Cardiometabolic risk factors and venous thrombosis

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Chapter 6

Association between hepatic triglyceride content and coagulation factor levels: The Netherlands Epidemiology of Obesity Study

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ABSTRACT

Background

Hepatic triglyceride content (HTGC) has been associated with levels of coagulation factors, which is relevant to improve understanding of the pathogenesis of venous thrombosis (VT). However, it is unclear whether HTGC contributes to coagulation factor levels beyond total body fat (TBF) and visceral adipose tissue (VAT).

Objective

To investigate the association between HTGC and levels of fibrinogen, and factors (F) VIII, IX and XI while taking into account TBF and VAT.

Methods

This is a cross-sectional analysis in a subset of participants of the NEO study (n=6671) who underwent magnetic resonance (MR) imaging and MR spectroscopy to assess VAT and HTGC (n=2580). We excluded participants without complete imaging and coagulation factor assessment, with liver disease, a history of VT, or on anticoagulation.

Results

1946 participants were included (53% women; median age 56 years). Coagulation factor levels increased dose-dependently across HTGC quartiles in linear regression models adjusted for age, sex, ethnicity, education, alcohol intake, physical activity, smoking, estrogen, and menopause. Mean differences between the fourth and first (reference) quartiles were 14.4 mg/dL (95% CI: 1.8, 26.9) for fibrinogen, 6.6 IU/dL (95% CI: 0.4, 12.8) for FVIII, 26.1 IU/dL (95% CI: 22.4, 29.8) for factor IX, and 8.4 IU/dL (95% CI 4.4, 12.5) for FXI. With further adjustment for TBF and VAT, the dose-response association of HTGC with factor IX levels persisted, whereas associations with the other factors disappeared.

Conclusions

HTGC was associated with levels of various coagulation factors, of which FIX remained associated with HTGC after adjustment for TBF and VAT. HTGC has the potential to contribute to VT risk beyond total body and visceral fat through FIX levels.

INTRODUCTION

The association between obesity and venous thrombosis is well established in epidemiological studies [1]. However, the mechanism underlying this association is not fully understood, and probably reflects the coexistence of multiple pathophysiological pathways. In particular, excess fat accumulation in the liver, also referred to as nonalcoholic fatty liver disease (NAFLD), is strongly associated with obesity and insulin resistance [2], and could be one of the mechanisms by which obesity increases the risk of venous thrombosis. NAFLD is a term used to describe a broad range of related disorders, initiating from simple steatosis (accumulation of triglycerides as lipid droplets in the cytoplasm of hepatocytes), which may progress to nonalcoholic steatohepatitis (NASH) (steatosis associated with inflammation, hepatocyte injury, and/or fibrosis), cirrhosis (replacement of hepatocytes by scar tissue), and liver cancer (hepatocellular carcinoma) [2]. NAFLD is a common disease, occurring in approximately 20 to 30% of adults in the general population in Western countries [3]. Its prevalence increases to 70 to 90% among those who are obese or have type 2 diabetes. The role of NAFLD in the risk of venous thrombosis is as yet largely unknown. In one previous study composed of 138 patients with unprovoked venous thrombosis and 276 controls, the prevalence of NAFLD was almost 3-fold higher in cases (81%) than in controls (30%) [4].

In the past two decades, several small studies have investigated the association between NAFLD and hemostatic factors, mainly in relation to plasminogen activator inhibitor-1 [5-19], a key inhibitor of the fibrinolytic system. However, results of these studies have often been inconsistent, probably due to limited statistical power of the individual studies and differences in study design, clinical characteristics of the study population, methods used to define NAFLD (liver biopsy vs. radiological imaging), or adjustments for potential confounding factors. It is of interest that in one of these studies (n=98) [18], liver fat content was positively correlated with levels of coagulation factors that are associated with an increased risk of venous thrombosis, i.e., factors VIII, IX and XI [20], independent of age, sex and body mass index (BMI). However, the amount of abdominal visceral adipose tissue (VAT) was not taken into account in the relationship between liver fat content and the aforementioned coagulation factors. VAT might be an important confounding factor, as it is strongly related to liver fat [21,22], while it has also been shown to be related to levels of some coagulation factors [6,23]. Moreover, since VAT is highly associated with total body fat (TBF) [24,25], adjustment for TBF should be performed when studying specific effects of VAT [26]. Hence, whether liver fat content is associated with levels of coagulation factors beyond total body and visceral fat is as yet unclear. Since obesity and fatty liver are potentially modifiable through lifestyle intervention [3], clarification of this question is a relevant clinical issue worth pursuing.

Localized hydrogen 1 (^{1}H) magnetic resonance spectroscopy (MRS) is a sensitive, quantitative, and noninvasive method for determining liver fat content, measured as hepatic triglyceride content (HTGC) [27]. The aim of our study was to investigate the association between HTGC, assessed by ^{1}H MRS, and levels of fibrinogen, and factors VIII, IX, and XI. We further hypothesized that this association could be explained, at least in part, by common causes (confounding variables), of both HTGC and coagulation factor levels, such as demographic and lifestyle factors [3,28-32], and total body and visceral fat [6,21-23]. As sex differences are observed in body fat distribution [33], liver fat content [22,34,35], and risk of venous thrombosis [36], we additionally performed subgroup analyses stratified by sex. For these purposes, we performed a cross-sectional analysis in the Netherlands Epidemiology of Obesity (NEO) study.

METHODS

Study design and study population

The NEO study is a population-based cohort study designed to investigate pathways that lead to obesity-related diseases. The NEO study includes 6671 participants, with an oversampling of individuals with overweight or obesity. Details on the design and study population were described elsewhere [37]. Briefly, between September 2008 and September 2012, men and women aged 45-65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged 45-65 years from one municipality (Leiderdorp) were invited to participate, irrespective of their BMI, in order to obtain a reference distribution of BMI.

The present study is a cross-sectional analysis of the baseline data from the NEO study. At the time of inclusion, a screening form was completed by all participants asking about contraindications to magnetic resonance imaging (MRI) [37]. Among the eligible participants, 2580 were randomly selected to undergo ^{1}H MRS to assess HTGC, and MRI to assess abdominal subcutaneous and visceral fat [37,38]. Of these, 2075 participants had complete imaging and quantification of HTGC, and of abdominal subcutaneous and visceral fat depots.We subsequently excluded participants with missing data on levels of coagulation factors (n=27), with a known history of liver disease (n=25) or venous thrombosis (n=53), or who reported anticoagulant treatment at the time of blood sampling (n=24), thus leaving 1946 participants for the current analysis. The NEO study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC), and all participants gave written informed consent.

Data collection and blood sampling

Participants were invited to visit the NEO study center after an overnight fast for baseline measurements, including blood sampling and anthropometry [37]. Prior to the baseline visit, participants completed questionnaires at home on demographic, lifestyle and clinical data [37]. In this study, we grouped demographic and lifestyle factors as follows: ethnicity into white and other, the level of education into high and other (according to the Dutch education system, participants with higher secondary education, higher vocational education, university, and PhD were categorized as highly educated), tobacco smoking into current and other (never and former smoker), estrogen use into current and other (never and former user), and menopause status into postmenopausal and premenopausal. Physical activity was expressed in metabolic equivalents of task (MET)-hours per week, and alcohol consumption was expressed as a continuous (g/day) or a categorical variable (<10g/day, 10-20g/day, 20-40g/day, and ≥40g/day).

Measurements of body fat and magnetic resonance studies

Body weight was measured and TBF (%) was estimated by the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) [37]. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference (WC) was measured mid-way between the lower costal margin and the iliac crest (cm).

MR imaging and spectroscopy were performed with a 1.5-T whole-body MR unit (Philips Medical Systems, Best, the Netherlands), details of which have been described elsewhere [26,37,38]. Briefly, abdominal VAT and subcutaneous adipose tissue (SAT) areas were quantified with a turbo spin-echo MRI protocol. At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during one breath-hold. Abdominal SAT and VAT were quantified by converting the number of pixels to square cm (MASS, Medis, Leiden, the Netherlands), and the average of the three slices was used for analyses.

HTGC was determined by ^{1}H MRS, as previously described in the NEO study [38]. 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. The resonances that were fitted and used for calculation of the triglycerides were methylene and methyl. The HTGC relative to water was calculated with the following formula: (signal amplitude of methylene + methyl) / (signal amplitude of water) x 100.

Laboratory measurements

Blood samples for coagulation factor measurements were drawn into tubes containing 0.106M trisodium citrate (Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 2500*g* for 10 min at room temperature and stored in aliquots at -80°C until testing. Fibrinogen activity was measured according to the method of Clauss [39]. Factor VIII activity, factor IX activity and factor XI activity were measured with a mechanical clot detection method on an ACL TOP 700 analyzer (Werfen, Barcelona, Spain). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by ultraviolet tests on a Cobas Integra 800 analyzer (Roche Diagnostics, Mannheim, Germany) [37]. All assays were performed by laboratory technicians who were unaware of the status of the samples.

Statistical analyses

In the NEO study, there is an oversampling of individuals with a BMI of 27 kg/m^2 or higher. As previously described [26], to correctly represent baseline associations in the general population, adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made. This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population aged 45-65 years. All results are based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2.

Baseline characteristics of the weighted study population are expressed as proportion instead of absolute numbers for categorical variables, and as mean (± standard deviation [SD]) or median (25th and 75th percentiles) for continuous variables.

Association between body fat measures and HTGC

We used linear regression to obtain insight into the degree to which the measures of body fat and body fat distribution (BMI, TBF, WC, VAT, abdominal SAT, and VAT/SAT ratio) were associated with HTGC. Because the distribution of HTGC and VAT/SAT ratio was skewed to the right, natural log-transformation was applied for both variables in all analyses (ln HTGC and ln VAT/SAT ratio). We calculated Z-scores of body fat measures to standardize the values of these measures to a mean of zero and a SD of one.

Weighted linear regression analyses were performed to assess the associations between each standardized measure of body fat (independent variable) and ln HTGC (dependent variable). The resulting regression coefficient (β) and its 95% confidence intervals (CIs) for a measure of body fat indicates the difference in ln HTGC when

that particular measure increases by one SD. We constructed scatter plots between Z-scores of body fat measures and ln HTGC, and observed that the assumption of linearity was met in all regression models. Crude associations (model 1) were adjusted for age and sex in model 2, and in model 3 for age, sex and for the other potential confounding factors, i.e., ethnicity (dichotomous value), education level (dichotomous value), alcohol intake (continuous value), physical activity (continuous value), tobacco smoking (dichotomous value), estrogen use (dichotomous value), and menopause status (dichotomous value).

Association between HTGC and coagulation factor levels

We calculated Z-scores of ln HTGC, and used weighted linear regression to examine the increase or decrease in levels of each coagulation factor (dependent variable) per one SD increase in ln HTGC (independent variable). Levels of coagulation factors were normally distributed and assumption of linearity was met in all regression models. Crude associations (model 1), were adjusted for age and sex (model 2), and further for the potential confounding factors described in model 3, adding VAT (continuous value) and TBF (continuous value) in a fourth model. We also investigated whether the association between HTGC and coagulation factor levels followed a dose-response relation. For this purpose, we *a-priori* categorized ln HTGC into quartiles, and used weighted linear regression to estimate mean differences and their 95% CIs in levels of coagulation factors for the second, third, and fourth quartile of ln HTGC compared with the first quartile (reference category). The regression coefficient for a ln HTGC quartile indicates the mean difference in levels of coagulation factors between that particular quartile and the reference category. We adjusted associations for the same aforementioned confounding factors described in models 2, 3 and 4. In subgroup analyses, we assessed the relationship between ln HTGC quartiles and levels of coagulation factors stratified by sex.

All analyses were repeated excluding participants with alcohol consumption ≥ 20g/day at the baseline study visit (sensitivity analyses). Statistical analyses were performed with STATA Statistical Software, version 12.0 (Statacorp, College Station, Texas, USA).

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics of the 1946 participants, of whom 53% were women. The median age was 56 years (interquartile range [IQR] 50, 61), 96% of participants were white, 47% were highly educated, and 14% were current smokers. Median alcohol consumption was 10.4 g/day (IQR 2.8, 21.4), and median physical

Table 1. Baseline characteristics of 1946 participants from the NEO study

Characteristics	
Demographic and lifestyle factors	
Sex (% women)	53
Age (years)	56 (50, 61)
Ethnicity (% whites)	96
Education level (% high)[a]	47
Alcohol consumption (g/day)	10.4 (2.8, 21.4)
Alcohol consumption (%)	
<10g/day	49
10-20g/day	21
20-40g/day	22
≥40g/day	8
Physical activity (MET-hours per week)	30.2 (15.8, 51.5)
Tobacco smoking (% current)	14
Estrogen (% current use, in women)	10
Menopause status (% postmenopausal, in women)	81
Measures of adiposity	
BMI (kg/m^2)	25.9 (3.9)
Total body fat (%)	30.7 (8.3)
Waist circumference (cm)	91.1 (12.6)
VAT (cm^2)	88.3 (54.5)
SAT (cm^2)	232.6 (96.5)
VAT/SAT	0.34 (0.22, 0.52)
Hepatic triglyceride content (%)	2.66 (1.34, 6.27)
Coagulation factors	
Fibrinogen (mg/dL)	289 (55)
Factor VIII (IU/dL)	122 (32)
Factor IX (IU/dL)	116 (20)
Factor XI (IU/dL)	116 (20)
Transaminases	
ALT (U/L)	25.1 (11.5)
AST (U/L)	24.7 (8.1)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missing for some participants in some subgroups. Data are shown as mean (± standard deviation), median (25th percentile -75th percentile) or percentage. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; MET, metabolic equivalents of task; SAT, abdominal subcutaneous adipose tissue; VAT, visceral adipose tissue; VAT/SAT, ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue; WC, waist circumference. [a] High educational level (according to Dutch educational system): higher secondary education, higher vocational education, university, and PhD.

activity during leisure time was 30.2 MET-hours per week (IQR 15.8, 51.5). Among women, 81% referred a postmenopausal status and 10% were current users of estrogen. Participants had a mean BMI of 25.9 kg/m^2 ± 3.9, and a median HTGC of 2.66% (IQR 1.34, 6.27). Supplementary Table 1 describes the baseline characteristics

separately in men and women. Men had higher WC, VAT, HTGC and transaminases levels, and lower abdominal SAT and TBF than women. When participants with alcohol consumption ≥20g/day were excluded (n=620) (Table S2), the proportion of men, current smokers and highly educated participants, and the amount of VAT and HTGC were slightly lower as compared with subjects included in the main analysis, with no substantial differences in the other variables.

Association between body fat measures and HTGC

Table 2 shows that all measures of body fat and body fat distribution were associated with ln HTGC, and upon adjustment for potential demographic and lifestyle confounding factors (model 3), the strongest associations were observed for TBF, VAT and WC. When participants with alcohol consumption ≥20g/day were excluded, results were virtually the same (Table S3).

Association between HTGC and coagulation factor levels

Associations between ln HTGC and coagulation factor levels are described in Table 3. For reporting and interpretation, values of ln HTGC were back transformed in Table 3. The results of the linear regression per SD of ln HTGC show that in crude analyses and in age- and sex-adjusted models, levels of all coagulation factors were associated with ln HTGC. With further adjustment for demographic and lifestyle factors (model 3), associations of ln HTGC with coagulation factors did not substantially change. In multivariate models, one SD in ln HTGC was associated with higher levels of fibrinogen (6.3 mg/dL, 95% CI: 2.6, 9.9), factor VIII (3.2 IU/dL, 95% CI: 1.1, 5.4), factor IX (9.7 IU/dL, 95% CI: 8.5, 10.9) and factor XI (3.1 IU/dL, 95% CI: 1.9, 4.4). With additional adjustment for VAT and TBF (model 4), the associations between ln HTGC and levels of coagulation factors disappeared for factor VIII (1.6 IU/dL, 95% CI: -0.9, 4.2) and factor XI (1.2, 95% CI: -0.3, 2.7), or even became negative for fibrinogen (-5.0 mg/dL, 95% CI: -9.2, -0.7). However, the association between ln HTGC and factor IX levels, albeit attenuated, persisted (6.6 IU/dL, 95% CI: 5.1, 8.1).

Next, we examined the mean differences in coagulation factor levels for each quartile of ln HTGC in comparison with the first quartile used as the reference category (Table 3). Levels of coagulation factors increased dose-dependently across ln HTGC quartiles compared with the reference in crude analyses (model 1), and after adjustment for demographic and lifestyle factors (models 2 and 3). With additional adjustment for TBF and VAT (model 4), the associations between ln HTGC and levels of fibrinogen, factor VIII and factor XI across quartiles disappeared, whereas the association between ln HTGC and factor IX levels persisted, as did the dose-response relation.

Table 2. Association between body fat measures and hepatic triglyceride content in 1946 participants from the NEO study

	Difference in hepatic triglyceride content (%) (95% CI)[a] per SD of:					
	BMI (SD = 3.9 kg/m²)	**TBF (SD = 8.3%)**	**WC (SD = 12.6 cm)**	**VAT (SD = 54.5 cm²)**	**SAT (SD = 96.5 cm²)**	**VAT/SAT (SD = 0.61)**
Model 1	0.54 (0.49, 0.58)	0.18 (0.12, 0.24)	0.61 (0.57, 0.66)	0.65 (0.60, 0.69)	0.34 (0.29, 0.40)	0.48 (0.43, 0.53)
Model 2	0.50 (0.46, 0.55)	0.73 (0.66, 0.80)	0.61 (0.55, 0.66)	0.62 (0.57, 0.68)	0.46 (0.41, 0.51)	0.49 (0.41, 0.57)
Model 3	0.48 (0.44, 0.53)	0.69 (0.62, 0.75)	0.58 (0.52, 0.63)	0.59 (0.54, 0.64)	0.43 (0.38, 0.48)	0.46 (0.38, 0.54)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missed for some participants in some subgroups.
Hepatic triglyceride content and the ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue were natural log-transformed.
BMI, body mass index; CI, Confidence Interval; SAT, abdominal subcutaneous adipose tissue; TBF, total body fat; VAT, visceral adipose tissue; VAT/SAT, ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue; WC, waist circumference.
[a]Beta coefficients (95% CI) from linear regression per weighted SD in BMI, TBF, WC, VAT, abdominal SAT, and VAT/SAT ratio.
Model 1: crude association.
Model 2: adjustment for age and sex.
Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use and menopause status.

Table 3. Association between hepatic triglyceride content and coagulation factor levels in 1946 participants from the NEO study

	Continuous scale	Reference (mean levels)	Mean difference (95% CI)[b] compared with the reference category		
	Difference in coagulation factor levels (95% CI)[a] per SD of HTGC (2.92%)[c]	**HTGC Quartile 1 <1.34%[c] (25%)**	**HTGC Quartile 2 1.34%-2.66%[c] (25%)**	**HTGC Quartile 3 2.66%-6.27%[c] (25%)**	**HTGC Quartile 4 ≥6.27%[c] (25%)**
Fibrinogen (mg/dL)					
Model 1	5.1 (2.0, 8.3)	281	7.3 (-3.5, 18.2)	11.1 (0.6, 21.6)	11.9 (2.2, 21.6)
Model 2	6.1 (2.8, 9.5)		7.0 (-4.2, 18.1)	12.7 (0.6, 24.9)	15.0 (3.8, 26.2)
Model 3	6.3 (2.6, 9.9)		6.3 (-5.3, 17.8)	13.7 (1.0, 26.3)	14.4 (1.8, 26.9)
Model 4	-5.0 (-9.2, -0.7)		-5.5 (-17.6, 6.7)	-10.3 (-25.0, 4.3)	-14.1 (-31.0, 2.2)
Factor VIII (IU/dL)					
Model 1	2.9 (0.9, 5.0)	119	2.0 (-4.6, 8.6)	2.7 (-3.5, 8.8)	7.1 (1.1, 13.1)
Model 2	3.0 (0.9, 5.0)		1.2 (-5.4, 7.9)	2.0 (-4.5, 8.6)	6.2 (0.1, 12.3)
Model 3	3.2 (1.1, 5.4)		1.4 (-5.4, 8.2)	2.5 (-4.1, 9.1)	6.6 (0.4, 12.8)
Model 4	1.6 (-0.9, 4.2)		-1.2 (-8.3, 5.9)	-1.9 (-9.2, 5.3)	3.2 (-5.1, 11.5)
Factor IX (IU/dL)					
Model 1	9.7 (8.5, 10.8)	103	9.8 (6.8, 12.8)	17.7 (14.4, 21.1)	26.2 (22.9, 29.5)
Model 2	9.9 (8.7, 11.0)		9.7 (6.6, 12.7)	18.6 (14.8, 22.3)	26.3 (23.0, 29.6)
Model 3	9.7 (8.5, 10.9)		9.8 (6.7, 12.9)	18.4 (14.8, 22.1)	26.1 (22.4, 29.8)
Model 4	6.6 (5.1, 8.1)		6.6 (3.6, 9.6)	11.7 (7.8, 15.6)	19.0 (13.8, 24.1)

Table 3. (continued)

	Continuous scale	Reference (mean levels)	Mean difference (95% CI)[b] compared with the reference category		
	Difference in coagulation factor levels (95% CI)[a] per SD of HTGC (2.92%)[c]	**HTGC Quartile 1 <1.34%[c] (25%)**	**HTGC Quartile 2 1.34%-2.66%[c] (25%)**	**HTGC Quartile 3 2.66%-6.27%[c] (25%)**	**HTGC Quartile 4 ≥6.27%[c] (25%)**
Factor XI (IU/dL)					
Model 1	2.0 (0.8, 3.1)	112	3.5 (-0.4, 7.4)	5.0 (1.2, 8.7)	5.8 (2.5, 9.2)
Model 2	3.3 (2.2, 4.5)		4.8 (1.0, 8.5)	7.7 (3.7, 11.7)	8.9 (5.3, 12.5)
Model 3	3.1 (1.9, 4.4)		4.2 (0.2, 8.1)	7.2 (2.9, 11.6)	8.4 (4.4, 12.5)
Model 4	1.2 (-0.3, 2.7)		2.4 (-1.8, 6.5)	2.1 (-2.7, 6.9)	4.9 (-0.3, 10.2)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missed for some participants in some subgroups.

CI, confidence interval; HTGC, hepatic triglyceride content; SD, standard deviation.

[a] Beta coefficients (95% CI) from linear regression per weighted SD in natural log-transformed HTGC.

[b] Beta coefficients (95% CI) obtained by linear regression in each weighted quartile of natural log-transformed HTGC compared with the lowest quartile (reference category).

[c] Values of SD and quartiles of HTGC were back transformed for interpretation.

Model 1: crude association.

Model 2: adjustment for age and sex.

Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use and menopause status.

Model 4: model 3 + adjustment for visceral adipose tissue and total body fat.

Table 4 presents mean differences in coagulation factor levels for each quartile of ln HTGC in comparison with the first quartile stratified by sex. For reporting and interpretation, values of ln HTGC were also back transformed in Table 4. As in overall analysis, upon adjustment for TBF and VAT, no associations between ln HTGC and levels of fibrinogen or factor VIII were observed in both men and women. Compared with the reference category, higher quartiles of ln HTGC were associated with higher levels of factor IX in a dose-response fashion and to a similar extent in men and women, also after adjustment for VAT and TBF. Interestingly, ln HTGC was consistently associated with factor XI levels in women, even with further adjustment for TBF and VAT, but not in men, in whom the associations were weak or absent across quartiles and regression models. After excluding participants with alcohol consumption ≥20g/day, results were similar for overall (Table S4) and subgroup analyses stratified by sex (Table S5).

DISCUSSION

In this large population-based cross-sectional study, HTGC was associated with levels of various coagulation factors (i.e., fibrinogen, factor VIII, factor IX or factor XI) in a dose-response fashion, even after adjustment for several demographic and lifestyle potential confounding factors. However, with further adjustment for total body and visceral fat, the associations between HTGC and levels of fibrinogen, factor VIII and factor XI disappeared, whereas the associations between HTGC and factor IX levels, albeit attenuated, persisted, as did the dose-response relation. This observation could be relevant, as high levels of factor IX related to liver fat content have the potential to be a critical pathway by which obesity increases the risk of venous thrombosis. Indeed, there is substantial body of evidence that factor IX, a vitamin K-dependent factor (VKDF), plays a pivotal role in thrombin generation [40,41]. Furthermore, high levels of factor IX have been associated with an increased risk of venous thrombosis in epidemiological studies [42-44].

To the best of our knowledge, this is the first study to show that HTGC and factor IX levels are associated in a dose-response fashion, also after adjustment for several potential confounding factors, including total body and visceral fat. Interestingly, our finding on the association of hepatic triglycerides with factor IX levels seems to be in line with previous studies on principal component analysis, in which serum triglycerides clustered together with factor IX and other procoagulant VKDFs, i.e., factors II, VII, and X [45,46]. Recently, we have assessed the interrelation between levels of several hemostatic factors and lipids in 2874 population controls from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study. We found that serum triglycerides clustered together with all VKDFs, including the procoagulant and anticoagulant factors [47]. Taken together,

Table 4. Association between hepatic triglyceride content and coagulation factor levels in 1946 participants from the NEO study according to sex

	Reference (mean levels)	Mean difference (95% CI)[a] compared with the reference category		
	HTGC Quartile 1 <1.34%[b]	**HTGC Quartile 2 1.34%-2.66%[b]**	**HTGC Quartile 3 2.66%-6.27%[b]**	**HTGC Quartile 4 ≥6.27%[b]**
Fibrinogen (mg/dL)				
Model 1				
Men	276	7.3 (-15.1, 29.7)	9.6 (-11.6, 30.7)	7.3 (-13.1, 27.6)
Women	283	9.5 (-3.3, 22.7)	18.8 (5.1, 32.6)	26.2 (13.2, 39.2)
Model 2				
Men	276	7.1 (-15.3, 29.4)	7.2 (-13.6, 28.1)	5.7 (-14.3, 25.8)
Women	283	6.6 (-6.1, 19.3)	16.8 (1.6, 32.1)	21.4 (8.2, 34.6)
Model 3				
Men	276	5.3 (-19.7, 30.3)	9.8 (-12.8, 32.5)	3.5 (-18.7, 25.8)
Women	283	6.6 (-5.7, 18.8)	17.2 (3.0, 31.3)	25.5 (12.3, 38.7)
Model 4				
Men	276	-4.2 (-30.3, 22.0)	-10.6 (-36.2, 15.1)	-16.0 (-44.4, 12.4)
Women	283	-6.1 (-19.0, 6.7)	-10.4 (-25.7, 4.9)	-11.2 (-29.2, 6.8)
Factor VIII (IU/dL)				
Model 1				
Men	120	-1.2 (-10.9, 8.5)	-2.1 (-10.8, 6.6)	4.5 (-4.1, 13.2)
Women	119	4.0 (-4.7, 12.7)	8.4 (-0.2, 17.0)	10.0 (1.8, 18.3)
Model 2				
Men	120	-1.4 (-11.0, 8.3)	-3.9 (-12.6, 4.8)	3.2 (-5.4, 11.7)
Women	119	2.3 (-6.6, 11.2)	6.2 (-3.4, 15.8)	9.0 (0.1, 17.9)
Model 3				
Men	120	-1.2 (-10.9, 8.4)	-3.6 (-12.0, 4.8)	3.3 (-5.1, 11.7)
Women	119	2.4 (-6.9, 11.6)	6.8 (-2.4, 16.0)	9.2 (0.6, 17.7)
Model 4				
Men	120	-2.7 (-12.5, 7.2)	-4.1 (-13.6, 5.3)	3.0 (-8.3, 14.2)
Women	119	-0.8 (-11.0, 9.3)	-2.1 (-12.2, 8.0)	2.0 (-10.3, 14.2)
Factor IX (IU/dL)				
Model 1				
Men	103	9.1 (4.0, 14.2)	14.0 (8.9, 19.1)	24.1 (18.7, 29.4)
Women	102	9.9 (6.1, 13.8)	22.1 (17.1, 27.1)	28.6 (24.5, 32.8)
Model 2				
Men	103	9.0 (4.0, 14.1)	13.4 (8.4, 18.3)	23.5 (18.2, 28.7)
Women	102	10.2 (6.4, 14.0)	22.4 (17.0, 27.8)	28.8 (24.6, 33.0)
Model 3				
Men	103	8.6 (3.2, 13.9)	11.8 (6.9, 16.7)	20.6 (14.4, 26.9)
Women	102	10.3 (6.3, 14.2)	22.7 (17.8, 27.6)	29.8 (26.0, 33.7)
Model 4				
Men	103	6.8 (1.5, 12.0)	8.0 (2.6, 13.4)	16.3 (8.1, 24.6)
Women	102	5.9 (2.1, 9.7)	12.7 (7.8, 17.5)	19.0 (13.2, 24.9)

Table 4. (continued)

	Reference (mean levels)	Mean difference (95% CI)[a] compared with the reference category		
	HTGC Quartile 1 <1.34%[b]	**HTGC Quartile 2 1.34%-2.66%[b]**	**HTGC Quartile 3 2.66%-6.27%[b]**	**HTGC Quartile 4 ≥6.27%[b]**
Factor XI (IU/dL)				
Model 1				
Men	109	1.1 (-5.7, 7.9)	2.4 (-3.5, 8.2)	4.6 (-0.9, 10.1)
Women	113	6.6 (2.1, 11.2)	12.2 (7.1, 17.3)	12.1 (7.4, 16.8)
Model 2				
Men	109	1.2 (-5.4, 7.9)	2.5 (-3.4, 8.4)	5.0 (-0.5, 10.5)
Women	113	5.8 (1.2, 10.3)	11.0 (5.5, 16.5)	11.3 (6.5, 16.2)
Model 3				
Men	109	1.0 (-6.5, 8.4)	1.0 (-5.6, 7.5)	2.9 (-3.2, 9.1)
Women	113	4.8 (0.2, 9.5)	11.5 (6.0, 17.0)	13.1 (8.1, 18.2)
Model 4				
Men	109	0.1 (-7.8, 7.9)	-3.7 (-10.9, 3.6)	-1.2 (-8.3, 5.9)
Women	113	2.1 (-2.6, 6.8)	6.3 (0.2, 12.5)	11.5 (4.2, 18.8)

Results were based on weighted analyses towards a normal body mass index distribution. Data were missed for some participants in some subgroups. HTGC, hepatic triglyceride content; CI, confidence interval.

[a] Beta coefficients (95% CI) obtained by linear regression in each weighted quartile of natural log-transformed HTGC compared with the lowest quartile (reference category).

[b] Values of HTCG quartiles were back transformed for interpretation.

Model 1: crude association. Model 2: adjustment for age. Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use (women only) and menopause status (women only). Model 4: model 3 + adjustment for visceral adipose tissue and total body fat.

results from these studies support the existence of an interrelation between serum triglycerides and VKDFs.

The mechanism underlying the relationship between factor IX levels and HTGC is as yet unknown. Still, since the liver is the main site of production of coagulation factors [48], it is biologically plausible to speculate that the observed association could be related to pathways involved in the synthesis of factor IX. Recently, gene expression of various coagulation factors has been investigated in NASH [49]. Compared with healthy individuals, NASH patients had increased hepatic triglyceride levels but reduced hepatic mRNA levels of factor IX and of several other coagulation factors. Although our results on the positive association between HTGC and plasma activity of factor IX do not seem to be in line with the above mentioned findings [49], it is important to address that gene expression of coagulation factors is not known in detail in NAFLD, and across the different stages of the disease (i.e., from simple steatosis

to NASH and cirrhosis). Alternatively, other mechanisms involved in the synthesis of factor IX could also influence its plasma activity without affecting its transcript levels, such as post-transcriptional or post-translational changes. For instance, Cleuren *et al*. [50] have recently demonstrated an increase in plasma activity of several coagulation factors (i.e., fibrinogen, and factors II, VII, VIII, IX, XI and XII) in mice kept on high fat diet for 14 days. However, with the exception of factor XI, the increase in plasma activity was not paralleled by changes in gene expression of these coagulation factors in the liver [50].

Here, one may also consider that the association between HTGC and factor IX could be explained by common mechanisms regulating both liver fat content and factor IX levels. Vanschoonbeek *et al*. [51] have shown in a murine model of type III hyperlipidemia that mice kept on fish oil diet (n-3 polyunsaturated fatty acids [n-3 PUFAs]) for 21 days had a reduction in plasma triglyceride levels, thrombin generation, and activity of VKDFs, but not in VKDF mRNA levels, which remained unchanged. Analysis of mouse livers showed that n-3 PUFA was associated with upregulation of genes related to lipid degradation, and downregulation of genes related to lipid synthesis and of γ-glutamyl carboxylase [51]. The latter gene encodes the enzyme responsible for the γ-carboxylation of VKDFs, which is a fundamental post-translational step for the activity of these factors in blood coagulation [52]. Consistent with the murine model study [51] and with the reduction in triglyceride levels by n-3 PUFA in clinical studies [53], n-3 PUFA supplementation has been suggested to decrease liver fat content in humans [54]. Furthermore, some observational studies have found an inverse association between fish/n-3 PUFA intake or n-3 PUFA blood levels and risk of venous thrombosis [55,56], including recurrent events [57]. The mechanism behind this inverse association is not fully understood, and may include downregulation of the activity of procoagulant VKDFs, such as factor IX. Taken together, there might be common mechanism(s) regulating the metabolism of lipids and the activity of VKDFs in the hepatocytes, which could explain, at least in part, our results on the strong association between HTGC and factor IX plasma activity. Therapeutic and lifestyle strategies targeting possible common mechanisms might decrease not only liver fat content and factor IX activity, but also the risk of venous thrombosis. Hence, further studies aimed to unravel the pathophysiology behind the association between HTGC and factor IX levels are important, both from a mechanistic and a clinical viewpoint.

In the present study, the associations between HTGC and levels of fibrinogen and factor VIII disappeared upon adjustment for TBF and VAT, thereby suggesting a close link of adipose tissue with both factors. Our results confirm previous studies, in which body fat measures were closely related to levels of fibrinogen [6,23,58] and factor VIII [23,59]. Adipose tissue may influence the regulation of fibrinogen and factor VIII levels possibly through the secretion of bioactive factors, such as pro-

inflammatory cytokines [60]. Fibrinogen, produced by hepatocytes [61], and factor VIII, produced in the liver by endothelial cells [62,63], are well-known for acting as acute-phase proteins [61,64]. For instance, interleukin-6 (IL-6), a pro-inflammatory cytokine secreted by adipose tissue [60], up-regulates the expression of both factors at the transcriptional level [61,64]. Furthermore, fibrinogen and factor VIII levels have been shown to cluster together with C-reactive protein (CRP), an inflammatory marker, rather than markers of procoagulant activity [45,47]. On the whole, based on our results, total body and visceral fat appear to largely explain the associations of HTGC with fibrinogen and factor VIII levels, and to a lesser extent, with factor XI levels. Notably, the association between HTGC and factor IX levels also appears to be partly explained by overall and visceral fat given the attenuation of this association upon adjustment for TBF and VAT. It is noteworthy that similarly to fibrinogen and factor VIII, factor IX levels were associated with IL-6 levels [65] and clustered together with CRP [45,47]. However, it remains open whether and how bioactive factors related to fat-cell biosynthesis mediate the effect of total body and visceral fat on fibrinogen and factors VIII, IX and XI levels, and further investigation on this topic is warranted.

In subgroup analyses stratified by sex, results were similar to the overall analysis, with the exception of factor XI, which levels were consistently associated with HTGC in women, in a dose-response fashion, even after adjustment for total body and visceral fat. As far as we know, the effect of sex on the association between HTGC and coagulation factors has not been studied before, and whether there is a biological reason behind the observed sex difference in factor XI levels remains to be clarified.

Strengths of the present study include the availability of ^{1}H MRS to quantify HTGC in combination with MRI to quantify VAT, and a more accurate measure of TBF (i.e., bioelectrical impedance analysis) than BMI. This enabled us to adjust all analyses for total body and visceral fat. Moreover, the strong associations of TBF and VAT with HTGC observed in this study underscores the need for taking into account both measures when studying specific effects of liver fat. Further strengths are the large study population and the information on multiple potential confounding factors. Because of the large sample size, we were able to categorize HTGC into quartiles, and assess a dose-response relation between HTGC and levels of coagulation factors; to adjust for several potential confounding factors; and to investigate possible sex differences.

Limitations of this study should also be addressed. First, the observational, cross-sectional nature of the present study precludes causal inferences related to our results. Second, as in all observational designs, we cannot exclude the presence of residual confounding due to unknown or unmeasured confounding factors. Third, since for obvious ethical reasons we could not perform liver biopsies, we were unable to determine whether levels of coagulation factors differ across the histological

stages of NAFLD. Fourth, among the VKDFs, we evaluated factor IX only. Whether HTGC is related to other VKDFs as well, either procoagulant (factors II, VII and X) or anticoagulant (protein C and protein S) factors, and whether the relationship between HTGC and VKDFs results in a hypercoagulable state may deserve further investigation. Finally, our study population consisted primarily of white individuals aged between 45-65 years, and our results may therefore not be generalizable to other ethnic or age groups.

In conclusion, HTGC was associated with levels of fibrinogen, and factors VIII, IX and XI, of which factor IX remained associated with HTGC after adjustment for TBF and VAT. Our results shed more light on the relation between obesity and venous thrombosis risk, including the potential that HTGC contributes to venous thrombosis risk beyond total body and visceral fat through factor IX levels.

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SUPPLEMENTAL MATERIAL

Supplementary Table 1. Baseline characteristics of 1946 participants from the NEO study according to sex

Characteristics	Men (n = 1017)	Women (n = 929)
Demographic and lifestyle factors		
Age (years)	56 (50, 61)	55 (51, 60)
Ethnicity (% whites)	96	96
Education level (% high)[a]	51	43
Alcohol consumption (g/day)	16.8 (5.2, 27.9)	7.7 (1.6, 14.4)
Alcohol consumption (%)		
<10g/day	36	62
10-20g/day	22	19
20-40g/day	28	16
≥40g/day	14	3
Physical activity (MET-hours per week)	31.0 (15.0, 52.8)	29.5 (16.5, 49.5)
Tobacco smoking (% current)	15	13
Estrogen (% current use)	NA	10
Menopause status (% postmenopausal)	NA	81
Measures of adiposity		
BMI (kg/m^2)	26.6 (3.4)	25.3 (4.3)
Total body fat (%)	24.5 (5.5)	36.2 (6.4)
Waist circumference (cm)	97.4 (10.2)	85.4 (11.9)
VAT (cm^2)	113.0 (56.3)	66.2 (42.1)
SAT (cm^2)	205.6 (80.8)	256.8 (102.9)
VAT/SAT	0.52 (0.39, 0.69)	0.23 (0.17, 0.32)
Hepatic triglyceride content (%)	3.78 (1.98, 8.44)	1.82 (1.09, 4.65)
Coagulation factors		
Fibrinogen (mg/dL)	283 (55)	294 (54)
Factor VIII (IU/dL)	121 (31)	123 (34)
Factor IX (IU/dL)	118 (19)	115 (21)
Factor XI (IU/dL)	111 (18)	120 (20)
Transaminases		
ALT (U/L)	29.3 (13.1)	21.2 (8.1)
AST (U/L)	26.7 (9.3)	22.9 (6.3)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missed for some participants in some subgroups. Data are shown as mean (± standard deviation), median (25th percentile -75th percentile) or percentage.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; MET, metabolic equivalents of task; NA, not applicable; SAT, abdominal subcutaneous adipose tissue; VAT, visceral adipose tissue; VAT/SAT, ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue; WC, waist circumference.

[a] High educational level (according to Dutch educational system): higher secondary education, higher vocational education, university, and PhD.

Supplementary Table 2. Baseline characteristics of 1326 participants from the NEO study with alcohol consumption <20g/day

Characteristics	All (n = 1326)	Men (n = 567)	Women (n = 759)
Demographic and lifestyle factors			
Age (years)	56 (50-60)	56 (50-61)	55 (51-60)
Ethnicity (% whites)	95	94	96
Education level (% high) [a]	42	50	37
Alcohol consumption (g/day)	4.8 (1.9-10.7)	7.0 (2.0-13.2)	4.1 (1.0-9.8)
Alcohol consumption (%)			
<10g/day	70	62	76
10-20g/day	30	38	24
Physical activity (MET-hours per week)	30.0 (15.3-51.2)	31.0 (14.7-54.0)	30.0 (16.1-49.0)
Tobacco smoking (% current)	10	11	10
Estrogen (% current use)	NA	NA	10
Menopause status (% postmenopausal)	NA	NA	81
Measures of adiposity			
BMI (kg/m^2)	25.8 (4.0)	26.3 (3.5)	25.4 (4.3)
Total body fat (%)	31.5 (8.5)	24.0 (5.6)	36.3 (6.3)
Waist circumference (cm)	90.0 (12.5)	96.6 (10.3)	85.7 (11.9)
VAT (cm^2)	82.0 (51.6)	106.6 (56.0)	66.4 (41.7)
SAT (cm^2)	238.3 (100.1)	202.6 (84.5)	260.9 (102.6)
VAT/SAT	0.31 (0.21-0.47)	0.50 (0.36-0.66)	0.23 (0.17-0.31)
Hepatic triglyceride content (%)	2.35 (1.22-5.8)	3.41 (1.82-7.75)	1.74 (1.08-4.54)
Coagulation factors			
Fibrinogen (mg/dL)	293 (55)	285 (56)	298 (54)
Factor VIII (IU/dL)	123 (32)	122 (30)	123 (34)
Factor IX (IU/dL)	115 (20)	116 (19)	114 (21)
Factor XI (IU/dL)	116 (20)	111 (18)	119 (20)
Transaminases			
ALT (U/L)	23.8 (10.2)	27.7 (11.9)	21.2 (8.0)
AST (U/L)	23.9 (6.6)	25.6 (6.6)	22.8 (6.4)

Results were based on weighted analyses towards a normal body mass index distribution. Data were missed for some participants in some subgroups. Data are shown as mean (± standard deviation), median (25th percentile -75th percentile) or percentage.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; MET, metabolic equivalents of task; NA, not applicable; SAT, abdominal subcutaneous adipose tissue; VAT, visceral adipose tissue; VAT/SAT, ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue; WC, waist circumference.

[a] High educational level (according to Dutch educational system): higher secondary education, higher vocational education, university, and PhD.

Supplementary Table 3. Association between body fat measures and hepatic triglyceride content in 1326 participants from the NEO study with alcohol consumption <20g/day

	Difference in hepatic triglyceride content (%) (95% CI)[a] per SD of:					
	BMI (SD = 4.0 kg/m²)	**TBF (SD = 8.5%)**	**WC (SD = 12.5 cm)**	**VAT (SD = 51.6 cm²)**	**SAT (SD = 100.1 cm²)**	**VAT/SAT (SD = 0.60)**
Model 1	0.55 (0.48, 0.59)	0.22 (0.15, 0.29)	0.61 (0.56, 0.66)	0.63 (0.57, 0.68)	0.36 (0.30, 0.43)	0.44 (0.37, 0.51)
Model 2	0.52 (0.47, 0.57)	0.76 (0.68, 0.84)	0.61 (0.55, 0.67)	0.61 (0.55, 0.67)	0.48 (0.42, 0.54)	0.44 (0.35, 0.54)
Model 3	0.50 (0.45, 0.56)	0.72 (0.64, 0.81)	0.58 (0.52, 0.65)	0.59 (0.54, 0.65)	0.45 (0.39, 0.52)	0.44 (0.35, 0.53)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missed for some participants in some subgroups.

Hepatic triglyceride content and the ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue were log-transformed.

BMI, body mass index; CI, Confidence Interval; SAT, abdominal subcutaneous adipose tissue; TBF, total body fat; VAT, visceral adipose tissue; VAT/SAT, ratio of visceral adipose tissue and abdominal subcutaneous adipose tissue; WC, waist circumference.

[a]Beta coefficients (95% CI) from linear regression per weighted SD in BMI, TBF, WC, VAT, abdominal SAT, and VAT/SAT ratio.

Model 1: crude association.

Model 2: adjustment for age and sex.

Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use and menopause status.

Supplementary Table 4. Association between hepatic triglyceride content and coagulation factor levels in 1326 participants from the NEO study with alcohol consumption <20g/day

	Continuous scale	Reference (mean levels)	Mean difference (95% CI)[b] compared with the reference category		
	Difference in coagulation factor levels (95% CI)[a] per SD of HTGC (2.89%)[c]	HTGC Quartile 1 <1.34%[c] (29%)	HTGC Quartile 2 1.34%-2.66%[c] (26%)	HTGC Quartile 3 2.66%-6.27%[c] (23%)	HTGC Quartile 4 ≥6.27%[c] (22%)
Fibrinogen (mg/dL)					
Model 1	7.8 (3.8, 11.8)	281	10.5 (-1.3, 22.3)	19.4 (7.0, 31.8)	18.9 (7.6, 30.2)
Model 2	8.3 (4.2, 12.4)		10.0 (-1.7, 21.7)	20.8 (7.2, 34.5)	21.0 (8.9, 33.1)
Model 3	7.8 (3.5, 12.1)		11.5 (0, 23.0)	20.6 (7.4, 33.8)	22.5 (9.9, 35.1)
Model 4	-2.8 (-7.6, 2.1)		-2.2 (-12.6, 9.2)	0.8 (-13.5, 15.0)	-9.4 (-25.9, 7.0)
Factor VIII (IU/dL)					
Model 1	3.6 (1.2, 6.1)	119	5.3 (-2.2, 12.8)	5.3 (-1.9, 12.5)	8.9 (1.7, 16.0)
Model 2	3.4 (0.9, 5.8)		4.4 (-3.3, 12.0)	4.0 (-3.9, 11.9)	7.2 (0, 14.3)
Model 3	3.5 (1.0, 6.0)		4.7 (-3.2, 12.6)	4.9 (-3.0, 12.7)	8.4 (1.4, 15.4)
Model 4	1.8 (-1.2, 4.9)		1.8 (-6.8, 10.4)	0.5 (-8.1, 9.2)	3.5 (-6.3, 13.3)
Factor IX (IU/dL)					
Model 1	9.4 (7.9, 10.8)	102	11.3 (8.0, 14.7)	18.4 (14.5, 22.2)	25.7 (21.5, 29.8)
Model 2	9.5 (8.0, 10.9)		11.0 (7.8, 14.3)	19.1 (14.8, 23.5)	25.6 (21.6, 29.5)
Model 3	9.8 (8.2, 11.3)		11.7 (8.5, 15.0)	20.0 (16.0, 24.0)	27.1 (22.9, 31.2)
Model 4	6.2 (4.4, 8.1)		8.0 (4.8, 11.1)	12.5 (8.5, 16.6)	19.1 (12.6, 25.6)

Supplementary Table 4. (continued)

	Continuous scale	Reference (mean levels)	Mean difference (95% CI)[b] compared with the reference category		
	Difference in coagulation factor levels (95% CI)[a] per SD of HTGC (2.89%)[c]	**HTGC Quartile 1 <1.34%[c] (29%)**	**HTGC Quartile 2 1.34%-2.66%[c] (26%)**	**HTGC Quartile 3 2.66%-6.27%[c] (23%)**	**HTGC Quartile 4 ≥6.27%[c] (22%)**
Factor XI (IU/dL)					
Model 1	2.2 (0.8, 3.6)	111	6.3 (1.9, 10.6)	6.7 (2.3, 11.1)	7.0 (3.1. 10.9)
Model 2	3.1 (1.7, 4.5)		6.9 (2.6, 11.2)	8.8 (4.2, 13.4)	9.2 (5.3, 13.1)
Model 3	3.2 (1.7, 4.8)		7.2 (2.8, 11.5)	9.5 (4.5, 14.4)	10.1 (5.8, 14.3)
Model 4	1.4 (-0.4, 3.2)		5.4 (0.8, 9.9)	4.8 (-0.7, 10.2)	6.7 (0.9, 12.5)

Results were based on analyses weighted towards a normal body mass index distribution. Data were missed for some participants in some subgroups.

CI, confidence interval; HTGC, hepatic triglyceride content; SD, standard deviation.

[a] Beta coefficients (95% CI) from linear regression per weighted SD in natural log-transformed HTGC.

[b] Beta coefficients (95% CI) obtained by linear regression in each weighted quartile of natural log-transformed HTGC compared with the lowest quartile (reference category).

[c] Values of SD and quartiles of HTGC were back transformed for interpretation.

Model 1: crude association.

Model 2: adjustment for age and sex.

Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use and menopause status.

Model 4: model 3 + adjustment for visceral adipose tissue and total body fat.

Supplementary Table 5. Association between hepatic triglyceride content and coagulation factor levels in 1326 participants from the NEO study with alcohol consumption <20g/day according to sex

	Reference (mean levels)	Mean difference (95% CI)[a] compared with the reference category		
	HTGC Quartile 1 <1.34%[b]	**HTGC Quartile 2 1.34%-2.66%[b]**	**HTGC Quartile 3 2.66%-6.27%[b]**	**HTGC Quartile 4 ≥6.27%[b]**
Fibrinogen (mg/dL)				
Model 1				
Men	265	20.8 (-3.0, 44.7)	26.7 (4.5, 48.9)	23.4 (2.9, 44.0)
Women	287	9.0 (-4.3, 22.3)	23.4 (7.7, 39.2)	26.2 (11.2, 41.3)
Model 2				
Men	265	22.2 (-1.2, 45.5)	23.0 (0.3, 45.7)	21.6 (1.0, 42.2)
Women	287	5.7 (-7.9, 19.2)	19.8 (2.6, 37.1)	20.1 (5.4, 34.9)
Model 3				
Men	265	27.5 (5.2, 49.9)	26.5 (4.2, 48.9)	24.1 (4.2, 44.0)
Women	287	5.2 (-8.1, 18.5)	18.4 (1.3, 35.4)	23.2 (8.4, 38.1)
Model 4				
Men	265	13.6 (-8.1, 35.2)	11.0 (-12.9, 34.9)	2.3 (-23.1, 27.6)
Women	287	-9.1 (-22.5, 4.4)	-6.7 (-24.5, 11.0)	-13.7 (-33.4, 6.0)
Factor VIII (IU/dL)				
Model 1				
Men	119	2.5 (-9.2, 14.2)	-1.8 (-8.8, 12.4)	6.7 (-4.7, 18.0)
Women	119	6.7 (-2.8, 16.3)	8.6 (-1.4, 18.5)	10.8 (1.9, 19.7)
Model 2				
Men	119	2.9 (-8.8, 14.5)	0.4 (-10.2, 11.1)	5.4 (-5.6, 16.5)
Women	119	4.9 (-5.2, 14.9)	6.0 (-5.2, 17.1)	8.3 (-1.4, 18.0)
Model 3				
Men	119	3.4 (-7.5, 14.4)	0 (-9.6, 9.5)	5.8 (-3.9, 15.2)
Women	119	4.2 (-6.0, 14.5)	6.2 (-4.4, 16.7)	9.9 (1.1, 18.8)
Model 4				
Men	119	3.0 (-8.4, 14.3)	0.2 (-11.2, 11.5)	6.3 (-8.9, 21.5)
Women	119	0.7 (-10.8, 12.2)	-1.8 (-13.1, 9.5)	1.7 (-10.4, 13.7)
Factor IX (IU/dL)				
Model 1				
Men	101	13.5 (7.8, 19.1)	15.2 (9.8, 20.6)	24.7 (17.4, 31.9)
Women	102	10.3 (6.2, 14.4)	22.0 (16.3, 27.8)	27.3 (22.7, 31.9)
Model 2				
Men	101	13.7 (8.1, 19.4)	14.2 (9.1, 19.4)	23.9 (16.9, 31.0)
Women	102	10.4 (6.2, 14.5)	22.1 (15.9, 28.4)	26.8 (22.1, 31.4)
Model 3				
Men	101	14.0 (8.8, 19.3)	13.5 (8.5, 18.6)	23.2 (15.3, 31.0)
Women	102	10.5 (6.4, 14.6)	23.0 (17.7, 28.3)	29.6 (25.4, 33.8)
Model 4				
Men	101	11.9 (6.9, 16.8)	8.6 (2.8, 14.3)	18.9 (6.1, 31.7)
Women	102	5.9 (1.9, 9.8)	13.4 (8.4, 18.3)	19.1 (12.7, 25.5)

Supplementary Table 5. (continued)

	Reference (mean levels)	Mean difference (95% CI)[a] compared with the reference category		
	HTGC Quartile 1 <1.34%[b]	**HTGC Quartile 2 1.34%-2.66%[b]**	**HTGC Quartile 3 2.66%-6.27%[b]**	**HTGC Quartile 4 ≥6.27%[b]**
Factor XI (IU/dL)				
Model 1				
Men	106	6.5 (-0.9, 13.9)	5.2 (-0.9, 11.4)	6.8 (0.9, 12.6)
Women	113	7.6 (2.5, 12.8)	11.9 (5.8, 17.9)	11.1 (6.0, 16.3)
Model 2				
Men	106	6.4 (-1.0, 13.7)	5.3 (-1.0, 11.6)	7.3 (1.5, 13.1)
Women	113	6.4 (1.1, 11.6)	10.6 (4.2, 17.0)	9.7 (4.4, 15.0)
Model 3				
Men	106	7.5 (0.1, 14.9)	4.4 (-2.3, 11.1)	6.6 (0.6, 12.7)
Women	113	6.6 (1.5, 11.8)	12.2 (5.8, 18.7)	12.8 (7.4, 18.2)
Model 4				
Men	106	7.3 (-0.6, 15.2)	0.2 (-7.5, 7.8)	2.7 (-4.2, 9.6)
Women	113	3.9 (-1.4, 9.2)	7.1 (0, 14.2)	11.0 (3.3, 18.7)

Results were based on weighted analyses towards a normal body mass index distribution. Data were missed for some participants in some subgroups. HTGC, hepatic triglyceride content; CI, confidence interval.

[a] Beta coefficients (95% CI) obtained by linear regression in each weighted quartile of natural log-transformed HTGC compared with the lowest quartile (reference category).

[b] Values of HTCG quartiles were back transformed for interpretation.

Model 1: crude association. Model 2: adjustment for age. Model 3: model 2 + adjustment for ethnicity, education level, alcohol intake, physical activity, tobacco smoking, estrogen use (women only) and menopause status (women only). Model 4: model 3 + adjustment for visceral adipose tissue and total body fat.