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The association between leptin concentration and blood coagulation: Results from the NEO study

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ABSTRACT

Background: The adipocyte-derived hormone leptin has been associated with altered blood coagulation in *in vitro* studies. However, it is unclear whether this association is relevant *in vivo* and to what extent this association is influenced by total body fat. Therefore, we aimed to examine the association between serum leptin and blood coagulation while taking total body fat into account in a population-based cohort study.

Methods: We performed a cross-sectional analysis with baseline measurements of 5797 participants of the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort of middle-aged men and women. We examined associations between serum leptin concentration and coagulation factor concentrations and parameters of platelet activation in linear regression analyses. All analyses were adjusted for multiple covariates, including total body fat.

Results: In multivariable adjusted analyses a 1 μg/L higher serum leptin concentration was associated with a 0.22 IU/dL (95% CI: 0.11, 0.32) higher FVIII concentration and a 0.20 IU/dL (95% CI: 0.14, 0.27) higher FIX concentration (3.5 IU/dL FVIII and 3.2 IU/dL FIX per SD leptin). Serum leptin concentration was not associated with FXI, fibrinogen, platelet count, mean platelet volume and platelet distribution width in multivariable adjusted analyses.

Discussion: This study showed that serum leptin concentration was associated with higher concentrations of FVIII and FIX in an observational study, which could be clinically relevant.

1. Introduction

The satiety hormone leptin is produced by adipocytes in fat tissue and functions as a negative feedback signal to the hypothalamus, informing the brain about the amount of peripheral fat storage and inhibiting food intake. Individuals with obesity develop a central resistance to leptin, which leads to increased leptin concentrations [1,2].

Leptin has previously been shown to promote platelet activation and aggregation *in vitro* and leads to both increased Tissue Factor and Plasma Activation Inhibitor-1 (PAI-1) expression [3–6], which effects may tilt the coagulation balance towards thrombosis. Therefore, leptin may have a clinically relevant role in blood coagulation, where a disturbed balance between the procoagulant and anticoagulant part of the coagulation system may result in bleeding or thrombotic disorders [7]. Several previous studies have reported that leptin is associated with an elevated risk of cardiovascular disease [8,9].

However, previous studies have mainly focused on *in vitro* effects of leptin on blood coagulation or were performed in small numbers of participants. Furthermore, most studies that estimated the association between leptin and incidence of cardiovascular disease adjusted for body mass index (BMI) in their analyses and not for total body fat. This may be insufficient as leptin is produced by adipose tissue and body mass index is also determined by lean body mass. Therefore, we aimed to examine the association between serum leptin and coagulation factors and parameters of platelet activation in the Netherlands Epidemiology of Obesity (NEO) Study (n = 5797) while taking into account the influence of adipose tissue on this association by adjusting for total body fat.

2. Methods

2.1. Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The study design and population is

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described in detail elsewhere [10]. The NEO study includes 6671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. For our analyses, we performed cross-sectional analyses using the baseline data of the NEO study.

Men and women living in the greater area of Leiden (in western Netherlands) were invited by letters sent by general practitioners, municipalities and local advertisements. They were invited to respond if they were between 45 and 65 years of age and had a self-reported body mass index of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent for participation and storage of blood samples.

For the present analyses, participants were excluded when they used platelet aggregation inhibitors, vitamin K antagonists or heparin. Furthermore, participants were excluded if they had missing values of serum leptin concentration, confounding variables or outcomes.

2.2. Data collection

Participants were invited to a baseline visit at the NEO study centre of the LUMC after an overnight fast. Prior to this study visit, participants completed a general questionnaire at home to report demographic, lifestyle and clinical information including age, sex, ethnicity, education level, smoking status, menopausal status and alcohol intake. Ethnicity was reported by participants in seven categories: white, black, Turkish, Moroccan, South-East Asian, Hindu and other. Highest level of education was reported in ten categories based on the Dutch educational system. Individuals with none, primary school or lower vocational education were categorized as less educated and others as highly educated. Smoking status was reported as current smoker, former smoker and never smoker. Menopausal status was reported in three categories: postmenopausal, premenopausal and perimenopausal. Alcohol intake was reported with a food frequency questionnaire and converted in grams per day. The medical history included the questions whether participants currently had a neoplasm or cancer or had one in the past and if yes, whether they had been declared “medically cured”. As a consequence, it was not possible to distinguish between participants who had a recurrent cancer or were diagnosed with a second form of cancer. In our study, active cancer was defined as currently having a neoplasm or cancer and not having been “medically cured”. Additionally, participants reported the frequency, duration, and intensity of their physical activity during leisure time on the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH), which was expressed in metabolic equivalent of task (MET) hours per week [11]. The participants were asked to bring all medication they were using to the study visit, including oral contraceptives and hormonal replacement therapy. Use of oral contraceptives was categorized in current user, former user and never user. Use of hormonal replacement therapy was categorized in current user, former user and never user.

At the baseline visit an extensive physical examination was performed. Height was measured with a vertically fixed, calibrated tape measure. Body weight and total body fat were determined using the Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without wearing shoes and one kilogram was subtracted to correct for the weight of clothing. Body mass index was computed by dividing the weight in kilograms by the height in meters squared.

At the baseline visit blood samples were drawn after an overnight fast of at least 10 h and serum, heparin-plasma, citrated-plasma and EDTA-plasma were collected. C-reactive protein (CRP) was measured in the central clinical haematology laboratory of the LUMC using standard methods. Platelet count, mean platelet volume and platelet distribution width were determined in a random subset of participants in the central clinical haematology laboratory of the LUMC *via* hydro dynamic focusing (DC detection) or flow cytometry method using semiconductor laser. The remaining blood was separated into plasma and serum. Aliquots were stored at −80 °C. Serum leptin concentration was measured using a human leptin competitive RadioImmunoAssay (RIA) (Cat Nr HL-81HK, Merck Millipore, Darmstadt, Germany). The leptin concentration was counted using a gamma counter (Wizard 2 3470, Perkin Elmer, StatLia software). Blood samples for measurement of concentrations of the coagulation factors VIII, IX, XI and fibrinogen were taken into tubes containing 0.106 M trisodium citrate (Sarstedt, Nümbrecht, Germany). Factor VIII, IX and XI serum concentrations were determined with a mechanical clot detection method on an ACL TOP 700 analyser (Werfen, Barcelona, Spain). Serum fibrinogen concentration was measured according to the method of Clauss [12]. ABO blood type was determined by genotyping the 21463C/G (rs7853989), 21867T/C (rs8176749) and 10721A/C (rs514659) blood group polymorphisms. Genomic DNA was isolated from the blood and subsequently genotyped by the Centre National de Génotypage (Paris, France), using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, CA, USA). Subsequently, genotypes were imputed to the 1000 Genome Project reference panel (v3 2011) using IMPUTE (v2.2) software. As 21463C/G (rs7853989) was imputed, we rounded the allelic dosage to the nearest integer, using 0.01, 0.99, 1.01 and 1.99 as thresholds. Using this method, we were able to distinguish between blood type antigens A, B, O1 and O2.

2.3. Statistical analyses

In the NEO study population individuals with a BMI of 27 kg/m^2 or higher were purposely oversampled. Adjustment was made for this oversampling by weighing all analyses towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population [10,13]. As a consequence, all results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 kg/m^2. Descriptive baseline characteristics of the study population were expressed as mean with standard deviation (SD) or as percentages (%).

We performed linear regression analyses to examine the associations between serum leptin concentration and coagulation factor concentrations and parameters of platelet activation. Mean differences in coagulation factor concentrations and platelet activation parameters were estimated per μg/L of leptin concentration with the corresponding 95% confidence interval (CI). We developed five different models to adjust for potential confounding factors. In each model extra covariates were added with respect to the previous model. First, we performed crude models (model 1). Second, analyses were adjusted for age and sex (model 2). Third, we additionally adjusted for total body fat (%) (model 3). Fourth, we adjusted for ethnicity, education level, smoking status, active cancer, current use of estrogen, current use of hormonal replacement therapy, menopausal status, physical activity and alcohol intake (model 4). Fifth, we adjusted for serum C-reactive protein (CRP) concentration in a separate model as CRP may be both a potential confounding variable and mediator of the association between serum leptin concentration and coagulation parameters (model 5). Subsequently, a sensitivity analysis was performed to check whether adjustment for non-O blood type, a known key determinant of FVIII concentration, influenced the association between serum leptin concentration and FVIII concentration [14]. ABO blood type was added as a covariate to model 5 of the regression analysis with serum leptin concentration as exposure and FVIII concentration as outcome. Residual errors were calculated and kernel density estimation was performed to check whether residual errors were normally distributed.

3. Results

After consecutive exclusion of individuals with current use of anti-

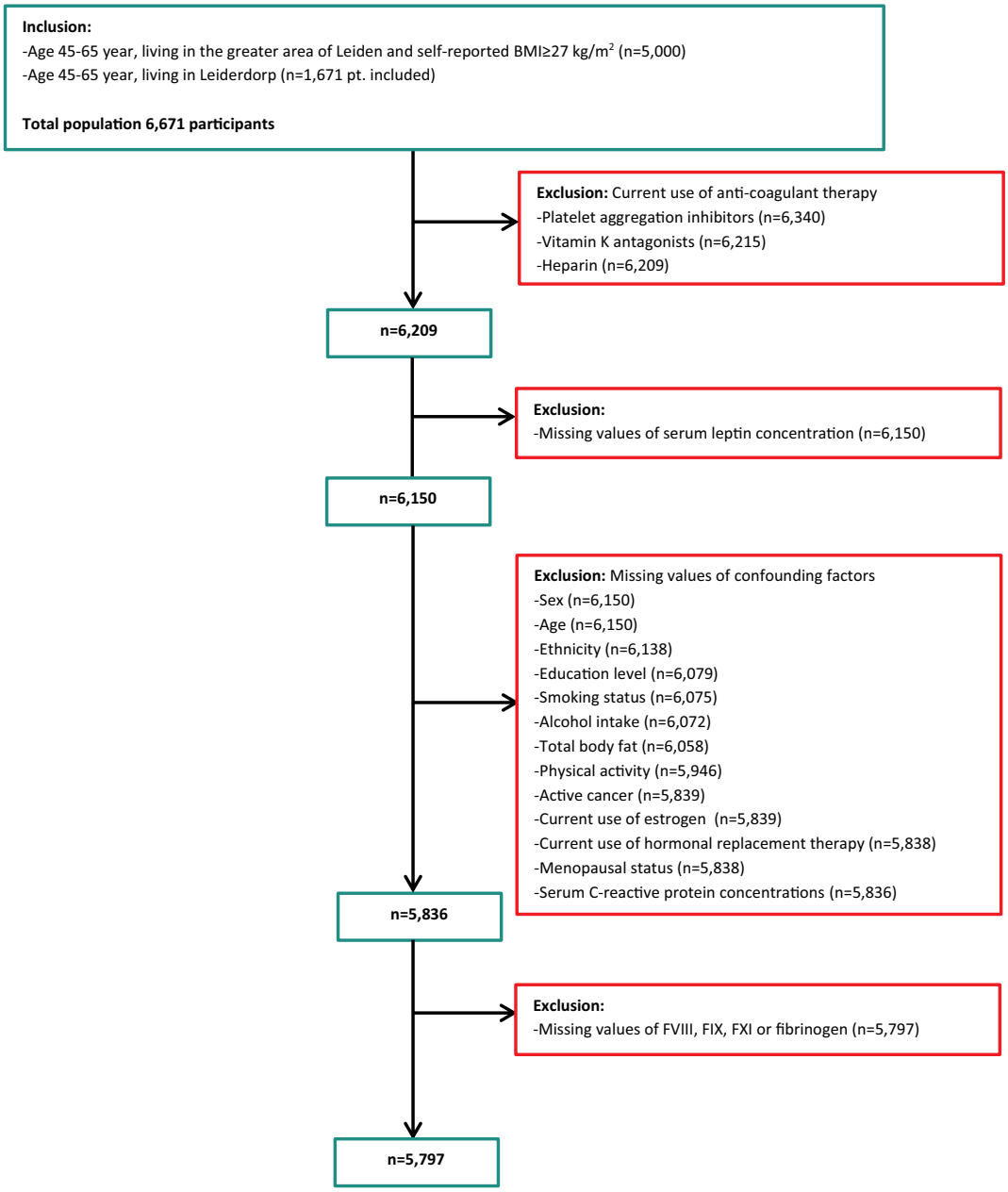

Fig. 1. Flow chart of the study for the observational analyses using coagulation factor concentrations as outcome.

coagulant therapy (n = 462), missing values of serum leptin concentration (n = 59), missing values of confounding factors (n = 314) and missing values of coagulation factor concentrations (n = 39), 5797 participants were included for all analyses using coagulation factor concentrations as outcome. For analyses using platelet activation parameters as outcome, 1645 participants were included. The flow chart of the study for the analyses with coagulation factor concentrations as outcome is displayed in Fig. 1.

Mean age was 55 years, 95% were white and the mean percentage of total body fat was 25% for men and 37% for women (Table 1).

3.1. Serum leptin and coagulation factor concentrations

Median serum leptin concentration was 19.1 μg/L (25th percentile: 9.8, 75th percentile: 34.3). Mean coagulation factor concentrations were 122.2 IU/dL (SD: 33.6) for FVIII, 116.6 IU/dL (SD: 19.8) for FIX, 117.1 IU/dL for FXI (SD: 20.0) and 292.7 mg/dL (SD: 58.0) for fibrinogen. Mean levels of platelet activation parameters were 237.2 10^9/L (SD: 51.6) for platelet count, 10.6 fL (SD: 0.8) for mean platelet volume and 12.6% (SD: 1.7) for platelet distribution width. Table 2 shows the association between serum leptin concentration and coagulation factor concentrations and platelet activation parameters. In crude analyses, serum leptin concentration was associated with higher FVIII, FIX, FXI and fibrinogen. These associations attenuated with adjustment for potential confounding variables. The crude association between FXI and serum leptin concentration largely attenuated after adjustment for total body fat. The crude association between serum leptin concentration and fibrinogen largely attenuated after adjustment for serum C-reactive protein concentration. Only FVIII and FIX remained associated with serum leptin concentration after adjustment for all potential confounding variables in model 5. Each μg/L higher serum leptin concentration was associated with a 0.22 IU/dL (95% CI: 0.11, 0.32) higher FVIII concentration and a 0.20 IU/dL (95% CI: 0.14, 0.27) higher FIX concentration. One standard deviation (SD: 16 μg/L) increase in serum leptin concentration was associated with a 3.5 IU/dL increase in FVIII concentration and a 3.2 IU/dL increase in FIX

Table 1
Baseline characteristics of 5797 participants from the NEO study population.

	Total study population
Demographic and lifestyle factors	
Sex (% women)	57
Age (years)	55 (6)
Ethnicity (% white)	95
Education level (% high)[a]	46
Alcohol consumption (g/day)	9 (2 – 22)
Physical activity (MET-hours per week)[b]	27 (14–47)
Tobacco smoking (% current)	16
Contraception (% current use, in women)	7
HRT (% current use, in women)	3
Menopause status (% postmenopausal, in women)	60
Comorbidity	
Active cancer (%)	1
Anthropometric factors	
BMI (kg/m^2)	
-Men	27 (4)
-Women	26 (5)
Total body fat (%)	
-Men	25 (6)
-Women	37 (7)
Serum leptin concentration	
-Men	8.9 (7.3)
-Women	23.2 (17.6)
C-reactive protein (mg/L)	2.1 (3.0)

Results are based on analyses weighted towards the BMI distribution of the general Dutch population. Results are based on data of the participants in whom coagulation factor concentrations were measured. Data are expressed as mean with standard deviation (SD) or percentage (%), except for alcohol consumption, physical activity and C-reactive protein which are expressed as median with interquartile range (IQR). NEO = Netherlands Epidemiology of Obesity. HRT = Hormonal Replacement Therapy. BMI = Body Mass Index. MET = Metabolic Equivalent of Task.

[a] Individuals with none, primary school or lower vocational education were categorized as poorly educated and others as highly educated.

[b] Physical activity was measured in MET-hours per week in leisure time only.

concentration. The estimated association between serum leptin concentration and FVIII concentration did not change after adding ABO blood type as an extra covariate in model 5 (results not shown). Platelet count was the only platelet activation parameter that was associated with serum leptin concentration in crude analyses. This association disappeared after adjustment for total body fat.

4. Discussion

In this population-based study of 5797 middle-aged men and women, serum leptin concentration was associated with higher concentrations of FVIII and FIX. Adjustment for total body fat had the largest impact on the association between serum leptin concentration and fibrinogen, FIX and FXI. Serum leptin concentration was not associated with the platelet activation parameters platelet count, mean platelet volume and platelet distribution width.

One cohort study has previously examined the association between leptin concentration and multiple parameters of blood coagulation. This study resulted in an association between increased leptin and higher platelet count, mean platelet volume, FVIII and fibrinogen [9]. However, in this study no adjustment was made for body mass index or for total body fat. These results may therefore have suffered from confounding by total body fat, which is supported by our results. Furthermore, multiple studies have investigated the association between leptin and blood coagulation *in vitro*. These studies found leptin capable of inducing platelet aggregation and activation [3,4,6]. These findings contrast with our results, which showed no associations *in vivo* between serum leptin concentration and mean platelet volume, a key parameter of platelet activation [15]. This might suggest leptin-induced platelet aggregation and activation is not relevant in physiological conditions or the effect might be too small to detect in our analyses.

Table 2
Association between serum leptin concentration and both coagulation factor concentrations and platelet activation parameters.

	Difference in coagulation factor concentration (95% CI) per µg/L of serum leptin concentration		Difference in platelet activation parameters (95% CI) per µg/L of serum leptin concentration
	n = 5797		*n* = 1645
Fibrinogen (mg/dL)		**Platelet count (billion/L)**	
Model 1	1.10 (0.99, 1.20)	Model 1	0.74 (0.56, 0,92)
Model 2	1.17 (1.05, 1.29)	Model 2	0.23 (0.03, 0.44)
Model 3	0.58 (0.43, 0.73)	Model 3	0.24 (−0.04, 0.53)
Model 4	0.45 (0.28, 0.62)	Model 4	0.17 (−0.15, 0.49)
Model 5	0.10 (−0.05, 0.24)	Model 5	0.09 (−0.24, 0.43)
Factor VIII (IU/dL)		**Mean Platelet Volume (fL)**	
Model 1	0.32 (0.25, 0.38)	Model 1	0.00 (0.00, 0.00)
Model 2	0.34 (0.27, 0.41)	Model 2	0.00 (0.00, 0.01)
Model 3	0.35 (0.26, 0.44)	Model 3	0.00 (0.00, 0.01)
Model 4	0.29 (0.18, 0.40)	Model 4	0.00 (0.00, 0.01)
Model 5	0.22 (0.11, 0.32)	Model 5	0.00 (0.00, 0.01)
Factor IX (IU/dL)		**Platelet Distribution Width** (%)	
Model 1	0.39 (0.35, 0.43)	Model 1	0.00 (0.00, 0.01)
Model 2	0.53 (0.48, 0.58)	Model 2	0.01 (0.00, 0.02)
Model 3	0.29 (0.23, 0.35)	Model 3	0.00 (0.00, 0.01)
Model 4	0.27 (0.21, 0.34)	Model 4	0.00 (−0.01, 0,02)
Model 5	0.20 (0.14, 0.27)	Model 5	0.00 (−0.01, 0.02)
Factor XI (IU/dL)			
Model 1	0.29 (0.24, 0.33)		
Model 2	0.19 (0.15, 0.24)		
Model 3	0.05 (−0,01, 0.11)		
Model 4	0.08 (0.01, 0.15)		
Model 5	0.06 (−0.01, 0.13)		

Results are based on linear regression analyses weighted towards the BMI distribution of the Dutch general population. Beta coefficients (95% CI) can be interpreted as differences in coagulation factor concentration per µg/L of leptin concentration.
Model 1 = crude model.
Model 2 = model 1 + age + sex.
Model 3 = model 2 + total body fat.
Model 4 = model 3 + ethnicity, education level, smoking status, active cancer, current use of estrogen, current use of hormonal replacement therapy, menopausal status, physical activity, alcohol intake.
Model 5 = model 4 + serum C-reactive protein concentration.

Previous studies confirmed that higher levels of FVIII and FIX are both risk factors for venous thrombosis and arterial cardiovascular disease [16–19]. Our findings suggest that one standard deviation (SD: 16 µg/L) increase in serum leptin concentration is associated with a 3.5 IU/dL increase in FVIII concentration and a 3.2 IU/dL increase in FIX concentration. Based on previous studies that assessed the association between coagulation factor concentrations and venous thrombosis risk these increases would correspond to respectively a 6% increase and 19% increase in venous thrombosis risk, which could be clinically relevant [14,18].

To our knowledge, there have been no previous studies to assess the association between leptin and venous thrombosis risk. As obesity is a known risk factor for venous thrombosis and the mechanisms behind this phenomenon are poorly understood, increased serum leptin concentration might at least partially explain this association [20,21]. Future research should therefore examine the association between leptin and clinical venous thrombosis risk.

Our findings are in line with previous studies that have suggested that leptin is associated with arterial vascular disease. A prospective

study in hypercholesterolemic men showed that leptin is associated with coronary heart disease and a case-control study demonstrated an association between leptin and stroke [22,23]. Both findings were confirmed in a subsequent meta-analysis [8]. However, these three studies merely adjusted for body mass index in their analyses rather than for total body fat. This raised the question whether residual confounding by body fat could have led to overestimated results in these studies, which is indeed suggested by our results.

The strengths of our study include the population-based design and extensive adjustment for potential confounding variables, including total body fat. Previous studies that investigated the association between serum leptin and cardiovascular disease adjusted for body mass index as a potential confounding factor in their analyses [8,22,23]. As leptin is produced by adipose tissue and body mass index is also influence by lean body mass, we adjusted our analyses for total body fat. However, total body fat is also a biologically plausible effect modifier and mediator in the association between serum leptin and blood coagulation. Therefore, adjustment for total body fat might lead to overadjustment and as a consequence the association between serum leptin and blood coagulation might be underestimated in our analyses [24]. We considered performing a Mendelian Randomization analysis, as a complementary approach in order to avoid possible residual confounding and reverse causality. However, sample size calculations showed that the required sample size exceeded the number of individuals available from the NEO study as well as from publicly available GWAS summary level datasets (Supplementary methods) [25–28].

Our study also has certain limitations. First, due to the observational cross-sectional study design, it was not possible to observe temporal associations. Furthermore, we included only individuals between 45 and 65 years old who were predominantly white. It is unclear whether our findings might generalize to different age and ethnic groups. Furthermore, it was not possible to distinguish between participants who had recurrent cancer or were diagnosed with a second form of cancer. Also, we relied on largely self-reported measures for our potential confounding factors.

Finally, in model 5 we adjusted for CRP in the association between serum leptin and blood coagulation. It should be noted that CRP could rather act as a mediator in the estimated association [29]. If so, adjustment for CRP has led to an underestimation of the association between serum leptin and blood coagulation.

In conclusion, the present study shows that in middle-aged men and women, serum leptin concentration was associated with higher concentrations of FVIII and FIX in observational analyses. Platelet count, mean platelet volume and platelet distribution width were not associated with serum leptin concentration.

Addendum

David T.P. Buis performed the analysis. David T.P. Buis, Tim Christen and Roelof A.J. Smit drafted the document. Renée de Mutsert, Johannes W. Jukema, Suzanne C. Cannegieter, Willem M. Lijfering and Frits R. Rosendaal interpreted the data, critically reviewed and revised the document before providing final approval.

Declaration of competing interest

The authors state that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2020.01.021.

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