Received: 8 November 2021 | Accepted: 7 March 2022

DOI: 10.1111/jth.15701

ORIGINAL ARTICLE

Elevated plasma levels of plasminogen activator inhibitor-1 are associated with risk of future incident venous thromboembolism

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

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

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

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

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

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

Correspondence
Tobias Frischmuth, Thrombosis Research Center, Department of Clinical Medicine, UiT—The Arctic University of Norway, Hansine Hansens veg 18, 9019 Tromsø, Norway.
Email: tobias.frischmuth@uit.no

Funding information
Helse Nord RHF; Stiftelsen Kristian Gerhard Jebsen

Abstract

Background: Plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of fibrinolysis, is frequently elevated in obesity and could potentially mediate the risk of venous thromboembolism (VTE) in obese subjects. However, whether PAI-1 is associated with VTE remains uncertain.

Objective: To investigate the association between plasma PAI-1 levels and risk of future incident VTE and whether PAI-1 could mediate the VTE risk in obesity.

Methods: A population-based nested case-control study, comprising 383 VTE cases and 782 age- and sex-matched controls, was derived from the Tromsø Study cohort. PAI-1 antigen levels were measured in samples collected at cohort inclusion. Logistic regression was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across PAI-1 tertiles.

Results: The VTE risk increased dose-dependently across PAI-1 tertiles (*P* for trend <.001) in the age- and sex-adjusted model. The OR of VTE for the highest versus lowest tertile was 1.73 (95% CI 1.27–2.35), and risk estimates were only slightly attenuated with additional stepwise adjustment for body mass index (BMI; OR 1.59, 95% CI 1.16–2.17) and C-reactive protein (CRP; OR 1.54, 95% CI 1.13–2.11). Similar results were obtained for provoked/unprovoked events, deep vein thrombosis, and pulmonary embolism. In obese subjects (BMI of ≥30 kg/m^2 vs. <25 kg/m^2), PAI-1 mediated 14.9% (95% CI 4.1%-49.4%) of the VTE risk in analysis adjusted for age, sex, and CRP.

Conclusion: Our findings indicate that plasma PAI-1 is associated with increased risk of future incident VTE and has the potential to partially mediate the VTE risk in obesity.

KEYWORDS
deep vein thrombosis, fibrinolysis, obesity, plasminogen activator inhibitor 1, pulmonary embolism, venous thromboembolism

Manuscript handled by: Sabine Eichinger

Final decision: Sabine Eichinger, 07 March 2022

1 | INTRODUCTION

Venous thromboembolism (VTE), an umbrella term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease affecting 1 to 2 per 1000 individuals each year.[1] Obesity, defined as a body mass index (BMI) ≥30 kg/m^2,[2] is a major risk factor for VTE,[3,4] contributing to a considerable burden of this disease in the general population.[5] Moreover, in Mendelian randomization studies, genetically elevated BMI is associated with a higher risk of VTE, implying a causal relationship.[6–9] Despite the well-established association between obesity and VTE in epidemiological studies, the underpinnings of the biology of VTE risk in obesity remain poorly understood.

Chronic low-grade inflammation and attenuated fibrinolysis are commonly observed in obesity,[10–12] potentially reflecting, at least in part, an altered adipose tissue expression of cytokines, hormones, and bioactive molecules, collectively termed adipokines.[12] One of the adipokines frequently elevated in obesity is plasminogen activator inhibitor-1 (PAI-1),[10,11] a member of the serine protease inhibitor (serpin) superfamily and the primary regulator of fibrinolysis.[13] By inhibiting plasma tissue plasminogen activator, PAI-1 downregulates the conversion of plasminogen to plasmin, and consequently the breakdown of fibrin clots. In addition to adipose tissue, where PAI-1 has been shown to be expressed by adipocytes and stromal cells,[11,14,15] several other sources may contribute to circulating PAI-1, including the liver, vascular endothelium, and platelets.[11,16,17]

As the main inhibitor of fibrinolysis, PAI-1 may presumably play a role in the pathogenesis of VTE and, in particular, obesity-related VTE. However, studies on the association between plasma PAI-1 and risk of VTE have yielded conflicting results, including two large population-based studies.[18–23] These inconsistencies could be due to limited statistical power of some studies, differences in study design and clinical characteristics of participants, poor harmonization between PAI-1 assays, and the biology of PAI-1. For instance, as a positive acute phase protein, PAI-1 levels can be affected by a variety of conditions associated with an inflammatory state,[16,24] which may be especially challenging to deal with in case-control studies as it may induce reverse causation. Given that attenuated fibrinolysis is considered an important feature in obesity,[11] clarification of the role of PAI-1 as a risk factor for VTE may provide novel insights into biological pathways that could serve as potential targets for VTE prevention in obese subjects.

In the present study, we hypothesized that plasma PAI-1 levels could, at least in part, mediate the risk of VTE in obese subjects. We therefore used data from a nested case-control study derived from the general population, with the aims (1) to investigate whether plasma PAI-1 levels were associated with risk of future incident VTE, and (2) to assess whether and to what extent PAI-1 could mediate the VTE risk in obesity.

Essentials

- The link of plasminogen activator inhibitor-1 (PAI-1) with venous thromboembolism (VTE) is unclear.
- We examined the association of PAI-1 with VTE and its potential to mediate the VTE risk in obesity.
- In a nested case-control study, PAI-1 was dose-dependently associated with VTE risk.
- PAI-1 mediated almost 15% of the VTE risk in obesity and may play a role in obesity-related VTE.

2 | METHODS

2.1 | Study population and study design

The source population for our nested case-control study was the Tromsø Study, a single-center, population-based cohort with repeated health surveys of inhabitants of Tromsø, Norway.[25] In 1994–1995, all inhabitants of the municipality of Tromsø aged ≥25 years were invited to take part in the fourth survey (Tromsø 4) and 77% (n = 27,158) participated. The participants were followed from the date of inclusion until incident VTE, migration, death, or end of follow-up (September 1, 2007). All incident VTE events occurring during follow-up (1994–2007) 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 providing diagnostic radiology and treatment for VTE in the region. Each potential VTE case was reviewed and recorded by trained personnel, as previously described in detail.[26] Briefly, the identification and adjudication process of VTEs included clinical signs and symptoms of DVT or PE, objective confirmation by radiological procedures, and treatment initiation. 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. We established a nested case-control study for the assessment of PAI-1 from blood samples collected at cohort baseline. In the design of a nested case control, the temporal sequence between exposure and outcome is preserved, allowing the investigation of biological variables as precursors of diseases. For each case, two age- and sex-matched controls, alive at the index date

of the VTE event, were randomly sampled from the source cohort (n = 924), as previously described.[27,28] Seventy-nine cases and 142 controls were excluded from the analyses because plasma samples were not available (64 cases and 113 controls) or were of inadequate quality due to hemolysis (15 cases and 29 controls). Thus, the final study population comprised 383 VTE cases and 782 controls (Figure 1). The regional committee for medical and health research ethics approved the study, and all participants provided written consent.

2.2 | 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). Self-administered questionnaires were used to obtain history on arterial cardiovascular disease (CVD; i.e., angina pectoris, stroke, and myocardial infarction) and cancer.

2.3 | Blood sampling and storage of blood products

At inclusion in Tromsø 4 (1994/1995), non-fasting blood was collected from an antecubital vein into 5-ml vacutainers (Becton Dickinson) containing ethylenediaminetetraacetic acid (K3-EDTA 40 µl, 0.37 mol/L per tube), as previously described.[27,28] Platelet-poor plasma was prepared by centrifugation at 3000 g for 10 min at room temperature, after which the supernatant was transferred into cryovials (Greiner Labortechnik) in 1-ml aliquots and stored at −80°C until further use.

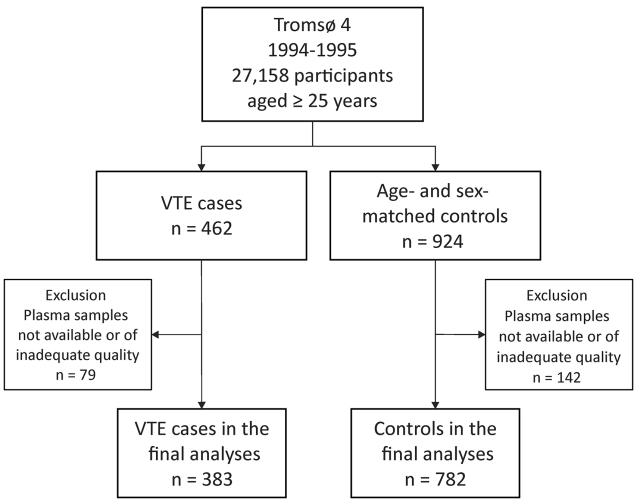

FIGURE 1 Flowchart of the study population. The flowchart illustrates the nested case-control study derived from the fourth survey of the Tromsø study (1994–1995). VTE, venous thromboembolism

2.4 | Measurements of plasma levels of PAI-1 and C-reactive protein

Measurement of PAI-1 and C-reactive protein (CRP) 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 min, followed by centrifugation for 2 min at 13,500 g to obtain platelet-free plasma. Plasma PAI-1 antigen levels were measured in duplicate by enzyme-immunoassay (EIA) with matched antibodies from R&D Systems designed to detect PAI-1 in its active and latent forms but not in complex with tissue plasminogen activator. EIA was performed in a 384-format using a combination of a SELMA (Jena) pipetting robot and a BioTek dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an ELISA plate reader (Bio-Rad). PAI-1 intra- and inter-assay coefficients of variation were <10%. CRP was measured by high-sensitivity CRP (hsCRP) using EIA, as previously described.[28]

2.5 | Statistical analysis

Statistical analysis was carried out with Stata (version 16; Stata Corporation) and R version 4.0.5 (The R Foundation for Statistical Computing). Plasma PAI-1 was categorized according to tertile cut-offs in the control population. Means (±standard deviation), medians (25th–75th percentiles), and proportions of baseline characteristics across tertiles of PAI-1 were calculated using descriptive statistics.

Unconditional logistic regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE according to tertiles of PAI-1 levels, with the lowest tertile serving as the reference group. The association between plasma PAI-1 levels and VTE was adjusted for age and sex in model 1 to take into account the matching variables,[29] for age, sex, and BMI in model 2, with the addition of CRP to model 3. Adjustment for BMI was carried out due to the association of obesity with both plasma PAI-1 levels[10,11] and VTE,[3,4] making it an important potential confounder for the association between PAI-1 and VTE. Analyses were additionally adjusted for CRP because inflammation, as reflected by CRP levels, is reported to be associated with PAI-1 levels[16,24] and VTE.[30,31] P values for linear trend across increasing tertiles of PAI-1 levels were estimated.

Subgroup analyses were conducted according to the presence of provoking factors (i.e., provoked and unprovoked VTE events) and VTE location (i.e., DVT and PE ± DVT). For sensitivity purposes, we evaluated the association between PAI-1 levels and overall VTE after excluding subjects with self-reported history of arterial CVD or cancer at the baseline.

As the follow-up time in the source cohort was long, the results based on baseline PAI-1 measurements could be influenced by regression dilution bias.[32] To investigate this, we performed analyses for overall VTE that restricted 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 10 VTE events, and ORs for highest versus lowest tertile were estimated at every time point a new VTE event occurred and were plotted as a function of this maximum time, with adjustment for age, sex, BMI, and CRP.

Next, we investigated whether PAI-1 could mediate the association between obesity and VTE. For this purpose, we estimated ORs with 95% CIs for overall VTE across clinical cut-offs of BMI established by the World Health Organization.[2] Overweight was defined as a BMI 25–30 kg/m^2 and obesity as a BMI ≥30 kg/m^2. Model 1 was adjusted for age and sex, with the addition of PAI-1, the mediator under investigation, to model 2. To further quantify the potential mediating effect of PAI-1, the Karlson, Holm, and Breen (KHB) method was applied.[33] The KHB method can be used for mediation analysis in nonlinear models, allowing the decomposition of the total effect of the exposure on the outcome into direct and indirect (i.e., mediating) effects.[33] The mediation analysis was performed with obesity as the exposure, overall VTE as the outcome, and PAI-1 as the mediator, with a stepwise adjustment for age and sex (model 1), and for CRP (model 2). We used bootstrapping with 10,000 resamples to calculate the 95% CIs for mediation percentages estimated by the KHB method.

3 | RESULTS

The distribution of baseline characteristics of the study participants across tertiles of plasma PAI-1 antigen levels is shown in Table 1. As expected, the mean BMI increased across tertiles of PAI-1 from 25.4 ± 3.9 kg/m^2 in the lowest tertile to 27.4 ± 4.6 kg/m^2 in the highest tertile. The mean age, median plasma levels of CRP, and the proportion of subjects with arterial CVD also increased across PAI-1 tertiles. The characteristics of the VTE events are described in Table 2. The mean age at the time of the VTE occurrence was 68 years, 48.8% were men, and most of the events were DVTs (63.4%) and provoked VTEs (59.0%).

The risk of overall VTE and subgroups (i.e., provoked and unprovoked VTE events, DVT, and PE) according to tertiles of plasma PAI-1 levels is shown in Table 3. The OR for overall VTE increased in a dose-response manner across tertiles of plasma PAI-1 levels in the age- and sex-adjusted model (*P* for trend <.001). Subjects with PAI-1 levels in the highest tertile (≥7.45 ng/ml) had an OR for VTE of 1.73 (95% CI 1.27–2.35) compared with those with PAI-1 in the lowest tertile (<4.08 ng/ml). The association was slightly attenuated after further adjustment for BMI (OR 1.59, 95% CI 1.16–2.17) and CRP (OR 1.54, 95% CI 1.13–2.11).

A dose-response relationship between plasma PAI-1 levels and thrombosis risk was also observed in all VTE subgroups (Table 3). The ORs for the highest versus lowest tertile were 1.71 (95% CI 1.18–2.47) for provoked VTE, 1.75 (95% CI 1.13–2.71) for unprovoked VTE, 1.75 (95% CI 1.22–2.51) for DVT, and 1.69 (95% CI 1.08–2.64) for PE in the age- and sex- adjusted models. As for overall VTE, risk estimates were marginally attenuated with additional adjustment for BMI and CRP. The sensitivity analysis, excluding participants with arterial CVD or cancer at baseline, yielded essentially similar results as the main analyses (Tables S1 and S2 in supporting information).

To consider the possibility of underestimating the true association due to regression dilution bias, the ORs for overall VTE (highest vs. lowest tertile of PAI-1) were calculated as a function of time between blood sampling and VTE events (Figure 2). Although the ORs for VTE were considerably higher with shortened time between blood sampling and VTE events, risk estimates remained significant even in the long term of the follow-up period of almost 13 years.

The risk of overall VTE according to categories of BMI is shown in Table 4. The OR for VTE was 1.92 (95% CI 1.34–2.75) in subjects with a BMI ≥30 kg/m^2 compared to those with a BMI <25 kg/m^2 in the age- and sex-adjusted model. In a mediation analysis, with addition of PAI-1 to the regression model, the OR was attenuated to 1.73 (95% CI 1.20–2.49). Further, the KHB method estimated that 16.8% (95% CI 5.6%–45.1%) of the association between obesity and

TABLE 1 Baseline characteristics according to tertiles of plasma levels of plasminogen activator inhibitor-1 (PAI-1)

Tertiles of PAI−1	<4.08 ng/ml	4.08–7.45 ng/ml	≥7.45 ng/ml
n	356	380	429
Sex (male)	42.7 (152)	50.3 (191)	48.7 (209)
Age (year)	59 ± 14	60 ± 14	62 ± 13
BMI (kg/m^2)	25.4 ± 3.9	26.1 ± 3.9	27.4 ± 4.6
hsCRP (mg/L)	0.92 (0.50–1.81)	1.14 (0.67–2.34)	1.85 (0.95–3.29)
CVD[a]	10.4 (37)	17.4 (66)	18.4 (79)
Cancer[b]	4.8 (17)	4.2 (16)	4.4 (19)

Note: Continuous variables are shown as mean (±standard deviation) or median (25th percentile–75th percentile). Categorical variables are shown as percentages with numbers in brackets.

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein.

[a]Self-reported history of cardiovascular disease (myocardial infarction, angina pectoris, stroke).

[b]Self-reported history of cancer.

TABLE 2 Characteristics of VTE events (*n* = 383)

Characteristics	Value
Age at VTE (years), mean ± SD	68 ± 14
Sex (males), % (*n*)	48.8 (187)
Deep vein thrombosis, % (*n*)	63.4 (243)
Pulmonary embolism, % (*n*)	36.6 (140)
Unprovoked VTE, % (*n*)	41.0 (157)
Provoked VTE, % (*n*)	59.0 (226)
Surgery/trauma, % (*n*)	22.5 (86)
Acute medical condition, % (*n*)	15.4 (59)
Cancer, % (*n*)	22.7 (87)
Immobilization, % (*n*)	18.8 (72)
Other factors, % (*n*)	4.4 (17)

Abbreviation: SD, standard deviation; VTE, venous thromboembolism.

TABLE 3 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism (VTE) and subgroups according to tertiles of plasma plasminogen activator inhibitor-1 (PAI-1) levels

Tertiles of PAI-1	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Overall VTE					
<4.08 ng/ml	260	96	1 (reference)	1 (reference)	1 (reference)
4.08–7.45 ng/ml	261	119	1.23 (0.89–1.69)	1.18 (0.86–1.63)	1.16 (0.84–1.61)
≥7.45 ng/ml	261	168	1.73 (1.27–2.35)	1.59 (1.16–2.17)	1.54 (1.13–2.11)
P for trend			<.001	.003	.006
Provoked VTE					
<4.08 ng/ml	260	58	1 (reference)	1 (reference)	1 (reference)
4.08–7.45 ng/ml	261	67	1.14 (0.77–1.69)	1.10 (0.74–1.64)	1.09 (0.74–1.63)
≥7.45 ng/ml	261	101	1.71 (1.18–2.47)	1.60 (1.10–2.33)	1.58 (1.08–2.30)
P for trend			.003	.01	.02
Unprovoked VTE					
<4.08 ng/ml	260	38	1 (reference)	1 (reference)	1 (reference)
4.08–7.45 ng/ml	261	52	1.35 (0.86–2.13)	1.30 (0.82–2.05)	1.27 (0.81–2.01)
≥7.45 ng/ml	261	67	1.75 (1.13–2.71)	1.55 (0.99–2.42)	1.48 (0.94–2.32)
P for trend			.01	.056	.09
Deep vein thrombosis					
<4.08 ng/ml	260	60	1 (reference)	1 (reference)	1 (reference)
4.08–7.45 ng/ml	261	78	1.30 (0.89–1.90)	1.26 (0.86–1.84)	1.24 (0.85–1.82)
≥7.45 ng/ml	261	105	1.75 (1.22–2.51)	1.63 (1.13–2.36)	1.60 (1.10–2.32)
P for trend			.002	.009	.01
Pulmonary embolism					
<4.08 ng/ml	260	36	1 (reference)	1 (reference)	1 (reference)
4.08–7.45 ng/ml	261	41	1.11 (0.69–1.80)	1.07 (0.66–1.73)	1.05 (0.65–1.71)
≥7.45 ng/ml	261	63	1.69 (1.08–2.64)	1.50 (0.95–2.37)	1.44 (0.91–2.29)
P for trend			.02	.07	.1

Note: Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and body mass index. Model 3: adjusted for age, sex, body mass index, and C-reactive protein.

VTE was mediated through PAI-1 in the age- and sex-adjusted model (Table S3 in supporting information), with a slight attenuation of the mediation percentage after additional adjustment for CRP (14.9%, 95% CI 4.1%–49.4%).

4 | DISCUSSION

In the present population-based nested case-control study, the risk of future VTE increased in a dose-dependent manner with increasing plasma levels of PAI-1, even after adjustment for BMI and hsCRP, a reliable and sensitive downstream marker of inflammation. Notably, a similar dose-response relationship between plasma PAI-1 and thrombosis risk was observed for provoked and unprovoked events, DVT and PE, and after excluding subjects with self-reported history of arterial CVD or cancer at baseline. Further, in a mediation analysis, we found that PAI-1 explained approximately 15% of the VTE risk in obesity. Even though the mediating effect was relatively modest, our results suggest that PAI-1 could play a role in the underlying biology of VTE risk in obesity.

To the best of our knowledge, this is the first study derived from a general population to show that elevated levels of plasma PAI-1 are associated with risk of future VTE. Several studies, often with limited sample sizes, have provided inconclusive results on the association between PAI-1 and VTE over the last decades.[18–20,23] It is worth noting that the findings from two large population-based studies were also conflicting.[21,22] In the Longitudinal Investigation of Thromboembolism Etiology (LITE) study, a nested case-control study comprising 308 VTE patients and 640 controls, Folsom et al. found no association between PAI-1 antigen levels and risk of future VTE,[21] contrasting our findings. However, the cohorts that served as the source population for the LITE study were composed of middle-aged and old individuals at baseline,[21] which could limit the comparability to our study. In the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a case control-study involving 770 VTE patients and 743

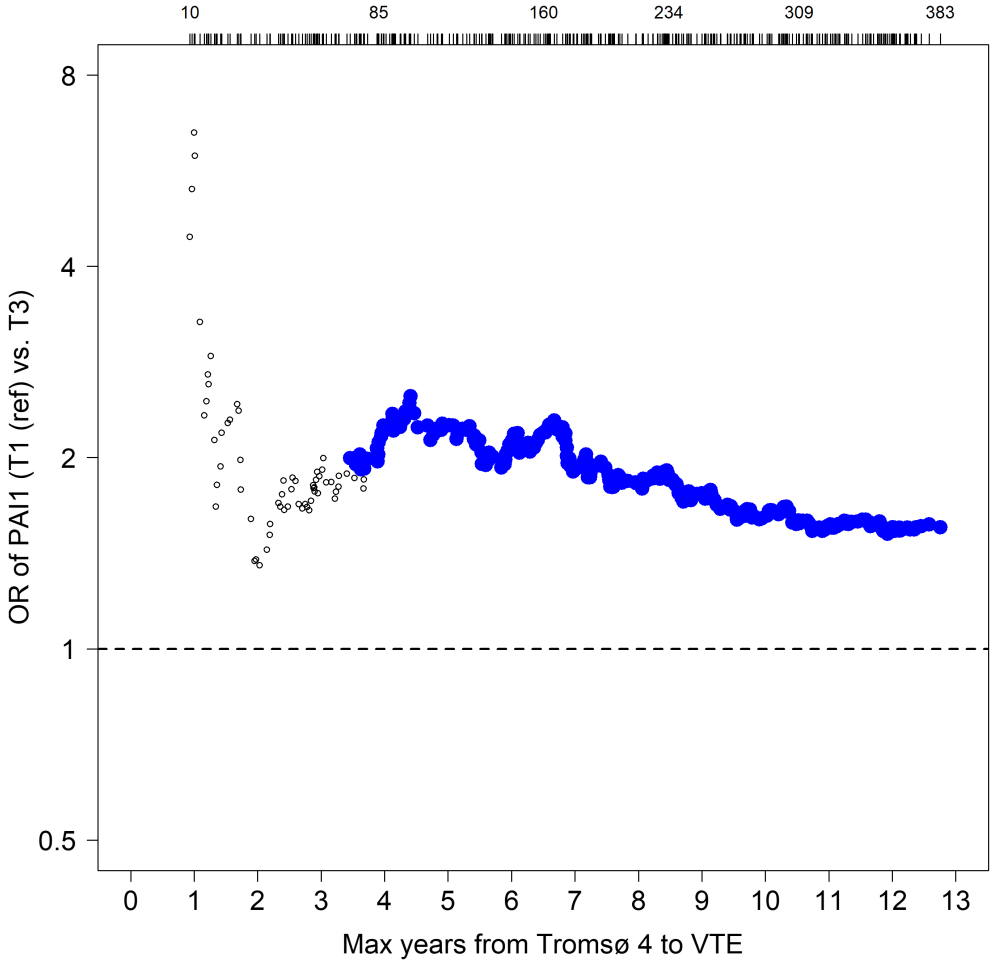

FIGURE 2 Plot of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time from blood sampling in Tromsø 4 (1994–1995) to VTE events. Participants with plasma levels of plasminogen activator inhibitor 1 (PAI-1) in the highest tertile (T3) were compared to those with levels in the lowest tertile (T1, reference category). Circles indicate ORs adjusted for age, sex, body mass index, and C-reactive protein. Solid blue circles indicate ORs with *P* values <.05. The number of VTE events are described above the plot

TABLE 4 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism according to clinical categories of body mass index (BMI)

Categories of BMI	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
<25 kg/m^2	326	129	1 (reference)	1 (reference)
25 – 30 kg/m^2	344	171	1.25 (0.95–1.65)	1.19 (0.90–1.57)
≥30 kg/m^2	110	82	1.92 (1.34–2.75)	1.73 (1.20–2.49)
p for trend			.001	.005

Note: Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, and plasminogen activator inhibitor-1. Note that two controls and one case had missing value on BMI.

controls, Meltzer et al. showed that high plasma levels of PAI-1 were associated with increased risk of VTE.[22] The association remained, albeit attenuated, after additional adjustment for BMI and markers of inflammation, which is in line with our results. Similar to VTE, PAI-1 has been extensively studied in relation to arterial CVD risk, with controversial findings.[34] However, two recently published meta-analyses point toward an association between elevated PAI-1 and arterial CVD.[35,36] Further, data from some, but not all, Mendelian randomization studies suggest a causal role of PAI-1 in arterial CVD.[35,37,38]

Here, we found that PAI-1 was not only associated with increased risk of VTE but also had the potential to mediate the thrombosis risk in obesity. Adipose tissue is likely a relevant contributor to elevated circulating levels of PAI-1 in obese subjects,[11] and visceral adipose tissue appears to be the main source of PAI-1 production according to some *in vitro* findings.[39–41] Consistent with this observation, imaging studies have shown that PAI-1 levels correlate more strongly with visceral adipose tissue than with subcutaneous adipose tissue.[42,43] In light of the close link between adipose tissue and PAI-1, we sought to investigate PAI-1 as a mediator of the VTE risk in

obese subjects. To date, only a few studies have pursued the identification of explanatory factors for the association between obesity and VTE using mediation analyses. For instance, in the Tromsø and REGARDS cohorts, CRP, a marker of inflammation, mediated approximately 15% to 20% of the VTE risk in obesity.[30,31] In a previous nested case-control study, we investigated the association between leptin, an adipocyte-derived hormone that is commonly elevated in obesity, and risk of future VTE.[44] Although leptin has been reported to upregulate the expression of tissue factor and PAI-1 *in vitro*,[45,46] our results suggest that leptin is not relevant for mediating the VTE risk among obese individuals.[44]

The present study supports the hypothesis that PAI-1 is in the causal path between obesity and VTE. PAI-1 mediated almost 15% of the VTE risk in obesity, which was largely independent of chronic low-grade inflammation, as reflected by CRP levels. Although the mediating effect was somehow modest, PAI-1 could be a potential target for intervention to decrease the risk of VTE in obese subjects. The relevance of PAI-1 in the pathogenesis of venous thrombosis is highlighted in a murine model of DVT, in which increasing expression levels of PAI-1 were associated with larger thrombus size and impaired thrombus resolution.[47]

To successfully target PAI-1, it is important to carefully consider its complex regulation. In a recent study, employing a targeted exome array in a cohort of 15,603 individuals with European ancestry, no genetic variant was significantly associated with PAI-1 plasma levels.[48] Further, a large genome-wide association study (GWAS) found that single nucleotide polymorphisms (SNPs) accounted for only 1% to 4% of the total variation in PAI-1 levels.[37] These results shed more light on the biology of PAI-1, suggesting that environmental factors might have a predominant role in the regulation of PAI-1. Indeed, some cardiometabolic factors associated with obesity have been reported to induce PAI-1 expression levels, such as angiotensin II, glucose, and insulin.[24] In interventional studies, treatment with angiotensin-converting enzyme inhibitors or with oral hypoglycemic medications was associated with decreased PAI-1 levels.[24,49–52] Notably, many efforts have been devoted to develop drugs that can modulate PAI-1 activity, the so-called PAI-1 inhibitors.[53] Several of these inhibitors, including low molecular weight molecules, peptides, monoclonal antibodies, and antibody fragments, have been extensively characterized in experimental studies.[24,53] Although some PAI-1 inhibitors are currently being investigated in clinical trials (for conditions other than obesity), none of them has been approved for clinical use to date to the best of our knowledge.[24,53]In view of the novel insights into PAI-1 biology, it may be speculated that targeting PAI-1 with inhibitors or indirectly via regulatory pathways (e.g., by the use of angiotensin-converting enzyme inhibitors or oral hypoglycemic agents) could emerge as promising therapeutic options to reduce the VTE risk in obesity.

The strengths of this 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 in which blood sampling took place before the VTE event, allowing assumptions on the direction of the association between exposure (PAI-1) and outcome (VTE). Another strength was the subgroup analyses according to the presence of provoking factors (i.e., provoked and unprovoked VTE events) and VTE location (i.e., DVT and PE). Some limitations merit attention. Although the number of plasma samples not available or of inadequate quality for the assessment of PAI-1 antigen level was somewhat high, missing data on PAI-1 was not related to the VTE status, occurring in 17% of the VTE cases and 15% of the controls (see Figure 1). Additionally, baseline characteristics of the study participants with and without measurement of PAI-1 were similar (data not shown). Thus, the missing data on PAI-1 was presumably completely at random. Plasma samples were frozen and stored at −80°C for up to 22 years, and the long storage time could have influenced plasma levels of PAI-1. However, because blood samples were stored in the same conditions in cases and controls, any potential misclassification would be non-differential with regard to VTE status, which could have led to an underestimation of the true associations. Moreover, intra-individual variation in PAI-1 levels during the long follow-up period could have contributed to attenuation of the true association.[32] This phenomenon is likely, because ORs for VTE by increased levels of PAI-1 were higher with shortened time between blood sampling and VTE events. However, it is important to address that ORs remained significant over time, reinforcing the association of PAI-1 with VTE. PAI-1 levels display a high biological variability, particularly between individuals,[54,55] and follow a circadian variation with a peak observed in the early morning.[56] In our study, blood samples were collected throughout the day, but 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 PAI-1 measurement would be nondifferential in relation to VTE status. Finally, measuring PAI-1 activity could have yielded additional information on the antifibrinolytic potential of the study population. Nevertheless, PAI-1 antigen and activity were reported to have a moderate to strong correlation,[18,23] and PAI-1 antigen was able to substantially explain the overall plasma fibrinolytic capacity measured as clot lysis time among healthy individuals.[22,57]

In conclusion, our results indicate that elevated plasma PAI-1 levels are associated with risk of future incident VTE. Further, PAI-1 mediated almost 15% of the VTE risk in obesity, suggesting that PAI-1 may play a role in the biology of VTE in obese subjects.

ACKNOWLEDGMENTS

The Thrombosis Research Center has received an independent grant from Stiftelsen Kristian Gerhard Jebsen (2014-2020). T. Frischmuth is supported by the Northern Norway Regional Health Authority.

CONFLICTS OF INTEREST

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 and 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.

ORCID

Tobias Frischmuth https://orcid.org/0000-0003-3203-3686
Thor Ueland https://orcid.org/0000-0001-5005-0784
Sigrid K. Brækkan https://orcid.org/0000-0002-9678-9696
Vânia M. Morelli https://orcid.org/0000-0002-0872-6645

REFERENCES

1. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J Thromb Haemost*. 2007;5:692-699.
2. World Health Organization (WHO). Fact sheet: obesity and overweight. 2018. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Accessed February 24, 2020.
3. Braekkan SK, Siegerink B, Lijfering WM, Hansen JB, Cannegieter SC, Rosendaal FR. Role of obesity in the etiology of deep vein thrombosis and pulmonary embolism: current epidemiological insights. *Semin Thromb Hemost*. 2013;39:533-540.
4. Rahmani J, Haghighian Roudsari A, Bawadi H, et al. Relationship between body mass index, risk of venous thromboembolism and pulmonary embolism: a systematic review and dose-response meta-analysis of cohort studies among four million participants. *Thromb Res*. 2020;192:64-72.
5. Heit JA, Ashrani A, Crusan DJ, McBane RD, Petterson TM, Bailey KR. Reasons for the persistent incidence of venous thromboembolism. *Thromb Haemost*. 2017;117:390-400.
6. Klovaite J, Benn M, Nordestgaard BG. Obesity as a causal risk factor for deep venous thrombosis: a Mendelian randomization study. *J Intern Med*. 2015;277:573-584.
7. Klarin D, Emdin CA, Natarajan P, Conrad MF, Invent Consortium, Kathiresan S. Genetic analysis of venous thromboembolism in UK biobank identifies the ZFPM2 locus and implicates obesity as a causal risk factor. *Circ Cardiovasc Genet*. 2017;10:e001643.
8. Lindstrom S, Wang L, Smith EN, et al. Genomic and transcriptomic association studies identify 16 novel susceptibility loci for venous thromboembolism. *Blood*. 2019;134:1645-1657.
9. Larsson SC, Back M, Rees JMB, Mason AM, Burgess S. Body mass index and body composition in relation to 14 cardiovascular conditions in UK Biobank: a Mendelian randomization study. *Eur Heart J*. 2020;41:221-226.
10. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. *Obes Rev*. 2002;3:85-101.
11. Skurk T, Hauner H. Obesity and impaired fibrinolysis: role of adipose production of plasminogen activator inhibitor-1. *Int J Obes Relat Metab Disord*. 2004;28:1357-1364.
12. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011;11:85-97.
13. Urano T, Suzuki Y, Iwaki T, Sano H, Honkura N, Castellino FJ. Recognition of plasminogen activator inhibitor type 1 as the primary regulator of fibrinolysis. *Curr Drug Targets*. 2019;20:1695-1701.
14. Samad F, Yamamoto K, Loskutoff DJ. Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-alpha and lipopolysaccharide. *J Clin Invest*. 1996;97:37-46.
15. Bastelica D, Morange P, Berthet B, et al. Stromal cells are the main plasminogen activator inhibitor-1-producing cells in human fat: evidence of differences between visceral and subcutaneous deposits. *Arterioscler Thromb Vasc Biol*. 2002;22:173-178.
16. Dellas C, Loskutoff DJ. Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thromb Haemost*. 2005;93:631-640.
17. Brogren H, Sihlbom C, Wallmark K, et al. Heterogeneous glycosylation patterns of human PAI-1 may reveal its cellular origin. *Thromb Res*. 2008;122:271-281.
18. Eriksson BI, Eriksson E, Risberg B. Impaired fibrinolysis and postoperative thromboembolism in orthopedic patients. *Thromb Res*. 1991;62:55-64.
19. Ridker PM, Vaughan DE, Stampfer MJ, et al. Baseline fibrinolytic state and the risk of future venous thrombosis. A prospective study of endogenous tissue-type plasminogen activator and plasminogen activator inhibitor. *Circulation*. 1992;85:1822-1827.
20. Bombeli T, Jutzi M, De Conno E, Seifert B, Fehr J. In patients with deep-vein thrombosis elevated levels of factor VIII correlate only with von Willebrand factor but not other endothelial cell-derived coagulation and fibrinolysis proteins. *Blood Coagul Fibrinolysis*. 2002;13:577-581.
21. Folsom AR, Cushman M, Heckbert SR, Rosamond WD, Aleksic N. Prospective study of fibrinolytic markers and venous thromboembolism. *J Clin Epidemiol*. 2003;56:598-603.
22. Meltzer ME, Lisman T, de Groot PG, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood*. 2010;116:113-121.
23. Bollen L, Peetermans M, Peeters M, et al. Active PAI-1 as marker for venous thromboembolism: case-control study using a comprehensive panel of PAI-1 and TAFI assays. *Thromb Res*. 2014;134:1097-1102.
24. Morrow GB, Whyte CS, Mutch NJ. A serpin with a finger in many PAIs: PAI-1's central function in thromboinflammation and cardiovascular disease. *Front Cardiovasc Med*. 2021;8:653655.
25. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso study. *Int J Epidemiol*. 2012;41:961-967.
26. Braekkan SK, Borch KH, Mathiesen EB, Njolstad I, Wilsgaard T, Hansen JB. Body height and risk of venous thromboembolism: the Tromso study. *Am J Epidemiol*. 2010;171:1109-1115.
27. Hoiland II, Liang RA, Braekkan SK, et al. Complement activation assessed by the plasma terminal complement complex and future risk of venous thromboembolism. *J Thromb Haemost*. 2019;17:934-943.
28. Liang RA, Hoiland II, Ueland T, et al. Plasma levels of mannose-binding lectin and future risk of venous thromboembolism. *J Thromb Haemost*. 2019;17:1661-1669.
29. Pearce N. Analysis of matched case-control studies. *BMJ*. 2016;352:i969.
30. Olson NC, Cushman M, Lutsey PL, et al. Inflammation markers and incident venous thromboembolism: the REasons for geographic and racial differences in stroke (REGARDS) cohort. *J Thromb Haemost*. 2014;12:1993-2001.
31. Horvei LD, Grimnes G, Hindberg K, et al. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. *J Thromb Haemost*. 2016;14:1561-1571.

32. Clarke R, Shipley M, Lewington S, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol*. 1999;150:341-353.
33. Breen R, Karlson KB, Holm A. Total, direct, and indirect effects in logit and probit models. *Sociol Methods Res*. 2013;42:164-191.
34. Pavlov M, Celap I. Plasminogen activator inhibitor 1 in acute coronary syndromes. *Clin Chim Acta*. 2019;491:52-58.
35. Song C, Burgess S, Eicher JD, O'Donnell CJ, Johnson AD. Causal effect of plasminogen activator inhibitor type 1 on coronary heart disease. *J Am Heart Assoc*. 2017;6:e004918.
36. Jung RG, Motazedian P, Ramirez FD, et al. Association between plasminogen activator inhibitor-1 and cardiovascular events: a systematic review and meta-analysis. *Thromb J*. 2018;16:12.
37. Huang J, Sabater-Lleal M, Asselbergs FW, et al. Genome-wide association study for circulating levels of PAI-1 provides novel insights into its regulation. *Blood*. 2012;120:4873-4881.
38. Zhao JV, Schooling CM. Coagulation factors and the risk of ischemic heart disease: a Mendelian randomization study. *Circ Genom Precis Med*. 2018;11:e001956.
39. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes*. 1997;46:860-867.
40. Gottschling-Zeller H, Birgel M, Rohrig K, Hauner H. Effect of tumor necrosis factor alpha and transforming growth factor beta 1 on plasminogen activator inhibitor-1 secretion from subcutaneous and omental human fat cells in suspension culture. *Metabolism*. 2000;49:666-671.
41. Barnard SA, Pieters M, De Lange Z. The contribution of different adipose tissue depots to plasma plasminogen activator inhibitor-1 (PAI-1) levels. *Blood Rev*. 2016;30:421-429.
42. Shimomura I, Funahashi T, Takahashi M, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med*. 1996;2:800-803.
43. Mertens I, Van der Planken M, Corthouts B, et al. Visceral fat is a determinant of PAI-1 activity in diabetic and non-diabetic overweight and obese women. *Horm Metab Res*. 2001;33:602-607.
44. Frischmuth T, Hindberg K, Aukrust P, et al. Plasma levels of leptin and risk of future incident venous thromboembolism. *Thromb Haemost*. 2021. doi:10.1055/s-0041-1732295
45. Napoleone E, Di santo A, Amore C, et al. Leptin induces tissue factor expression in human peripheral blood mononuclear cells: a possible link between obesity and cardiovascular risk? *J Thromb Haemost*. 2007;5:1462-1468.
46. Singh P, Peterson TE, Barber KR, et al. Leptin upregulates the expression of plasminogen activator inhibitor-1 in human vascular endothelial cells. *Biochem Biophys Res Commun*. 2010;392:47-52.
47. Klarin D, Busenkell E, Judy R, et al. Genome-wide association analysis of venous thromboembolism identifies new risk loci and genetic overlap with arterial vascular disease. *Nat Genet*. 2019;51:1574-1579.
48. Thibord F, Song C, Pattee J, et al. FGL1 as a modulator of plasma D-dimer levels: exome-wide marker analysis of plasma tPA, PAI-1, and D-dimer. *J Thromb Haemost*. 2021;19:2019-2028.
49. Brown NJ, Kumar S, Painter CA, Vaughan DE. ACE inhibition versus angiotensin type 1 receptor antagonism: differential effects on PAI-1 over time. *Hypertension*. 2002;40:859-865.
50. Cefalu WT, Schneider DJ, Carlson HE, et al. Effect of combination glipizide GITS/metformin on fibrinolytic and metabolic parameters in poorly controlled type 2 diabetic subjects. *Diabetes Care*. 2002;25:2123-2128.
51. Fonseca VA, Theuma P, Mudaliar S, Leissinger CA, Clejan S, Henry RR. Diabetes treatments have differential effects on non-traditional cardiovascular risk factors. *J Diabetes Complications*. 2006;20:14-20.
52. Fogari R, Zoppi A, Mugellini A, Maffioli P, Lazzari P, Derosa G. Role of angiotensin II in plasma PAI-1 changes induced by imidapril or candesartan in hypertensive patients with metabolic syndrome. *Hypertens Res*. 2011;34:1321-1326.
53. Sillen M, Declerck PJ. Targeting PAI-1 in cardiovascular disease: structural insights into PAI-1 functionality and inhibition. *Front Cardiovasc Med*. 2020;7:622473.
54. Banfi G, Del Fabbro M. Biological variation in tests of hemostasis. *Semin Thromb Hemost*. 2009;35:119-126.
55. Jung RG, Simard T, Di Santo P, et al. Performance of plasminogen activator inhibitor-1 as a biomarker in patients undergoing coronary angiography: analytical and biological considerations. *Diab Vasc Dis Res*. 2019;16:478-482.
56. Sakata K, Hoshino T, Yoshida H, et al. Circadian fluctuations of tissue plasminogen activator antigen and plasminogen activator inhibitor-1 antigens in vasospastic angina. *Am Heart J*. 1992;124:854-860.
57. Siudut J, Iwaniec T, Plens K, Pieters M, Undas A. Determinants of plasma fibrin clot lysis measured using three different assays in healthy subjects. *Thromb Res*. 2021;197:1-7.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Frischmuth T, Hindberg K, Aukrust P, et al. Elevated plasma levels of plasminogen activator inhibitor-1 are associated with risk of future incident venous thromboembolism. *J Thromb Haemost*. 2022;20:1618–1626. doi:10.1111/jth.15701