had higher subcutaneous adipose tissue compared with those who successfully underwent MR spectroscopy (mean, 738 cm^3 ± 298 vs. 701 cm^3 ± 290, respectively; $P = 0.04$). Ultimately, 926 participants successfully underwent cardiovascular and abdominal MR imaging and ^{1}H MR spectroscopy of the liver. Participants with a history of cardiovascular disease ($n = 52$), liver disease ($n = 9$), and alcohol consumption of more than 10 units per day ($n = 7$) and those taking statins and other lipid-lowering drugs ($n = 100$) were consecutively excluded. Furthermore, participants with missing data ($n = 44$) were excluded. Finally, 714 participants (45% men, 98% white) were included in the present analysis (**Figure 1**). The subjects had a mean age of 55.3 years ± 6.2 (men, 54.8 years ± 6.6; women, 55.6 years ± 5.8; $P > 0.05$), a median BMI of 25.6 kg/m^2 (25^{th} and 75^{th} percentiles, 22.9 kg/m^2 and 27.9 kg/m^2), and a median hepatic triglyceride content of 2.60% (25^{th} and 75^{th} percentiles, 1.30% and 6.05%). The hepatic triglyceride content ranged from 0.3% to 62.9%. Mean line widths of the spectra were 44.7 Hz ± 14.9 (water, 4.7 ppm), 46.7 Hz ± 18.9 (methylene, 1.3 ppm), and 43.2 Hz ± 18.4 (methyl, 0.9 ppm).

Participant characteristics are shown in **Table 1** and **Figure 2**. The median hepatic triglyceride content was highest in the obese subgroup. Furthermore, the prevalence of the metabolic syndrome was markedly higher in the obese subgroup ($P < 0.05$).

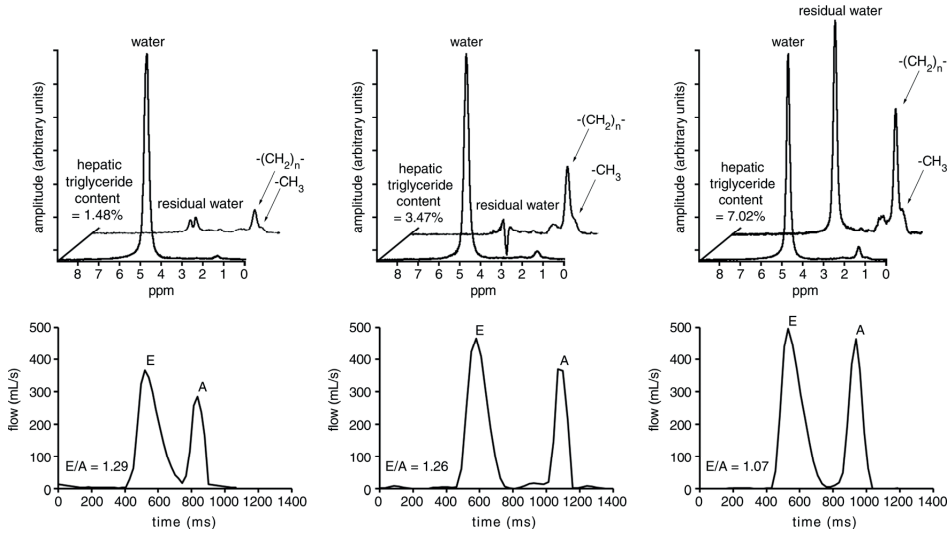

Figure 2. Representative examples of MR spectral data of hepatic triglyceride content (upper row) and MR imaging-derived LV diastolic function (lower row) in normal-weight (left), overweight (middle), and obese (right) participant. Spectra were obtained without (front) and with (back) water suppression. Resonances from protons of methylene (peak at 1.3 ppm, $[CH_2]_n$) and methyl (peak at 0.9 ppm, CH_3) are highlighted. *A* = atrial contraction, *E* = early filling phase.

Table 1. Participant characteristics according to BMI (n = 714)

Parameter	Normal-weight (BMI < 25 kg/m^2)	Overweight (BMI 25-30 kg/m^2)	Obese (BMI ≥ 30 kg/m^2)
Proportion of study population (%)	43	44	13
Men (%)	34	57*	42†
White subjects (%)	99	97	96
Age (y)	55.5 ± 3.5	55.2 ± 6.2	54.8 ± 10.5
Median BMI (kg/m^2)‡	22.4 (21.4, 23.8)	26.9 (25.9, 27.9)*	32.3 (31.0, 34.5)†§
Mean BSA (m^2)	1.8 ± 0.1	2.0 ± 0.2*	2.2 ± 0.3†§
Total body fat (%)	27.7 ± 3.9	31.3 ± 7.8*	40.1 ± 13.0†§
Men	18.9 ± 1.5	25.5 ± 3.3*	32.8 ± 7.8†§
Women	32.2 ± 2.0	39.1 ± 3.7*	45.5 ± 6.2†§
Median HTG content (%)‡	1.49 (0.93, 3.08)	3.44 (1.84, 7.43)*	7.64 (3.47, 15.54)†§
Mean VAT (cm^2)	161 ± 49	302 ± 136*	436 ± 320†§
Mean SAT (cm^2)	527 ± 89	734 ± 204*	1166 ± 554†§
Mean heart rate (beats/min)	66 ± 5	66 ± 11	68 ± 17†§
Mean pack-years of smoking	3.5 ± 3.7	8.4 ± 15.5*	11.1 ± 26.0†§
Mean systolic blood pressure (mmHg)	130 ± 11	131 ± 17	134 ± 30†§
Mean diastolic blood pressure (mmHg)	82 ± 6	85 ± 11*	86 ± 17†§
Metabolic syndrome (%)	6.6	23.9*	60.9†§
Mean waist circumference (cm)$^{\|}$	80.9 ± 4.2	94.9 ± 7.7*	108.2 ± 15.6†§
Men	87.6 ± 2.9	98.4 ± 6.2*	113.0 ± 11.2†§
Women	77.5 ± 3.5	90.4 ± 6.9*	104.7 ± 15.6†§
Median fasting triglyceride level (mmol/L)$^{\ddagger\|}$	0.8 (0.7, 1.1)	1.2 (0.9, 1.5)*	1.4 (1.0, 1.9)†§
Mean fasting HDL cholesterol level (mmol/L)$^{\|}$	1.7 ± 0.3	1.4 ± 0.4*	1.3 ± 0.6†§
Mean fasting total cholesterol level (mmol/L)	5.7 ± 0.5	5.9 ± 1.0	5.8 ± 1.8
Mean fasting glucose level (mmol/L)$^{\|}$	5.1 ± 0.2	5.5 ± 0.9*	5.7 ± 1.6†§
Hypertension (%)$^{\|}$	53.8	59.8	75.1†§
Diabetes (%)	0.0	3.8*	8.2†§

Results are based on weighted analyses. BSA = body surface area, HDL = high-density lipoprotein, HTG = hepatic triglyceride, SAT = subcutaneous adipose tissue.

* P < 0.05 normal-weight versus overweight subjects.

† P < 0.05 overweight versus obese subjects.

‡ Numbers in parentheses are the 25th and 75th percentiles.

§ P < 0.05 normal-weight versus obese subjects.

$^{\|}$ Component of the metabolic syndrome.

LV end-diastolic volume and LV mass indexed to body surface area were higher in the obese subgroup (P < 0.05) (**Table 2**). Although cardiac output was higher in the overweight and obese subgroups compared with the normal-weight subgroup, the cardiac index was similar among groups. Ejection fraction was similar between the normal-weight participants and overweight participants but was slightly higher in normal-weight compared with

Table 2. Left ventricular dimensions and function according to BMI ($n = 714$)

Parameter	Normal-weight (BMI < 25 kg/m^2)	Overweight (BMI 25-30 kg/m^2)	Obese (BMI ≥ 30 kg/m^2)
Dimensions			
End-diastolic volume (mL)	137.1 ± 16.1	153.7 ± 33.8*	161.1 ± 63.7†‡
End-diastolic volume index (mL/m^2)	76.9 ± 7.7	76.5 ± 13.3	73.9 ± 22.9‡
End-systolic volume (mL)	49.2 ± 7.1	56.8 ± 18.0*	60.9 ± 33.3†‡
End-systolic volume index (mL/m^2)	27.6 ± 3.7	28.2 ± 7.9	27.9 ± 13.6
Mass (g)	92.6 ± 11.8	107.4 ± 28.4*	111.4 ± 58.1†
Mass index (g/m^2)	51.7 ± 5.2	53.3 ± 11.6	50.8 ± 21.7‡
Systolic function			
Stroke volume (mL)	87.9 ± 10.6	96.9 ± 21.4*	100.2 ± 39.0†
Cardiac output (L/min)	5.8 ± 0.7	6.3 ± 1.4*	6.8 ± 2.7†‡
Cardiac index (L/min/m^2)	3.2 ± 0.3	3.2 ± 0.6	3.1 ± 1.0
Ejection fraction (%)	64.2 ± 2.7	63.4 ± 6.4	62.5 ± 10.9†
Diastolic function			
Peak flow rate of early filling phase (mL/s)	432.9 ± 53.4	452.6 ± 114.7	467.4 ± 209.9†
Peak flow rate of atrial contraction (mL/s)	330.1 ± 43.0	366.6 ± 104.0*	404.5 ± 159.4†‡
E/A ratio	1.36 ± 0.19	1.32 ± 0.49	1.20 ± 0.61†‡

Data are means ± standard deviations. Results are based on weighted analyses.

* $P < 0.05$ normal-weight versus overweight subjects.

† $P < 0.05$ normal-weight versus obese subjects.

‡ $P < 0.05$ overweight versus obese subjects.

obese participants. Diastolic function was lower in the obese subgroup, as demonstrated by a lower E/A ratio compared with the normal-weight and overweight subgroups ($P < 0.05$).

There was no statistically significant interaction between log hepatic triglyceride content and the three World Health Organization categories or with BMI as a continuous variable in their association with LV E/A ratio. However, there was a significant interaction between log hepatic triglyceride content and the binary variable BMI less than and greater than 27 kg/m^2 ($P = 0.012$), meaning that the association between log hepatic triglyceride content and LV E/A ratio is different below and above a BMI of 27 kg/m^2. Because of this arbitrary cutoff, we show the results stratified according to the World Health Organization categories of normal weight, overweight, and obesity.

Table 3 shows the association between hepatic triglyceride content and LV diastolic function in the entire study population and according to BMI. A significant crude inverse association was observed in the total study population (β: −0.170; 95% CI: −0.273, −0.068), normal-weight participants (β: −0.336; 95% CI: −0.509, −0.163), and obese participants (β: −0.209; 95% CI: −0.298, −0.121) (model 1). These associations diminished after adjusting for confounding factors in model 2. The addition of all components of the metabolic syndrome (model 3) or the metabolic syndrome as a single variable (model 3a) to the multivariate linear regression

model did not alter the results. After additional adjustment for VAT and total body fat in the final model (model 4), the inverse association between hepatic triglyceride content and E/A ratio was significant only in the obese subgroup (β:–0.109; 95% CI: –0.186, –0.032), which represents a decrease in mean E/A ratio of 0.109 for a 10-fold increase in hepatic triglyceride content. In other words, in two otherwise-identical obese individuals with hepatic triglyceride contents of 1.5% and 15%, the difference in E/A ratio is estimated to be 0.109. This applies on any 10-fold difference in hepatic triglyceride content. Additional adjustment for the presence of the metabolic syndrome above the five individual components did not alter the results.

Table 3. Association between hepatic triglyceride content and LV diastolic function in total study population and in normal-weight, overweight, and obese individuals

Model and subject group	β logHTGC*	95% CI	*P* value	R^2
Model 1 (crude)				
Total study population	-0.170	-0.273, -0.068	0.001	0.036
Normal-weight	-0.336	-0.509, -0.163	0.000	0.134
Overweight	-0.002	-0.180, 0.177	0.986	0.000
Obese	-0.209	-0.298, -0.121	0.000	0.067
Model 2 (model 1 + age, sex, heart rate, alcohol use, and pack-years)				
Total study population	-0.068	-0.160, 0.025	0.150	0.345
Normal-weight	-0.159	-0.319, 0.002	0.053	0.448
Overweight	0.059	-0.112, 0.230	0.501	0.323
Obese	-0.112	-0.185, -0.039	0.003	0.386
Model 3 (model 2 + all components metabolic syndrome)				
Total study population	0.001	-0.130, 0.131	0.994	0.379
Normal-weight	-0.180	-0.417, 0.057	0.134	0.474
Overweight	0.095	-0.077, 0.268	0.279	0.398
Obese	-0.118	-0.197, -0.040	0.003	0.408
Model 3a (model 2 + metabolic syndrome)				
Total study population	-0.028	-0.136, 0.081	0.618	0.354
Normal-weight	-0.120	-0.319, 0.078	0.232	0.453
Overweight	0.084	-0.094, 0.262	0.353	0.333
Obese	-0.118	-0.191, -0.045	0.002	0.387
Model 4 (model 3 + VAT + total body fat)				
Total study population	-0.004	-0.134, 0.125	0.946	0.381
Normal-weight	-0.194	-0.430, 0.042	0.106	0.476
Overweight	0.079	-0.090, 0.248	0.357	0.409
Obese	-0.109	-0.186, -0.032	0.006	0.411

Reported differences represent differences in E/A ratio associated with a 10-fold increase in hepatic triglyceride content. Corresponding 95% CIs and *P* values are shown. R^2 indicates the explained variance of E/A ratio according to the applied model. Results are based on weighted analyses.

* LogHTGC is the log-transformation of hepatic triglyceride content.

DISCUSSION

After stratification in BMI categories according to the World Health Organization, hepatic triglyceride content was significantly associated with diastolic function independent of confounding factors including the metabolic syndrome, VAT, and total body fat in obese adults aged 45-65 years. This association was not statistically significant in normal-weight and overweight subgroups. Future studies with larger sample sizes should reveal to what extent associations between hepatic triglyceride content and diastolic function exist and differ in normal-weight, overweight, and obese persons.

A large Korean epidemiologic study recently reported that NAFLD was associated with subclinical diastolic dysfunction independent of the metabolic syndrome[17]. Furthermore, LV diastolic function was impaired in normotensive nondiabetic patients with NAFLD compared with control subjects[18], and NAFLD was associated with the early diastolic velocity of the mitral annulus at tissue Doppler imaging in a multivariate stepwise regression analysis including metabolic and multiple echocardiographic variables[19]. In addition, patients with hypertension and NAFLD had a higher prevalence of diastolic dysfunction (E/A ratio, < 1) compared with hypertensive patients without NAFLD, and NAFLD was associated with diastolic function[20]. NAFLD was also associated with early diastolic dysfunction in patients with well-controlled type 2 diabetes mellitus after adjustment for age, sex, triglyceride level, hemoglobin A_{1c} level, and hypertension[21]. Most studies assessed NAFLD by using criteria for abdominal ultrasonography. Quantification of hepatic steatosis could therefore often not be assessed. Furthermore, most studies were limited by small sample sizes, whereas the current study aimed to investigate the relationship between hepatic triglyceride content and diastolic function in a large sample of the general population. The association between hepatic triglyceride content and LV E/A ratio in the normal-weight subgroup could not be detected with statistical significance, probably because of the small number of individuals in the normal-weight subgroup (n = 91). Therefore, future studies with larger sample sizes should reveal to what extent associations between hepatic triglyceride content and diastolic function exist and differ in normal-weight, overweight, and obese persons.

The complex interrelationships among NAFLD, the metabolic syndrome, visceral obesity, and cardiovascular complications make it difficult to distinguish the causal links underlying the increased risk of cardiovascular disease among patients with NAFLD and/or the metabolic syndrome[3]. For example, subjects who meet the diagnostic criteria for the metabolic syndrome have multiple risk factors for cardiovascular disease[22]. The findings from our study suggest that hepatic triglyceride content contributes to subclinical impairment of LV diastolic function independently of the components of the metabolic syndrome, at least in obese persons. Further study is required to unravel the clinical relevance of the observed small association.

Causal pathways between fatty liver and diastolic function are speculative, but inflammatory cytokines, lipids, and advanced glycation end-products may play an important role[23]. An important link between hepatic steatosis and cardiac disease has been described by Rijzewijk et al[6], who reported an association between hepatic steatosis and decreased myocardial perfusion, glucose uptake, and high-energy phosphate metabolism, but not with LV function, in type 2 diabetes. In addition, interstitial myocardial fibrosis has been associated with myocardial systolic and diastolic function in patients with diabetes[24]. These findings may indicate an early alteration in myocardial tissue and/or vascular properties. Perseghin et al[25] reported that fatty liver was associated with changes in LV energy metabolism in healthy obese individuals. An animal study provided evidence that decreased adenosine triphosphate synthesis may be responsible for LV dysfunction in obesity[26]. It may be hypothesized that NAFLD causes myocardial tissue alterations that lead to diastolic dysfunction. Diastolic dysfunction reflects increased LV diastolic stiffness, which is recognized as the earliest manifestation of LV dysfunction in diabetes mellitus, and is caused by fibrosis, deposition of advanced glycation end-products, and increased cardiomyocyte resting tension[27]. Structural myocardial changes have been found in this study and are reflected by decreased LV end-diastolic volume index and increased LV mass in obesity.

Our study adds to the present knowledge that fatty liver was inversely associated with LV diastolic function independent of the metabolic syndrome and abdominal visceral adiposity, which may suggest subclinical impaired LV relaxation. The importance of subclinical effects is the potential reversibility of the pathophysiologic process, and the possibility to detect and follow up before overt cardiovascular failure is apparent. Increased intrahepatic cytokine expression is likely to play a key role in the progression of NAFLD[3, 23, 28] and cardiovascular disease[29, 30].

Strengths of this study are the large study population and the availability of ^{1}H MR spectroscopy to quantify hepatic steatosis in combination with cardiac MR imaging. To the best of our knowledge, the current study is the first to report associations between hepatic triglyceride content and diastolic dysfunction in a general Western population. Further strengths are the availability of information about the components of the metabolic syndrome and other potential confounding variables, including total body fat and VAT.

A few limitations of this study should be addressed. No imaging modality is currently able to depict subtle histologic changes of inflammation and thus help differentiate simple steatosis from nonalcoholic steatohepatitis. Therefore, liver biopsy is the standard of reference for differentiating these two stages of NAFLD[31]. For ethical reasons, we could not perform liver biopsies in this study and therefore could not classify nonalcoholic steatohepatitis. To assess NAFLD, we measured hepatic triglyceride content by using localized ^{1}H MR spectroscopy. Because of limited MR protocol time per participant in this large population-based cohort study, we did not correct for individual T2 relaxation times and we could not perform repeat imaging when technical failures were recognized. Proton-density fat fraction measurement

with MR imaging recently showed good diagnostic accuracy for quantifying steatosis[32, 33]. Unfortunately, this technique was not yet validated at the time our study started in 2008. Optimally, such an advanced MR imaging technique would be better than MR spectroscopy, not least because MR spectroscopy is technically demanding. Participants in whom MR spectroscopy was unsuccessful had slightly higher amounts of subcutaneous adipose tissue, which potentially could have introduced selection bias. Finally, the observational, cross-sectional nature of our study precludes a causal interpretation of our results.

In conclusion, we showed that hepatic triglyceride content was associated with decreased diastolic function; however, adjustments for confounding factors attenuated this association. Only in persons with obesity could an association independent of the metabolic syndrome and abdominal visceral adiposity be demonstrated significantly. Therefore, confounding factors seem to largely explain the relationship between hepatic triglyceride content and diastolic function, but fatty liver itself could, at least in obesity, pose a risk of myocardial dysfunction above and beyond known cardiovascular risk factors that are clustered within the metabolic syndrome. Prospective follow-up research is required to study the effect of hepatic steatosis on incident cardiovascular events.

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APPENDIX

METHODS

Study design

Detailed information about the study design of the NEO study and data collection has been described elsewhere[34]. In short, enrolled participants completed a questionnaire about demographic and clinical information and came to the NEO study site for one morning after an overnight fast for several baseline measurements, including anthropometric measurements and blood sampling. In a random subset of approximately 30% of the study population without contraindications to MR imaging (metallic devices, claustrophobia, or a body circumference > 170 cm), MR imaging of the abdomen and ^{1}H MR spectroscopy of the liver were performed. The MR imaging data of abdominal visceral and subcutaneous adipose tissue of our study population have been described in previously published articles[35-37]; however, this is the first article reporting results of ^{1}H MR spectroscopy of the liver and cardiovascular MR imaging.

Data collection

Information about alcohol consumption, smoking behavior, history of cardiovascular disease, and liver disease was collected with a baseline questionnaire. Alcohol consumption was classified in six categories (0 units per day, ≤ 1 unit per week, 2-6 units per week, 1 unit per day, 2-4 units per day, and 5-9 units per day). Smoking data were converted to pack-years, defined as mean packs per day times years of smoking. 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. Medication use in the month preceding the study visit was recorded at the study site. Body weight and total body fat (in percentage) were measured by using the Tanita bioimpedance balance (TBF-310; Tanita International Division, Yiewsley, United Kingdom) without shoes; 1 kilogram was subtracted to correct for the weight of clothing. BMI was calculated by dividing weight in kilograms by the height in meters squared. Body surface area was calculated according to the Mosteller formula[38,39], as follows: (weight [in kilograms] × height [in centimeters]/3600)$^{0.5}$. Waist circumference was measured between the border of the lower costal margin and the iliac crest, and measurements were rounded to the nearest millimeter. Blood samples were taken after an overnight fast of at least 10 hours. Serum concentrations of total cholesterol and high-density lipoprotein (HDL) were determined by using standard enzymatic methods (Roche Modular Analytics P800; Roche Diagnostics, Mannheim, Germany). Serum alanine aminotransferase and aspartate aminotransferase were measured by using ultraviolet tests

(Cobas Integra 800 Analyzer, Roche Diagnostics). All analyses were performed in the central clinical chemistry laboratory of Leiden University Medical Center[34].

Metabolic syndrome

According to the National Cholesterol Education Program Adult Treatment Panel III definition, the metabolic syndrome is defined as having any three of the following five measures: (a) waist circumference of at least 102 cm in men or 88 cm in women, (b) triglyceride level of at least 1.7 mmol/L or receiving drug treatment for elevated triglyceride level, (c) HDL level less than 1.03 mmol/L in men or less than 1.3 mmol/L in women or receiving drug treatment for reduced HDL, (d) systolic blood pressure of at least 130 mmHg and/or diastolic blood pressure of at least 85 mmHg or on antihypertensive drug treatment, and (e) elevated fasting glucose level of at least 5.6 mmol/L or on drug treatment for elevated glucose.

MR spectroscopy

Gross vascular structures and adipose tissue depots were avoided when positioning the 8-mL voxel in the right lobe of the liver. Sixty-four signals were acquired with water suppression (repetition time = 2900 ms; echo time = 23 ms [2900/23]). Data points (1024) were collected by using a 1000-Hz spectral line. Without changing any parameters, spectra without water suppression (repetition time = 10 seconds; four signals acquired) were obtained as an internal reference. Spectra were not corrected for frequency drift. Spectral data were analyzed while blinded to all study parameters, including age, sex, LV function and dimensions, BMI, waist circumference, visceral adipose tissue, and total body fat. 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.

MR imaging

Imaging parameters of the turbo spin-echo protocol to assess abdominal VAT and subcutaneous adipose tissue were as follows: 300/20; flip angle, 90˚; section thickness, 10 mm, section gap, 2 mm. VAT and subcutaneous adipose tissue areas were quantified by converting the number of pixels to square centimeters and totaling the areas of the three sections, using in-house-developed software (MASS; Leiden University Medical Center, Leiden, the Netherlands)[40]. In the cardiovascular imaging protocol, the parameters of the balanced steady-state free precession imaging for measuring LV dimensions were as follows: 3.4/1.7; flip angle, 35˚; section thickness, 10 mm, section gap, 0 mm; field of view, 400 × 400 mm; and reconstructed matrix size, 256 × 256. LV dimensions (end-diastolic volume and end-systolic volume), mass, and cardiac output were indexed to body surface area. Imaging parameters of the gradient-echo sequence for measuring blood flow across the mitral valve were as follows: 6.5/1; flip angle, 20˚; section thickness, 8 mm; field of view, 350 × 350 mm; matrix size, 256 × 256; velocity encoding gradient, 150 cm/s; and imaging percentage, 80%.

Statistical analyses

In the NEO study, individuals with a BMI of 27 kg/m^2 and greater were oversampled. To correctly represent associations in the general population[41], adjustments for the oversampling of individuals with a BMI of at least 27 kg/m^2 were made. This was done by weighting individuals toward the BMI distribution of participants from the Leiderdorp municipality[42], whose BMI distribution was similar to the BMI distribution in the general Dutch population[43]. All results were based on weighted analyses. Consequently, results apply to a population-based study without an oversampling of BMI of at least 27 kg/m^2. Participants were stratified into subgroups according to the World Health Organization criteria (< 25 kg/m^2, 25-30 kg/m^2, ≥ 30 kg/m^2). Because there was only one participant with a BMI of less than 18.5 kg/m^2, this category could not be studied separately. The following multivariate linear regression analyses were used to study the association between hepatic triglyceride content and E/A ratio: We adjusted the crude (model 1) association between hepatic triglyceride content and E/A ratio for age, sex, heart rate, alcohol consumption, pack-years of smoking (model 2), and, additionally for all components of the metabolic syndrome, waist circumference, serum triglycerides, high-density lipoprotein cholesterol, systolic and diastolic blood pressure, and fasting plasma glucose level (model 3). It may be possible that the metabolic syndrome represents more than the sum of its parts. Therefore, we also adjusted for the metabolic syndrome as a dichotomous variable instead of the individual components (model 3a). Subsequently, VAT and total body fat were added to the regression model (model 4). Finally, the metabolic syndrome as a single variable was added to the regression model.

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