Plasma levels of leptin and risk of future incident venous thromboembolism

Running head: Leptin, obesity and venous thromboembolism

Tobias Frischmuth,[1] Kristian Hindberg,[1] Pål Aukrust,[1,2,3,4] Thor Ueland,[1,2,3] Sigrid K. Brækkan,[1,5] John-Bjarne Hansen,[1,5] and Vânia M. Morelli[1]

[1]Thrombosis Research Center, Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, Norway

[2]Faculty of Medicine, University of Oslo, Oslo, Norway

[3]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway

[4]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway

[5]Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

Corresponding author:

Tobias Frischmuth, MD

Thrombosis Research Center, Department of Clinical Medicine, UiT - The Arctic University of Norway, N-9037 Tromsø, Norway

Phone: +47 77625105; Fax: +47 77625105; E-mail: tobias.frischmuth@uit.no

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Abstract

Background: Circulating levels of leptin, an adipocyte-derived hormone, are frequently elevated in obesity. Leptin has been reported to upregulate prothrombotic hemostatic factors *in vitro* and could potentially mediate venous thromboembolism (VTE) risk in obesity. However, whether leptin is associated with VTE remains uncertain.

Objective: To investigate the association between plasma leptin and risk of incident VTE, and the potential of leptin to mediate VTE risk in obesity.

Methods: A population-based nested case-control study with 416 VTE cases and 848 age- and sex-matched controls was derived from the Tromsø Study. Logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across leptin quartiles. Analyses were performed separately in men and women using sex-specific quartile cut-offs determined in controls.

Results: In the age-adjusted model, the VTE risk increased across leptin quartiles, particularly in men. Compared with the lowest quartile, the ORs for VTE in the highest quartile were 1.70 (95% CI 1.04-2.79) in men and 1.36 (95% CI 0.85-2.17) in women. However, with additional adjustment for body mass index (BMI), risk estimates were markedly attenuated in men (OR 1.03, 95% CI 0.55-1.93) and women (OR 0.82, 95% CI 0.45-1.48). The ORs for VTE were increased in obese men and women (BMI $\geq$30kg/m^2) and were only marginally affected after adjustment for leptin.

Conclusion: Our results indicate that the apparent association between plasma leptin levels and VTE risk is confounded by BMI and that leptin is not a relevant mediator for VTE risk in obesity.

Keywords: adipokines; deep vein thrombosis; leptin; obesity; venous thromboembolism.

Introduction

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease affecting 1 to 2 per 1,000 individuals each year.[1,2] Obesity, defined as a body mass index (BMI) ≥30 kg/m^2,[3] is a major modifiable risk factor for VTE,[4,5] where almost one-third of the unprovoked VTE events are attributed to obesity in the general population.[5] Obesity is associated with a two- to three-fold increased risk of VTE, which increases linearly with increasing BMI,[4,6] and weight gain over time is associated with additional VTE risk, particularly in obese.[7] Mendelian randomization studies have shown that genetically elevated BMI is associated with a higher risk of VTE,[8-11] supporting a causal link between obesity and VTE.

The mechanism underlying the association between obesity and VTE remains poorly understood. Obesity may increase thrombosis risk because of venous stasis,[4,12] and associated chronic low-grade inflammation could promote a prothrombotic state.[13,14] However, inflammation, assessed by C-reactive protein, appears to explain only a small fraction of the VTE risk among obese.[15,16] Notably, adipose tissue is an active endocrine organ that secretes cytokines, hormones, and other bioactive molecules, collectively termed adipokines.[14] Leptin is a hormone, mainly produced by adipocytes,[14] that has been reported to upregulate the expression of key hemostatic factors *in vitro*, including tissue factor (TF) [17,18] and plasminogen activator inhibitor-1 (PAI-1).[19] Although the primary function of leptin is appetite control,[14] plasma leptin is usually elevated in obese subjects, which is related to a phenomenon described as leptin resistance.[20] Given the potential of leptin to shift the balance of hemostasis towards a prothrombotic state, this adipokine could serve as a mediator for the VTE risk in obese subjects.

Data on the role of leptin in VTE are scarce, with previous studies reporting an association of increased leptin levels with DVT after total knee arthroplasty,[21] peripheral

chronic venous disease,[22] and post thrombotic syndrome.[23] The clarification of the association between leptin and VTE may shed more light on the molecular pathways in the pathogenesis of VTE in obese, which could thereby serve as potential targets for VTE prevention. However, the assessment of the relationship between leptin and VTE may be challenging because leptin, as a satiety hormone, can influence body weight and BMI.[14] As BMI is a measure of body fat related to both leptin levels [24] and VTE risk,[4,6] it would act as a confounder in the association between leptin and VTE. In the present study, we set out to investigate the association between plasma leptin levels and risk of incident VTE while adjusting for BMI, using a population-based nested case-control study. Further, we assessed whether leptin could mediate the association between obesity and VTE. Plasma leptin levels have been consistently shown to be 2-3 times higher in women than in men,[25,26] which is likely due to differences in total body fat percentage between sexes.[26] Accordingly, all analyses were performed separately for men and women.

Methods

Study population

The Tromsø Study is a single-center, population-based cohort with repeated health surveys of inhabitants of Tromsø, Norway.[27] In 1994-95, all inhabitants of the municipality of Tromsø aged ≥25 years were invited to participate in the fourth survey of the Tromsø Study (Tromsø 4). With a high response rate (77%), a total of 27,158 individuals participated and were followed from the date of inclusion until an incident VTE, migration, death, or end of follow-up (September 1, 2007). All incident VTE events were identified by searching the hospital discharge registry, the autopsy registry and the radiology procedure registry of the University Hospital of North Norway (UNN), which is the only hospital in the Tromsø region. Trained personal extensively reviewed the medical records to adjudicate each VTE event. A VTE

event was confirmed if there were signs and symptoms of DVT or PE combined with objective confirmation by radiological procedures, leading to treatment initiation, as previously described.[28] The VTE events were classified as provoked if one or more of the following provoking factors were present: surgery, trauma, or acute medical condition (acute myocardial infarction, acute ischemic stroke, and acute infections) within 8 weeks before the event, immobilization (bed rest >3 days or confinement to wheelchair within the last 8 weeks, or long-distance travel ≥4 h within the last 14 days), active cancer at the time of VTE diagnosis, or other factors specifically described as provoking by a physician in the medical record (e.g., intravascular catheter).

During the follow-up period (1994-2007), 462 individuals experienced a VTE event. For each case, two age- and sex-matched controls, who were alive at the index date of the VTE event, were randomly sampled from the source cohort (n=924), as previously described.[29,30] From this population, 46 cases and 76 controls did not have plasma samples of sufficient quality available for the analyses, thus leaving 416 VTE cases and 848 controls for the final analyses in our nested case-control study (Fig. 1). The regional committee for medical and health research ethics approved the study, and all participants provided written informed consent.

Baseline measurements

Baseline information was collected by physical examination, questionnaires and blood samples. The height (to the nearest centimeter) and weight (to the nearest 0.5 kilograms) were measured with subjects wearing light clothing and no shoes. BMI was calculated as weight in kilogram per square of height in meters (kg/m^2). A self-administered questionnaire was used to collect a detailed history of previous chronic diseases, including arterial cardiovascular disease (CVD) (i.e. angina pectoris, stroke, and myocardial infarction) and cancer.

Blood sample collection and storage of blood products

Procedures for blood collection and storage of blood products have been previously described elsewhere.[29,30] In short, at inclusion in Tromsø 4 (1994/95), non-fasting blood was collected from an antecubital vein into 5-mL vacutainers (Becton Dickinson, Le Pont de Claix, France) containing EDTA (K3-EDTA 40 µL, 0.37 mol/L per tube) as an anticoagulant. Platelet-poor plasma was prepared by centrifugation at 3000*g* for 10 minutes at room temperature, after which the supernatant was transferred into cryovials (Greiner Labortechnik, Nürtingen, Germany) in 1-mL aliquots and stored at −80°C until further use.

Measurement of leptin

Measurement of leptin was performed at the Research Institute of Internal Medicine at Oslo University Hospital, Rikshospitalet. Plasma samples were thawed in a water bath at 37°C for 5 minutes, followed by centrifugation for 2 minutes at 13500*g* to obtain platelet-free plasma. Plasma levels of leptin were measured in duplicates by enzyme-immunoassay (EIA) using commercially available reagents (R&D Systems, Minneapolis, MN) in a 384 format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, VT) dispenser/washer (EL406). Absorption was read at 450 nm with a wavelength correction set to 540 nm using an EIA plate reader (Synergy H1 Hybrid, BioTek, Vinooski, VT). The intra- and inter-assay coefficients of variation were 1.7% and 6.4%, respectively.

Statistical analysis

Statistical analysis was carried out with STATA (version 16; Stata Corporation, College Station, TX, USA) and R version 4.0.4. (The R Foundation for Statistical Computing, Vienna, Austria). Means (± standard deviation), medians (25th-75th percentiles) and proportions of

baseline characteristics were calculated for cases and controls using descriptive statistics. Spearman's correlation coefficients were calculated to assess the relationship between leptin levels and BMI in control subjects.

Sex-specific leptin categories were defined according to quartile cut-offs determined in the control population. Unconditional logistic regression was used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to quartiles of plasma leptin levels, and the lowest quartile served as the reference category. The association between leptin levels and VTE was adjusted for age in model 1, with the addition of BMI to model 2. We calculated the *P* value for linear trend for VTE risk across increasing quartiles of leptin levels. Additionally, subgroup analyses according to the presence of provoking factors (i.e. provoked and unprovoked VTE events) and VTE location (i.e. DVT and PE) were carried out for each model. Leptin has been reported to be related to arterial CVD in some studies.[31,32] Because arterial CVD is associated with increased risk of VTE,[33,34] we evaluated, for sensitivity purposes, the association between leptin levels and overall VTE after excluding subjects with self-reported history of arterial CVD at baseline.

As the follow-up time in the source cohort was long, the results based on baseline leptin measurements could be subject to regression dilution bias.[35] To address this issue, analyses were performed by restricting the maximum time from blood sampling in Tromsø 4 to the VTE events, while keeping all controls in the analyses. The logistic regression analyses on time restrictions were set to require at least 15 VTE events for the first estimation, and ORs were generated at each time a new VTE event occurred since blood sampling and plotted as a function of this maximum time.

Next, we investigated the potential of leptin to mediate the association between obesity and VTE in both sexes. For this purpose, we estimated ORs with 95% CIs for overall VTE across clinical cut-offs of BMI established by the World Health Organization (WHO):

BMI <25 kg/m^2 (reference category), BMI 25-30 kg/m^2 (overweight) and BMI ≥30 kg/m^2 (obesity).[3] To assess the mediating effect of leptin levels on the relationship between obesity and VTE, we initially performed analyses adjusted for age in model 1, adding leptin to model 2.

Results

The sex-specific distribution of baseline characteristics in VTE cases and controls is shown in Table 1. As expected, median leptin levels in controls were almost 3 times higher in women (25.5 ng/mL; interquartile range, 15.8-36.7 ng/mL) than in men (9.2 ng/mL; interquartile range, 5.6-13.4 ng/mL), with higher levels of leptin also being observed in women among cases. Mean age between cases and controls was virtually the same, as age was a matching variable. In both sexes, median plasma leptin levels and mean BMI, in particular, were higher in cases than in controls. The proportion of subjects with self-reported arterial CVD was higher in men than in women, but there was no substantial difference between cases and controls. The proportion of subjects with self-reported cancer was slightly higher in cases than in controls, mainly in women. When baseline characteristics were presented according to quartiles of plasma leptin levels, we observed that mean age and BMI, and the proportion of subjects with self-reported arterial CVD increased across leptin quartiles in both sexes (Supplementary Table1). Among controls, there was a moderate to strong correlation between BMI and leptin levels in men (r_s = 0.63, P<0.001) and women (r_s = 0.72, P<0.001).

The characteristics of the VTE events are shown in Table 2. The mean age at the time of the VTE occurrence was 68 years and 48.1% were men. The majority of the subjects presented with DVT (62.3%) and provoked events (57.9%). The most common provoking factors were surgery or trauma (22.4%), cancer (21.4%), and immobilization (18.0%).

The ORs for VTE according to quartiles of plasma leptin levels are shown for men and women in Tables 3 and 4, respectively. In men, the ORs for overall VTE increased with increasing levels of leptin in the age-adjusted model (*P* for trend = 0.04) (Table 3). Male subjects with leptin levels in the highest quartile (≥13.4 ng/mL) had an OR of 1.70 (95% CI 1.04-2.79) for VTE compared with those with leptin in the lowest quartile (<5.6 ng/mL). However, the association disappeared after further adjustment for BMI in model 2 (OR 1.03, 95% CI 0.55-1.93). In women, the ORs for overall VTE slightly increased across leptin quartiles in the age-adjusted model, but associations were not significant (*P* for trend = 0.2) (Table 4), with an OR of 1.36 (95% CI 0.85-2.17) for the highest (≥36.7 ng/mL) vs the lowest (<15.8 ng/mL) quartile. Similar to men, ORs were substantially attenuated in women when BMI was added to model 2 (OR 0.82, 95% CI 0.45-1.48 for the highest vs the lowest quartile).

The ORs for VTE comparing the highest vs the lowest quartile of plasma leptin levels were calculated as a function of time between blood sampling and VTE to assess the possibility of regression dilution bias (Fig. 2). The ORs for VTE were higher in men as compared with women during all follow-up time in the age-adjusted model, and risk estimates tended to be slightly increased with shortened time between blood sampling and VTE events, mainly in men. Consistent with previous analyses, there was a marked attenuation in ORs after further adjustment for BMI during the entire follow-up period in men and women.

In subgroups (provoked VTE, unprovoked VTE, DVT and PE), the effect of leptin on thrombosis risk was most pronounced for DVT (OR 2.06, 95% CI 1.10-3.88) in men (Table 3) and for PE (OR 2.57, 95% CI 1.18-5.60) in women (Table 4), when comparing the highest with the lowest quartile in models adjusted for age. In line with overall VTE, no associations between leptin levels and subgroups of VTE were observed in the age- and BMI-adjusted models (Tables 3 and 4). Similarly, no associations between leptin levels and VTE were

found in the sensitivity analysis (Supplementary Tables 2 and 3) where participants with self-reported history of arterial CVD at baseline were excluded.

The ORs for overall VTE according to BMI categories are shown in Table 5. Compared with subjects with a BMI <25 kg/m^2, the ORs for VTE among those with a BMI ≥30 kg/m^2 (obese) were 2.04 (95% CI 1.17-3.57) in men and 2.09 (95% CI 1.34-3.26) in women in the age-adjusted model. The addition of leptin to a second model resulted in marginal changes in risk estimates for both sexes.

Discussion

In this population-based nested case-control study, we investigated the association between plasma leptin levels and risk of future incident VTE. We found that the risk for VTE increased across quartiles of leptin levels in models adjusted for age, particularly in men. However, risk estimates were markedly attenuated with additional adjustment for BMI in both sexes, and no association between leptin and VTE was observed in the fully adjusted models. Similar results were obtained for VTE subgroups, and after excluding participants with self-reported history of arterial CVD at baseline. Furthermore, adjustment for leptin levels had only a marginal effect on risk estimates for VTE in obese men and women. Our findings indicate that the apparent association between plasma leptin levels and VTE risk is confounded by BMI and that leptin is not a relevant mediator for VTE risk in obese subjects.

This is the first study to investigate the association between plasma leptin levels and risk of incident VTE in the general population. To our knowledge, only one study has assessed the impact of leptin levels on VTE risk, but this report was restricted to patients with osteoarthritis (n= 203) undergoing total knee arthroplasty.[21] The authors found that preoperative serum leptin levels were associated with increased risk of DVT after surgery in multivariable-adjusted analyses that included sex and BMI. However, several factors limit the

comparison of this report with our results, such as the inclusion of a highly selected population, and an outcome involving only provoked DVTs that were detected by systematic postoperative screening. In the other two studies that addressed the role of leptin levels in venous disease, VTE was not the outcome of interest. In a case-control study derived from the San Diego Population Study, higher plasma leptin levels were associated with the presence of peripheral chronic venous disease.[22] It is worth noting that the risk estimates were attenuated with additional adjustment for BMI, a finding that is in line with our results. In a prospective cohort study comprising 320 patients with a first DVT, higher leptin levels, independent of obesity, predicted the occurrence of post thrombotic syndrome.[23]

In contrast to the scarce clinical data on the relationship between leptin and VTE risk, several *in vitro* studies have investigated the effect of leptin on platelet activation parameters [36] and on the expression of key hemostatic factors for blood coagulation and fibrinolysis.[17-19,37,38] In human peripheral mononuclear cells, leptin was shown to induce TF activity, antigen and mRNA expression,[17,18] as well as the release of TF-bearing microparticles. [37] Interestingly, Ayer *et al*. [38] demonstrated that the upregulation of TF activity in mononuclear cells was only achieved at supra-physiological concentrations of leptin, such as those used in the aforementioned studies,[17,18,37] but not at the lower concentrations generally observed in obese subjects. Thus, the *in vitro* findings may not reflect the ability of physiological levels of leptin to upregulate TF expression *in vivo*. Moreover, leptin was implicated in the upregulation of PAI-1 mRNA and protein expression in human coronary endothelial cells.[19] However, this is in contrast with findings in leptin-deficient *ob/ob* mice, which were characterized by high levels of PAI-1 [39] and with an *in vitro* study, where leptin was reported to have no effect on PAI-1 secretion from human adipocytes.[40] In experimental studies, leptin was also assessed in a mouse model of venous thrombosis, in which PE was induced by the injection of collagen and epinephrine (well-known agonists of platelet activation) into the

jugular vein.[41] The authors found that prior administration of leptin-neutralizing antibodies was associated with improved survival and reduced number of occlusive thrombi in the pulmonary vessels. However, since thrombosis is essentially driven by platelet activation in this model, it poorly reproduces the mechanism of venous thrombus formation in humans, which frequently occurs in the presence of stasis and absence of endothelial injury.[42]

In previous epidemiological studies, involving a small number of participants, circulating leptin levels have been reported to be associated with a broad range of hemostatic factors, such as platelet activation parameters, and procoagulant and fibrinolytic factors.[43-46] In two larger studies (the British Regional Heart Study and the Netherlands Epidemiology of Obesity Study), leptin levels were associated with several hemostatic factors, including fibrinogen, factor VIII, factor IX, von Willebrand factor, tissue plasminogen activator and D-dimer, even after adjustment for measures of body fat.[47,48] However, a main limitation of the above mentioned studies [43-48] is that they are not designed to reveal the direction of the associations given their cross-sectional nature, which precludes any inference on potential causality owing to the undetermined temporal sequence between exposure (i.e. leptin) and outcome (i.e. hemostatic factors).

In light of our findings, the association of leptin with hemostatic factors reported *in vitro* [17-19,37,38] and in epidemiological studies [43-48] does not seem to be clinically relevant for the underlying biology of VTE risk in obese. Even though plasma leptin levels were associated with increased ORs for VTE, particularly in men, risk estimates were largely attenuated after adjustment for BMI and were about 1.0 in both sexes. Similar results were obtained when analyses were extended for specific subgroups (i.e. DVT, PE, and provoked VTE), and with the exclusion of participants with self-reported history of arterial CVD at baseline. Taken together, our findings indicate that leptin does not contribute to the VTE risk beyond body fat, as reflected by BMI. Moreover, in the mediation analysis, risk estimates for

VTE in obese men and women were only marginally changed when the regression models were adjusted for leptin. Even though it cannot be completely ruled out that leptin could contribute to some extent to the mechanism of venous thrombus formation in obesity, our mediation analysis suggests that any possible role of leptin in the causal path between obesity and VTE is minor at most, and probably not clinically relevant.

The strengths of our study include the recruitment of VTE patients from a population-based cohort with age- and sex-matched controls from the same source population. The nested case-control study is derived from a large prospective cohort where blood sampling took place before the VTE event, allowing assumptions on the direction of the association between exposure (leptin) and outcome (VTE). Another strength was the conduction of separate analysis for men and women, which enabled to account for the large difference in plasma leptin levels between sexes. Some limitations merit attention. Plasma samples were frozen and stored at -80 °C for up to 22 years, and the long storage time could have influenced plasma leptin levels. However, it is unlikely that this would affect the results, since the potential storage effect would be similar in VTE cases and controls. Plasma leptin levels are influenced by the circadian rhythm and potentially by food intake.[49] In our study, blood samples were collected throughout the day and in a non-fasting state. However, the approach for blood sampling did not differ for cases and controls and was performed without knowledge of future case-control status. Therefore, any potential misclassification of leptin measurement would be non-differential with regards to VTE status. It is important to address that our finding of a moderate to strong correlation between BMI and plasma leptin levels was similar to previous studies, in which blood collection was carried out under optimal pre-analytical conditions (i.e. fasting state and same time of the day).[43,45] In addition, plasma leptin levels that we found in men and women were similar to the levels described in other population-based studies.[25,48] Of note, our results were based on a single measurement of

leptin at baseline, and changes during the long follow-up (up to 13 years) could have resulted in an underestimation of the association because of regression dilution.[35] We addressed this by performing a time restricted analysis and observed that the risk of VTE was higher with shortened time between blood sampling and VTE events, especially in men. Nevertheless, after adjustment for BMI, no association between leptin and VTE was observed in both sexes during the entire period of observation, implying that regression dilution is unlikely to explain our findings.

In conclusion, our results indicate that the apparent association between plasma leptin levels and VTE risk is attributed to confounding by BMI. Our findings suggest that the association of leptin with hemostatic factors reported *in vitro* and in epidemiological studies is not clinically relevant for the mediation of VTE risk in obese subjects.

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Disclosures

There are no conflicts of interest reported by any of the authors.

Author contributions

T. Frischmuth analyzed data, interpreted the results and drafted the manuscript. K. Hindberg provided statistical support, interpreted the results, and revised the manuscript. P. Aukrust and T. Ueland performed the laboratory analysis, interpreted the results, and revised the manuscript. S.K. Brækkan designed the study, organized data collection, interpreted the

results, and revised the manuscript. J-B Hansen designed the study, organized data collection, interpreted the results, and revised the manuscript. V.M. Morelli designed the study, interpreted the results, contributed to the manuscript draft, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Figures

Figure 1 Flowchart of the study population. The flowchart illustrates the nested case-control study derived from the fourth survey of the Tromsø Study (1994-1995). VTE, venous thromboembolism.

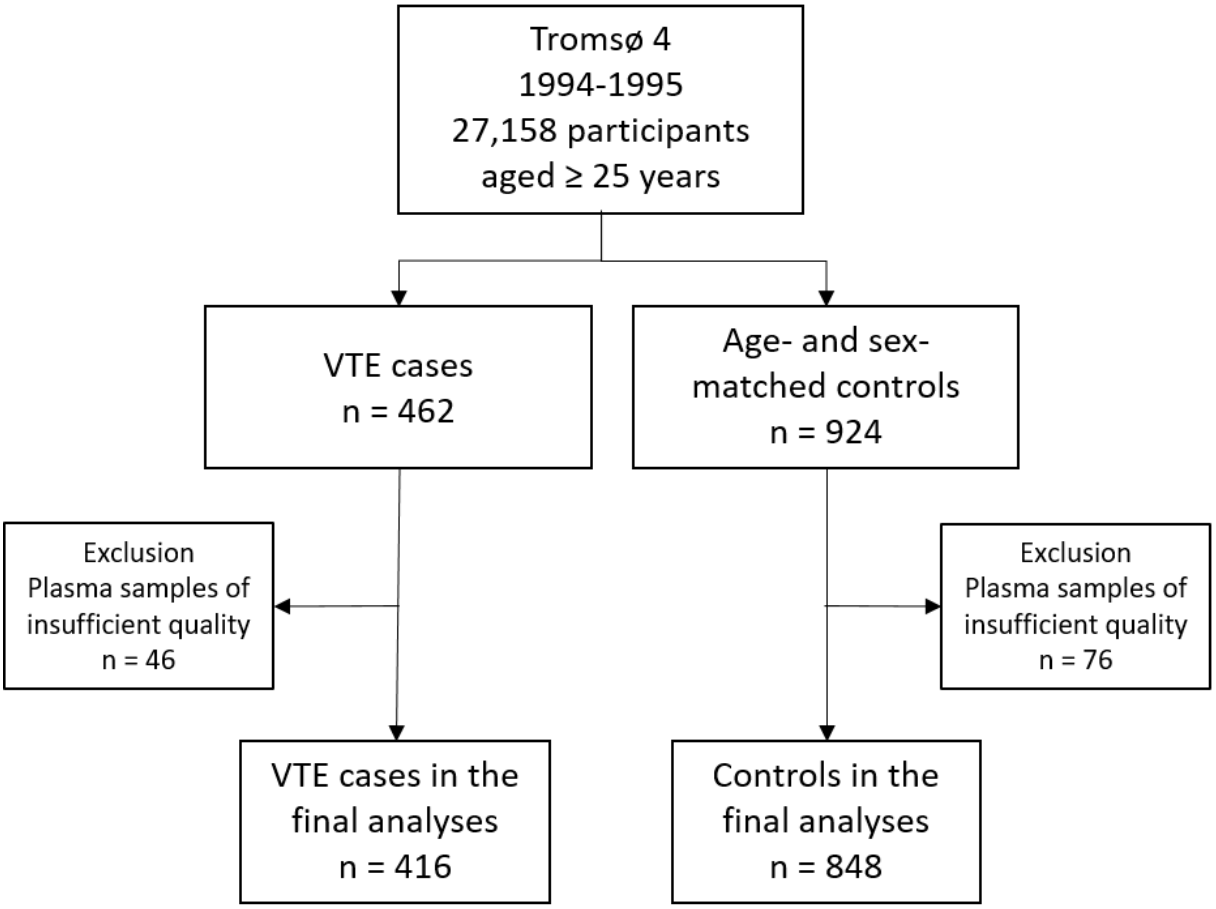
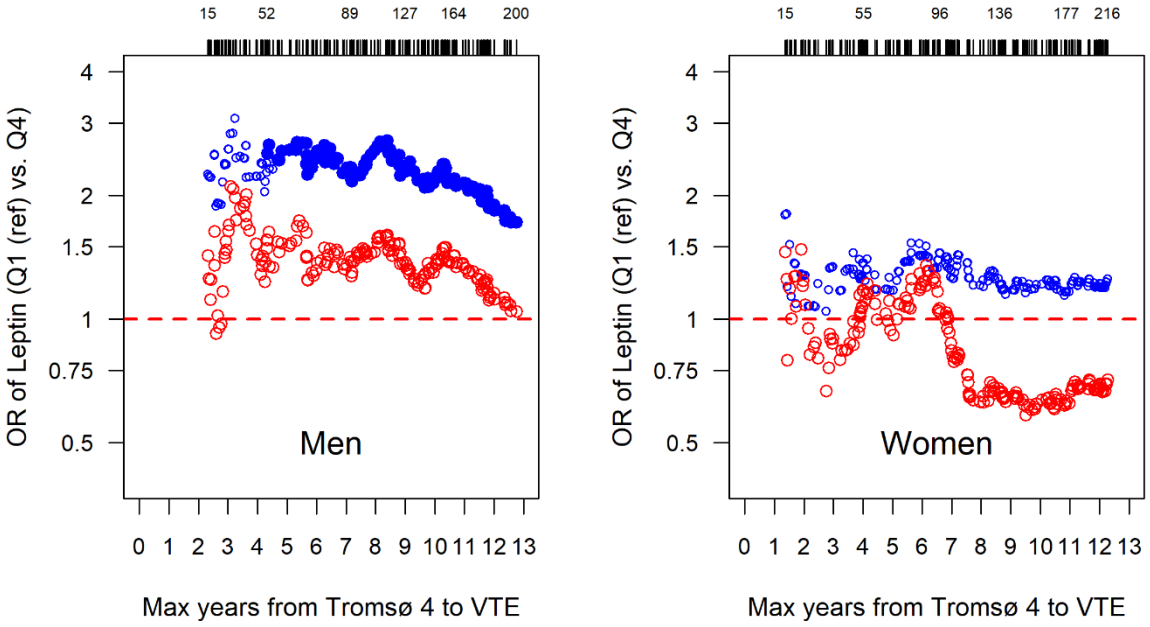


Figure 2 Plots of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time from blood sampling in Tromsø 4 to VTE events. Participants with plasma leptin levels in the highest quartile (Q4) were compared with those with leptin levels in the lowest quartile (Q1, reference category). Blue circles indicate ORs adjusted for age (model 1) and red circles are ORs adjusted for age and body mass index (model 2). Solid circles indicate ORs with *P* values < 0.05.



Tables

Table 1 Baseline characteristics in venous thromboembolism (VTE) cases and controls for men and women.

	VTE Cases	Controls
Men		
n	200	394
Age (years)	58 ± 12	58 ± 12
BMI (kg/m^2)	26.8 ± 3.3	25.8 ± 3.4
Leptin (ng/mL)	10.1 (6.0-14.2)	9.2 (5.6-13.4)
CVD*	20.0 (40)	18.8 (74)
Cancer†	3.5 (7)	2.3 (9)
Women		
n	216	454
Age (years)	62.2 ± 14.8	61.8 ± 14.9
BMI (kg/m^2)	27.5 ± 5.4	26.3 ± 4.6
Leptin (ng/mL)	26.4 (18.2-38.1)	25.5 (15.8-36.7)
CVD*	12.5 (27)	13.0 (59)
Cancer†	9.3 (20)	4.9 (22)

Continuous variables are shown as mean (± standard deviation) or median (25th percentile -75th percentile). Categorical are shown as percentages with numbers in brackets.
Abbreviations: BMI, body mass index; CVD, cardiovascular disease; SD, standard deviation.
*Self-reported history of arterial cardiovascular disease (myocardial infarction, angina pectoris, stroke) at baseline.
†Self-reported history of cancer at baseline.

Table 2 Characteristics of venous thromboembolism (VTE) events (n=416).

Characteristics	Value
Age at VTE (years), mean ± SD	68 ± 14
Male sex, % (n)	48.1 (200)
Deep vein thrombosis, % (n)	62.3 (259)
Pulmonary embolism, % (n)	37.7 (157)
Unprovoked VTE, % (n)	42.1 (175)
Provoked VTE, % (n)	57.9 (241)
Surgery/trauma, % (n)	22.4 (93)
Acute medical condition, % (n)	15.6 (65)
Cancer, % (n)	21.4 (89)
Immobilization, % (n)	18.0 (75)
Other factors, % (n)	4.1 (17)

Abbreviations: SD, standard deviation.

Table 3 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) and subgroups according to quartiles of plasma leptin levels in men.

Quartiles of leptin	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
< 5.6 ng/mL	98	37	1 (reference)	1 (reference)
5.6 - 9.2 ng/mL	99	50	1.34 (0.81 - 2.23)	1.13 (0.67 - 1.92)
9.2 - 13.4 ng/mL	98	50	1.36 (0.82 - 2.27)	1.03 (0.59 - 1.79)
≥ 13.4 ng/mL	99	63	1.70 (1.04 - 2.79)	1.03 (0.55 - 1.93)
P for trend			0.04	0.9
Provoked VTE				
< 5.6 ng/mL	98	17	1 (reference)	1 (reference)
5.6 - 9.2 ng/mL	99	29	1.69 (0.87 - 3.27)	1.46 (0.74 - 2.89)
9.2 - 13.4 ng/mL	98	36	2.12 (1.12 - 4.03)	1.69 (0.84 - 3.39)
≥ 13.4 ng/mL	99	31	1.81 (0.98 - 3.49)	1.22 (0.54 - 2.76)
P for trend			0.07	0.6
Unprovoked VTE				
< 5.6 ng/mL	98	20	1 (reference)	1 (reference)
5.6 - 9.2 ng/mL	99	21	1.04 (0.53 - 2.04)	0.84 (0.42 - 1.70)
9.2 - 13.4 ng/mL	98	14	0.71 (0.34 -1.49)	0.51 (0.23 - 1.12)
≥ 13.4 ng/mL	99	32	1.60 (0.86 - 3.00)	0.86 (0.38 - 1.96)
P for trend			0.2	0.5
Deep vein thrombosis				
< 5.6 ng/mL	98	18	1 (reference)	1 (reference)
5.6 - 9.2 ng/mL	99	33	1.82 (0.96 - 3.44)	1.54 (0.79 - 2.98)
9.2 - 13.4 ng/mL	98	33	1.87 (0.98 - 3.54)	1.43 (0.71 - 2.86)
≥ 13.4 ng/mL	99	37	2.06 (1.10 - 3.88)	1.29 (0.59 - 2.84)
P for trend			0.04	0.7
Pulmonary embolism				
< 5.6 ng/mL	98	19	1 (reference)	1 (reference)
5.6 - 9.2 ng/mL	99	17	0.88 (0.43 - 1.8)	0.73 (0.35 - 1.52)
9.2 - 13.4 ng/mL	98	17	0.88 (0.43 - 1.8)	0.65 (0.30 - 1.41)
≥ 13.4 ng/mL	99	26	1.33 (0.69 - 2.57)	0.76 (0.32 - 1.80)
P for trend			0.4	0.5

Model 1: adjusted for age. Model 2: adjusted for age and body mass index.

Table 4 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) and subgroups according to quartiles of plasma leptin levels in women.

Quartiles of leptin	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Overall VTE				
< 15.8 ng/mL	113	45	1 (reference)	1 (reference)
15.8 - 25.5 ng/mL	114	52	1.14 (0.71 - 1.84)	0.99 (0.61 - 1.61)
25.5 - 36.7 ng/mL	113	57	1.26 (0.79 - 2.02)	0.98 (0.59 - 1.63)
≥ 36.7 ng/mL	114	62	1.36 (0.85 - 2.17)	0.82 (0.45 - 1.48)
P for trend			0.2	0.5
Provoked VTE				
< 15.8 ng/mL	113	28	1 (reference)	1 (reference)
15.8 - 25.5 ng/mL	114	28	0.99 (0.59 - 1.77)	0.89 (0.49 - 1.62)
25.5 - 36.7 ng/mL	113	36	1.25 (0.71 - 2.18)	1.05 (0.57 - 1.91)
≥ 36.7 ng/mL	114	36	1.23 (0.70 - 2.16)	0.84 (0.42 - 1.71)
P for trend			0.3	0.8
Unprovoked VTE				
< 15.8 ng/mL	113	17	1 (reference)	1 (reference)
15.8 - 25.5 ng/mL	114	24	1.40 (0.71 - 2.75)	1.13 (0.56 - 2.26)
25.5 - 36.7 ng/mL	113	21	1.26 (0.63 - 2.52)	0.86 (0.41 - 1.81)
≥ 36.7 ng/mL	114	26	1.56 (0.8 - 3.04)	0.74 (0.31 - 1.76)
P for trend			0.3	0.4
Deep vein thrombosis				
< 15.8 ng/mL	113	35	1 (reference)	1 (reference)
15.8 - 25.5 ng/mL	114	33	0.93 (0.54 -1.61)	0.79 (0.45 - 1.39)
25.5 - 36.7 ng/mL	113	34	0.97 (0.56 - 1.66)	0.74 (0.41 - 1.35)
≥ 36.7 ng/mL	114	36	1.01 (0.59 - 1.73)	0.59 (0.28 - 1.21)
P for trend			0.9	0.2
Pulmonary embolism				
< 15.8 ng/mL	113	10	1 (reference)	1 (reference)
15.8 - 25.5 ng/mL	114	19	1.88 (0.84 - 4.23)	1.65 (0.73 - 3.74)
25.5 - 36.7 ng/mL	113	23	2.30 (1.04 - 5.05)	1.76 (0.78 - 4.01)
≥ 36.7 ng/mL	114	26	2.57 (1.18 - 5.60)	1.50 (0.61 - 3.70)
P for trend			0.02	0.5

Model 1: adjusted for age. Model 2: adjusted for age and body mass index.

Table 5 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) according to clinical categories of body mass index (BMI) in men and women.

Categories of BMI	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
Men				
< 25 kg/m^2	169	64	1 (reference)	1 (reference)
25 - 30 kg/m^2	185	106	1.53 (1.05 - 2.22)	1.45 (0.95 - 2.22)
≥ 30 kg/m^2	39	30	2.04 (1.17 - 3.57)	1.86 (0.96 - 3.60)
P for trend			0.005	0.045
Women				
< 25 kg/m^2	186	71	1 (reference)	1 (reference)
25 - 30 kg/m^2	188	82	1.16 (0.79 - 1.70)	1.25 (0.81 - 1.92)
≥ 30 kg/m^2	79	62	2.09 (1.34 - 3.26)	2.40 (1.36 - 4.25)
P for trend			0.002	0.003

Model 1: adjusted for age. Model 2: adjusted for age and quartiles of plasma levels of leptin. Note that two controls (one man, one woman) and one case (woman) had missing value in BMI.