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CLINICAL AND POPULATION STUDIES

Association Between Hepatic Triglyceride Content and Coagulation Factors

The Netherlands Epidemiology of Obesity Study

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OBJECTIVE: Whether hepatic triglyceride content (HTGC) contributes to hypercoagulability beyond total body fat (TBF) and visceral adipose tissue (VAT) is unclear. We, therefore, aimed to investigate the association between HTGC and coagulation factors (F)I (fibrinogen), VIII, IX, and XI while adjusting for TBF and VAT.

APPROACH AND RESULTS: In this cross-sectional analysis of the NEO study (Netherlands Epidemiology of Obesity; n=6671), a random subset of participants underwent magnetic resonance imaging and magnetic resonance spectroscopy to assess VAT and HTGC (n=2580). We excluded participants without complete imaging and coagulation assessment, and with history of liver disease, venous thrombosis, or on anticoagulation. Mean differences in coagulation factor levels across HTGC quartiles were estimated by linear regression adjusted for age, sex, ethnicity, education, alcohol intake, physical activity, smoking, estrogen, and menopause, in addition to TBF and VAT. Among the 1946 participants included, median HTGC was 2.66% (interquartile range: 1.34%–6.27%). Coagulation factor levels increased dose-dependently across HTGC quartiles. Mean differences between the fourth and first quartiles were 14.7 mg/dL (95% CI, 2.1–27.2) for fibrinogen, 6.7 IU/dL (95% CI, 0.5–12.9) for FVIII, 26.1 IU/dL (95% CI, 22.4–29.8) for FIX, and 8.6 IU/dL (95% CI, 4.6–12.6) for FXI. With further adjustment for TBF and VAT, the dose-response association of HTGC with FIX persisted, whereas associations with other factors disappeared.

CONCLUSIONS: HTGC was associated with various coagulation factors, of which FIX remained associated with HTGC after adjustment for TBF and VAT. HTGC might contribute to venous thrombosis risk beyond total body and visceral fat through FIX levels.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: coagulation factors ■ liver ■ obesity ■ triglycerides ■ venous thrombosis

Obesity has consistently been associated with increased risk of venous thrombosis (VT).[1] However, the mechanisms underlying this association are not fully understood and likely reflect multiple coexisting 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 contribute to the increased risk of VT in obesity. NAFLD is a term used to describe a broad range of related disorders, initiating from simple steatosis (accumulation of triglycerides in the cytoplasm of hepatocytes), which may progress to nonalcoholic steatohepatitis (steatosis associated with inflammation, hepatocyte injury, and fibrosis), cirrhosis (replacement of hepatocytes by scar tissue), and hepatocellular carcinoma.[2] The prevalence of NAFLD, defined as an hepatic triglyceride content (HTGC) of >5.5%,[3] is increasing and occurs in ≈20% to 30% of adults in the general population in Western countries.[3,4] The role of NAFLD in the risk of VT is largely unknown. In one previous study with 138 patients with VT and 276 controls, the prevalence of NAFLD was almost 3-fold higher in cases (81%) than in controls (30%).[5]

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Nonstandard Abbreviations and Acronyms

^{1}H	hydrogen 1
BMI	body mass index
HNF4	hepatocyte nuclear factor 4
HTGC	hepatic triglyceride content
IL	interleukin
MEGA	Multiple Environmental and Genetic Assessment of Risk Factors for VT
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
n-3 PUFAs	n-3 polyunsaturated fatty acids
NAFLD	nonalcoholic fatty liver disease
NEO	Netherlands Epidemiology of Obesity
PAI-1	plasminogen activator inhibitor-1
SAT	subcutaneous adipose tissue
TBF	total body fat
TNF	tumor necrosis factor
VAT	visceral adipose tissue
VKDF	vitamin K-dependent factor
VT	venous thrombosis

Highlights

- In a cross-sectional analysis of the NEO study (Netherlands Epidemiology of Obesity), liver fat, assessed as hepatic triglyceride content, is associated with increased plasma levels of fibrinogen, and factors VIII, IX, and XI in a dose-response fashion, even after adjustment for several potential demographic and lifestyle confounding factors.
- With further adjustment for total body fat and visceral adipose tissue, the associations of hepatic triglyceride content with levels of fibrinogen, factor VIII, and factor XI disappear, whereas the association between hepatic triglyceride content and factor IX levels persists, as well as the dose-response relationship.
- Hepatic triglyceride content has the potential to contribute to venous thrombosis risk beyond total body and visceral fat through factor IX levels.

In the past 2 decades, several small studies have investigated the association between NAFLD and hemostatic factors.[6–11] 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 participants, methods used to define NAFLD, or adjustments for potential confounding factors. To gain enhanced knowledge on the potential of NAFLD to contribute to a hypercoagulable state and possibly thrombosis risk, it would be particularly relevant to assess the relationship of liver fat content with coagulation factors that not only play key roles in the pathways that lead to clot formation but are also associated with an increased risk of VT, such as factors VIII (FVIII), IX, and XI and fibrinogen.[12–19] Notably, liver fat content has been previously reported to be positively associated with levels of FVIII, IX, and XI, independent of age, sex, and body mass index (BMI).[8] However, the amount of visceral adipose tissue (VAT) was not taken into account in the relationship between liver fat and the aforementioned coagulation factors.[8] VAT may be an important confounding factor, as it is strongly related to liver fat,[20,21] while it has also been shown to be related to levels of some coagulation factors.[6,22] Moreover, since VAT and liver fat are strongly associated with total body fat (TBF),[20,21,23,24] adjustment for TBF should be performed when studying specific effects of VAT or liver fat. Hence, whether liver fat is associated with levels of coagulation factors after adjustment for total body and visceral fat remains unclear. In addition to obtaining more mechanistic insight, clarification of this question is a relevant clinical issue worth pursuing since obesity and fatty liver may be modifiable through lifestyle intervention.[25]

The aim of our study was to investigate the association between HTGC, assessed by localized hydrogen 1 (^{1}H) magnetic resonance spectroscopy (MRS; ^{1}H MRS), and levels of fibrinogen and FVIII, 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,[25–28] and TBF and VAT.[6,20–22] As sex differences are observed in body fat distribution, liver fat content,[20,21] and risk of VT,[29] we additionally performed subgroup analyses stratified by sex.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population and Study Design

The NEO study (Netherlands Epidemiology of Obesity) is a population-based cohort study designed to investigate pathways that lead to obesity-related diseases, details of which have been described elsewhere.[30] Briefly, the NEO study includes 6671 participants, with an oversampling of individuals with overweight or obesity. Between September 2008 and September 2012, men and women aged 45 to 65 years with a self-reported BMI of 27 kg/m^2 or higher living in the greater area of Leiden (West of The Netherlands) were eligible to participate in the NEO study. Additionally, all inhabitants aged 45 to 65 years from one municipality (Leiderdorp) were invited to participate, irrespective of their BMI, to obtain a reference distribution of BMI.

The present study is a cross-sectional analysis of the baseline data from the NEO study. At study inclusion, all participants were asked about contraindications to magnetic resonance imaging (MRI).[30] Among the eligible participants, 2580 were randomly

selected to undergo ^{1}H MRS to assess HTGC, and MRI to assess abdominal subcutaneous adipose tissue (SAT) and VAT.[30,31] In 11 participants, the images were of insufficient quality to quantify SAT and VAT. Among the remaining 2569 participants, 494 had ^{1}H MRS of insufficient quality to quantify HTGC and were excluded. We subsequently excluded participants with missing data on coagulation factors (n=27), with a known history of liver disease (n=25) or VT (n=53), or who reported anticoagulant treatment at blood sampling (n=24), thus leaving 1946 participants for the present analysis. The NEO study was approved by the medical ethics committee of the Leiden University Medical Center, 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.[30] Before the baseline visit, participants completed questionnaires on demographic, lifestyle, and clinical data. 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, tobacco smoking into current and other (never/former smoker), estrogen use into current and other (never/former user), and menopause status into postmenopausal/perimenopausal and premenopausal. Physical activity during leisure time was expressed in metabolic equivalents of task-hours per week, and alcohol consumption as a continuous or categorical variable (<10, 10–20, 20–40, and ≥40 g/d).

Assessment of Measures of Body Fat and HTGC

Body weight and TBF (%) were determined with the Tanita bioimpedance balance (TBF-310, Tanita International Division, United Kingdom).[30] BMI was calculated by dividing the weight in kilograms by the height in meters squared. Waist circumference was measured mid-way between the lower costal margin and the iliac crest (cm).

MRI and spectroscopy were performed with a 1.5-T whole-body MR unit (Philips Medical Systems, Best, the Netherlands).[30,31] Abdominal SAT and VAT areas were quantified with a turbo spin-echo MRI protocol. At the level of the fifth lumbar vertebra, 3 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 3 slices was used for analyses. HTGC was determined by ^{1}H MRS, as previously described.[31] In short, an 8-mL voxel was positioned in the right lobe of the liver, while avoiding major vascular structures and adipose tissue depots. A point-resolved spectroscopy sequence was used to acquire hepatic spectral data without respiratory motion correction. Spectra were obtained with and without water suppression. The HTGC relative to water was calculated with the following formula: (signal amplitude of methylene + methyl)/(signal amplitude of water)×100.

Laboratory Measurements

Blood samples for coagulation factor measurements were drawn into tubes containing 0.106 mol/L trisodium citrate (Sarstedt, Nümbrecht, Germany). Plasma was obtained by centrifugation at 2500*g* for 10 minutes at room temperature and stored in aliquots at −80 °C until testing. Fibrinogen activity was measured using the method of Clauss.[32] Activity of FVIII, IX, and XI was measured with factor-specific clotting assays based on the activated partial thromboplastin time using a mechanical clot detection method on an ACL TOP 700 analyzer (Werfen, Barcelona, Spain). Serum alanine aminotransferase, aspartate aminotransferase, and triglycerides were assessed as previously described in the NEO study.[30]

Statistical Analyses

In the NEO study, there is an oversampling of individuals with a BMI of 27 kg/m^2 or higher. To correctly represent baseline associations in the general population, adjustments for the oversampling of individuals with a BMI≥27 kg/m^2 were made.[33] This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality,[34] whose BMI distribution was similar to the BMI distribution of the Dutch general population.[35] 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 (±SD) or median (25th–75th percentiles) for continuous variables.

Association Between Measures of Body Fat and HTGC

We used linear regression to evaluate the association between measures of body fat (BMI, TBF, waist circumference, VAT, abdominal SAT, and VAT/SAT ratio) and HTGC. Because the distribution of HTGC and VAT/SAT ratio was skewed to the right, natural logarithm transformation was applied for both variables (natural logarithm of HTGC [ln HTGC] and of VAT/SAT ratio [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 and ln HTGC. The resulting regression coefficient (β) and its 95% CIs for a measure of body fat indicate the difference in ln HTGC when that particular measure increases by one SD. We constructed scatter plots between *Z* scores of the measures of body fat 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, that is, ethnicity (dichotomous variable), education level (dichotomous variable), alcohol intake (continuous variable), physical activity (continuous variable), tobacco smoking (dichotomous variable), estrogen use (dichotomous variable), and menopause status (dichotomous variable).

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 per one SD increase in ln HTGC. 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 variable) and TBF (continuous variable) to a fourth model. We also investigated whether the association between HTGC and coagulation factor levels followed a dose-response relation. To this end, we categorized 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 HTGC compared with the first quartile (reference category). The regression coefficient for a 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 variables included in models 2 to 4. In subgroup analyses, we assessed the relationship between HTGC quartiles and coagulation factor levels stratified by sex.

Serum triglycerides have been shown to be associated with liver fat content independently of VAT[20] and with VKDFs (vitamin K-dependent factors).[36] As FIX is a VKDF, we also adjusted associations between HTGC and FIX for serum triglycerides. Serum triglyceride was not normally distributed and was log-transformed (ln TG) for regression analyses.

As a sensitivity analysis, all analyses were repeated excluding participants with alcohol consumption ≥20 g/d at baseline. Statistical analyses were performed with STATA Statistical Software, version 15.0 (Stata Corporation, College Station, TX).

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, 50–61), 96% were White participants, 47% were highly educated, and 14% were current smokers. Median alcohol consumption was 10.4 g/d (interquartile range, 2.8–21.4), and median physical activity during leisure time was 30.2 metabolic equivalents of task-hours per week (interquartile range, 15.8–51.5). Among women, 81% referred a postmenopausal status and 10% current estrogen use. Participants had a mean BMI of 25.9±3.9 kg/m^2 and a median HTGC of 2.66% (interquartile range, 1.34–6.27). Men had higher waist circumference, VAT, HTGC, and transaminases levels, and lower abdominal SAT and TBF than women. When participants with alcohol consumption ≥20 g/d were excluded (n=620; Table I in the Data Supplement), the proportion of men, current smokers, and highly educated participants, and the amount of VAT and HTGC were slightly lower compared with the main analysis, with no substantial differences in the other variables.

Association Between Measures of Body Fat and HTGC

The difference in ln HTGC per SD of measures of body fat was back transformed for easier interpretation (Table 2). All measures of body fat were associated with HTGC and upon adjustment for potential demographic and lifestyle confounding factors (model 3) the strongest associations were observed for TBF, VAT, and waist circumference. HTGC was 1.99% (95% CI, 1.86–2.12) higher per one SD of TBF (8.3%), and 1.80% (95% CI, 1.72–1.90) higher per one SD of VAT (54.5 cm^2). When participants with alcohol consumption ≥20 g/d were excluded, results were virtually the same as the main analysis (Table II in the Data Supplement).

Association Between HTGC and Coagulation Factor Levels

The results of the linear regression per SD of ln HTGC are described in Table 3, and for interpretation, the SD of ln HTGC was back transformed. 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 of ln HTGC (corresponding to 2.91% HTGC) was associated with higher levels of fibrinogen (6.3 mg/dL [95% CI, 2.6–9.9]), FVIII (3.2 IU/dL [95% CI, 1.1–5.4]), FIX (9.7 IU/dL [95% CI, 8.5–10.9]), and FXI (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 FVIII (1.6 IU/dL [95% CI, −0.9 to 4.2]) and FXI (1.2 [95% CI, −0.3 to 2.7]) or even became negative for fibrinogen (−5.0 mg/dL [95% CI, −9.2 to −0.7]). However, the association between ln HTGC and FIX levels, albeit attenuated, persisted (6.6 IU/dL [95% CI, 5.1–8.1]). The associations between ln HTGC and FIX levels were marginally attenuated when serum triglyceride (ln TG) was added to model 3 (7.5 IU/dL [95% CI, 6.2–8.8]) or model 4 (5.4 IU/dL [95% CI, 3.9–6.9]).

Next, we examined the mean differences in coagulation factor levels for each HTGC quartile in comparison with the first quartile used as the reference category (Table 3). Levels of coagulation factors increased dose-dependently across 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 HTGC and levels of fibrinogen, FVIII, and FXI across quartiles disappeared, whereas the association between HTGC and FIX levels persisted, as did the dose-response relation.

Table 4 shows the mean differences in coagulation factor levels for each quartile of HTGC compared with the first quartile stratified by sex. As in the overall analysis, upon adjustment for TBF and VAT, no associations between HTGC and levels of fibrinogen or FVIII were observed in either men or women. Consistent with the

Table 1. Baseline Characteristics of 1946 Participants From the Netherlands Epidemiology of Obesity Study

Characteristics	All participants	Men (47%)	Women (53%)
Demographic and lifestyle factors			
Age, y	56 (50–61)	56 (50–61)	55 (51–60)
Ethnicity (% Whites)	96	96	96
Education level (% high)*	47	51	43
Alcohol consumption, g/d	10.4 (2.8–21.4)	16.8 (5.2–27.9)	7.7 (1.6–14.4)
Alcohol consumption (%)			
<10 g/d	49	36	62
10–20 g/d	21	22	19
20–40 g/d	22	28	16
≥40 g/d	8	14	3
Physical activity (MET-hours per week)	30.2 (15.8–51.5)	31.0 (15.0–52.8)	29.5 (16.5–49.5)
Tobacco smoking (% current)	14	15	13
Estrogen (% current use)	NA	NA	10
Menopause status (% postmenopausal/perimenopausal)	NA	NA	81
Measures of adiposity			
BMI, kg/m^2	25.9 (3.9)	26.6 (3.4)	25.3 (4.3)
Total body fat (%)	30.7 (8.3)	24.5 (5.5)	36.2 (6.4)
Waist circumference, cm	91.1 (12.6)	97.4 (10.2)	85.4 (11.9)
VAT, cm^2	88.3 (54.5)	113.0 (56.3)	66.2 (42.1)
SAT, cm^2	232.6 (96.5)	205.6 (80.8)	256.8 (102.9)
VAT/SAT	0.34 (0.22–0.52)	0.52 (0.39–0.69)	0.23 (0.17–0.32)
Hepatic triglyceride content (%)	2.66 (1.34–6.27)	3.78 (1.98–8.44)	1.82 (1.09–4.65)
Coagulation factors			
Fibrinogen, mg/dL	289 (55)	283 (55)	294 (54)
Factor VIII, IU/dL	122 (32)	121 (31)	123 (34)
Factor IX, IU/dL	116 (20)	118 (19)	115 (21)
Factor XI, IU/dL	116 (20)	111 (18)	120 (20)
Transaminases			
ALT, U/L	25.1 (11.5)	29.3 (13.1)	21.2 (8.1)
AST, U/L	24.7 (8.1)	26.7 (9.3)	22.9 (6.3)

Results were based on analyses weighted towards the BMI distribution of the general population (n=1946, 1017 men and 929 women). Data are shown as mean (± SD), median (25th percentile–75th percentile) or percentage. Missing data: n=2 for ethnicity, n=16 for educational level, n=1 for tobacco smoking, n=42 for physical activity, n=1 for total body fat, and n=4 for transaminases. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; MET, metabolic equivalents of task; NA, not applicable; SAT, abdominal subcutaneous adipose tissue; and VAT, visceral adipose tissue.

*High educational level (according to the Dutch educational system): higher secondary education, higher vocational education, university, or PhD.

previous finding, FIX levels increased dose-dependently across HTGC quartiles in men and women, also after adjustment for VAT and TBF. With the addition of ln TG to models 3 and 4 in overall and stratified analyses, the associations between HTGC and FIX levels were slightly attenuated (data not shown). Of note, HTGC was consistently associated with FXI levels in women, even with further adjustment for TBF and VAT, while in men these associations were weak or absent across quartiles (Table 4).

Exclusion of participants with alcohol consumption of ≥20 g/d yielded similar results to the main analysis for the overall population and subgroups stratified by sex (Tables III and IV in the Data Supplement).

DISCUSSION

In this large population-based cross-sectional study, HTGC was associated with levels of various coagulation factors (ie, fibrinogen, FVIII, FIX, or FXI) in a dose-response fashion, even after adjustment for several demographic and lifestyle confounding factors. However, after further adjustment for total body and visceral fat, the positive associations of HTGC with levels of fibrinogen, FVIII, and FXI disappeared, whereas the associations between HTGC and FIX levels persisted, as did the dose-response relation. This observation could be relevant, as a potentially critical pathway by which obesity increases the risk of VT.

Table 2. Association Between Measures of Body Fat and Hepatic Triglyceride Content in 1946 Participants From the Netherlands Epidemiology of Obesity Study

	Difference in HTGC (%) (95% CI)* per SD of:					
	BMI	TBF	WC	VAT	SAT	ln VAT/SAT
	(SD=3.9 kg/m²)	(SD=8.3%)	(SD=12.6 cm)	(SD=54.5 cm²)	(SD=96.5 cm²)	(SD=0.61)
Model 1	1.72 (1.63–1.79)	1.20 (1.13–1.27)	1.84 (1.77–1.93)	1.92 (1.82–1.99)	1.40 (1.34–1.49)	1.62 (1.54–1.70)
Model 2	1.65 (1.58–1.73)	2.08 (1.93–2.23)	1.84 (1.73–1.93)	1.86 (1.77–1.97)	1.58 (1.51–1.66)	1.63 (1.51–1.77)
Model 3	1.62 (1.55–1.70)	1.99 (1.86–2.12)	1.79 (1.68–1.88)	1.80 (1.72–1.90)	1.54 (1.46–1.62)	1.58 (1.46–1.72)

Results were based on analyses weighted towards the body mass index distribution of the general population. Missing data: n=2 for ethnicity, n=16 for educational level, n=1 for tobacco smoking, n=42 for physical activity, and n=1 for total body fat. 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. BMI indicates body mass index; HTGC, hepatic triglyceride content; SAT, abdominal subcutaneous adipose tissue; TBF, total body fat; VAT, visceral adipose tissue; and WC, waist circumference.

*Beta coefficients (95% CI) from linear regression; expressed per SD of BMI, TBF, WC, VAT, abdominal SAT, and the natural logarithm of VAT/SAT ratio (ln VAT/SAT ratio). Linear regression was performed using the natural logarithm of HTGC (ln HTGC). For interpretation, the difference in ln HTGC (%) and the corresponding 95% CI per SD of measures of body fat were back transformed.

FIX plays a pivotal role in thrombin generation,[37] with high levels of FIX being associated with increased risk of VT in epidemiological studies,[14–17] including the MEGA study (Multiple Environmental and Genetic Assessment of Risk Factors for VT), a large population-based case-control study on the cause of VT.[14] A unit (IU/dL) increase in FIX level is associated with a 1.4% (95% CI, 1.1%–1.7%) increase in the risk of VT in the MEGA study. If we assume a linear relationship, the 18.9 IU/dL increase of FIX that we found for the highest quartile of HTGC after adjustment for TBF and VAT would be translated into a 26.5% increase in the risk of VT. Our findings are clinically relevant, as HTGC could contribute to VT risk among obese subjects through FIX levels independently of total body and visceral fat.

To the best of our knowledge, this is the first study to show that HTGC and FIX levels are associated in a dose-response fashion, also after adjustment for several potential confounding factors, including TBF and VAT. However, the mechanism underlying this relationship is as yet unclear. Since the liver is the main site of production of coagulation factors,[38] it is plausible to consider that the observed association between HTGC and FIX levels could be related to pathway(s) involved in the hepatic synthesis of FIX. Of note, gene expression of various coagulation factors has been investigated in nonalcoholic steatohepatitis.[39] Compared with healthy individuals, nonalcoholic steatohepatitis patients had increased levels of hepatic triglycerides but reduced hepatic mRNA levels of HNF4 (hepatocyte nuclear factor 4) and several HNF4 target genes, including those that encode coagulation factors, such as FIX.[39] Although our results on the positive association between HTGC and plasma activity of FIX do not seem to be in line with the above-mentioned findings,[39] a detailed understanding of the effects of NAFLD and its various stages on the transcription of hepatically expressed coagulation factors is lacking thus far. Alternatively, other mechanisms involved in either the production, clearance, or posttranslational modifications of FIX could mediate its plasma activity levels independent of transcription. Cleuren et al[40] have demonstrated an increase in plasma activity of several coagulation factors (ie, fibrinogen and FII, VII, VIII, IX, XI, and XII) in mice kept on a high-fat diet. However, with the exception of FXI, the increase in plasma activity was not paralleled by an upregulated gene expression of these coagulation factors in the liver.[40]

Given that the liver plays a central role in the regulation of lipid metabolism, one may also speculate that the association between HTGC and FIX could be explained by common mechanism(s) regulating both liver fat content and FIX levels. For instance, Vanschoonbeek et al[41] demonstrated a reduction in plasma triglyceride levels, thrombin generation, and plasma activity of VKDFs in type III hyperlipidemic mice that were fed a fish oil diet (n-3 polyunsaturated fatty acids [n-3 PUFAs]). While the hepatic VKDF mRNA levels remained unchanged, the n-3 PUFA diet was associated with upregulated expression of genes related to lipid degradation and downregulated expression of lipid synthesis genes and the γ-glutamyl carboxylase gene.[41] The latter enzyme is responsible for the γ-carboxylation of VKDFs, which is a posttranslational process essential to the generation of properly functioning VKDFs. Consistent with the murine model study[41] and with the n-3 PUFA-associated reduction in triglyceride levels observed in clinical studies,[42] n-3 PUFA supplementation has been suggested to decrease liver fat in humans.[43] Furthermore, n-3 PUFA intake or n-3 PUFA blood levels have been reported to be inversely associated with risk of VT.[44,45] The mechanism that is at the basis of this inverse association remains unclear, and we can only speculate that it may include a downregulated plasma activity of procoagulant VKDFs, such as FIX. Taken together, common mechanism(s) may regulate the metabolism of lipids and levels of VKDFs, which could explain, at least in part, the strong association between HTGC and plasma levels of FIX as observed here. As such, interventions targeting common mechanisms could have the potential to decrease not only liver fat content and FIX levels but also the risk of VT. However, our study

Table 3. Association Between Hepatic Triglyceride Content and Coagulation Factor Levels in 1946 Participants From the Netherlands Epidemiology of Obesity Study

	Continuous scale	Reference (mean levels)	Mean difference (95% CI)* compared with the reference category		
	Difference in coagulation factor levels (95% CI)† per SD of ln HTGC (corresponding to 2.91% HTGC)	HTGC quartile 1	HTGC quartile 2	HTGC quartile 3	HTGC quartile 4
		<1.34%	1.34%–2.66%	2.66%–6.27%	≥6.27%
Fibrinogen, mg/dL					
Model 1	5.1 (2.0 to 8.3)	281	7.3 (–3.5 to 18.2)	10.7 (0.2 to 21.4)	12.4 (2.7 to 22.1)
Model 2	6.1 (2.8 to 9.5)		7.0 (–4.2 to 18.1)	12.4 (0.2 to 24.6)	15.3 (4.1 to 26.5)
Model 3	6.3 (2.6 to 9.9)		6.3 (–5.3 to 17.8)	13.5 (0.8 to 26.1)	14.7 (2.1 to 27.2)
Model 4	–5.0 (–9.2 to –0.7)		–5.5 (–17.6 to 6.7)	–10.5 (–25.1 to 4.2)	–14.1 (–30.8 to 2.6)
Factor VIII, IU/dL					
Model 1	2.9 (0.9 to 5.0)	119	2.0 (–4.6 to 8.6)	2.6 (–3.5 to 8.7)	7.2 (1.2 to 13.2)
Model 2	3.0 (0.9 to 5.0)		1.2 (–5.4 to 7.9)	2.0 (–4.6 to 8.6)	6.3 (0.1 to 12.4)
Model 3	3.2 (1.1 to 5.4)		1.4 (–5.4 to 8.2)	2.5 (–4.1 to 9.1)	6.7 (0.5 to 12.9)
Model 4	1.6 (–0.9 to 4.2)		–1.2 (–8.3 to 5.9)	–1.9 (–9.1 to 5.3)	3.3 (–5.1 to 11.7)
Factor IX, IU/dL					
Model 1	9.7 (8.5 to 10.8)	103	9.8 (6.8 to 12.8)	17.8 (14.4 to 21.1)	26.3 (23.0 to 29.6)
Model 2	9.9 (8.7 to 11.0)		9.7 (6.6 to 12.7)	18.6 (14.9 to 22.3)	26.3 (23.0 to 29.6)
Model 3	9.7 (8.5 to 10.9)		9.8 (6.7 to 12.9)	18.5 (14.8 to 22.1)	26.1 (22.4 to 29.8)
Model 4	6.6 (5.1 to 8.1)		6.6 (3.6 to 9.6)	11.8 (7.9 to 15.7)	18.9 (13.7 to 24.1)
Factor XI, IU/dL					
Model 1	2.0 (0.8 to 3.1)	112	3.5 (–0.4 to 7.4)	4.8 (1.1 to 8.6)	6.0 (2.7 to 9.4)
Model 2	3.3 (2.2 to 4.5)		4.8 (1.0 to 8.5)	7.6 (3.5 to 11.6)	9.0 (5.4 to 12.6)
Model 3	3.1 (1.9 to 4.4)		4.2 (0.2 to 8.1)	7.1 (2.7 to 11.5)	8.6 (4.6 to 12.6)
Model 4	1.2 (–0.3 to 2.7)		2.4 (–1.8 to 6.5)	1.9 (–3.0 to 6.7)	5.3 (0.1 to 10.6)

Results were based on analyses weighted towards the body mass index distribution of the general population. Missing data: n=2 for ethnicity, n=16 for educational level, n=1 for tobacco smoking, n=42 for physical activity, and n=1 for total body fat. 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. HTGC indicates hepatic triglyceride content.

*Beta coefficients (95% CI) from linear regression in each quartile of HTGC compared with the lowest quartile (reference category).

†Beta coefficients (95% CI) from linear regression; expressed per SD of the natural logarithm of HTGC (ln HTGC). For interpretation, the SD of ln HTGC (1.07%) was back transformed (2.91%).

was not designed to investigate the mechanistic link between HTGC and FIX levels. Future studies aimed at unraveling the pathophysiology behind the association between HTGC and FIX levels are important, both from mechanistic and clinical viewpoints.

In the present study, the associations of HTGC with FVIII levels disappeared or even became negative in the case of fibrinogen upon adjustment for TBF and VAT, thereby suggesting a close link of adipose tissue with both factors. This is consistent with previous studies, in which body fat measures were related to levels of fibrinogen[6,22]and FVIII.[22,46] Fibrinogen and FVIII are well-known for acting as acute-phase proteins, and the gene transcription of both factors has been shown to be upregulated by IL (interleukin)-6,[47] a key proinflammatory cytokine. It is worth noting that adipose tissue is a main source of proinflammatory cytokines in human obesity, including IL-6 and TNF (tumor necrosis factor)-α.[48–50] Furthermore, adipose tissue was found to have a much higher mRNA expression of IL-6 and TNF-α compared with the liver in subjects with severe obesity, and extensive weight loss was associated with a significant decrease of this expression, manly in adipose tissue.[50] Our results analyzed in light of these findings suggest that total body and visceral fat, most likely via inflammation, is a driving force for raised fibrinogen and FVIII levels in NAFLD and not the liver fat content. In addition, we found that the association between HTGC and FXI levels was largely explained by total body and visceral fat, which also appeared to partially contribute to FIX levels, given the attenuation of the association with HTGC after adjustment for TBF and VAT. To what extent proinflammatory cytokines and other bioactive factors related to a dysfunctional adipose tissue may contribute to coagulation factor levels in NAFLD requires further investigation.

In subgroup analyses stratified by sex, results were similar to the overall analysis, with the exception of FXI, 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

Table 4. Association Between Hepatic Triglyceride Content and Coagulation Factor Levels in 1946 Participants From the Netherlands Epidemiology of Obesity Study Stratified by Sex

	Reference (mean levels)	Mean difference (95% CI)* compared with the reference category		
	HTGC quartile 1	HTGC quartile 2	HTGC quartile 3	HTGC Quartile 4
	<1.34%	1.34%–2.66%	2.66%–6.27%	≥6.27%
Fibrinogen, mg/dL				
Model 1				
Men	276	7.3 (−15.1 to 29.7)	8.9 (−12.2 to 30.0)	7.9 (−12.5 to 28.2)
Women	283	9.5 (−3.3 to 22.7)	18.8 (5.1 to 32.6)	26.2 (13.2 to 39.2)
Model 2				
Men	276	7.1 (−15.3 to 29.4)	6.5 (−14.4 to 27.3)	6.4 (−13.6 to 26.4)
Women	283	6.6 (−6.1 to 19.3)	16.8 (1.6 to 32.1)	21.4 (8.2 to 34.6)
Model 3				
Men	276	5.3 (−19.7 to 30.3)	9.4 (−13.4 to 32.1)	4.1 (−18.1 to 26.4)
Women	283	6.6 (−5.7 to 18.8)	17.2 (3.0 to 31.3)	25.5 (12.3 to 38.7)
Model 4				
Men	276	−4.2 (−30.3 to 22.0)	−11.0 (−36.6 to 14.6)	−15.2 (−43.9 to 13.5)
Women	283	−6.1 (−19.0 to 6.7)	−10.4 (−25.7 to 4.9)	−11.2 (−29.2 to 6.8)
Factor VIII, IU/dL				
Model 1				
Men	120	−1.2 (−10.9 to 8.5)	−2.2 (−10.9 to 6.5)	4.7 (−4.0 to 13.4)
Women	119	4.0 (−4.7 to 12.7)	8.4 (−0.2 to 17.0)	10.0 (1.8 to 18.3)
Model 2				
Men	120	−1.4 (−11.0 to 8.3)	−3.9 (−12.6 to 4.8)	3.3 (−5.3 to 11.9)
Women	119	2.3 (−6.6 to 11.2)	6.2 (−3.4 to 15.8)	9.0 (0.1 to 17.9)
Model 3				
Men	120	−1.2 (−10.9 to 8.4)	−3.6 (−12.0 to 4.8)	3.5 (−4.9 to 11.9)
Women	119	2.4 (−6.9 to 11.6)	6.8 (−2.4 to 16.0)	9.2 (0.6 to 17.7)
Model 4				
Men	120	−2.7 (−12.5 to 7.2)	−4.1 (−13.5 to 5.3)	3.3 (−8.1 to 14.6)
Women	119	−0.8 (−11.0 to 9.3)	−2.1 (−12.2 to 8.0)	2.0 (−10.3 to 14.2)
Factor IX, IU/dL				
Model 1				
Men	103	9.1 (4.0 to 14.2)	14.1 (9.0 to 19.2)	24.1 (18.7 to 29.5)
Women	102	9.9 (6.1 to 13.8)	22.1 (17.1 to 27.1)	28.6 (24.5 to 32.8)
Model 2				
Men	103	9.0 (4.0 to 14.1)	13.5 (8.6 to 18.4)	23.5 (18.2 to 28.8)
Women	102	10.2 (6.4 to 14.0)	22.4 (17.0 to 27.8)	28.8 (24.6 to 33.0)
Model 3				
Men	103	8.6 (3.2 to 13.9)	11.9 (7.0 to 16.7)	20.7 (14.4 to 27.0)
Women	102	10.3 (6.3 to 14.2)	22.7 (17.8 to 27.6)	29.8 (26.0 to 33.7)
Model 4				
Men	103	6.8 (1.5 to 12.0)	8.2 (2.8 to 13.6)	16.4 (8.0 to 24.8)
Women	102	5.9 (2.1 to 9.7)	12.7 (7.8 to 17.5)	19.0 (13.2 to 24.9)
Factor XI, IU/dL				
Model 1				
Men	109	1.1 (−5.7 to 7.9)	2.2 (−3.7 to 8.0)	4.9 (−0.6 to 10.4)
Women	113	6.6 (2.1 to 11.2)	12.2 (7.1 to 17.3)	12.1 (7.4 to 16.8)
Model 2				
Men	109	1.2 (−5.4 to 7.9)	2.2 (−3.7 to 8.1)	5.3 (−0.1 to 10.8)
Women	113	5.8 (1.2 to 10.3)	11.0 (5.5 to 16.5)	11.3 (6.5 to 16.2)

(*Continued*)

Table 4. Continued

	Reference (mean levels)	Mean difference (95% CI)* compared with the reference category		
	HTGC quartile 1	HTGC quartile 2	HTGC quartile 3	HTGC Quartile 4
	<1.34%	1.34%–2.66%	2.66%–6.27%	≥6.27%
Model 3				
Men	109	1.0 (−6.5 to 8.4)	0.7 (−5.8 to 7.3)	3.3 (−2.9 to 9.4)
Women	113	4.8 (0.2 to 9.5)	11.5 (6.0 to 17.0)	13.1 (8.1 to 18.2)
Model 4				
Men	109	0.1 (−7.8 to 7.9)	−4.0 (−11.2 to 3.2)	−0.5 (−7.6 to 6.5)
Women	113	2.1 (−2.6 to 6.8)	6.3 (0.2 to 12.5)	11.5 (4.2 to 18.8)

Results were based on analyses weighted towards the body mass index distribution of the general population. Missing data (men): n=1 for ethnicity, n=7 for educational level, n=1 for tobacco smoking, n=16 for physical activity, and n=1 for total body fat. Missing data (women): n=1 for ethnicity, n=9 for educational level, n=26 for physical activity. 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. HTGC indicates hepatic triglyceride content.

*Beta coefficients (95% CI) from linear regression in each quartile of HTGC compared with the lowest quartile (reference category).

whether there is a biological reason behind the observed sex difference in FXI levels remains to be clarified.

Strengths of the present study include the availability of ^{1}H MRS to quantify HTGC in combination with MRI for VAT quantification and a more accurate measure of TBF (ie, 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 both measures into account when studying specific effects of liver fat. Further strengths are the large study population and information on multiple potential confounding factors. Because of the large sample size, we were able to 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. The observational, cross-sectional nature of the present study precludes causal inferences related to our results. Since for ethical reasons we could not perform liver biopsies, we were unable to determine whether coagulation factor levels differ across the histological stages of NAFLD. Among the VKDFs, only FIX was available in the present study. Whether HTGC is related to other VKDFs as well, either procoagulant (FII, VII, and X) or anticoagulant (protein C and protein S), and whether the relationship between HTGC and VKDFs results in a hypercoagulable state would require further study. Finally, NAFLD has been associated with higher levels of PAI-1 (plasminogen activator inhibitor-1),[6,9,51] the main inhibitor of fibrinolysis. In the study by Verrijken et al,[9] PAI-1 levels increased with increasing severity of steatosis and inflammation in liver biopsy of NAFLD patients, even after adjustment for VAT. As high levels of PAI-1 have been reported to be associated with an increased risk of VT,[52] assessment of PAI-1 in relation to HTGC would be relevant to gain a deeper insight into the potential mechanisms of VT in obesity, but unfortunately, measurement of PAI-1 levels was not available in this study.

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

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Disclosures

None.

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