UiT The Arctic University of Norway

Faculty of Health Sciences

Department of Clinical Medicine

Obesity-related venous thromboembolism

Tobias Frischmuth

A dissertation for the degree of Philosophiae Doctor (PhD) December 2022

Table of Contents

Acknowledgments

The work presented in this thesis was carried out at the K.G. Jebsen Thrombosis Research and Expertise Center (TREC), later named Thrombosis Research Center (TREC) at the department of Clinical Medicine, UiT – The Arctic University of Norway between February 2020 and December 2022. I was enrolled as full time PhD student at the UiT and funded by the Northern Norway Regional Heath Authorities.

First and foremost, I would like to thank my main supervisor Vania Maris Morelli. You introduced me to epidemiology when I first came to TREC and you taught me how to work systematically and to think methodologically. I appreciate all the help and guidance you provided through the course of my PhD. I am especially impressed by your focus on the details and how thorough you work, always striving to improve each and every aspect of a research project.

I am deeply grateful to my co-supervisors Professor John-Bjarne Hansen and Professor Sigrid K. Brækkan. You have built an excellent research group here in Tromsø consisting of people with different backgrounds who have complementary skills. I am astonished by the endless well-designed research projects concerning VTE and the continuous flow of new ideas. Whenever I was in doubt how to go on further with one of my projects, I could rely on you to bring new ideas into the projects and to guide me in the right direction.

Further, I would like to extend my sincere thanks to my co-authors Kristian Hindberg, Pål Aukrust, Thor Ueland, Maiken E. Gabrielsen, Ben Brumpton, Kristian Hveem and Birgitte G. Tøndel for your contributions. I would like to thank especially Kristian Hindberg for your support with statistical software in the beginning of my time as a PhD student, which helped me greatly in all my projects. In addition, I would also like to express my gratitude to all past and present TREC-members creating a positive and stimulating work environment making my stay in Tromsø and TREC a pleasant experience.

Finally, I would like to thank my family for your support that encouraged me along the way. I would especially like to thank my wife Luam, for her unwavering support and belief in me.

Tromsø, December 2022

Summary

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and multicausal disease. Obesity is a major and likely causal risk factor for VTE. However, to what extent obesity contributes to VTE risk in the general population and the mechanism of obesity-related VTE remain poorly understood. The overall aim of this thesis was (i) to determine the risk of VTE attributed to obesity at population level and (ii) to reveal biomarkers of obesity-related VTE.

The study population in all papers was recruited from the 4th-7th surveys of the Tromsø Study (enrolment: 1994-2016), a population-based cohort study. Paper II also included participants from the Trøndelag Health Study (HUNT 2). Exposure information was obtained at survey inclusion through self-administered questionnaires, physical examination, and blood samples. Incident VTE events during follow-up were registered and objectively validated.

In paper I, to assess the VTE risk attributed to overweight and obesity, we calculated the population attributable fraction (PAF) using a cohort design and repeated measurements of body mass index (BMI). The PAF of incident VTE due to overweight (BMI 25-30 kg/m^2) and obesity (BMI ≥30 kg/m^2) was 24.6% (12.9% was attributed to overweight and 11.7% to obesity). In paper II, the joint effect of obesity and established prothrombotic genotypes (rs8176719 in *ABO*, rs6025 in *F5*, rs1799963 in *F2*, rs2066865 in *FGG*, and rs2036914 in *F11*) on VTE risk was investigated. Using a case-cohort design, it was observed that the combination of obesity and prothrombotic genotypes, assessed either individually or as a genetic risk score, had an additive effect on VTE risk (i.e., no biological interaction). However, the combination of obesity and some prothrombotic genotypes appeared to have a supra-additive effect on the risk of DVT and unprovoked VTE. In papers III and IV, a nested case-control design was used to investigate whether plasma leptin and plasminogen activator inhibitor-1 (PAI-1) were associated with VTE risk and their potential to mediate the VTE risk in obesity. The VTE risk increased with increasing levels of leptin, and particularly of PAI-1. Additional adjustment for BMI markedly attenuated risk estimates for leptin, while PAI-1 remained associated with VTE. In a mediation analysis, PAI-1 mediated almost 15% of the VTE risk in obesity, while no apparent mediation was observed for leptin.

In conclusion, the main findings of this thesis indicate that obesity is a major risk factor for VTE at population level and that PAI-1 levels mediate part of the VTE risk in obesity.

Sammendrag

Venøs tromboembolisme (VTE) omfatter dyp venetrombose (DVT) og lungeemboli (LE) og er en vanlig og multifaktoriell sykdom. Fedme er en viktig og sannsynligvis kausal risikofaktor for VTE. Til tross for dette er mekanismen bak fedme-relatert VTE lite kjent. Det overordnede formålet for denne avhandlingen var (i) å beregne hvor stor andel av VTE-tilfellene som kan tilskrives fedme i den generelle befolkningen og (ii) å finne biomarkører for fedmerelatert VTE.

Studiepopulasjonen i alle fire publikasjonene ble rekruttert fra Tromsøundersøkelsen (Tromsø 4-7, 1994-2016) som er en populasjonsbasert kohortstudie. Artikkel II inkluderte i tillegg deltakere fra Helseundersøkelsen i Trøndelag (HUNT 2). Informasjon om eksposisjon ble samlet inn ved deltakelse i helseundersøkelsene gjennom spørreskjemaer, fysisk undersøkelse og blodprøver. Alle førstegangs VTE tilfeller i løpet av oppfølgingsperioden ble registrert og objektivt validert.

Artikkel I undersøkte hvor stor andel av VTE-tilfellene i den generelle befolkningen som kan tilskrives overvekt og fedme. En kohortstudie med gjentatte målinger av kroppsmasseindeks (KMI) ble benyttet, og tilskrivbar risiko i befolkningen (PAF) ble beregnet. Total PAF for førstegangs VTE som skyldtes overvekt og fedme var 24,6%, hvorav 12,9% kunne tilskrives overvekt og 11,7% kunne tilskrives fedme. I artikkel II ble kombinasjonen av fedme og flere velkjente protrombotiske genotyper (rs8176719 i *ABO*, rs6025 i *F5*, rs1799963 i *F2*, rs2066865 i *FGG*, and rs2036914 i *F11*) for VTE risiko undersøkt i en kasus-kohortstudie. Vi fant ingen synergistisk effekt mellom fedme og de ulike genotypene, hverken enkeltvis eller kombinert i en genetisk risikoskår. Kombinasjonen av fedme og noen av de protrombotiske genotypene var imidlertid forbundet med forhøyet risiko for DVT og uprovosert VTE, noe som muligens kan skyldes synergi. I artikkel III og IV brukte vi en kasus-kontroll studie basert på Tromsøundersøkelsen til å undersøke om konsentrasjonen av leptin og PAI-1 i blodet var assosiert med økt risiko for VTE. Videre ble det undersøkt om disse biomarkørene kunne forklare den økte VTE risikoen forbundet med fedme. Risikoen for VTE økte med økende leptin og PAI-1 verdier, men etter justering for BMI var det bare PAI-1 som fortsatt var assosiert med VTE risiko. I en medieringsanalyse fant vi at PAI-1 kunne forklare cirka 15% av VTE risikoen ved fedme.

Kort oppsummert er fedme en svært viktig risiko faktor for VTE i den generelle befolkningen, og PAI-1 ser ut til å forklare en viss andel av VTE-risikoen relatert til fedme.

List of Papers

I. The risk of incident venous thromboembolism attributed to overweight and obesity: The Tromsø Study
Frischmuth T, Birgitte G. Tøndel, Brækkan SK, Hansen JB, and Morelli VM
Submitted

II. Joint Effect of Multiple Prothrombotic Genotypes and Obesity on the Risk of Incident Venous Thromboembolism
Frischmuth T, Hindberg K, Gabrielsen ME, Brumpton B, Hveem K, Brækkan SK, Hansen JB, Morelli VM
Thromb Haemost. 2022; 122(02): 267-276.

III. Plasma Levels of Leptin and Risk of Future Incident Venous Thromboembolism
Frischmuth T, Hindberg K, Aukrust P, Ueland T, Brækkan SK, Hansen JB, Morelli VM
Thromb Haemost. 2022; 122(04): 560-569.

IV. Elevated plasma levels of plasminogen activator inhibitor-1 are associated with risk of future incident venous thromboembolism
Frischmuth T, Hindberg K, Aukrust P, Ueland T, Brækkan SK, Hansen JB, Morelli VM
J Thromb Haemost. 2022; 20: 1618– 1626.

Abbreviations

AP	Attributable proportion
APC	Activated protein C
AT	Antithrombin
BMI	Body mass index
CFD	Complement factor D
CI	Confidence interval
CRP	C-reactive protein
CTPA	Computer tomographic pulmonary angiography
CVD	Cardiovascular disease
DVT	Deep vein thrombosis
EIA	Enzyme-immunoassay
EV	Extracellular vesicle
FGG	Fibrinogen gamma gene
FVL	Factor V Leiden
GRS	Genetic risk score
GWAS	Genome-wide association studies
HC	Hip circumference
HR	Hazard ratio
HRT	Hormone replacement
HUNT	Trøndelag Health Study
ICD	International Classification of Diseases
IL-6	Interleukin-6
KHB	Karlson, Holm and Breen
LETS	Leiden Thrombophilia Study
LITE	Longitudinal Investigation of Thromboembolism Etiology
MAR	Missing at random
MC	Monocytes
MCAR	Missing completely at random
MEGA	Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis
MI	Myocardial infarct
MNAR	Missing not at random

MR	Mendelian randomization
OC	Oral contraceptive
OR	Odds ratio
PAF	Population attributable fraction
PAI-1	Plasminogen activator inhibitor 1
PE	Pulmonary embolism
Plt	Platelets
PPL	Procoagulant phospholipid
PTS	Post thrombotic syndrome
RCT	Randomized controlled trial
REGARDS	Reasons for Geographic And Racial Differences in Stroke
RERI	Relative excess risk attributable to interaction
SI	Synergy index
SNP	Single nucleotide polymorphism
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator;
UNN	University Hospital of North Norway
VTE	Venous thromboembolism
VWF	Von Willebrand factor.
WC	Waist circumference
WHO	World Health Organization

1 Introduction

Venous thromboembolism (VTE), an umbrella term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a disease that primarily results from a disturbed regulation of hemostasis. DVT is the formation of blood clots in the deep veins, which generally occurs in the deep veins of the lower extremities. More rarely, DVT is observed in the deep veins of the upper extremities, the splanchnic veins, and the cerebral veins.[1] DVT classically manifests clinically as swelling, redness, and pain in the affected limb, caused by obstruction of venous return to the heart.[2] PE is traditionally regarded as a complication of DVT, where parts of the thrombus break away, travel via the venous system through the right side of the heart, and lodge in the pulmonary arterial circulation. However, in approximately 50% of PE cases, no apparent DVT can be detected.[3] It has therefore been suggested that emboli could originate in the right atrium as a consequence of atrial fibrillation,[4,5] or that thrombi could arise *de novo* in the pulmonary circulation.[3] Typical symptoms of PE include dyspnea, pleuritic chest pain, and hemoptysis, while severe cases may lead to circulatory collapse and sudden death.[6,7]

VTE is a common and multicausal disease usually occurring as the result of the interplay of genetic and acquired risk factors.[8] Several hereditary and acquired risk factors have been discovered in the past decades, and acquired risk facts may be divided into persistent or transient.[9] While VTE events occurring in the presence of a provoking risk factor, either of transient (e.g., surgery, trauma, and prolonged immobilization) or persistent (e.g., active cancer) nature, are classified as provoked VTEs, those events without an apparent risk factor are classified as unprovoked.[10] Acquired risk factors may also be acknowledged as non-modifiable (e.g., advancing age) and modifiable factors (e.g., obesity, immobilization, hormone therapy), where the latter may be subjected to change in order to reduce VTE risk.

Obesity is a major modifiable risk factor for VTE.[11] Indeed, Mendelian randomization (MR) studies showed that genetically elevated body mass index (BMI) is associated with an increased risk of VTE, supporting a causal link between obesity and VTE.[12-15] The prevalence of obesity has increased dramatically over the last decades and is expected to increase continuously in the coming years,[16-18] despite an increased focus on obesity as a major public health concern. As obesity is a major risk factor for VTE, this may partly explain the persistent or even slight increase in the incidence of VTE in the past decades.[19-21] However, the pathophysiological mechanisms underlying the association between obesity and VTE remain poorly understood.

The focus of this thesis is to provide updated estimates of the contribution of overweight and obesity to the incidence of VTE in the general population, to investigate biological interactions between established prothrombotic genotypes and obesity, and to reveal further insights into the pathophysiology of obesity-related VTE. Unraveling causal pathways may lead to the identification of novel biomarkers and potential preventive therapeutic targets for obesity-related VTE that could ultimately contribute to reduce the incidence of VTE in the general population.

1.1 Epidemiology of VTE

VTE is the third most common cardiovascular disease (CVD) after myocardial infarct (MI) and ischemic stroke.[22] The incidence rate of VTE in a population with European ancestry is 1-2 per 1,000 persons annually,[19,21,23,24] which corresponds to approximately 1.1 million affected individuals in Europe per year.[25] VTE is primarily a disease of older age and the incidence of VTE increases exponentially with age.[19,24,26-28] Whether a true sex difference with regards to VTE incidence exists is under debate, due to contrasting findings from observational studies.[28-33] It appears that women have higher incidence rates in the fertile age, while men have higher rates in the older age groups,[24] and when accounting for reproductive factors, men and women seem to have a similar life-time risk of VTE.[28]

The incidence of VTE has been stable or even slightly increased over the past decades, which was mainly due to an increase in PE rates.[19,21,29] Awareness among clinicians and improved diagnostic approaches, such as the use of computer tomographic pulmonary angiography (CTPA), have been considered as possible explanations for the increase in the incidence of PE.[19] It is worth noting that the overall trend for VTE incidence is in contrast to a declining trend for arterial CVD incidence in the same time period.[34-36] The decrease in incident arterial CVD can be mainly explained by the development of effective preventive strategies against major modifiable cardiovascular risk factors, such as smoking, hypertension, and hypercholesterolemia.[36] In contrast, major risk factors for VTE, including older age,[19] surgery,[20] cancer,[37] and obesity,[11,38] have increased concomitantly in the general population,[20] likely explaining, at least in part, the observed trends in the VTE incidence.

VTE is a potentially fatal disease and the 30-day all-cause mortality after a VTE event is between 6% and 11%, the 1-year all-cause mortality is approximately 21-24%,[23,39,40] which

translates into roughly 540,000 VTE-related deaths in Europe annually.[25] Furthermore, VTE is associated with serious short- and long-term complications, which represent a considerable socioeconomic burden not only related to treatment of the thrombotic event,[41,42] but also to the detrimental impact on quality of life[43,44] and increased risk of work-related disability.[45,46] Recurrence is a known complication of VTE and approximately 30% of patients will experience a recurrence within 10 years after a first event.[47-49] The risk of recurrence is highest within the first 6-12 months,[49] and recurrent events are more likely to affect the same location as the index event. Recurrence rates are higher in patients with initial unprovoked VTEs compared with provoked VTEs.[24,47] This is likely explained by the fact that individuals experiencing provoked VTEs associated with major transient risk factors (e.g., surgery) have a lower baseline risk of VTE and are consequently at lower risk of recurrence.[50] Other serious long-term complications of VTE include the post-thrombotic syndrome (PTS),[51,52] and the post-PE syndrome.[53,54] The PTS develops in approximately 50% of DVT patients within two years after the event and is characterized by swelling, pain, edema, venous ectasia, skin induration, and the development of venous leg ulcers in severe cases.[51,52,55] The post-PE syndrome is a relatively new concept and develops in approximately 50% of PE patients.[53] It may be defined as reduced cardiac function or pulmonary artery flow dynamics, and dyspnea, exercise intolerance, or lowered quality of life, with no alternative explanation.[54] The most severe presentation of the post-PE syndrome is the chronic thromboembolic pulmonary hypertension (CTEPH), which is characterized by hemodynamic compromise and persistent pulmonary perfusion defects. CTEPH is found in 0.6-3% of PE patients and presents commonly with rest dyspnea, hypoxemia, and right sided heart failure.[56]

1.1.1 Risk factors for VTE

In a broader definition, anything that may affect the incidence of disease occurrence is considered a risk factor.[57] VTE is regarded a multicausal disease where the interplay of several risk factors is required in order for an event to occur.[8] In the past decades an extensive list of hereditary and acquired risk factors for VTE has been established, and some of them are summarized in Table 1.

Table 1. Risk factors for venous thromboembolism.[57-59]

Acquired risk factors	Hereditary risk factors
• Old age • Obesity • Oral contraceptives and hormone replacement therapy • Pregnancy and post-partum • Cancer • Surgery • Trauma • Infection • Immobilization • Acute medical conditions* • Chronic inflammatory diseases	• Deficiency of antithrombin • Deficiency of protein C • Deficiency of protein S • Factor V Leiden • Prothrombin G2021A • Other prothrombotic genotypes in *ABO*, *FGG* and *F11*

FGG, fibrinogen gamma gene. *Acute stroke and myocardial infarction.

1.1.1.1 Hereditary risk factors

Family-based studies showed that VTE has a strong genetic component and approximately 50-60% of variance in VTE incidence can be attributed to genetics.[59-61] The well-known inherited thrombophilias can be divided into loss-of-function mutations in genes encoding natural anticoagulants and gain-of-function mutations in genes encoding coagulation factors. Deficiencies of natural anticoagulants (i.e., antithrombin [AT], protein C, and protein S) are rare in the general population (<1%) and associated with high relative risk of VTE.[62] The well-known gain of function thrombophilias are the common single nucleotide polymorphisms (SNPs) factor V Leiden (FVL) and prothrombin G2021A mutation. Heterozygous FVL and prothrombin mutation are seen in 5% and 1-2% of the general Caucasian population, respectively, and are associated with a moderate 2-3 fold increased relative risk of VTE.[59]

Technical advances, such as high-throughput microarray-based genotyping used in genome-wide association studies (GWAS), confirmed already known thrombophilias and led to the discovery of a large number of SNPs associated with the risk of VTE. According to the NHGRI-EBI Catalog of published GWAS (GWAS Catalog), the search term "venous thromboembolism" retrieves hundreds of SNPs associated with VTE.[63] It is important to address that the effect sizes for most individual SNPs identified in GWAS are small, or relatively modest (for instance, in case of the well-known SNPs FVL and prothrombin G2021A mutation).[64-68] In light of the discovery of new genetic variants, De Haan *et al*. developed a genetic risk score (GRS) comprising five SNPs (out of 31) that individually showed the strongest association with VTE.[69] This 5-SNP score included rs8176719 (non-O blood group) in *ABO*, rs6025 (FVL) in *F5*, rs1799963 (prothrombin G20210A) in *F2*, rs2066865 in the fibrinogen gamma gene (*FGG)*, and rs2036914 in *F11*, and identified subjects at increased risk of VTE similarly to the score including all 31 SNPs.[69] The five SNPs and their associated phenotypes and VTE risk estimates are displayed in Table 2.

Table 2. Established prothrombotic single nucleotide polymorphisms (SNPs) and odds ratios (ORs) for venous thromboembolism (VTE) derived from genome wide association studies.[13,70,71]

Gene	SNP	Associated phenotype	VTE ORs
F5	rs6025	APC resistance[72]	3.0-3.5
F2	rs1799963	↑ FII[73]	2.3-2.6
ABO	rs8176719	↑ VWF, ↑ FVIII[74]	1.5
F11	rs2036914	↑ FXI[75]	1.35
FGG	rs2066865	↓ Fibrinogen γ′[76]	1.2-1.5

Abbreviations: APC, activated protein C; VWF, von Willebrand factor

1.1.1.2 Acquired risk factors

Increasing age is one of the strongest risk factors for VTE. While VTE events in children and adolescence are extremely rare,[24,39,77,78] the incidence rate in the age group of 25-30 years is approximately 1 per 10,000 person-years.[39,78,79] An exponential increase is seen with increasing age, reaching an incidence rate of 8 per 1,000 person-years in the population over 85 years.[23,39,79,80] An explanation for the exponential rise is likely the concomitant increase of

traditional risk factors (e.g., immobility, malignancy, and co-morbidities), increased levels of coagulation factors,[80] as well as stasis and changes in the venous vasculature.[81,82]

Another major risk factor for VTE is cancer, which is associated with a 4-7-fold increased risk of VTE compared with the general population.[83-85] In population-based studies, approximately 20-30% of incident VTE events are cancer-related.[19,24] However, cancer is a heterogenous disease and the risk of VTE varies largely across cancer types, localization, stage, and cancer treatment.[83-85]

Major surgery,[86] major trauma,[87] immobilization,[88] and acute medical conditions (which include myocardial infarction,[89] ischemic stroke,[90] or infections) are highly relevant transient risk factors.[20,85,91] Hospitalization in connection with these risk factors accounts for more than 50% of VTE events.[92]

Pregnancy is associated with a 4-5 fold increased VTE risk, where a up to 20-fold increased risk is observed in the postpartum period, compared with non-pregnant women in the same age.[93,94] Additionally, exogenous hormone supplements, including combined oral contraceptives (OCs) and hormone replacement therapy (HRT), are associated with an 2-3 fold increased risk of VTE.[95,96]

Another major modifiable risk factor for VTE is obesity, which will be discussed in detail in the following sections, including its epidemiology and role as a risk factor for VTE, along with the potential mechanisms underlying the association between obesity and VTE.

1.2 Epidemiology of Obesity

Overweight and obesity are major public health concerns, often described as a "global epidemic". The World Health Organization (WHO) defines overweight and obesity as excessive fat accumulation that has the potential to cause health impairment.[18] The BMI, calculated as weight in kilograms divided by the square of height in meters (kg/m^2), is used to classify adults into overweight and obesity, defined as a BMI between 25 and 30 kg/m^2 and a BMI $\geq$ 30 kg/m^2, respectively.[18] BMI is a somewhat suboptimal measure of total body fat and has a poor sensitivity in assessing excess fatness on individual level,[97] because it does not take factors such as muscle mass or sex-dependent fat distribution into consideration.

Nevertheless, on a population level, BMI correlates well with body fat and its simplicity makes it the preferred anthropometric measure in epidemiological studies.[97]

The cause of obesity can be simplified as excess energy consumption in relation to energy expenditure (i.e., a positive caloric balance).[98] Frequent causes in high-income countries are related to a sedentary lifestyle, and highly processed, energy dense food.[98] However, the etiology of obesity is complex and includes genetic, physiological, environmental, psychological, social, and economic factors.[99]

The worldwide prevalence of overweight and obesity nearly tripled from 1975 to 2016 according to data from the WHO.[18] In 2016, the prevalence of overweight and obesity in adulthood was 39% and 13%, respectively.[18] However, the prevalence of obesity differs largely between countries and ranged from over 40% in high-income countries like the USA[100] to less than 5% in some low-income countries.[101] Currently, as much as two-thirds of the adult population is overweight or obese in high-income countries.[101]

Simulation studies estimated that the prevalence of obesity in the USA will increase continuously, reaching up to 50% in 2030.[16,17] If trends continue unchanged, it was estimated that the entire US population may be overweight or obese by 2048.[17] Based on survey data from Europe, the prevalence of obesity is also projected to increase in most European countries.[102] Additionally, the development of overweight and obesity in children (5-19 years) is concerning, as the prevalence increased from 4% in 1975 to 18% in 2018.[18] The dramatic increase in the prevalence of overweight and obesity in adults and children, initially seen in high-income countries, is currently also observed in low- and middle-income countries, particularly in urban settings.[18] Despite global public health efforts by the WHO supporting interventions to mitigate the increasing prevalence of overweight and obesity, the obesity epidemic seems far from being reversed. Potential explanations include the ineffectiveness of structured weight-loss programs in a long-term perspective and the relapsing nature of obesity.[103-106]

Obesity is a major contributor to mortality and morbidity as it is a major risk factor for a broad spectrum of noncommunicable diseases, including CVD, diabetes mellitus type 2, gastrointestinal disease, musculoskeletal disorders, and several types of cancers.[18] In fact, the majority of the world's population lives in countries where more deaths can be attributed to overweight and obesity than to underweight.[18,107]

1.2.1 Obesity as a risk factor for VTE

A large number of observational studies have investigated obesity as a risk factor for VTE over the past decades. Obesity is consistently associated with a two- to three-fold increased risk of VTE compared with normal weight,[11,108-110] and the risk increases linearly with an increasing BMI.[11,38,111] While all anthropometric measures of obesity, including waist circumference (WC) and hip circumference (HC), are associated with increased risk of VTE,[112-114] WC shows the strongest association and identifies most people at risk.[113]

In addition to the well-established association between obesity and VTE, weight gain over time, especially in the presence of obesity, is associated with increased VTE risk.[115,116] Further, MR studies show that genetically elevated BMI is associated with a higher risk of VTE, thereby strengthening the notion that obesity is causally related to VTE.[12-15] MR is an elegant method to make inference on causality, when clinical trials are impossible or unethical. Genetic variants robustly associated with the exposure under investigation are used as natural experiments resembling randomization in order to investigate causal relationships between modifiable exposures and diseases from observational data.[117]

Obesity, as a modifiable and likely causal risk factor for VTE, can be analyzed in the context of the thrombosis potential model conceived by Rosendaal, which illustrates the dynamics between risk factors leading to a thromboembolic event (Figure 1).[8]

The impact of obesity on incident VTE in the general population has been scarcely investigated thus far. In an American community-based cohort, 33% of unprovoked VTE were attributable to overweight and obesity.[20] However, estimates were based on data covering a time period from 1980-2012, and updated estimates are needed in light of the rising prevalence of overweight and obesity.

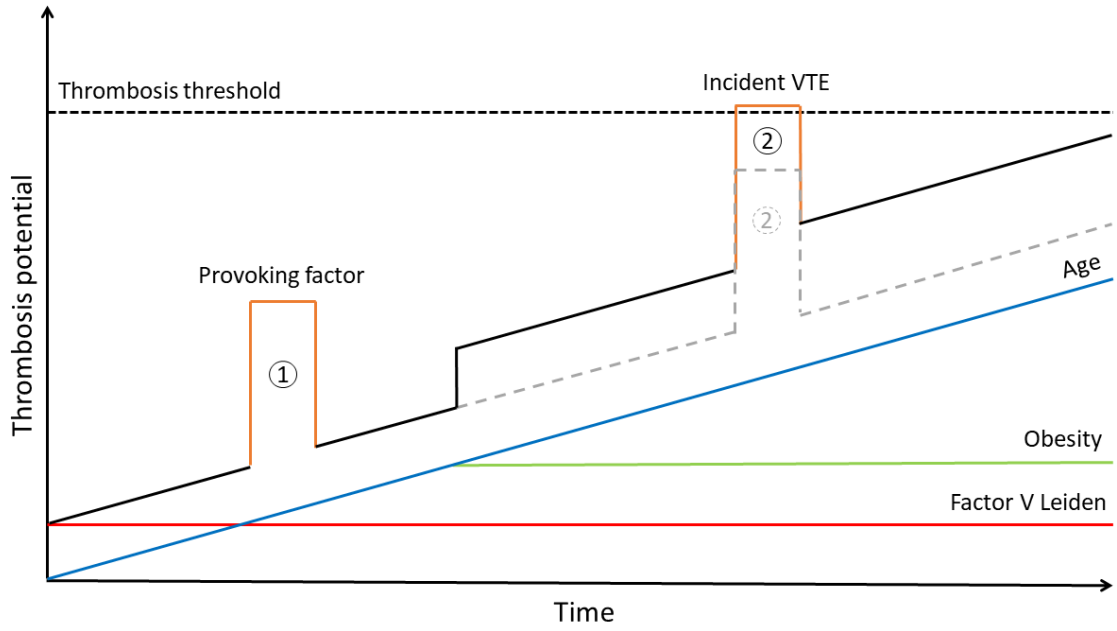

Figure 1. Thrombosis potential model. Factor V Leiden (red) and obesity (green) represent a hereditary and an acquired risk factor, respectively. Age (blue) shows the increasing risk of VTE with increasing age. The black line represents an individuals' thrombosis potential. The dotted black line represents the thrombosis potential threshold, which has to be reached in order for an event to occur. While provoking factor 1 does not lead to a VTE event, provoking factor 2 in the presence of obesity and an advancing age triggers an incident VTE. The gray dashed line represents the thrombosis potential in a theoretical case where the thrombosis risk attributed to obesity is removed. (Adapted from Rosendaal, Lancet, 1999)[8]

1.2.2 Interaction between obesity and other risk factors

Given the multicausal nature of VTE, including genetic and environmental risk factors, interactions between risk factors leading to a VTE event are likely to occur and clinically relevant. Biological interactions can be measured in epidemiological studies and in literature numerous terms like joint or combined effect, synergy, and effect modification are used.[118] Previous studies have investigated the interaction between obesity and both genetic and environmental risk factors.

In obese subjects, the presence of genetic variants associated with a prothrombotic state, such as FVL, prothrombin G20210A, and non-O blood group, has been suggested to synergistically increase the risk of VTE because of biological interaction.[109,119-121] More recently, a synergistic effect between obesity and a GRS based on 16 VTE-associated SNPs was reported.[122] Further, the combined effect of obesity and increasing body height showed a

synergistic effect on VTE risk.[123] In addition, obesity is associated with a broad spectrum of diseases, which themselves are risk factors for VTE, like arterial CVD and cancer.[15,83,124,125] Indeed, the combination of obesity and MI has been shown to have a supra-additive effect on VTE risk.[126] Interestingly, a synergism between obesity and OCs was observed, and obese women taking OCs had a 24-fold increased risk of VTE when compared with normal weight women not taking OCs.[109]

1.3 Pathophysiology of VTE

1.3.1 General VTE pathophysiology

In a simplified overview, a VTE develops as result of one or more of the following characteristics: (i) changes in blood flow (stasis), (ii) changes in blood composition (hypercoagulability), (iii) changes in the vessel wall.[127] These components are known as the Virchow's Triad, named after the German physician Rudolf Virchow, who described the components already in his works from 1856.[127] The Virchow's Triad is still used today to explain the basic concept that underlies venous thrombus formation (Figure 2), and VTE risk factors can be generally ascribed to one or more of these components. It is important to address that vessel wall injury, as initially suggested by Virchow, does not seem to play a role in venous thrombosis since thrombi are generally found in the presence of intact endothelium.[81] However, upon activation, the endothelium is converted from a surface with anticoagulant properties to one with procoagulant properties, and thereby become dysfunctional and favoring venous thrombosis.

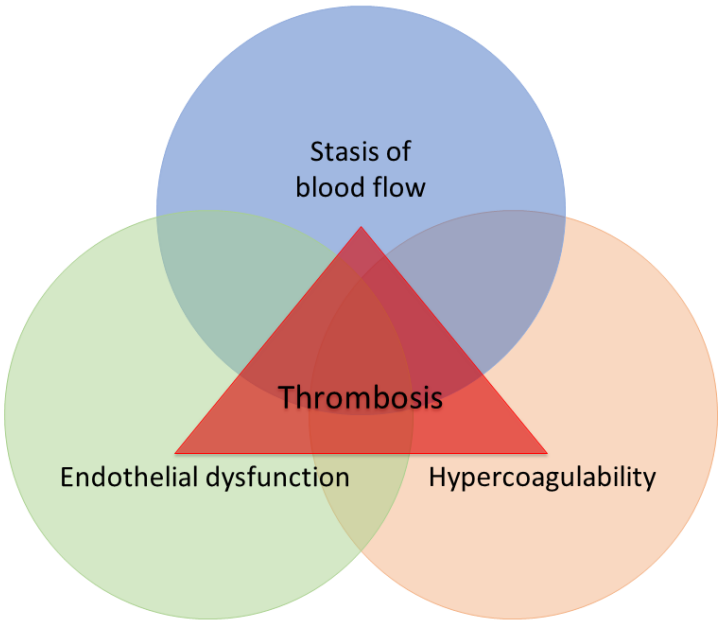

Figure 2. Virchow's Triad

Even though the understanding of VTE pathophysiology has advanced since the concept of Virchow's Triad, the exact mechanism of venous thrombus initiation remains largely unknown. Venous thrombi originate commonly in the pockets of venous valves of the deep leg veins,[2,81] as suggested by autopsy and venography studies.[128,129] The initial thrombus, rich in fibrin and erythrocytes, is formed adjacent to intact endothelial surface.[81] Experimental observations suggest a tissue factor (TF) dependent fibrin deposition as the primary trigger of thrombus formation,[130] whereas it was suggested by others that activation of the intrinsic coagulation system may be the primary trigger of thrombus formation.[131,132] In physiological conditions, laminar flow is observed in the lumen of veins, while reduced blood flow or stasis can be present in the pockets of venous valves. Two counterrotating vortices are observed in the deep recesses of valvular pockets (Figure 3),[133-135] where blood cells are trapped. Because of the vortical blood flow, cycling or intermittent hypoxia develops, which is a strong proinflammatory and procoagulant stimulus, activating endothelial cells, monocytes, and platelets.[81] Activated cells express TF and secrete extracellular vesicles (EVs) coated with TF bound to negatively charged phospholipids (PPLs). TF binds FVIIa, triggering the activation of coagulation and thrombin generation, which initiates thrombus formation.[81,136]

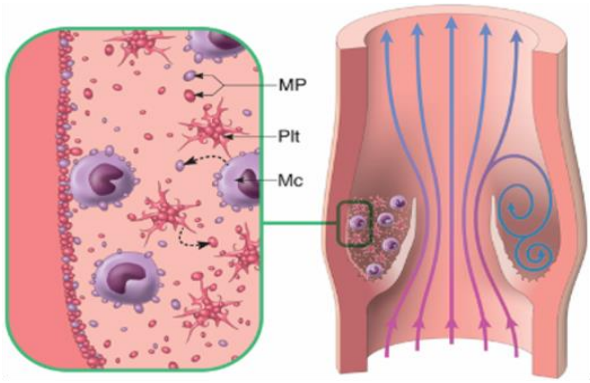

Figure 3. Proposed pathophysiology of venous thrombosis in venous valve pockets. Hypoxic conditions due to vortical blood flow in the valvular sinuses trigger the activation of endothelial cells. Leukocytes, such as monocytes (Mc), and platelets (Plt) are recruited. These cells shed extracellular vesicles that are tissue factor-positive. This process may lead to thrombus formation.

1.3.2 Pathophysiology of VTE in obesity

The underlining pathophysiological mechanism by which obesity increases the risk of VTE is poorly understood. However, the mechanism appears to be different from arterial thrombotic disease, as cardiometabolic risk factors associated with obesity, such as increased lipid levels, hypertension and impaired glucose tolerance or diabetes, were not associated with VTE risk in population-based cohort studies.[137-139] In the Tromsø Study, the association of obesity with MI was essentially explained by the aforementioned cardiometabolic risk factors, as

adjustments for these factors largely attenuated the association.[112] In contrast, the association between obesity and VTE remained unchanged after adjustment for these factors, indicating that the increased VTE risk observed in obese individuals is not mediated by cardiometabolic disorders induced by obesity.[112] Several pathways have been suggested to underlie the pathophysiology of VTE in obesity including venous stasis,[11,140] chronic low-grade inflammation and associated hypercoagulability,[141,142] and impaired fibrinolysis.[143,144]

A straightforward explanation for obesity-associated VTEs is venous stasis. Obese individuals tend to be more immobile, which could lead to stasis and thrombus formation.[11] Additionally, the accumulation of visceral adipose tissue has been shown to increase intra-abdominal pressure, which is transmitted to the lower extremities by the femoral veins.[145] Accordingly, altered venous blood flow parameters were detected in the lower extremities of obese individauls,[140] potentially causing venous stasis and VTE.

Stasis as a sole explanation for the increased risk of VTE in obesity seems unlikely, and other mechanisms related to the secretory activity of adipose tissue may contribute to thrombosis risk. While adipose tissue was traditionally viewed as an energy storage organ, it is now well-recognized that adipose tissue is an active endocrine organ, playing a key role in the integration of systemic metabolism.[142] Adipose tissue exerts its regulatory function by secreting various bioactive substances, collectively termed adipokines.[142,146] In fact, proteomic studies suggest that adipose tissue may secrete more than 600 different proteins.[146]

It is well-established that obesity is linked to a state of chronic low-grade inflammation.[142] With the development of obesity, the cellular composition of adipose tissue changes, including the number, phenotype, and localization of immune, vascular, and structural cells,[142] creating a local inflammatory environment with differential expression of adipokines, as illustrated in Figure 4.[142]

Adipose tissue, in particular visceral adipose tissue, expresses several pro-inflammatory cytokines such as Interleukin-6 (IL-6) and tumor necrosis factor (TNF).[142] Local proinflammatory stimuli elicit a systemic response, which may explain why obesity is associated with increased circulating levels of C-reactive protein (CRP),[147] a sensitive downstream marker of inflammation. In the Tromsø and the REasons for Geographic And

Racial Differences in Stroke (REGARDS) cohort studies, CRP, as a proxy for inflammation, mediated approximately 15%-20% of VTE risk in obesity.[148,149]

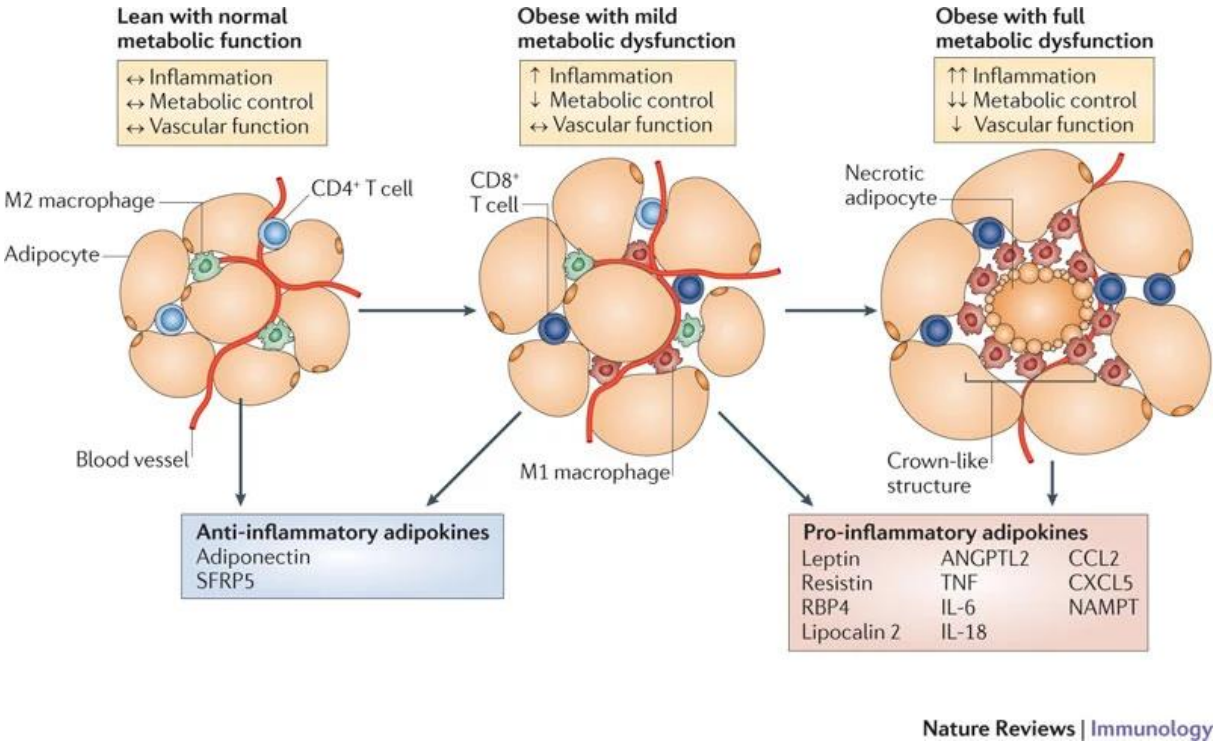

Figure 4. Obesity, adipose tissue, adipokines, and inflammation. (Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Immunology, Adipokines in inflammation and metabolic disease. Ouchi, N., Parker, J., Lugus, J. et al., Nat Rev Immunol 11, 85–97 (2011). https://doi.org/10.1038/nri2921. Permission conveyed through Copyright Clearance Center, Inc.)

Chronic inflammation is closely related to hypercoagulability, a well-established risk factor for VTE.[120] Although not consistent across studies, obesity is associated with increased levels of fibrinogen, FVII, FVIII, FIX, FXI, FXII, and VWF.[120,150-153] Of these, increased levels of FVIII are particularly interesting, as high FVIII is associated with the so-called activated protein C (APC) resistance, a well-recognize cause of hypercoagulability and risk factor for VTE.[120,154] Nevertheless, in the Leiden Thrombophilia Study (LETS), risk estimates for VTE according to BMI were only slightly attenuated after adjustment for APC resistance, indicating a marginal mediating effect at most.[120]

Hypofibrinolysis, describing a state of impaired clot resolution, is associated with increased VTE risk.[155-157] Of note, obesity is associated with hypofibrinolysis,[143,144] likely due

to increased levels of plasminogen activator inhibitor 1 (PAI-1), the primary regulator of fibrinolysis.[158] PAI-1 downregulates the conversion of plasminogen to plasmin by inhibiting plasma tissue plasminogen activator, which consequently inhibits the breakdown of fibrin clots. As adipose tissue was reported to contribute to circulating PAI-1 levels,[144,159,160] PAI-1 could therefore be a mediator of VTE risk in obesity.

The hemostatic and complement system are closely linked by intensive crosstalk.[161,162] Indeed, some factors of the complement system are associated with VTE risk, as recently demonstrated in the Tromsø Study.[163,164] Adipose tissue is able to express several complement factors,[165] of which complement factor D (CFD), formerly called adipsin, appears to be closely related to obesity.[166,167] CFD is a serine protease that catalyzes the rate-limiting step of the alternative complement pathway,[168] required in the amplification of complement activation.[169] Given the close link between the complement and hemostatic system, CFD may be involved in the pathogenesis of obesity-related VTE.

EVs have emerged as another proposed mechanism in the pathogenesis of obesity-related VTE. EVs include exosomes, microvesicles, and apoptotic bodies, which are released from cells upon activation and apoptosis.[170] EVs are highly procoagulant in certain disease states like sepsis, disseminated intravascular coagulation and cancer because of the large quantity of surface-exposed negatively charged PPLs and TF.[170-172] Increased levels of EVs have been reported in obesity[173] and in VTE patients.[174-177]

The well-known adipokines leptin and adiponectin could potentially play a role in the mechanism of VTE in obesity. Leptin, a hormone mainly produced by adipocytes[142] was reported to upregulate the expression of key hemostatic factors *in vitro*, including TF[178,179] and PAI-1.[180] Although the primary role of leptin is appetite control, plasma leptin is generally elevated in obesity, which could be attributed to a phenomenon known as leptin resistance.[181] 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. In contrast, the anti-inflammatory adipokine adiponectin, which primarily regulates glucose and lipid metabolism, improving whole-body energy homeostasis,[182] was shown to inhibit TF expression and increase tissue factor pathway inhibitor (TFPI) expression *in vitro*.[183,184] As adiponectin levels are reduced in obesity, this could promote venous

thrombosis.[185,186] A simplified overview of potential mediators of VTE risk in obesity is described in Figure 5.

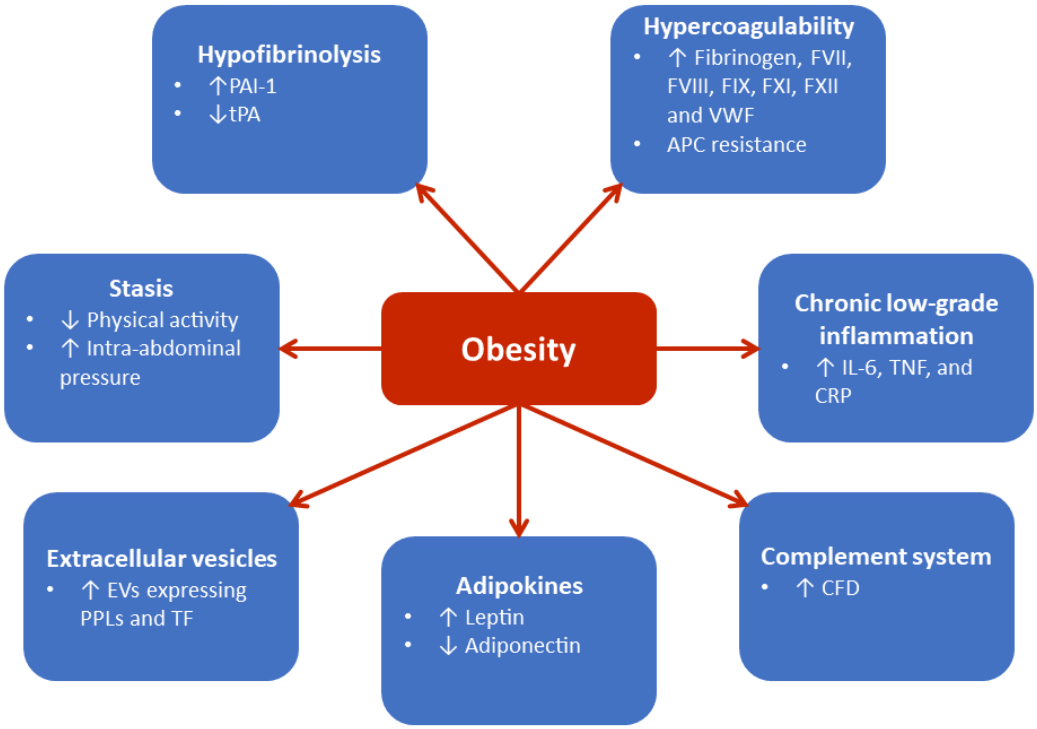

Figure 5. Potential mediators of obesity-related VTE. Abbreviations: APC, activated protein C; CFD, complement factor D; CRP, C-reactive protein; EVs; extracellular vesicles; IL-6, interleukin 6; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; VWF, von Willebrand factor.

1.4 Major knowledge gaps in obesity-related VTE

The prevalence of obesity has increased dramatically during the last decades and is expected to continue to rise in the future. As obesity is a major, likely causal risk factor for VTE, the increase in obesity prevalence will probably have an impact on the incidence of VTE in the coming years. At present, it is not well described to what extent overweight and obesity contribute to the incidence of VTE in the general population. Using updated data from a large population-based cohort may improve characterizing obesity-related VTE in the general population. Since intra-individual BMI may fluctuate over time, the impact of overweight and obesity on VTE risk may be underestimated in cohort studies with a single assessment of BMI and long follow-up because of regression dilution.[187] By using a population-based cohort study with repeated measurements of BMI, our findings will provide updated estimates of the

proportion of VTE events attributed to overweight and obesity at population level, having the potential to aid public health interventions aimed to mitigate the incidence of obesity-related VTE.

As described briefly, it has been observed that obesity may interact with some prothrombotic genotypes, synergistically increasing the VTE risk. Nevertheless, a comprehensive investigation of biological interactions between obesity and multiple established prothrombotic genotypes is missing. This may provide insights into obesity-related VTE pathophysiology and could help to identify individuals at substantially high risk of VTE, guiding targeted interventions.

Likewise, identifying mediators on the causal path between obesity and VTE could facilitate targeted prevention for obesity-related VTE. However, most mechanisms proposed for the pathophysiology of VTE in obesity are based on *in vitro* findings, such as the effect of leptin on the expression of hemostatic factors, and their clinical relevance remains uncertain. It is therefore vital to expand our understanding of clinically relevant mechanisms in obesity-related VTE. Indeed, targeting mediators, directly or indirectly via associated pathways, could emerge as viable therapeutic options. Additionally, mediators could serve as biomarkers helping to identify obese individuals at particularly high risk of VTE.

2 Aim of the thesis

The overall aim of the thesis is to improve the understanding of obesity-related VTE by providing estimates of the impact of obesity on incident VTE in the general population and unravelling biomarkers of incident VTE in obesity.

Specific aims:

i. To investigate the population attributable fraction (PAF) of overweight and obesity for the risk of VTE in a population-based cohort study with repeated measurements of BMI (the Tromsø Study).

ii. To investigate the joint effect of obesity and established prothrombotic genotypes on the risk of VTE in a population-based case-cohort study derived from the Tromsø Study and the Trøndelag Health Study (HUNT).

iii. To investigate the potential of adipose-tissue enriched plasma proteins, such as leptin and PAI-1, to mediate the association between obesity and VTE risk in a population-based nested case-control study derived from the Tromsø Study.

3 Methods

The papers included in this thesis were all built on population-based cohort studies and applied three different study designs. In brief, Paper I included participants from surveys 4-7 of the Tromsø Study and a cohort design was employed. In paper II, participants were recruited from the fourth survey of the Tromsø Study and the second survey of the HUNT Study to conceive a case-cohort study. A nested case-control design was used in Papers III and IV, and participants were recruited from the fourth survey of the Tromsø Study.

3.1 Study population

3.1.1 The Tromsø Study

The Tromsø Study is a single-center population-based prospective cohort study with repeated health surveys, which was initiated in 1974 in order to combat the high cardiovascular mortality in middle-aged Norwegian men.[188] To date, seven surveys of the Tromsø Study have been conducted. The study objectives have changed over the years, leading to the collection of extensive information of participants, facilitating the investigation of a wide range of diseases. In total, more than 45,000 unique individuals were included into the Tromsø Study.[189] A unique feature of the Tromsø Study is the fact that the University Hospital of North Norway (UNN) is the sole healthcare provider for Tromsø municipality, which allows a complete follow-up and cross-linkage to hospital discharge registries and national disease registries.

The papers of this thesis included surveys 4-7 of the Tromsø Study.[188,190] Tromsø 4, conducted in 1994/95, was the largest survey, where all inhabitants of Tromsø aged 25 years or older were invited. The participation rate was high (77%) and a total of 27,158 individuals were included. Enrollment of participants in Tromsø 5 was conducted in 2001/02 and 8,130 individuals aged 30-89 years participated (79% of participation rate). Tromsø 6 (2007/08) included 12,984 participants aged 30-87 years, and Tromsø 7 (2015/16) included 21,083 participants aged 40-99 years, with participation rates of 66% and 65%, respectively.

3.1.2 The Trøndelag Health Study

The HUNT Study was initiated in 1984-86 and was originally designed to investigate hypertension, diabetes, lung disease, and quality of life.[191] To date, four surveys have been completed. The first three surveys included only inhabitants of Nord-Trøndelag County and encompassed health information, anthropometric measures, and biological material. The fourth survey included additionally the population of Sør-Trøndelag County. In all four surveys together, 230,000 unique individuals were enrolled and approximately 120,000 have provided biological material and anthropometric measures.[192]

In paper II, the second survey of the HUNT Study (HUNT 2) was used to conceive a case-cohort study. In this survey, all inhabitants of Nord-Trøndelag County aged 20 years or older were invited in 1995-1997 and 66,140 participated, corresponding to a participation rate of 71%.

3.2 Study design

3.2.1 Cohort study

The Tromsø Study is a prospective cohort study, which follows a defined population from inclusion until the outcome of interest (e.g., VTE), a censoring event (e.g., death or loss to follow-up), or end of follow-up, as shown in Figure 6. A cohort study permits the calculation of absolute and relative risks and is mainly useful for the investigation of common outcomes. In paper I, a cohort design was employed using the study population from surveys 4-7 of the Tromsø Study. Of note, participants were invited to participate in consecutive surveys, and this is the reason why some of the participants attended more than one survey. Participants were followed from the date of enrollment to the date of incident VTE, migration, death, or end of follow-up (31 December 2020), whichever came first.

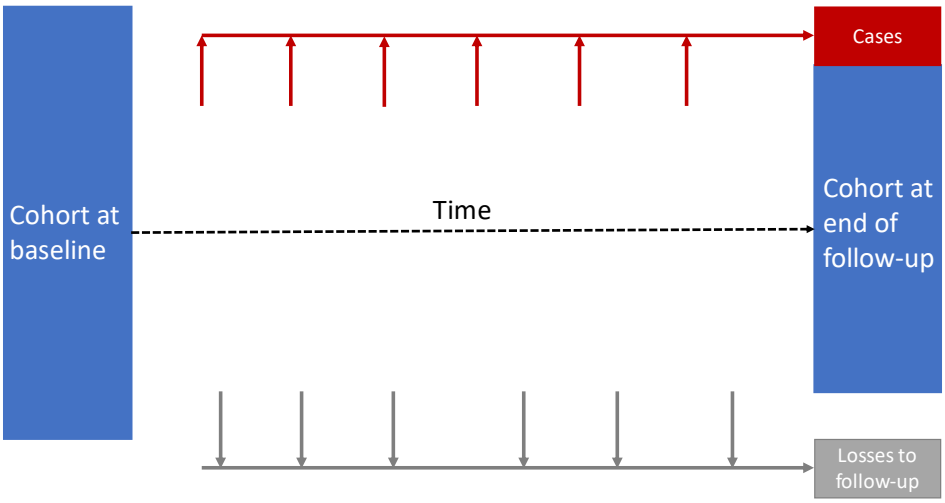

Figure 6 Cohort study design. Cases are represented by red arrows. Gray arrows represent losses to follow-up and that is why the number of subjects at the end of follow-up is lower than the number of subjects at cohort baseline.

3.2.2 Case-cohort study

In paper II, a case-cohort study was conceived, which included participants from Tromsø 4 and HUNT 2. Participants were followed from the date of enrolment until the date of incident VTE, migration, death, or end of follow-up. The follow-up ended in December 31, 2012 in Tromsø 4 and December 31, 2008 in HUNT 2. All participants experiencing an incident VTE event during the follow-up period were included as cases. A subcohort consisting of a random sample of subjects, which was age-weighted based on the age-distribution of cases, was derived from the parent cohort at baseline. In a case–cohort design, every participant in the cohort has the same probability of being selected to the subcohort, and some subcohort members can also be cases. The concept of a case-cohort design is exemplified in Figure 7A.

3.2.3 Nested case-control study

In papers III and IV, a nested case-control study was conceived. Participants from Tromsø 4 were included and followed from the date of enrolment until the date of incident VTE, migration, death, or end of follow-up (September 1, 2007). All individuals experiencing an incident VTE event during the follow-up period (1994-2007) were included as cases into the nested case-control study. 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. The concept of a nested case-control study is shown in Figure 7B.

Figure 7A

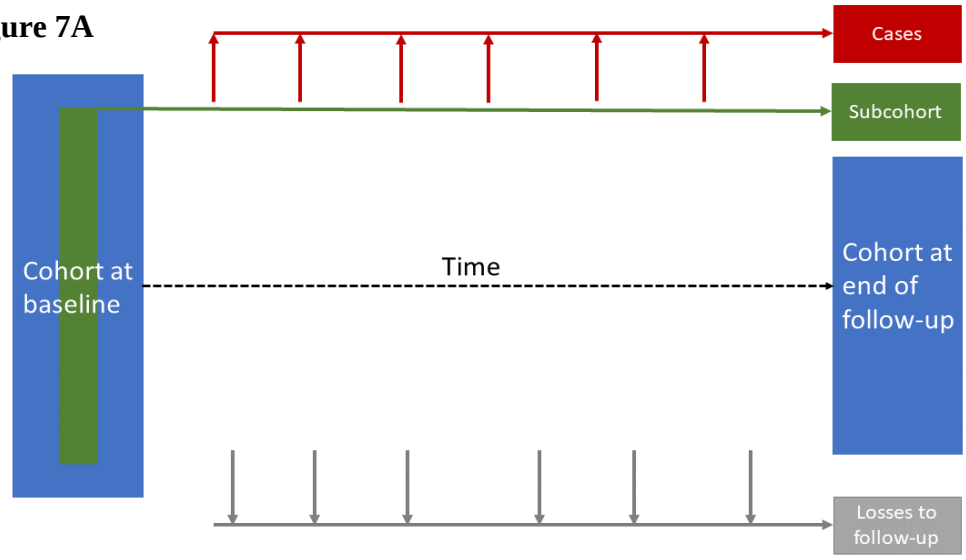

Figure 7B

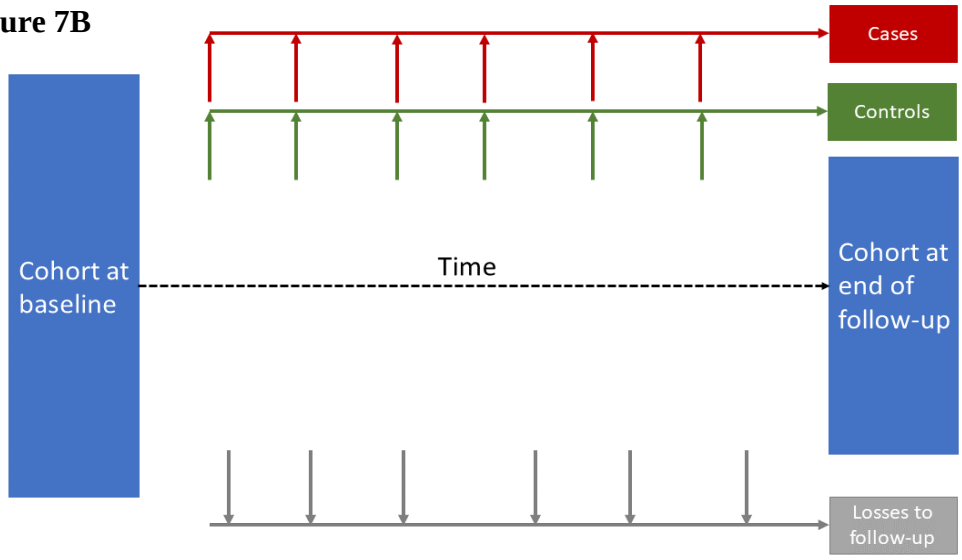

Figure 7. Case-cohort study design (**Figure 7A**). Subcohort members represented by a green box are selected from the baseline cohort. Nested case-control study design (**Figure 7B**). Controls represented by green arrows are selected each time a case occurs. In figures 7A and 7B, cases are represented by red arrows. Gray arrows represent losses to follow-up.

3.3 Exposure assessment

3.3.1 Baseline measurements

Baseline information in the Tromsø and HUNT studies was obtained from physical examinations, blood samples and self-administered questionnaires. Body height (to the nearest centimeter) and weight (to the nearest 0.5 kilograms) were measured with subjects wearing light clothes and no shoes. BMI was calculated as weight in kilogram per square of height in meters (kg/m^2). A detailed self-reported questionnaire was used to obtain information on smoking habits, use of oral contraceptives or hormone replacement therapy, and chronic diseases, including arterial CVD (i.e., angina pectoris, stroke, and myocardial infarction), cancer, and diabetes mellitus. Blood pressure measurement, blood sampling and analyses were previously described for Tromsø 4-6[188,190] and were conducted in the same way for Tromsø 7. In brief, systolic and diastolic blood pressure were measured three times with an automated device and the average of the two last measures was used. Non-fasting blood samples were analyzed by standard methods for biochemical variables (e.g., total cholesterol) at the UNN.

3.3.2 Blood sampling, storage, and laboratory analysis of plasma leptin, PAI-1, and CRP

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, 0.37mol/L per tube), as previously described.[163,164] 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, PAI-1 and 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 minutes, followed by centrifugation for 2 minutes at 13500*g* to obtain platelet-free plasma. Plasma levels of leptin, PAI-1 and high-sensitivity (hs) CRP were measured in duplicates by enzyme-immunoassay (EIA) using commercially available reagents (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation of leptin, PAI-1 and CRP were <10%.

3.3.3 Genetic analysis - prothrombotic genotypes

Samples from the Tromsø Study were genotyped using the Sequenom and the TaqMan platforms, as previously described.[193] The HUNT samples were genotyped using the Illumina HumanCore Exome array. The following SNPs were assessed: rs8176719 in *ABO* (non-O blood group), rs6025 in *F5* (FVL), rs1799963 in *F2* (prothrombin G20210A), rs2066865 in *FGG*, and rs2036914 in *F11*. Individuals were classified as carriers of a prothrombotic SNP if one or two risk alleles were present, and as non-carriers if no risk allele was present. For rs2036914 in *F11*, the minor allele is associated with a reduced risk of VTE, and we therefore considered the common allele as the risk allele.[75] A GRS was created by summing up the number of risk alleles of the five aforementioned SNPs, with a theoretical maximum number of ten risk alleles for an individual.

3.4 Outcome assessment

In the Tromsø Study, incident VTE events were identified by searching the hospital discharge registry, the autopsy registry, and the radiology procedure registry of the UNN, which is the only hospital providing diagnostic radiology and treatment for VTE in the region. The relevant discharge codes were International Classification of Diseases (ICD), Ninth Revision, codes 325, 415.1, 451, 452, 453, 671.3, 671.4, and 671.9 from 1994 to1998, and ICD, Tenth Revision, codes I80.0–I80.3, I80.8, I80.9, I81, I82.0–I82.3, I82.8, I82.9, I67.6, O22.3, O22.5, O87.1, O87.3, I26.0, and I26.9 from 1999 to 2020. Each potential VTE case was reviewed by trained personnel and a VTE was confirmed if the following 4 conditions were satisfied: i) confirmation by diagnostic procedures (i.e., compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography, or autopsy); ii) diagnosis of DVT or PE in the medical record; iii) Signs and symptoms consistent with DVT or PE; and iv) treatment initiation with anticoagulants, thrombolytic therapy, or vascular surgery. For patients derived from the autopsy registry, a VTE event was recorded when the autopsy record indicated pulmonary embolism as cause of death or as a significant condition contributing to death.[194]

In the HUNT Study, incident VTE events were identified by searching the electronic patient registry of the Levanger and Namsos hospital. Additionally, a case-finding search in the electronic discharge registry of the tertiary-care center, St. Olav's Hospital in Trondheim,

was performed.[23] VTEs were identified by hospital discharge codes; ICD-9 codes 415.x, 451.x, 452, 453.x, 325, 362.3, 433, 557.0, 634–638 (with decimals 6 and 7), 639.6, 639.8, 639.9, 671.x, 673.x, 674, and 997.2, and ICD-10 codes I26.x, I80.x, I81, I82.x, I 63.6, I67.6, K55, H34.8, O08.x, O22.x, O87.x, and O88.x. Two physicians reviewed and validated potential VTE cases. DVT cases and PEs were objectively confirmed by radiological procedures.[195,196]

All events were classified as either PE (with or without DVT) or isolated DVT and as provoked or unprovoked based on the presence of provoking factors at the time of diagnosis. In the Tromsø Study, the VTE events were classified as provoked if one or more of the following provoking factors were present: surgery, trauma or acute medical conditions (acute myocardial infarction, acute ischemic stroke, or acute infection) within 8 weeks prior to 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). In the HUNT Study, provoking factors included trauma or surgery, cancer (active malignancy at the time of the event or within 6 months after the event), marked immobilization (paresis, paralysis, prolonged bedrest due to acute medical illness, or travel >8 hours) within the previous 3 months, pregnancy or puerperium at the time of the event, or use of oral contraceptives at the time of the event or up to one month prior to the event.

3.5 Statistical analysis

Statistical analysis was carried out with Stata (version 15 and 16; Stata Corporation, College Station, TX, USA) and R version 4.0.4. (The R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics of study participants and characteristics of VTE events were presented as means (± standard deviation), medians (25th-75th percentiles) and proportions, and were calculated using descriptive statistics. In papers I and II, Cox proportional hazards regression models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for incident VTE. The main exposure was BMI and study participants were divided into three BMI categories according to cutoff values defined by the WHO: BMI <25 kg/m^2, BMI 25-30 kg/m^2 (overweight) and BMI ≥30 kg/m^2 (obesity).[18] In paper I, a time-varying analysis was used, where subjects who participated in more than one survey contributed with one observation period per attended survey. Such approach allowed for update of the BMI

measurement for those who were re-measured during follow-up. In paper II, combined categories were formed according to BMI categories and prothrombotic SNPs or a GRS. In paper III and IV, unconditional logistic regression models were used to estimate odds ratios (ORs) with 95% CIs for incident VTE. Main exposures (i.e., plasma leptin and PAI-1) were analyzed as categorical variables according to cutoff values established in the control population. All analyses, encompassing papers I to IV, were adjusted for predefined potential confounders.

3.5.1 Population attributable fraction (PAF)

The PAF is defined as the proportion of cases of a particular disease (e.g., VTE) in a population that is attributable to a risk factor (e.g., overweight or obesity).[197,198] The concept of PAF has a causal interpretation as it indicates the proportion of which the incidence of a disease would decrease if a specific risk factor could hypothetically be removed. Hence, to obtain meaningful PAF estimates, a risk factor must be modifiable.[198,199] The PAF is dependent on the prevalence and the relative risk of a risk factor. The formula used to calculate PAF in paper I took into account both the prevalence of overweight or obesity in VTE cases (p) and the age-and sex-adjusted HRs of VTE (PAF = p[1-1/HR]). With this formula, internally valid estimates are generated when adjusted relative risk is used.[199]

3.5.2 Interaction analysis

Paper II had the aim to investigate biological interaction between obesity and multiple established prothrombotic SNPs. The presence of biological interaction between the exposures was assessed on an additive scale by calculating the relative excess risk attributable to interaction (RERI), the attributable proportion (AP) due to interaction, and the synergy index (SI) with corresponding 95% CIs.[200,201] In order to calculate these measures, participants were stratified into categories according to their exposure status, and the reference category included participants with no exposure under investigation (e.g. BMI < 25Kg/m^2 and no risk allele). RERI was calculated as $HR_{11} – HR_{10} – HR_{01} + 1$, where AP was calculated as RERI / HR_{11}.[201] HR_{11} = combined category with both exposures (e.g., obesity and risk allele), HR_{10} = exposure 1 only (e.g., obesity and no risk allele), HR_{01} = exposure 2 only (e.g., BMI < 25kg/m^2 and presence of risk allele). Briefly, the RERI can be interpreted as

part of the total effect on the outcome that is attributable to interaction, and the AP as the proportion of the combined effect that is due to interaction between the two exposures.[200] For interpretation, a RERI >0, an AP >0 and a SI >1 indicate a departure from additivity of effects, suggesting positive interaction, i.e. the effect of the joint exposure (having both risk factors) on the outcome is greater than the sum of the two separate effects.[200,201]

3.5.3 Mediation analysis

The aim of a mediation analysis is to disentangle the casual pathways linking an exposure and an outcome.[202] In the framework of a mediation analysis in papers III and IV, there were 3 components; the main exposure (i.e., obesity), the potential mediator (i.e., leptin in paper III and PAI-1 in paper IV), and the outcome (i.e., VTE). In statistical modeling aiming to explore the mechanism that underlies the association between exposure and outcome, risk estimates are expected to be attenuated when a variable that is in the causal pathway between the exposure and outcome is introduced into the model.[202] To quantify this attenuation (i.e., the potential mediating effect), the Karlson, Holm and Breen (KHB) method was applied.[203] 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, while adjusting for potential confoundres.[203]

3.6 Ethics

All participants included in the Tromsø and HUNT Study provided written consent prior to inclusion, and are free to withdraw consent at any time. The present thesis and papers included are approved by the Regional Committees for Medical and Health Research Ethics.

4 Main results

4.1 Paper I: The risk of incident venous thromboembolism attributed to overweight and obesity: The Tromsø Study

Data on the contribution of overweight and obesity on incident VTE in the general population is scarce. In this study, we aimed to estimate the PAF of VTE attributable to overweight and obesity in a population-based cohort study with repeated measurements of BMI.

A population-based cohort study was conceived by including 36,341 unique participants from the 4-7th surveys of the Tromsø Study (enrolment: 1994-2016). Participants were followed until December 31, 2020, and all incident VTEs were recorded. BMI was objectively measured at survey inclusion, and measurement was updated in subsequent surveys for those attending more than one. BMI was categorized according to the WHO cut-off values as BMI <25 kg/m^2, BMI 25-30 kg/m^2 (overweight) and BMI ≥30 kg/m^2 (obesity). Time-varying Cox regression models were used to calculate HRs with 95% CIs. The PAF for overweight and obesity was estimated based on age- and sex- adjusted HRs and the prevalence of BMI categories in VTE cases.

During a median follow-up of 13.9 years, 1,051 VTEs occurred. The age-and sex-adjusted HRs of VTE were 1.40 (95% CI 1.21-1.61) for overweight and 1.86 (95% CI 1.58-2.20) for obesity compared with subjects with BMI <25 kg/m^2. The PAF of VTE due to overweight and obesity was 24.6% (95% CI 16.6-32.9), with 12.9% (95% CI 6.6-19.0) being attributed to overweight and 11.7% (95% CI 8.5-14.9) to obesity. Similar PAFs were obtained in analyses stratified by sex and VTE subtypes (provoked/unprovoked events, deep vein thrombosis, pulmonary embolism).

In conclusion, in this population-based cohort study, almost 25% of all VTE events could be attributed to overweight and obesity. Our findings suggest that public health efforts dedicated to develop strategies that can effectively fight the obesity epidemic along with targeted interventions aimed to reduce the thrombosis risk in overweight and obese subjects may substantially lower the incidence of VTE in the general population.

4.2 Paper II: Joint Effect of Multiple Prothrombotic Genotypes and Obesity on the Risk of Incident Venous Thromboembolism

The combined effect of obesity and multiple prothrombotic genotypes on the risk of VTE and subgroups (i.e., DVT, PE, provoked and unprovoked events) remains unclear. We aimed to investigate the joint effect of obesity and established prothrombotic SNPs, either individually or in a GRS, on the risk of overall VTE and subgroups using a population-based case-cohort study.

Cases with incident VTE (n = 1,470) and randomly selected subcohort members (n = 12,826) were derived from Tromsø 4 (1994–2012) and HUNT 2 (1995–2008) cohorts. Participants were genotyped for *ABO* (rs8176719), *F5* (rs6025), *F2* (rs1799963), *FGG* (rs2066865), and *F11* (rs2036914) SNPs. A GRS was conceived by summing up the number of risk alleles of the five aforementioned SNPs. Age- and sex-adjusted HRs with 95% CIs were estimated according to BMI categories and number of risk alleles for individual SNPs and the GRS (0-1, 2, 3, ≥4 alleles). The presence of interaction on an additive scale was evaluated with the RERI, AP and SI.

We found that the VTE risk increased with both the number of risk alleles and an increasing BMI, and the highest risk estimates were obtained when obesity (BMI ≥30kg/m^2) was jointly present with prothrombotic risk alleles, for the individual SNPs (i.e., ≥1 risk alleles) and the high-risk category of the GRS (i.e., ≥4 risk alleles). The combination of obesity and risk alleles, either as individual SNPs or as a GRS, had an additive effect on VTE risk (i.e., no biological interaction). Obese subjects who were carriers of ≥4 risk alleles had a 2.85-fold (95% CI: 2.05–3.96) increased risk of overall VTE compared with those with BMI <25 kg/m^2 and 0 to 1 risk allele, with a RERI of 0.11 (95% CI -0.77 to 0.99), an AP of 0.04 (95%CI -0.27 to 0.34) and a SI of 1.06 (95% CI 0.65 to 1.74). However, in subgroups, the combination of obesity and ≥4 risk alleles was more pronounced for DVT (HR: 3.20; 95% CI: 2.09–4.90) and unprovoked VTE (HR: 3.82; 95% CI: 2.25–6.47), suggesting a supra-additive effect on thrombosis risk.

Our findings indicate that the combination of obesity and GRS has an additive effect on the risk of overall VTE. However, it may have a supra-additive effect on the risk of DVT and unprovoked VTE.

4.3 Paper III: Plasma Levels of Leptin and Risk of Future Incident Venous Thromboembolism

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 the VTE risk in obesity. However, whether leptin is associated with VTE remains uncertain.

This study aimed to investigate the association between plasma leptin levels and risk of incident VTE, and the potential of leptin to mediate the VTE risk in obesity. A population-based nested case–control study with 416 VTE cases and 848 age- and sex-matched controls was derived from the Tromsø Study cohort (4th survey). Leptin antigen levels were measured in plasma samples collected at cohort inclusion. Logistic regression was used to calculate ORs with 95% CIs for VTE across leptin quartiles. Analyses were performed separately in men and women using sex-specific quartile cut-offs determined in controls, since plasma leptin has been consistently reported to be two to three times higher in women than in men.

Among controls, there was a moderate to strong correlation between BMI and leptin levels in men (Spearman's rho = 0.63, $p<0.001$) and women (Spearman's rho = 0.72, $p<0.001$). 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 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 ≥30 kg/m2) and were only marginally affected after adjustment for leptin.

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.

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

PAI-1, the main inhibitor of fibrinolysis, is reported to be expressed by adipose tissue and is frequently elevated in obesity. Hence, PAI-1 could potentially mediate the risk of VTE in obese subjects. However, whether PAI-1 is associated with VTE remains uncertain. We aimed 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.

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 (4^{th} survey). PAI-1 antigen levels were measured in plasma samples collected at cohort inclusion. Logistic regression was used to calculate ORs with 95% CIs for VTE across PAI-1 tertiles, defined in the control population. Mediation analysis was carried out using the KHB method.

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 vs. lowest tertile was 1.73 (95% CI 1.27-2.35), and risk estimates were only slightly attenuated with additional stepwise adjustment for BMI (OR 1.59, 95% CI 1.16-2.17) and CRP (OR 1.54, 95% CI 1.13-2.11). Similar results were obtained for provoked/unprovoked events, DVT, and PE. In obese subjects (BMI of $\geq$30kg/m^2 vs. <25kg/m^2), the KHB method revealed that PAI-1 mediated 14.9% (95% CI 4.1%-49.4%) of the VTE risk in analysis adjusted for age, sex, and CRP.

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.

5 General discussion

5.1 Methodological considerations

5.1.1 Study design

The four papers included in this thesis were epidemiological studies. According to Rothman, the cohort and the case-control study, with their variants,[204] are the two typical observational study designs. Studies included in this thesis fall into these categories and are originated from population-based cohort studies. Other study designs in epidemiology include case-series, cross-sectional studies, case-crossover studies, randomized controlled trials (RCTs), and MR studies. Epidemiological studies might be ranked according to their ability to provide causal interference and RCTs are generally viewed as the gold standard. However, in RCTs the exposure/intervention is applied by the researchers, and in some instances, this type of study can be unfeasible or unethical to be carried out. MR studies have emerged as an elegant approach to infer causality in some of the situations where RCTs cannot be applied.[205,206]

Paper I, as described previously, employed a cohort study design. The Tromsø Study can be classified as a prospective cohort, following a defined population from inclusion until the outcome of interest (e.g., VTE), a censoring event (e.g., death), or end of follow-up. Cohort studies are suited to investigate common outcomes and permit the calculation of absolute and relative risks. Major strengths include the ability to investigate multiple exposures and outcomes, where a temporal sequence between exposure and outcome is established. Although temporal sequence is a prerequisite for causal inferences, it is not sufficient.[204] Main disadvantages concerning cohort studies include a considerable demand for resources and time, and their ineffectiveness when investigating rare or latent outcomes. Further limitations, such as a differential loss to follow-up (i.e., informative censoring), long period of follow-up leading to changes in exposure status, and threats to validity, including bias and confounding will be discussed in detail in the following sections.

Another commonly used epidemiological study design is the case-control study. “Classical” case-control studies consist of a group of individuals with the outcome of interest (i.e., cases), which is compared with a group without the outcome (i.e., controls). Case-control studies are cost-efficient, can investigate multiple exposures, do not require follow-up, and are particularly useful in investigating rare outcomes. Nevertheless, case-control studies

have disadvantages compared with cohort studies, as only one outcome can be studied, absolute risk measures cannot be readily calculated, and no temporal sequence between exposure and outcome is given (i.e., the exposure is assessed after the occurrence of the outcome). This can be troublesome in some instances because it may lead to reverse causation, which describes a situation where the outcome affects the exposure, and the investigator may faultily conclude the opposite. A major challenge in case-control studies is the selection of the controls, as they should be selected from the same source population that gives rise to the cases. The controls serve to estimate the distribution of the exposure in the population that originates the cases, which is why the selection of the controls must be independent of the exposure status.[204,207] Other challenges include recall bias and confounding. In papers II, III, and IV, two variants of a case-control design were employed, i.e., the case-cohort and the nested case-control study.

Case-cohort and nested case-control studies are both derived from cohort studies and are fundamentally efficient designs when additional information that was not obtained or measured for the whole cohort is needed.[208] This may be done in order to reduce costs and to preserve biological material (e.g., blood samples) for future analysis. The case-cohort study, as implied by the name, includes features of both study designs. The case-cohort consists of all cases occurring in the parent cohort during follow-up and a randomly sampled subcohort. Random sampling is carried out at baseline, and therefore cases are also eligible to be included into the subcohort, as exemplified in Figure 8. An important feature of this design is that it permits time-to-event analysis (e.g., Cox regression). Of note, the subcohort once sampled can be used for several studies investigating different diseases, thereby further improving cost-effectiveness and usage of biological material.

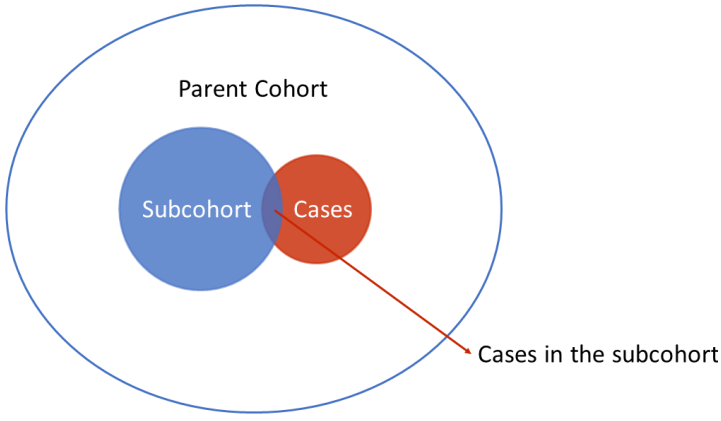

Figure 8. Sampling of a case-cohort study.

In papers III and IV, a nested case-control study design was employed, describing a cases-control study that is "nested" within a parent cohort, where cases and controls are sampled from the same parent cohort study. Each time a case occurs, controls, who are alive

at the index time, are randomly sampled from the parent cohort. The main difference between the case-cohort and the nested case-control design is the sampling of controls. Controls are selected at baseline in the case-cohort and at the time an index event occurs in the nested case-control. Because of the differences in sampling strategies, the case-cohort design permits time-to-event analysis, while logistic regression is generally used in the nested design.

Both study designs have the ability to overcome some of the drawbacks associated with "classical" case-control studies. Controls are sampled from the same source population as the cases, thus decreasing the probability of selection bias. Importantly, in this thesis, cases and controls are derived from the general population, which may increase the generalizability of study results. Furthermore, exposures are measured at inclusion of individuals into the parent cohort, thereby preserving the temporal sequence between the exposure and the outcome.

Of note, all observational epidemiological studies come with an inborne limitation, namely confounding. Even if strategies to deal with confounding are extensively employed, residual or unmeasured confounding may still distort study results. As stated previously, the gold standard to infer causality is the RCT due to its ability to circumvent confounding. In RCTs, study participants are randomly allocated to groups (intervention or control), and the effect of the exposure on the outcome is measured. Randomization, if conducted properly, allocates all covariates randomly between the groups, minimizing confounding. Nevertheless, RCTs are time- and resource-demanding and may be impossible or unethical, and studies using an MR approach emerge as possible alternatives in some cirumstances.[205,206] MR studies are based on the principle that inheritance of genetic variants by random segregation during meiosis resembles randomization in a trial, which theoretically is thought to overcome confounding.[117] Genetic instruments used in MR have their own set of assumptions and MR studies come with some limitations (e.g. large sample size is required, linkage disequilibrium between variants, population stratification and pleiotropy).[209,210]

5.1.2 Validity

The validity of epidemiological studies may be divided into internal validity and external validity. The internal validity implies how accurate study findings are in perspective of "true" findings, free from errors. Internal validity is a prerequisite for external validity, which

describes the generalizability or representativeness of study results outside the setting of the study, e.g., different study population, study settings, or time-period when the study is conducted.[211]

5.1.2.1 External validity - Generalizability

The external validity of the Tromsø Study is generally viewed as high, as it is a large population-based cohort study comprising a representative sample of the adult population of the Tromsø municipality. Complete birth cohorts or representative samples were invited, and attendance rates were high (77%-65%). However, in accordance with a declining attendance in other population-based studies,[212] attendance rates have also been decreasing in each subsequent survey of the Tromsø Study. Still, attendance was somewhat higher compared with similar population-based cohort studies.[190] In general, non-attendees in health surveys have a lower socioeconomic status and higher mortality rates.[212]Aditionally, in the Tromsø Study, the non-attendees were often young men, singles and the very old ones,[188] and caution is therefore warranted when generalizing study findings to these groups.

In the past, representativeness of the study population was viewed as a cornerstone in epidemiological research. In contrast to this, Rothman argued that representativeness is mainly required for the purpose of descriptive epidemiology, while in etiological research, high representativeness might hinder internal validity due to higher risk of confounding and difficulty to obtain information.[213] RCTs are good examples of studies with limited representativeness and high internal validity. Even though RCTs generally involve a highly selected population, results can be extrapolated to the general population, when the underlying disease pathophysiology is well understood and considered.

5.1.2.2 Bias

In epidemiology, errors are commonly divided into systematic or random. The latter can be decreased by increasing the sample size, while the former is not affected by the sample size.[204] Another term for systematic error is bias. Bias describes the tendency of study results to differ from a “true” effect and can arise in virtually any step while conducting

epidemiological studies, including the recruiting phase (selection bias), the data collection or analysis phase (information bias), or the publication phase (publication bias).

Selection bias may result from procedures used to enroll participants or factors influencing study participation and it can distort study results.[213] A type of selection bias is the non-responder bias, which has already been briefly discussed in section 5.1.2.1 in the framework of external validity. In addition to potentially affect external validity, when non-participation is associated with either the exposure or outcome, it may introduce bias, compromising the internal validity. In the present papers, participation appears to be associated with age, as young men and the very old were underrepresented in the Tromsø Study.[188] Age is a major risk factor for VTE and the association of age with non-participation may have biased risk estimates as the absolute risk for VTE is very low in the young, while it is high in the old.[24] Even though we cannot exclude that non-participation biased risk estimates, it is unlikely that this limitation had a substantial effect on the results and conclusions drawn from the papers of this thesis as participation rates were considerably high in all surveys of the Tromsø Study.

Information bias is a systematic error occurring when information is collected, recorded, or handled.[213] Information bias may lead to classification error or misclassification of the exposure, outcome, or confounder status. Misclassification may be differential or non-differential, depending on whether it is dependent on another variable.[214] Differential misclassification arises when the misclassification is related to another variable (e.g., assessment of exposure is dependent on the outcome). Examples of differential misclassification in case-control studies include recall bias (participants who experienced an outcome are more likely to remember hazardous exposures) and interviewer bias (interviewers might obtain information differentially when outcome status is known). In the present study, we can exclude these types of biases as information on exposure was collected prior to the disease occurrence. Additionally, observer bias (outcome assessors' knowledge of exposure status) can be virtually disregarded as VTE diagnosis was entirely based on objective criteria and knowledge of exposure status is not expected to impact the identification of VTE cases.

In contrast to differential misclassification, non-differential misclassification arises when misclassification of a variable is completely independent of other variables. BMI was used as a measure of total body fat in all papers and was assessed objectively by measurement

of height and weight at the physical examination. Hence, we expect that misclassification due to the approach of measuring BMI to be a minor concern for this variable. Nevertheless, as mentioned in the introduction, BMI does not seem to be the most appropriate measure for the classification of obesity, since the numerator in the BMI calculation is total body weight, which does not discriminate between lean and fat mass.[97] For example, adults with high levels of lean body mass (i.e., muscle mass) may be misclassified as overweight or obese. Further, waist circumference, a measure of abdominal obesity that reflects visceral adiposity, seems to yield the highest risk estimates for VTE and identify most people at risk.[215-217]Assessing obesity also based on waist circumference or other anthropometric measures could have been helpful in addressing potential misclassification.

In paper II, high-throughput analysis techniques were used to detect prothrombotic SNPs. These techniques including the TaqMan and Illumina platforms produce accurate and reliable genotyping assessment,[218-220] and we expect that misclassification would be minimal in paper II. In papers III and IV, variability in the pre-analytical and analytical conditions for the assessment of leptin and PAI-1 and the biology of these proteins (e.g., circadian variation) may have led to misclassification. We do acknowledge that potential measurement errors in BMI, SNPs, leptin, and PAI-1, even if minor, could have led to misclassification. Nevertheless, misclassification would be non-differential with regards to the outcome of interest (i.e., VTE) as anthropometric measurements, blood sample handling and laboratory analyses were carried out without the knowledge of future case-control status (i.e. VTE vs no VTE) for all participants. In general, non-differential misclassification may lead to an underestimation of true effects, biasing results towards the null hypothesis.[214]

5.1.2.3 Modifiable risk factors and regression-dilution bias

Except for prothrombotic SNPs, all other exposure variables were modifiable and susceptible to change during the follow-up. In prospective studies with long follow-up times, measurement of modifiable exposures at baseline may lead to an underestimation of the strength of the real association, which is also called regression dilution bias.[187]

BMI is susceptible to both change over time and change with an advancing age. In paper I, we addressed this issue by including BMI as a time-varying variable into the analyses, as repeated measures of BMI were available for a considerable proportion of the

population. Of note, HRs were only slightly different when comparing time-varying vs. time-fixed analysis, which is in line with previous observations regarding BMI and VTE risk in the Tromsø Study.[221] In papers III and IV, which were based one single measurements of biomarkers (i.e., leptin and PAI-1) and a follow-up time up to 13 years, we included analyses that restricted the maximum time from blood sampling in Tromsø 4 to the VTE events, while keeping all controls in the analyses. It was observed for both biomarkers that ORs for VTE were higher with shortened time between blood sampling and VTE events, and this was especially pronounced for PAI-1. We concluded that the association for the entire follow-up time might have been underestimated due to regression dilution.

5.1.2.4 Confounding and mediation

Confounding is a central issue in epidemiological studies investigating causal associations. A simple definition of confounding is the confusion of effects, which implies that the effect of an exposure is mixed with the effect of another variable.[204] This mixing of effects may change estimates by strengthening, weaking, or reversing the direction of the association.[208] A confounder must fulfill the following criteria: it must be associated with the exposure; it must be associated with the outcome; and it must not be in the causal path between the exposure and outcome,[204] as shown in Figure 9. The determination of confounders to be assessed in a study should be done *a priori* based on the existing literature and requires knowledge of the disease pathophysiology. Of note, a variable which lies on the causal path between the exposure and outcome is defined as a mediator. Adjusting for a mediator is wrong when the total effect of the exposure on the outcome is the objective of the investigation.

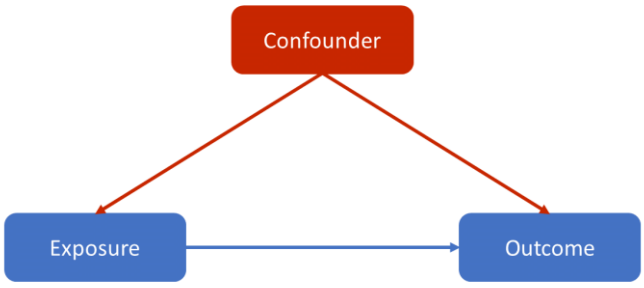

Figure 9. Graphical illustration of a confounder

Confounding may be addressed by several techniques implemented in the study design such as randomization, restriction, and matching.[204] Randomization is used in RCTs to facilitate that confounders are randomly distributed between the exposed and unexposed groups (e.g., the intervention and placebo groups). As defined by Rothman, restriction describes a situation where all subjects are confined to a single value or narrow range of

values of a particular factor that is recognized to be a potential confounder (e.g., a study comprising only subjects with a BMI <30 kg/m^2, because obesity is suspected to be a possible confounder in the association between the exposure under investigation and the outcome).[204] Matching is another technique to control for confounding, mainly used in case-control studies, where controls are chosen based on the value of a potential confounding variable in order to achieve an equal distribution of this variable between cases and controls. For instance, one approach to perform matching in a case-control study is to obtain the age distribution of cases and then select control subjects to replicate that age distribution (the so-called "frequency matching").[204] In observational studies, several methods can be employed in the analytic phase, after study completion, to control for confounding. These methods include stratification, standardization, and multivariable analyses in regression models. In the papers included in this thesis, stratification and mainly multivariable techniques were used.

VTE was the outcome of interest in all papers and VTE incidence increases exponentially with age.[19,28] BMI may also change with age, making age a major confounder in the relationship between obesity and VTE. In papers I and II, Cox proportional hazard regression models were used and adjustment for age was carried out by using age as a time scale.[222] This was done since age is an important confounder and using age as the time scale is therefore more meaningful than using time on study.[222] In papers III and IV, age was a matching variable for cases and controls, and analyses were adjusted for the matching variables. This adjustment was done because the matching process can make the controls more similar to the cases not only for the matching factor but also for the exposure itself, which may introduce bias that needs to be controlled for in the analysis, when unpaired analyses are performed.[223] Sex might confound the association between obesity and VTE, and analyses were adjusted for sex, with the exception of paper III, where all analyses were stratified by sex, as plasma levels of leptin are 2-3 times higher in women than in men.[224,225] In paper IV, chronic inflammation was considered to be a confounder, as it may induce PAI-1 expression [226,227] and it is associated with VTE.[148,149] Therefore, analyses were adjusted for CRP, as a proxy for inflammation. Despite careful consideration of potential confounders, residual confounding due to unmeasured or unknown confounders cannot be excluded in any observational study.

The aim of papers III and IV was to unravel casual pathways in the pathophysiology of obesity-related VTE. We aimed to detect mediators that are in the causal pathway between obesity and VTE. For a variable to be considered a mediator it must be a consequence of the

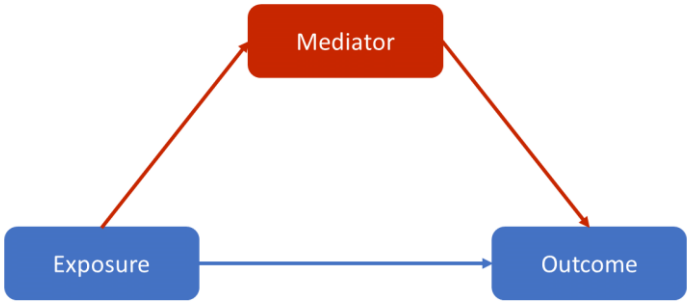

Figure 10. Graphical illustration of a mediator

exposure and associated with the outcome (Figure 10). In paper III, we initially tested whether leptin was associated with VTE. After adjustment for BMI, which was defined *a priori* as a major confounder, no association was found between leptin and risk of VTE. It is worth noting, however, that the relationship between leptin and BMI is complex and likely bidirectional. Leptin, as a satiety hormone, can influence body weight and consequentially BMI,[142] while BMI in turn has an effect on circulating levels of leptin.[228] Increasing adipose tissue is probably responsible for increased levels of leptin in obesity, which might be related to a phenomenon called leptin resistance.[181] Due to this, we decided to define BMI as a confounder for the association between leptin and VTE. When we tested whether leptin could act as a mediator in the association between BMI and VTE, risk estimates were only marginally changed upon including leptin in the regression models, and no further formal mediation analysis to quantify a potential mediating effect was pursued. In paper IV, on the other hand, PAI-1 was associated with risk of VTE even after adjusting for confounders, and a formal mediation analysis was conducted to evaluate the mediating effect of PAI-1 on the relationship between BMI and VTE using the KHB method.

5.1.2.5 Biological interaction

Biological interactions are present when the combined effect of two exposures on a given outcome exceeds the sum of their separate effects (i.e., more than additivity).[200] As elaborated by Rothman, biological interactions should be measured on an additive scale.[200] Measuring interactions on an additive scale is also possible when the underlying model is on a multiplicative scale (e.g., logistic regression and Cox regression), by using the RERI, the AP and the SI.[118] Interaction assessed on a multiplicative scale is commonly called statistical interaction.[200] In paper II, all interactions were presented in a transparent way to provide the reader sufficient information to evaluate interaction on additive and multiplicative scales, as

recommended by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).[229]

Of note, interaction analyses have inherent statistical limitations since they require division of the study population into smaller subgroups. This way, statistical tests of additivity have limited power at typical study sizes, and the corresponding estimates of departures from additivity may have little precision.[213] Although our interaction analyses lack statistical significance, some of our results may still reveal biological significance for the combination of genotypes and obesity because of the magnitude and direction of the point estimates of measures of biological interaction. This would be the case for the interaction analysis of obesity with factor V Leiden or with the high-risk category of the GRS for DVT, and the interaction analysis of obesity with rs2036914 (*F11*) for unprovoked VTE. As highlighted in several papers, emphasis should be focused on the size of the risk estimates, rather than the p-value and whether the 95% CI crosses unity, when interpreting potential associations in epidemiological studies.[230-232]

5.1.2.6 Missing data

Missing data is a common phenomenon in epidemiological studies and clinical research, which is defined as missingness of values for variables of interest, not recoded for every participant.[233] Missing data is categorized in three categories: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR).[233] Data is considered to be MCAR if the probability of missingness is independent of observed and unobserved variables for that subject. As an example, if a blood sample is damaged on the way to the laboratory, the cause of missingness would be completely at random. In this case, the subsample of all subjects with complete data is representative of the overall sample. MAR means that if after accounting for all observed variables, the probability of missingness is independent of all unobserved variables. This would be the case, for example, if measurements of human choriogonadotropin in women were dependent of age, thus missingness is dependent on an observed variable (i.e., age). MNAR occurs when even after accounting for observed variables, missingness is dependent on the value of the missing variable (e.g., reporting income in surveys is dependent on the income of the subject). A common approach to deal with MCAR and MAR data is the complete case analysis, which

was also applied in the present thesis. Another possibility, if the number of missingness is of concern, would be the use of imputation methods.

Missingness of information on BMI was of minor concern, as it was low in all studies (<1%). In papers III and IV, the number of missing measurements of leptin and PAI-1, respectively, was somewhat high, particularly for PAI-1. An explanation for missingness is that blood samples from the nested case-control study were used to measure several biological variables, not only leptin and PAI-1 antigens. Thus, some participants did not have enough remaining plasma for laboratory analyses. Additionally, some samples were hemolyzed, which precluded further laboratory analysis. Nevertheless, the proportion of missing samples was similar in cases and controls, and the reason for missingness was not related to the VTE status. Additionally, missingness was not associated with any specific baseline characteristic or comorbidity of the study participants. Thus, the missing data on leptin and PAI-1 was presumably MCAR and a complete case analysis likely produced valid estimates, albeit with reduced precision.

5.2 Discussion of main results

5.2.1 Risk of VTE attributable to overweight and obesity

Obesity is a well-known, likely causal risk factor for VTE.[11-15] Still to what extent overweight and obesity contribute to incident VTE in the general population had been scarcely investigated prior to this thesis. In paper I, it was observed that overweight and obesity accounted for almost 25% of all incident VTE events in the general adult population. Subgroup analyses yielded essentially similar results to the overall analyses, irrespective of the anatomical location of the thrombus (i.e., DVT or PE), presence of provoking factors, sex and age group (below or above 70 years), with PAF estimates ranging from 21% to 29%. Results from paper I demonstrate that overweight and obesity are major contributors to the VTE burden in the general population. From a public health perspective, these findings suggest that strategies pursuing an effective mitigation of the obesity epidemic as well as targeted interventions aimed to reduce the thrombosis risk in overweight and obese subjects have the potential to substantially lower the incidence of VTE at the population level.

In a previous study, the PAF of overweight and obesity for VTE was assessed in a Swedish cohort, where 12.4% of the VTE cases were attributed to a BMI ≥25 kg/m^2.[215] The estimate is lower compared with the PAF reported in paper I (~25%) but some reasons might explain this difference. The PAF is calculated based on risk estimates and the prevalence of the exposure, and consequently inaccuracy or underestimation of these estimates will influence the accuracy of the PAF. In the Swedish cohort, height and weight used to calculate BMI were self-reported and the diagnosis of VTE was based on the International Classification of Diseases codes,[215] which could have resulted in some degree of misclassification of both exposure and outcome. Further, estimates were based on a single baseline assessment of anthropometrics in 1997.[215] Given that BMI is a modifiable exposure, intra-individual change of BMI over time among participants of the Swedish cohort could have introduced regression dilution, which can lead to an underestimation of the results compared with the true associations.[187] In the present study, height and weight were objectively assessed at each physical examination, and all VTEs were thoroughly identified and validated. Further, we sought to mitigate regression dilution bias by taking repeated measurements of BMI into account. Moreover, because our cohort study is composed of several surveys conducted over time (1994/95-2015/16), we were able to account for the rising prevalence of overweight and obesity in the last two decades when calculating the PAF. For instance, data from the Tromsø study showed that the age-adjusted prevalence of obesity increased from about 11% in 1994/95 to 24% in 2015/16.[234,235] Such data is consistent with estimates from the WHO that indicate that almost one quarter (23%) of adults in the European Region were obese in 2016.[18]

It is worth noting that regardless of advancing age, sex or presence of VTE provoking factors, an excess of total body fat seems to contribute to at least 20% of the incident VTE events occurring in the general population. Hence, it is reasonable to assume that the promotion of a healthy lifestyle to fight the obesity epidemic could lower the incidence of VTE in the general population. While population-based strategies to improve lifestyle have contributed to an important decline in smoking in several countries in recent decades,[236-238] current population strategies have not been successful in reversing the obesity epidemic.[18,238] Obesity is recognized to have a chronic, relapsing and multicausal nature.[106] Even though weight loss is achievable by most lifestyle and dietary interventions, long-term maintenance of lost weight is challenging and weight regain is typical.[104,106] For instance, a meta-analysis comprising 29 studies on long-term weight loss showed that more than 50% of the lost weight

was regained in an individual within 2 years, and by 5 years, the proportion of lost weight that was regained was approximately 80%.[105]

Interestingly, the PAF of provoked VTE (21%) was slightly lower when compared with the estimate of unprovoked VTE (29%). These findings can be interpreted using the thrombosis potential model conceived by Rosendaal.[8] In this model, each risk factor contributes to increase the thrombosis potential of an individual, and when sufficient risk factors have been accumulated in a patient, the thrombosis potential exceeds the so-called thrombosis threshold, and an event occurs.[8] In this study, some of the provoking factors used to categorize an event as provoked are strong risk factors for VTE, including major surgery and active cancer,[10] which occurred in almost 15% and 25% of the events in our study, respectively. In the presence of these strong factors, the thrombosis potential would more likely exceed the thrombosis threshold leading to an incident VTE in both normal weight and overweight/obese subjects, which might explain why the proportion of thrombotic events due to an excess of total body fat was lower for provoked as compared with unprovoked VTE. Our estimate that 29% of the unprovoked VTEs were attributed to overweight and obesity was similar to the estimate of 33% reported in a population-based cohort study from Olmsted County in the USA.[20]

As demonstrated in paper I, excess of total body fat is an important contributor to incident VTE. Still, despite using repeated measures of BMI for a substantial proportion of the study population, we may have underestimated the contribution of an excess of total body fat to VTE risk because of the marked increase in the prevalence of overweight and obesity in recent years.[239] According to the World Obesity Federation, by 2030, it is predicted that 1 in 5 women and 1 in 7 men will be living with obesity, corresponding to over 1 billion people globally.[239] Due to this, it likely that the proportion of VTE cases attributable to above normal body weight (i.e., BMI $\geq$ 25 kg/m^2) will increase further in the coming years. As stated above, a global reduction of the prevalence of overweight and obesity seems unlikely, and it is therefore crucial to understand the underpinnings of the pathophysiological mechanisms of obesity-related VTE. This may reveal novel biomarkers for the identification of overweight and obese individuals at a substantially high risk of VTE who would benefit most from VTE preventive strategies. Further, unraveling the pathophysiology may help identify targetable pathways for intervention aimed to reduce the risk of VTE among overweight and obese people.

5.2.2 Joint effect of multiple prothrombotic genotypes and obesity on VTE risk

Paper II was set out to investigate the combined effect of overweight or obesity and multiple prothrombotic genotypes on VTE risk. Whether and to what extent the combination of overweight or obesity with multiple established prothrombotic SNPs affects the VTE risk in the general population was uncertain prior to this thesis. The rationale behind this study was to detect individuals at especially high-risk of VTE, who would potentially benefit from targeted interventions, and to improve our understanding of obesity-related VTE mechanisms. We found that the combination of obesity and individual prothrombotic SNPs had an additive effect on the risk of overall VTE, as the risk in the combined category approximated the sum of the separate effects of the two exposures (i.e., no biological interaction). This contrasts with previous studies as the presence of genetic variants associated with a prothrombotic state, such as FVL, prothrombin G20210A, and non-O blood group in obesity, has been suggested to synergistically increase the risk of VTE because of biological interaction.[109,119-121]

Previous studies investigating individual SNPs include the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) case-control study and a case-cohort derived from the Danish Diet, Cancer and Health study.[109,119] It was found that the combination of obesity with FVL or prothrombin G20210A resulted in a VTE risk that was higher than the sum of the separate effects.[109,119] Several reasons may account for the distinct findings between paper II and the previous reports. In the MEGA and Danish studies, the population was mostly comprised of subjects younger than 70 years at study inclusion, who would have a lower baseline risk and presumably less comorbidities associated with risk of VTE, which could result in higher relative effects of prothrombotic genotypes and obesity. In the MEGA and Danish studies, the combination of FVL and obesity resulted in a 7.86-fold (95% CI 4.70-13.15) and in a 5.27-fold (95% CI 2.74-10.14) increased risk of VTE, respectively.[109,119] These risk estimates are somewhat higher than the HR of 3.30 (95% CI 2.42-4.50) observed in paper II. Of note, investigating interactions in epidemiological studies comes with the drawback of low precision, which is indicated by the wide 95% CIs, especially noted in the MEGA and Danish studies.

Further, two studies, conducted by Crous-Bou *et al.*[240] and Kim *et al.*,[122] evaluated interaction between obesity and multiple SNPs with regards to VTE risk. In both studies, no interaction on a multiplicative scale was detected,[122,240] but Kim *et al*. reported interaction on

an additive scale for the combination of a high GRS and a high BMI.[122] The authors conceived their nested case-control studies using the same cohorts consisting of health care professionals. In these studies, the exposure (BMI) and the outcome (incident VTE) were self-reported in questionnaires, and a weighted GRS was built using 16 SNPs associated with VTE in genome-wide association studies.[122,240] Only FVL and prothrombin G20210A overlapped with the SNPs included in our genetic score, and not all the SNPs were related to hemostatic factor genes. Differences in study population, the way information was collected, and analyses conducted could explain conflicting results. Taken together, results from paper II suggest that the combination of obesity and prothrombotic genotypes has an additive effect on VTE risk.

It is worth noting, however, that when subgroup analysis was conducted, it was observed that obesity in combination with the SNPs in *F5* (FVL), *ABO* (non-O blood group), or *F11* or with the high-risk category of the GRS (i.e., ≥ 4 alleles) had a stronger impact on the risk of DVT and unprovoked VTE than on the risk of PE and provoked VTE. Measures of biological interaction suggested that the combination of obesity and some of the prothrombotic genotypes had a slight supra-additive effect on the risk of DVT (obesity plus FVL or non-O blood group) and unprovoked VTE (obesity plus rs2036914 in *F11*). These specific interactions might provide some mechanistical insights, as a potential synergism between obesity, FVL, and non-O blood groups could be explained by a common pathway associated with a prothrombotic state, namely APC resistance.[72,120,154,241] Of note, individuals with non-O blood group have higher levels of factor VIII than individuals with O blood group, and factor VIII in known to increase APC resistance.[120,241] A synergism between obesity and rs2036914 in *F11* is also biologically plausible, since both obesity and rs2036914 were reported to be associated with factor XI levels.[75,153] It is important to address that a substantial proportion of VTE cases in our study were provoked events (56%) and PE (41%), which may help explain why the analyses of the overall population did not reveal any consistent finding of biological interaction between obesity and prothrombotic genotypes. In summary, our subgroup analyses suggest that obese individuals who are carriers of some prothrombotic SNPs may be at a particularly high risk of developing DVT or unprovoked events.

5.2.3 Mediators of VTE risk in obesity

Papers III and IV aimed to investigate potential mediators on the casual path between obesity and VTE in order to expand our understanding of the pathophysiology of obesity-related VTE.

In paper III, we found that the adipokine leptin was associated with VTE risk, particularly in men. However, when BMI was taken into account, the apparent association disappeared. Further, when leptin was introduced in the regression models as a potential mediator for the association between obesity and VTE, the risk estimates were only marginally affected in both sexes, suggesting no mediating effect. This was somewhat surprising as previous findings based on *in vitro* studies speculated that leptin could play a role in obesity related-VTE. Several *in vitro* studies have investigated the effect of leptin on platelet activation parameters [186] and on the expression of key hemostatic factors for coagulation and fibrinolysis.[178-180,242,243] In human peripheral mononuclear cells, leptin was shown to induce TF activity, antigen and mRNA expression,[178,179] as well as the release of TF-bearing microparticles. [242] Interestingly, Ayer *et al*. [243] 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,[178,179,242] but not at the lower concentrations generally observed in obese subjects. Thus, *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.[180] However, this is in contrast with findings in leptin-deficient *ob/ob* mice, which were characterized by high levels of PAI-1[244] and with an *in vitro* study, where leptin was reported to have no effect on PAI-1 secretion from human adipocytes.[245] 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.[246] 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 resembles the mechanism of venous thrombus formation in humans, which frequently occurs in the presence of stasis and absence of endothelial injury.[247]

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.[248-251] 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.[252,253] However, a main limitation of the above mentioned studies[248-253] 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). Finally, the association between leptin and venous disease was investigated in few epidemiological studies, which suggested a potential role of leptin in DVT after knee arthroplasty, peripheral chronic venous disease, and post-thrombotic syndrome.[254-256]

In light of our findings, the association of leptin with hemostatic factors reported *in vitro* [178-180,242,243] and in epidemiological studies [248-253] does not seem to be clinically relevant for the underlying pathophysiology of VTE in obese, and interventions aimed at targeting leptin would unlikely impact the incidence of obesity-related VTE.

In paper IV, PAI-1 was investigated as a potential mediator of VTE risk in obesity. In this paper, we found that the risk of future VTE increased in a dose-dependent manner with increasing plasma levels of PAI-1, even after adjustment for BMI and CRP, a reliable and sensitive downstream marker of inflammation. Several studies, often with limited sample sizes, have provided inconclusive results on the association between PAI-1 and VTE over the last decades.[257-260] It is worth noting that the findings from two large population-based studies were also conflicting.[156,261] 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,[261] 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,[261] which could limit the comparability with our study. In the MEGA study, a case control-study involving 770 VTE patients and 743 controls, Meltzer *et al.* showed that high plasma levels of PAI-1 were associated with increased risk of VTE.[156] The association remained, albeit attenuated,

after additional adjustment for BMI and markers of inflammation, which is in line with our results.

We found that PAI-1 mediated almost 15% of the VTE risk in obesity, which was largely independent of chronic low-grade inflammation, 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 was highlighted by a murine model of DVT, in which increased expression levels of PAI-1 were associated with larger thrombus size and impaired thrombus resolution.[68] Targeting PAI-1 in order to reduce VTE risk in obesity might be suitable as PAI-1 seems to be predominantly regulated by environmental factors.[262,263] It was shown that angiotensin II, glucose, and insulin may induce the expression of PAI-1.[227 227] In interventional studies, treatment with angiotensin-converting enzyme inhibitors or with oral hypoglycemic medications was associated with decreased PAI-1 levels.[227,264-267] Notably, many efforts have been devoted to develop drugs that can modulate PAI-1 activity, the so-called PAI-1 inhibitors.[268] Several of these inhibitors, including low molecular weight molecules, peptides, monoclonal antibodies and antibody fragments, have been extensively characterized in experimental studies.[227,268] 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.

6 Conclusions

- In the general population, almost 25% of all incident VTE cases were attributed to overweight and obesity. Given the likely causal relationship between obesity and VTE, the rising prevalence of overweight and obesity may substantially contribute to the incidence of VTE in the coming years.

- The combination of obesity and established prothrombotic genotypes, evaluated either as individual SNPs or as a GRS, had an additive effect on the risk of overall VTE. However, such combination may have a supra-additive effect on the risk of DVT and unprovoked VTE.

- The apparent association between plasma leptin levels and VTE risk is confounded by BMI and leptin does not mediate the VTE risk in obese. These findings suggest that the effect of leptin on prothrombotic hemostatic factors reported *in vitro* is not clinically relevant for the mediation of VTE risk in obesity.

- Elevated PAI-1 levels, the main inhibitor of fibrinolysis, were associated with increased risk of future incident VTE and mediated almost 15% of the VTE risk in obesity in our study. Targeting PAI-1 may emerge as a promising therapeutic approach in obesity-related VTE.

7 Future perspectives

The aim of this thesis was to expand our knowledge concerning obesity-related VTE. We showed that one of four VTEs in the general population are attributable to overweight and obesity. Nevertheless, due to the increasing prevalence of overweight and obesity, we would expect this proportion to increase even further in the future, which may be evaluated in future studies to guide public health authorities.

We could not detect a synergism between obesity and established prothrombotic genotypes. Future research should aim to investigate the interaction between obesity and other genetic and environmental factors using large prospective population-based studies. Findings from these studies may facilitate the identification of overweight and obese individuals at a substantially high risk of VTE who would benefit most from public health interventions for disease prevention.

We identified PAI-1 as a mediator of VTE risk in obesity. PAI-1 might be a promising candidate to be investigated in future studies, especially because PAI-1 inhibitors have been extensively explored in experimental studies and even tested in some clinical trials, yet for conditions other than obesity and VTE.[227] Nevertheless, the search for other mediators has to be pursued in the coming years as only a small proportion of the VTE risk in obesity seems to be explained by specific mediators. Investigating single plasma proteins, as we did in this thesis, may not be the most efficient way to detect further causal biomarkers or pathways in obesity-related VTE and the use of somewhat "wide-spectrum" techniques such as proteomics, transcriptomics or metabolomics may assist in improving efficiency by which new mediators can be detected in the future.

Even though we could shed some light on obesity-related VTE mechanism, much is still unknow and obesity remains a major and challenging risk factor for VTE.

References

1. Di Nisio M, van Es N, Büller HR. Deep vein thrombosis and pulmonary embolism. Lancet. 2016;388:3060-3073
2. Line BR. Pathophysiology and diagnosis of deep venous thrombosis. Semin Nucl Med. 2001;31:90-101
3. van Langevelde K, Srámek A, Vincken PW, van Rooden JK, Rosendaal FR, Cannegieter SC. Finding the origin of pulmonary emboli with a total-body magnetic resonance direct thrombus imaging technique. Haematologica. 2013;98:309-315
4. Sørensen HT, Horvath-Puho E, Lash TL, Christiansen CF, Pesavento R, Pedersen L, Baron JA, Prandoni P. Heart disease may be a risk factor for pulmonary embolism without peripheral deep venous thrombosis. Circulation. 2011;124:1435-1441
5. Enga KF, Rye-Holmboe I, Hald EM, Løchen ML, Mathiesen EB, Njølstad I, Wilsgaard T, Braekkan SK, Hansen JB. Atrial fibrillation and future risk of venous thromboembolism:the Tromsø study. J Thromb Haemost. 2015;13:10-16
6. Dunnick NR, Newman GE, Perlmutt LM, Braun SD. Pulmonary embolism. Curr Probl Diagn Radiol. 1988;17:197-237
7. Goldhaber SZ, Elliott CG. Acute pulmonary embolism: part I: epidemiology, pathophysiology, and diagnosis. Circulation. 2003;108:2726-2729
8. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet. 1999;353:1167-1173
9. Wolberg AS, Rosendaal FR, Weitz JI, Jaffer IH, Agnelli G, Baglin T, Mackman N. Venous thrombosis. Nat Rev Dis Primers. 2015;1:15006
10. Kearon C, Ageno W, Cannegieter SC, Cosmi B, Geersing GJ, Kyrle PA. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. J Thromb Haemost. 2016;14:1480-1483
11. 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
12. 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
13. 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
14. Lindstrom S, Germain M, Crous-Bou M, Smith EN, Morange PE, van Hylckama Vlieg A, de Haan HG, Chasman D, Ridker P, Brody J, de Andrade M, Heit JA, Tang W, DeVivo I, Grodstein F, Smith NL, Tregouet D, Kabrhel C, Consortium I. Assessing the causal relationship between obesity and venous thromboembolism through a Mendelian Randomization study. Hum Genet. 2017;136:897-902
15. 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
16. Finkelstein EA, Khavjou OA, Thompson H, Trogdon JG, Pan L, Sherry B, Dietz W. Obesity and severe obesity forecasts through 2030. Am J Prev Med. 2012;42:563-570
17. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring). 2008;16:2323-2330
18. World Health Organization (WHO). Fact sheet: Obesity and overweight. 2018. Available at: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Accessed February 24, 2020
19. Arshad N, Isaksen T, Hansen JB, Braekkan SK. Time trends in incidence rates of venous thromboembolism in a large cohort recruited from the general population. Eur J Epidemiol. 2017;32:299-305
20. 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

21. Münster AM, Rasmussen TB, Falstie-Jensen AM, Harboe L, Stynes G, Dybro L, Hansen ML, Brandes A, Grove EL, Johnsen SP. A changing landscape: Temporal trends in incidence and characteristics of patients hospitalized with venous thromboembolism 2006–2015. Thrombosis Research. 2019;176:46-53
22. Glynn RJ, Rosner B. Comparison of Risk Factors for the Competing Risks of Coronary Heart Disease, Stroke, and Venous Thromboembolism. American Journal of Epidemiology. 2005;162:975-982
23. 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
24. Heit JA. Epidemiology of venous thromboembolism. Nature Reviews Cardiology. 2015;12:464-474
25. Cohen AT, Agnelli G, Anderson FA, Arcelus JI, Bergqvist D, Brecht JG, Greer IA, Heit JA, Hutchinson JL, Kakkar AK, Mottier D, Oger E, Samama MM, Spannagl M, Europe VTEIAGi. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. Thromb Haemost. 2007;98:756-764
26. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998;158:585-593
27. Spencer FA, Emery C, Lessard D, Anderson F, Emani S, Aragam J, Becker RC, Goldberg RJ. The Worcester Venous Thromboembolism study: a population-based study of the clinical epidemiology of venous thromboembolism. J Gen Intern Med. 2006;21:722-727
28. Arnesen CAL, Veres K, Horváth-Puhó E, Hansen JB, Sørensen HT, Brækkan SK. Estimated lifetime risk of venous thromboembolism in men and women in a Danish nationwide cohort: impact of competing risk of death. Eur J Epidemiol. 2021
29. Huang W, Goldberg RJ, Anderson FA, Kiefe CI, Spencer FA. Secular Trends in Occurrence of Acute Venous Thromboembolism: The Worcester VTE Study (1985-2009). The American Journal of Medicine. 2014;127:829-839.e825
30. Cushman M, Tsai AW, White RH, Heckbert SR, Rosamond WD, Enright P, Folsom AR. Deep vein thrombosis and pulmonary embolism in two cohorts: the longitudinal investigation of thromboembolism etiology. Am J Med. 2004;117:19-25
31. Severinsen MT, Johnsen SP, Tjønneland A, Overvad K, Dethlefsen C, Kristensen SR. Body height and sex-related differences in incidence of venous thromboembolism: a Danish follow-up study. Eur J Intern Med. 2010;21:268-272
32. Roach RE, Cannegieter SC, Lijfering WM. Differential risks in men and women for first and recurrent venous thrombosis: the role of genes and environment. J Thromb Haemost. 2014;12:1593-1600
33. Roach RE, Lijfering WM, Rosendaal FR, Cannegieter SC, le Cessie S. Sex difference in risk of second but not of first venous thrombosis: paradox explained. Circulation. 2014;129:51-56
34. Yeh RW, Sidney S, Chandra M, Sorel M, Selby JV, Go AS. Population trends in the incidence and outcomes of acute myocardial infarction. N Engl J Med. 2010;362:2155-2165
35. Vangen-Lønne AM, Wilsgaard T, Johnsen SH, Carlsson M, Mathiesen EB. Time trends in incidence and case fatality of ischemic stroke: the tromsø study 1977-2010. Stroke. 2015;46:1173-1179
36. Mannsverk J, Wilsgaard T, Mathiesen EB, Løchen ML, Rasmussen K, Thelle DS, Njølstad I, Hopstock LA, Bønaa KH. Trends in Modifiable Risk Factors Are Associated With Declining Incidence of Hospitalized and Nonhospitalized Acute Coronary Heart Disease in a Population. Circulation. 2016;133:74-81
37. Timp JF, Braekkan SK, Versteeg HH, Cannegieter SC. Epidemiology of cancer-associated venous thrombosis. Blood. 2013;122:1712-1723
38. Rahmani J, Haghighian Roudsari A, Bawadi H, Thompson J, Khalooei Fard R, Clark C, Ryan PM, Ajami M, Rahimi Sakak F, Salehisahlabadi A, Abdulazeem HM, Jamali MR, Mirzay Razaz J. 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

39. Tagalakis V, Patenaude V, Kahn SR, Suissa S. Incidence of and mortality from venous thromboembolism in a real-world population: the Q-VTE Study Cohort. Am J Med. 2013;126:832.e813-821
40. Bikdeli B, Wang Y, Jimenez D, Parikh SA, Monreal M, Goldhaber SZ, Krumholz HM. Pulmonary Embolism Hospitalization, Readmission, and Mortality Rates in US Older Adults, 1999-2015. Jama. 2019;322:574-576
41. Raskob GE, Angchaisuksiri P, Blanco AN, Buller H, Gallus A, Hunt BJ, Hylek EM, Kakkar A, Konstantinides SV, McCumber M, Ozaki Y, Wendelboe A, Weitz JI. Thrombosis: a major contributor to global disease burden. Arterioscler Thromb Vasc Biol. 2014;34:2363-2371
42. Grosse SD, Nelson RE, Nyarko KA, Richardson LC, Raskob GE. The economic burden of incident venous thromboembolism in the United States: A review of estimated attributable healthcare costs. Thromb Res. 2016;137:3-10
43. van Korlaar IM, Vossen CY, Rosendaal FR, Bovill EG, Cushman M, Naud S, Kaptein AA. The impact of venous thrombosis on quality of life. Thromb Res. 2004;114:11-18
44. Kahn SR, Shbaklo H, Lamping DL, Holcroft CA, Shrier I, Miron MJ, Roussin A, Desmarais S, Joyal F, Kassis J, Solymoss S, Desjardins L, Johri M, Ginsberg JS. Determinants of health-related quality of life during the 2 years following deep vein thrombosis. J Thromb Haemost. 2008;6:1105-1112
45. Braekkan SK, Grosse SD, Okoroh EM, Tsai J, Cannegieter SC, Naess IA, Krokstad S, Hansen JB, Skjeldestad FE. Venous thromboembolism and subsequent permanent work-related disability. J Thromb Haemost. 2016;14:1978-1987
46. Jørgensen H, Horváth-Puhó E, Laugesen K, Brækkan S, Hansen JB, Sørensen HT. Risk of a permanent work-related disability pension after incident venous thromboembolism in Denmark: A population-based cohort study. PLoS Med. 2021;18:e1003770
47. Arshad N, Bjøri E, Hindberg K, Isaksen T, Hansen JB, Brækkan SK. Recurrence and mortality after first venous thromboembolism in a large population‐based cohort. Journal of Thrombosis and Haemostasis. 2017;15:295-303
48. Schulman S, Lindmarker P, Holmstrom M, Larfars G, Carlsson A, Nicol P, Svensson E, Ljungberg B, Viering S, Nordlander S, Leijd B, Jahed K, Hjorth M, Linder O, Beckman M. Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. Journal of Thrombosis and Haemostasis. 2006;4:734-742
49. Heit JA, Lahr BD, Ashrani AA, Petterson TM, Bailey KR. Predictors of venous thromboembolism recurrence, adjusted for treatments and interim exposures: a population-based case-cohort study. Thromb Res. 2015;136:298-307
50. Cannegieter SC, van Hylckama Vlieg A. Venous thrombosis: understanding the paradoxes of recurrence. J Thromb Haemost. 2013;11 Suppl 1:161-169
51. Ashrani AA, Heit JA. Incidence and cost burden of post-thrombotic syndrome. J Thromb Thrombolysis. 2009;28:465-476
52. Kahn SR. The post-thrombotic syndrome. Hematology Am Soc Hematol Educ Program. 2016;2016:413-418
53. Klok FA, van der Hulle T, den Exter PL, Lankeit M, Huisman MV, Konstantinides S. The post-PE syndrome: a new concept for chronic complications of pulmonary embolism. Blood Rev. 2014;28:221-226
54. Sista AK, Klok FA. Late outcomes of pulmonary embolism: The post-PE syndrome. Thromb Res. 2018;164:157-162
55. Kahn SR, Solymoss S, Lamping DL, Abenhaim L. Long-term outcomes after deep vein thrombosis: postphlebitic syndrome and quality of life. J Gen Intern Med. 2000;15:425-429
56. Ende-Verhaar YM, Cannegieter SC, Vonk Noordegraaf A, Delcroix M, Pruszczyk P, Mairuhu AT, Huisman MV, Klok FA. Incidence of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: a contemporary view of the published literature. Eur Respir J. 2017;49
57. Lijfering WM, Rosendaal FR, Cannegieter SC. Risk factors for venous thrombosis - current understanding from an epidemiological point of view. Br J Haematol. 2010;149:824-833

58. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost. 2005;3:1611-1617
59. Crous-Bou M, Harrington LB, Kabrhel C. Environmental and Genetic Risk Factors Associated with Venous Thromboembolism. Semin Thromb Hemost. 2016;42:808-820
60. Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM, Coll I, Felices R, Stone W, Fontcuberta J, Blangero J. Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. Am J Hum Genet. 2000;67:1452-1459
61. Heit JA, Phelps MA, Ward SA, Slusser JP, Petterson TM, De Andrade M. Familial segregation of venous thromboembolism. J Thromb Haemost. 2004;2:731-736
62. Martinelli I, De Stefano V, Mannucci PM. Inherited risk factors for venous thromboembolism. Nat Rev Cardiol. 2014;11:140-156
63. MacArthur J, Bowler E, Cerezo M, Gil L, Hall P, Hastings E, Junkins H, McMahon A, Milano A, Morales J, Pendlington ZM, Welter D, Burdett T, Hindorff L, Flicek P, Cunningham F, Parkinson H. The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res. 2017;45:D896-d901
64. Trégouët DA, Heath S, Saut N, Biron-Andreani C, Schved JF, Pernod G, Galan P, Drouet L, Zelenika D, Juhan-Vague I, Alessi MC, Tiret L, Lathrop M, Emmerich J, Morange PE. Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. Blood. 2009;113:5298-5303
65. Germain M, Saut N, Greliche N, et al. Genetics of venous thrombosis: insights from a new genome wide association study. PLoS One. 2011;6:e25581
66. Hinds DA, Buil A, Ziemek D, Martinez-Perez A, Malik R, Folkersen L, Germain M, Mälarstig A, Brown A, Soria JM, Dichgans M, Bing N, Franco-Cereceda A, Souto JC, Dermitzakis ET, Hamsten A, Worrall BB, Tung JY, Sabater-Lleal M. Genome-wide association analysis of self-reported events in 6135 individuals and 252 827 controls identifies 8 loci associated with thrombosis. Hum Mol Genet. 2016;25:1867-1874
67. 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
68. 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
69. de Haan HG, Bezemer ID, Doggen CJ, Le Cessie S, Reitsma PH, Arellano AR, Tong CH, Devlin JJ, Bare LA, Rosendaal FR, Vossen CY. Multiple SNP testing improves risk prediction of first venous thrombosis. Blood. 2012;120:656-663
70. Morange PE, Tregouet DA. Lessons from genome-wide association studies in venous thrombosis. Journal of Thrombosis and Haemostasis. 2011;9:258-264
71. Germain M, Chasman DI, de Haan H, et al. Meta-analysis of 65,734 individuals identifies TSPAN15 and SLC44A2 as two susceptibility loci for venous thromboembolism. Am J Hum Genet. 2015;96:532-542
72. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-67
73. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood. 1996;88:3698-3703
74. Franchini M, Makris M. Non-O blood group: an important genetic risk factor for venous thromboembolism. Blood Transfus. 2013;11:164-165
75. Li Y, Bezemer ID, Rowland CM, Tong CH, Arellano AR, Catanese JJ, Devlin JJ, Reitsma PH, Bare LA, Rosendaal FR. Genetic variants associated with deep vein thrombosis: the F11 locus. J Thromb Haemost. 2009;7:1802-1808
76. Uitte De Willige S, De Visser MCH, Houwing-Duistermaat JJ, Rosendaal FR, Vos HL, Bertina RM. Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen γ' levels. Blood. 2005;106:4176-4183

77. Mahajerin A, Croteau SE. Epidemiology and Risk Assessment of Pediatric Venous Thromboembolism. Front Pediatr. 2017;5:68
78. White RH. The Epidemiology of Venous Thromboembolism. Circulation. 2003;107:4I--8
79. Lazo-Langner A, Liu K, Shariff S, Garg AX, Ray JG. Immigration, region of origin, and the epidemiology of venous thromboembolism: A population-based study. Res Pract Thromb Haemost. 2018;2:469-480
80. Engbers MJ, van Hylckama Vlieg A, Rosendaal FR. Venous thrombosis in the elderly: incidence, risk factors and risk groups. J Thromb Haemost. 2010;8:2105-2112
81. Bovill EG, van der Vliet A. Venous valvular stasis-associated hypoxia and thrombosis: what is the link? Annu Rev Physiol. 2011;73:527-545
82. McLachlin AD, McLachlin JA, Jory TA, Rawling EG. Venous stasis in the lower extremities. Ann Surg. 1960;152:678-685
83. Walker AJ, Card TR, West J, Crooks C, Grainge MJ. Incidence of venous thromboembolism in patients with cancer - a cohort study using linked United Kingdom databases. Eur J Cancer. 2013;49:1404-1413
84. Cronin-Fenton DP, Søndergaard F, Pedersen LA, Fryzek JP, Cetin K, Acquavella J, Baron JA, Sørensen HT. Hospitalisation for venous thromboembolism in cancer patients and the general population: a population-based cohort study in Denmark, 1997-2006. Br J Cancer. 2010;103:947-953
85. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, 3rd. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. Arch Intern Med. 2000;160:809-815
86. White RH, Zhou H, Romano PS. Incidence of symptomatic venous thromboembolism after different elective or urgent surgical procedures. Thromb Haemost. 2003;90:446-455
87. Geerts WH, Code KI, Jay RM, Chen E, Szalai JP. A prospective study of venous thromboembolism after major trauma. N Engl J Med. 1994;331:1601-1606
88. Pottier P, Hardouin JB, Lejeune S, Jolliet P, Gillet B, Planchon B. Immobilization and the risk of venous thromboembolism. A meta-analysis on epidemiological studies. Thromb Res. 2009;124:468-476
89. Sejrup JK, Børvik T, Grimnes G, Isaksen T, Hindberg K, Hansen JB, Morelli VM, Brækkan SK. Myocardial Infarction as a Transient Risk Factor for Incident Venous Thromboembolism: Results from a Population-Based Case-Crossover Study. Thromb Haemost. 2019;119:1358-1364
90. Morelli VM, Sejrup JK, Småbrekke B, Rinde LB, Grimnes G, Isaksen T, Hansen JB, Hindberg K, Brækkan SK. The Role of Stroke as a Trigger for Incident Venous Thromboembolism: Results from a Population-based Case-Crossover Study. TH Open. 2019;3:e50-e57
91. Samama MM, Dahl OE, Quinlan DJ, Mismetti P, Rosencher N. Quantification of risk factors for venous thromboembolism: a preliminary study for the development of a risk assessment tool. Haematologica. 2003;88:1410-1421
92. Heit JA, O'Fallon WM, Petterson TM, Lohse CM, Silverstein MD, Mohr DN, Melton LJ, 3rd. Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study. Arch Intern Med. 2002;162:1245-1248
93. Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. J Thromb Haemost. 2008;6:632-637
94. Heit JA, Kobbervig CE, James AH, Petterson TM, Bailey KR, Melton LJ, 3rd. Trends in the incidence of venous thromboembolism during pregnancy or postpartum: a 30-year population-based study. Ann Intern Med. 2005;143:697-706
95. Stegeman BH, de Bastos M, Rosendaal FR, van Hylckama Vlieg A, Helmerhorst FM, Stijnen T, Dekkers OM. Different combined oral contraceptives and the risk of venous thrombosis: systematic review and network meta-analysis. Bmj. 2013;347:f5298
96. Jacobsen AF, Sandset PM. Venous thromboembolism associated with pregnancy and hormonal therapy. Best Pract Res Clin Haematol. 2012;25:319-332

97. Cornier MA, Després JP, Davis N, Grossniklaus DA, Klein S, Lamarche B, Lopez-Jimenez F, Rao G, St-Onge MP, Towfighi A, Poirier P. Assessing adiposity: a scientific statement from the American Heart Association. Circulation. 2011;124:1996-2019
98. Wright SM, Aronne LJ. Causes of obesity. Abdom Imaging. 2012;37:730-732
99. Aronne LJ, Nelinson DS, Lillo JL. Obesity as a disease state: a new paradigm for diagnosis and treatment. Clin Cornerstone. 2009;9:9-25; discussion 26-29
100. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017-2018. NCHS Data Brief. 2020:1-8
101. Ritchie H, Roser M. Obesity. OurWorldInData.org. 2017. Available at: https://ourworldindata.org/obesity. Accessed January 13 2022
102. Pineda E, Sanchez-Romero LM, Brown M, Jaccard A, Jewell J, Galea G, Webber L, Breda J. Forecasting Future Trends in Obesity across Europe: The Value of Improving Surveillance. Obes Facts. 2018;11:360-371
103. Mann T, Tomiyama AJ, Westling E, Lew AM, Samuels B, Chatman J. Medicare's search for effective obesity treatments: diets are not the answer. Am Psychol. 2007;62:220-233
104. Hall KD, Kahan S. Maintenance of Lost Weight and Long-Term Management of Obesity. Med Clin North Am. 2018;102:183-197
105. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr. 2001;74:579-584
106. Bray GA, Kim KK, Wilding JPH. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Obes Rev. 2017;18:715-723
107. Collaborators GBDRF. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1923-1994
108. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular Risk Factors and Venous Thromboembolism Incidence. 2002;162:1182
109. Pomp ER, le Cessie S, Rosendaal FR, Doggen CJ. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. Br J Haematol. 2007;139:289-296
110. Lutsey PL, Virnig BA, Durham SB, Steffen LM, Hirsch AT, Jacobs DR, Jr., Folsom AR. Correlates and consequences of venous thromboembolism: The Iowa Women's Health Study. Am J Public Health. 2010;100:1506-1513
111. Holst AG, Jensen G, Prescott E. Risk Factors for Venous Thromboembolism. Circulation. 2010;121:1896-1903
112. Horvei LD, Brækkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Hansen J-B. Obesity measures and risk of venous thromboembolism and myocardial infarction. European Journal of Epidemiology. 2014;29:821-830
113. Borch KH, Brækkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Størmer J, Hansen J-B. Anthropometric Measures of Obesity and Risk of Venous Thromboembolism. Arteriosclerosis, Thrombosis, and Vascular Biology. 2010;30:121-127
114. Severinsen MT, Kristensen SR, Johnsen SP, Dethlefsen C, Tjønneland A, Overvad K. Anthropometry, Body Fat, and Venous Thromboembolism. Circulation. 2009;120:1850-1857
115. Horvei LD, Braekkan SK, Hansen JB. Weight Change and Risk of Venous Thromboembolism: The Tromso Study. PLoS One. 2016;11:e0168878
116. French SA, Lutsey PL, Rosamond W, Maclehose RF, Cushman M, Folsom AR. Weight change over 9 years and subsequent risk of venous thromboembolism in the ARIC cohort. International Journal of Obesity. 2020
117. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. Bmj. 2018;362:k601
118. De Mutsert R, Jager KJ, Zoccali C, Dekker FW. The effect of joint exposures: examining the presence of interaction. Kidney International. 2009;75:677-681
119. Severinsen MT, Overvad K, Johnsen SP, Dethlefsen C, Madsen PH, Tjonneland A, Kristensen SR. Genetic susceptibility, smoking, obesity and risk of venous thromboembolism. Br J Haematol. 2010;149:273-279

120. Christiansen SC, Lijfering WM, Naess IA, Hammerstrom J, van Hylckama Vlieg A, Rosendaal FR, Cannegieter SC. The relationship between body mass index, activated protein C resistance and risk of venous thrombosis. J Thromb Haemost. 2012;10:1761-1767
121. Ribeiro DD, Lijfering WM, Rosendaal FR, Cannegieter SC. Risk of venous thrombosis in persons with increased body mass index and interactions with other genetic and acquired risk factors. J Thromb Haemost. 2016;14:1572-1578
122. Kim J, Kraft P, Hagan KA, Harrington LB, Lindstroem S, Kabrhel C. Interaction of a genetic risk score with physical activity, physical inactivity, and body mass index in relation to venous thromboembolism risk. Genet Epidemiol. 2018;42:354-365
123. Borch KH, Nyegaard C, Hansen JB, Mathiesen EB, Njølstad I, Wilsgaard T, Brækkan SK. Joint effects of obesity and body height on the risk of venous thromboembolism: the Tromsø Study. Arterioscler Thromb Vasc Biol. 2011;31:1439-1444
124. Rinde LB, Lind C, Smabrekke B, Njolstad I, Mathiesen EB, Wilsgaard T, Lochen ML, Hald EM, Vik A, Braekkan SK, Hansen JB. Impact of incident myocardial infarction on the risk of venous thromboembolism: the Tromso Study. J Thromb Haemost. 2016;14:1183-1191
125. Larsson SC, Burgess S. Causal role of high body mass index in multiple chronic diseases: a systematic review and meta-analysis of Mendelian randomization studies. BMC Med. 2021;19:320
126. Sejrup JK, Tøndel BG, Morelli VM, Løchen ML, Njølstad I, Mathiesen EB, Wilsgaard T, Hansen JB, Braekkan SK. Joint effect of myocardial infarction and obesity on the risk of venous thromboembolism: The Tromsø Study. J Thromb Haemost. 2022
127. Bagot CN, Arya R. Virchow and his triad: a question of attribution. Br J Haematol. 2008;143:180-190
128. Lund F, Diener L, Ericsson JL. Postmortem intraosseous phlebography as an aid in studies of venous thromboembolism. With application on a geriatric clientele. Angiology. 1969;20:155-176
129. Sevitt S. The structure and growth of valve-pocket thrombi in femoral veins. J Clin Pathol. 1974;27:517-528
130. Hoffman M, Monroe DM, 3rd. A cell-based model of hemostasis. Thromb Haemost. 2001;85:958-965
131. Kannemeier C, Shibamiya A, Nakazawa F, Trusheim H, Ruppert C, Markart P, Song Y, Tzima E, Kennerknecht E, Niepmann M, von Bruehl ML, Sedding D, Massberg S, Günther A, Engelmann B, Preissner KT. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. Proc Natl Acad Sci U S A. 2007;104:6388-6393
132. Müller F, Mutch NJ, Schenk WA, Smith SA, Esterl L, Spronk HM, Schmidbauer S, Gahl WA, Morrissey JH, Renné T. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. Cell. 2009;139:1143-1156
133. Karino T, Goldsmith HL, Motomiya M, Mabuchi S, Sohara Y. Flow patterns in vessels of simple and complex geometries. Ann N Y Acad Sci. 1987;516:422-441
134. Karino T, Motomiya M. Flow through a venous valve and its implication for thrombus formation. Thromb Res. 1984;36:245-257
135. Lurie F, Kistner RL, Eklof B, Kessler D. Mechanism of venous valve closure and role of the valve in circulation: a new concept. J Vasc Surg. 2003;38:955-961
136. Zhou L, Qi XL, Xu MX, Mao Y, Liu ML, Song HM. Microparticles: new light shed on the understanding of venous thromboembolism. Acta Pharmacol Sin. 2014;35:1103-1110
137. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365:1415-1428
138. Steffen LM, Cushman M, Peacock JM, Heckbert SR, Jacobs Jr DR, Rosamond WD, Folsom AR. Metabolic syndrome and risk of venous thromboembolism: Longitudinal Investigation of Thromboembolism Etiology. Journal of Thrombosis and Haemostasis. 2009;7:746-751
139. Borch KH, Braekkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Størmer J, Hansen JB. Abdominal obesity is essential for the risk of venous thromboembolism in the metabolic syndrome: the Tromsø study. 2009;7:739-745
140. Willenberg T, Schumacher A, Amann-Vesti B, Jacomella V, Thalhammer C, Diehm N, Baumgartner I, Husmann M. Impact of obesity on venous hemodynamics of the lower limbs. J Vasc Surg. 2010;52:664-668

141. Faber DR, De Groot PG, Visseren FLJ. Role of adipose tissue in haemostasis, coagulation and fibrinolysis. Obesity Reviews. 2009;10:554-563
142. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85-97
143. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. Obes Rev. 2002;3:85-101
144. 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
145. Arfvidsson B, Eklof B, Balfour J. Iliofemoral venous pressure correlates with intraabdominal pressure in morbidly obese patients. Vasc Endovascular Surg. 2005;39:505-509
146. Fasshauer M, Blüher M. Adipokines in health and disease. Trends in Pharmacological Sciences. 2015;36:461-470
147. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. Jama. 1999;282:2131-2135
148. Olson NC, Cushman M, Lutsey PL, McClure LA, Judd S, Tracy RP, Folsom AR, Zakai NA. Inflammation markers and incident venous thromboembolism: the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. J Thromb Haemost. 2014;12:1993-2001
149. Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njolstad I, Wilsgaard T, Brox J, Braekkan SK, Hansen JB. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. J Thromb Haemost. 2016;14:1561-1571
150. Cushman M, Yanez D, Psaty BM, Fried LP, Heiss G, Lee M, Polak JF, Savage PJ, Tracy RP. Association of fibrinogen and coagulation factors VII and VIII with cardiovascular risk factors in the elderly: the Cardiovascular Health Study. Cardiovascular Health Study Investigators. Am J Epidemiol. 1996;143:665-676
151. Rosito G, D'Agostino R, Massaro J, Lipinska I, Mittleman M, Sutherland P, Wilson P, Levy D, Muller J, Tofler G. Association between obesity and a prothrombotic state: the Framingham Offspring Study. Thrombosis and Haemostasis. 2004;91:683-689
152. Abdollahi M, Cushman M, Rosendaal F. Obesity: risk of venous thrombosis and the interaction with coagulation factor levels and oral contraceptive use. Thrombosis and Haemostasis. 2003;89:493-498
153. Kaye SM, Pietilainen KH, Kotronen A, Joutsi-Korhonen L, Kaprio J, Yki-Jarvinen H, Silveira A, Hamsten A, Lassila R, Rissanen A. Obesity-related derangements of coagulation and fibrinolysis: a study of obesity-discordant monozygotic twin pairs. Obesity (Silver Spring). 2012;20:88-94
154. Lowe GD, Rumley A, Woodward M, Reid E, Rumley J. Activated protein C resistance and the FV:R506Q mutation in a random population sample--associations with cardiovascular risk factors and coagulation variables. Thromb Haemost. 1999;81:918-924
155. Lisman T, de Groot PG, Meijers JC, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. Blood. 2005;105:1102-1105
156. Meltzer ME, Lisman T, de Groot PG, Meijers JC, le Cessie S, Doggen CJ, Rosendaal FR. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. Blood. 2010;116:113-121
157. Meltzer M, Doggen C, De Groot P, Rosendaal F, Lisman T. The Impact of the Fibrinolytic System on the Risk of Venous and Arterial Thrombosis. Seminars in Thrombosis and Hemostasis. 2009;35:468-477
158. 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
159. 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
160. Bastelica D, Morange P, Berthet B, Borghi H, Lacroix O, Grino M, Juhan-Vague I, Alessi MC. 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
161. Markiewski MM, Nilsson B, Nilsson Ekdahl K, Mollnes TE, Lambris JD. Complement and coagulation: strangers or partners in crime? Trends in Immunology. 2007;28:184-192
162. Oikonomopoulou K, Ricklin D, Ward PA, Lambris JD. Interactions between coagulation and complement—their role in inflammation. 2012;34:151-165
163. Hoiland, II, Liang RA, Braekkan SK, Pettersen K, Ludviksen JK, Latysheva N, Snir O, Ueland T, Hindberg K, Mollnes TE, Hansen JB. Complement activation assessed by the plasma terminal complement complex and future risk of venous thromboembolism. J Thromb Haemost. 2019;17:934-943
164. Liang RA, Hoiland, II, Ueland T, Aukrust P, Snir O, Hindberg K, Braekkan SK, Garred P, Mollnes TE, Hansen JB. Plasma levels of mannose-binding lectin and future risk of venous thromboembolism. J Thromb Haemost. 2019;17:1661-1669
165. Vlaicu SI, Tatomir A, Boodhoo D, Vesa S, Mircea PA, Rus H. The role of complement system in adipose tissue-related inflammation. 2016
166. White RT, Damm D, Hancock N, Rosen BS, Lowell BB, Usher P, Flier JS, Spiegelman BM. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. J Biol Chem. 1992;267:9210-9213
167. Fain JN, Nesbit AS, Sudlow FF, Cheema P, Peeples JM, Madan AK, Tichansky DS. Release in vitro of adipsin, vascular cell adhesion molecule 1, angiotensin 1–converting enzyme, and soluble tumor necrosis factor receptor 2 by human omental adipose tissue as well as by the nonfat cells and adipocytes. Metabolism. 2007;56:1583-1590
168. Volanakis JE, Narayana SV. Complement factor D, a novel serine protease. Protein Science. 1996;5:553-564
169. Suankratay C, Zhang XH, Zhang Y, Lint TF, Gewurz H. Requirement for the alternative pathway as well as C4 and C2 in complement-dependent hemolysis via the lectin pathway. J Immunol. 1998;160:3006-3013
170. van Es N, Hisada Y, Di Nisio M, Cesarman G, Kleinjan A, Mahe I, Otten HM, Kamphuisen PW, Berckmans RJ, Buller HR, Mackman N, Nieuwland R. Extracellular vesicles exposing tissue factor for the prediction of venous thromboembolism in patients with cancer: A prospective cohort study. Thromb Res. 2018;166:54-59
171. Campello E, Spiezia L, Radu CM, Bulato C, Castelli M, Gavasso S, Simioni P. Endothelial, platelet, and tissue factor-bearing microparticles in cancer patients with and without venous thromboembolism. Thromb Res. 2011;127:473-477
172. Gheldof D, Haguet H, Dogné JM, Bouvy C, Graux C, George F, Sonet A, Chatelain C, Chatelain B, Mullier F. Procoagulant activity of extracellular vesicles as a potential biomarker for risk of thrombosis and DIC in patients with acute leukaemia. J Thromb Thrombolysis. 2017;43:224-232
173. Pardo F, Villalobos-Labra R, Sobrevia B, Toledo F, Sobrevia L. Extracellular vesicles in obesity and diabetes mellitus. Molecular Aspects of Medicine. 2018;60:81-91
174. Bucciarelli P, Martinelli I, Artoni A, Passamonti SM, Previtali E, Merati G, Tripodi A, Mannucci PM. Circulating microparticles and risk of venous thromboembolism. Thrombosis Research. 2012;129:591-597
175. Chirinos JA, Heresi GA, Velasquez H, Jy W, Jimenez JJ, Ahn E, Horstman LL, Soriano AO, Zambrano JP, Ahn YS. Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. J Am Coll Cardiol. 2005;45:1467-1471
176. Snir O, Wilsgård L, Latysheva N, Wahlund CJE, Braekkan SK, Hindberg K, Hansen JB. Plasma levels of platelet-derived microvesicles are associated with risk of future venous thromboembolism. J Thromb Haemost. 2022
177. Jamaly S, Basavaraj MG, Starikova I, Olsen R, Braekkan SK, Hansen JB. Elevated plasma levels of P-selectin glycoprotein ligand-1-positive microvesicles in patients with unprovoked venous thromboembolism. J Thromb Haemost. 2018
178. Napoleone E, A DIS, Amore C, Baccante G, di Febbo C, Porreca E, de Gaetano G, Donati MB, Lorenzet R. 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
179. Rafail S, Ritis K, Schaefer K, Kourtzelis I, Speletas M, Doumas M, Giaglis S, Kambas K, Konstantinides S, Kartalis G. Leptin induces the expression of functional tissue factor in human neutrophils and peripheral blood mononuclear cells through JAK2-dependent mechanisms and TNFalpha involvement. Thromb Res. 2008;122:366-375
180. Singh P, Peterson TE, Barber KR, Kuniyoshi FS, Jensen A, Hoffmann M, Shamsuzzaman AS, Somers VK. Leptin upregulates the expression of plasminogen activator inhibitor-1 in human vascular endothelial cells. Biochem Biophys Res Commun. 2010;392:47-52
181. Myers MG, Cowley MA, Munzberg H. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol. 2008;70:537-556
182. Fang H, Judd RL. Adiponectin Regulation and Function. Compr Physiol. 2018;8:1031-1063
183. Okamoto Y, Ishii S, Croce K, Katsumata H, Fukushima M, Kihara S, Libby P, Minami S. Adiponectin inhibits macrophage tissue factor, a key trigger of thrombosis in disrupted atherosclerotic plaques. Atherosclerosis. 2013;226:373-377
184. Chen YJ, Zhang LQ, Wang GP, Zeng H, Lü B, Shen XL, Jiang ZP, Chen FP. Adiponectin inhibits tissue factor expression and enhances tissue factor pathway inhibitor expression in human endothelial cells. Thromb Haemost. 2008;100:291-300
185. Vilahur G, Ben-Aicha S, Badimon L. New insights into the role of adipose tissue in thrombosis. Cardiovascular Research. 2017;113:1046-1054
186. Schafer K, Konstantinides S. Adipokines and thrombosis. Clin Exp Pharmacol Physiol. 2011;38:864-871
187. Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, Peto R. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. Am J Epidemiol. 1999;150:341-353
188. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. Int J Epidemiol. 2012;41:961-967
189. The Tromsø Study Available at: https://uit.no/research/tromsostudy. Accessed March 23, 2022
190. Eggen AE, Mathiesen EB, Wilsgaard T, Jacobsen BK, Njølstad I. The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. Scand J Public Health. 2013;41:65-80
191. Holmen J, Midthjell K, Krüger Ø, Langhammer A, Holmen TL, Bratberg GH, Vatten L, Lund-Larsen PG. The Nord-Trøndelag Health Study 1995-97 (HUNT 2). Norsk Epidemiologi. 2011;13:19-32
192. About HUNT. Available at: https://www.ntnu.edu/web/hunt/about-hunt. Accessed March 23 2022
193. Horvei LD, Braekkan SK, Smith EN, Solomon T, Hindberg K, Frazer KA, Rosendaal FR, Hansen JB. Joint effects of prothrombotic genotypes and body height on the risk of venous thromboembolism: the Tromso study. J Thromb Haemost. 2018;16:83-89
194. 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
195. Gottschalk A, Sostman HD, Coleman RE, Juni JE, Thrall J, McKusick KA, Froelich JW, Alavi A. Ventilation-perfusion scintigraphy in the PIOPED study. Part II. Evaluation of the scintigraphic criteria and interpretations. J Nucl Med. 1993;34:1119-1126
196. PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). Jama. 1990;263:2753-2759
197. Miettinen OS. Proportion of disease caused or prevented by a given exposure, trait or intervention. Am J Epidemiol. 1974;99:325-332
198. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. Am J Public Health. 1998;88:15-19

199. Mansournia MA, Altman DG. Population attributable fraction. Bmj. 2018;360:k757
200. Rothman KJ. Measuring interactions. In: Epidemiology: An Introduction. New York: Oxford University Press; 2002:168–180
201. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol. 2005;20:575-579
202. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. Int J Epidemiol. 2013;42:1511-1519
203. Breen R, Karlson KB, Holm A. Total, Direct, and Indirect Effects in Logit and Probit Models. Sociological Methods & Research. 2013;42:164-191
204. Rothman KJ. *Epidemiology : an introduction.* 2nd ed. Oxford: Oxford University Press; 2012
205. Euser AM, Zoccali C, Jager KJ, Dekker FW. Cohort studies: prospective versus retrospective. Nephron Clin Pract. 2009;113:c214-217
206. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Human Molecular Genetics. 2014;23:R89-R98
207. Waldram A, McKerr C, Gobin M, Adak G, Stuart JM, Cleary P. Control selection methods in recent case-control studies conducted as part of infectious disease outbreaks. Eur J Epidemiol. 2015;30:465-471
208. Szklo M, Nieto FJ. *Epidemiology : beyond the basics.* 3rd ed. Burlington, Mass: Jones & Bartlett Learning; 2014
209. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?*. International Journal of Epidemiology. 2003;32:1-22
210. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. International Journal of Epidemiology. 2015;44:512-525
211. Ferguson L. External validity, generalizability, and knowledge utilization. J Nurs Scholarsh. 2004;36:16-22
212. Galea S, Tracy M. Participation Rates in Epidemiologic Studies. Annals of Epidemiology. 2007;17:643-653
213. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology.* Lippincott Williams & Wilkins.; 2008
214. Tripepi G, Jager KJ, Dekker FW, Zoccali C. Selection bias and information bias in clinical research. Nephron Clin Pract. 2010;115:c94-99
215. Yuan S, Bruzelius M, Xiong Y, Håkansson N, Åkesson A, Larsson SC. Overall and abdominal obesity in relation to venous thromboembolism. J Thromb Haemost. 2021;19:460-469
216. Borch KH, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J, Hansen JB. Anthropometric measures of obesity and risk of venous thromboembolism: the Tromso study. Arterioscler Thromb Vasc Biol. 2010;30:121-127
217. Horvei LD, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Hansen JB. Obesity measures and risk of venous thromboembolism and myocardial infarction. Eur J Epidemiol. 2014;29:821-830
218. Behrens M, Lange R. A highly reproducible and economically competitive SNP analysis of several well characterized human mutations. Clin Lab. 2004;50:305-316
219. Benson JM, Ellingsen D, El-Jamil M, Jenkins M, Miller CH, Dilley A, Evatt BL, Hooper WC. Factor V Leiden and factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb Haemost. 2001;86:1188-1192
220. Hong H, Xu L, Liu J, et al. Technical reproducibility of genotyping SNP arrays used in genome-wide association studies. PLoS One. 2012;7:e44483
221. Smabrekke B, Rinde LB, Hindberg K, Hald EM, Vik A, Wilsgaard T, Lochen ML, Njolstad I, Mathiesen EB, Hansen JB, Braekkan S. Atherosclerotic Risk Factors and Risk of Myocardial Infarction and Venous Thromboembolism; Time-Fixed versus Time-Varying Analyses. The Tromso Study. PLoS One. 2016;11:e0163242
222. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol. 1997;145:72-80

223. Pearce N. Analysis of matched case-control studies. BMJ. 2016;352:i969
224. Marshall JA, Grunwald GK, Donahoo WT, Scarbro S, Shetterly SM. Percent body fat and lean mass explain the gender difference in leptin: analysis and interpretation of leptin in Hispanic and non-Hispanic white adults. Obes Res. 2000;8:543-552
225. Christen T, Trompet S, Noordam R, van Klinken JB, van Dijk KW, Lamb HJ, Cobbaert CM, den Heijer M, Jazet IM, Jukema JW, Rosendaal FR, de Mutsert R. Sex differences in body fat distribution are related to sex differences in serum leptin and adiponectin. Peptides. 2018;107:25-31
226. 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
227. 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
228. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334:292-295
229. Vandenbroucke JP, von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Epidemiology. 2007;18:805-835
230. Rosendaal FR, Reitsma PH. No P Please. J Thromb Haemost. 2016;14:1493
231. Sterne JA, Davey Smith G. Sifting the evidence-what's wrong with significance tests? Bmj. 2001;322:226-231
232. van Rijn MHC, Bech A, Bouyer J, van den Brand J. Statistical significance versus clinical relevance. Nephrol Dial Transplant. 2017;32:ii6-ii12
233. Austin PC, White IR, Lee DS, van Buuren S. Missing Data in Clinical Research: A Tutorial on Multiple Imputation. Can J Cardiol. 2021;37:1322-1331
234. Løvsletten O, Jacobsen BK, Grimsgaard S, Njølstad I, Wilsgaard T, Løchen ML, Eggen AE, Hopstock LA. Prevalence of general and abdominal obesity in 2015-2016 and 8-year longitudinal weight and waist circumference changes in adults and elderly: the Tromsø Study. BMJ Open. 2020;10:e038465
235. Jacobsen BK, Aars NA. Changes in body mass index and the prevalence of obesity during 1994-2008: repeated cross-sectional surveys and longitudinal analyses. The Tromsø Study. BMJ Open. 2015;5:e007859
236. National Institutes of Health State-of-the-Science conference statement: tobacco use: prevention, cessation, and control. Ann Intern Med. 2006;145:839-844
237. Mozaffarian D, Afshin A, Benowitz NL, Bittner V, Daniels SR, Franch HA, Jacobs DR, Jr., Kraus WE, Kris-Etherton PM, Krummel DA, Popkin BM, Whitsel LP, Zakai NA. Population approaches to improve diet, physical activity, and smoking habits: a scientific statement from the American Heart Association. Circulation. 2012;126:1514-1563
238. Folsom AR, Cushman M. Exploring Opportunities for Primary Prevention of Unprovoked Venous Thromboembolism: Ready for Prime Time? J Am Heart Assoc. 2020;9:e019395
239. World Obesity Federation. World Obesity Atlas 2022. 2022. Available at: https://www.worldobesity.org/resources/resource-library/world-obesity-atlas-2022. Accessed 30.06., 2022
240. Crous-Bou M, De Vivo I, Camargo CA, Jr., Varraso R, Grodstein F, Jensen MK, Kraft P, Goldhaber SZ, Lindstrom S, Kabrhel C. Interactions of established risk factors and a GWAS-based genetic risk score on the risk of venous thromboembolism. Thromb Haemost. 2016;116:705-713
241. Morelli VM, de Visser MC, van Tilburg NH, Vos HL, Eikenboom JC, Rosendaal FR, Bertina RM. ABO blood group genotypes, plasma von Willebrand factor levels and loading of von Willebrand factor with A and B antigens. Thromb Haemost. 2007;97:534-541
242. Petrini S, Neri T, Lombardi S, Cordazzo C, Balia C, Scalise V, Paggiaro P, Pedrinelli R, Celi A. Leptin induces the generation of procoagulant, tissue factor bearing microparticles by human peripheral blood mononuclear cells. Biochim Biophys Acta. 2016;1860:1354-1361

243. Ayer JG, Song C, Steinbeck K, Celermajer DS, Ben Freedman S. Increased tissue factor activity in monocytes from obese young adults. Clin Exp Pharmacol Physiol. 2010;37:1049-1054
244. Samad F, Loskutoff DJ. Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. Mol Med. 1996;2:568-582
245. Aprath-Husmann I, Rohrig K, Gottschling-Zeller H, Skurk T, Scriba D, Birgel M, Hauner H. Effects of leptin on the differentiation and metabolism of human adipocytes. Int J Obes Relat Metab Disord. 2001;25:1465-1470
246. Konstantinides S, Schafer K, Neels JG, Dellas C, Loskutoff DJ. Inhibition of endogenous leptin protects mice from arterial and venous thrombosis. Arterioscler Thromb Vasc Biol. 2004;24:2196-2201
247. Mackman N. New insights into the mechanisms of venous thrombosis. J Clin Invest. 2012;122:2331-2336
248. Soderberg S, Olsson T, Eliasson M, Johnson O, Ahren B. Plasma leptin levels are associated with abnormal fibrinolysis in men and postmenopausal women. J Intern Med. 1999;245:533-543
249. Gómez-Ambrosi J, Salvador J, Páramo JA, Orbe J, de Irala J, Diez-Caballero A, Gil MaJ, Cienfuegos JA, Frühbeck G. Involvement of leptin in the association between percentage of body fat and cardiovascular risk factors. Clinical Biochemistry. 2002;35:315-320
250. Skurk T, van Harmelen V, Lee YM, Wirth A, Hauner H. Relationship between IL-6, leptin and adiponectin and variables of fibrinolysis in overweight and obese hypertensive patients. Horm Metab Res. 2002;34:659-663
251. Csongradi E, Kaplar M, Nagy B, Jr., Koch CA, Juhasz A, Bajnok L, Varga Z, Seres I, Karanyi Z, Magyar MT, Olah L, Facsko A, Kappelmayer J, Paragh G. Adipokines as atherothrombotic risk factors in obese subjects: Associations with haemostatic markers and common carotid wall thickness. Nutr Metab Cardiovasc Dis. 2017;27:571-580
252. Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Kelly A, Rumley A, Wallace AM, Sattar N. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. Atherosclerosis. 2007;191:418-426
253. Buis DTP, Christen T, Smit RAJ, de Mutsert R, Jukema JW, Cannegieter SC, Lijfering WM, Rosendaal FR. The association between leptin concentration and blood coagulation: Results from the NEO study. Thromb Res. 2020;188:44-48
254. Lu W, Zhou S, Xue L, Dai B, Qiao L, Chen D, Xu Z, Yao Y, Teng H, Jiang Q. High preoperative serum leptin level is an independent risk factor for deep vein thrombosis after total knee arthroplasty in osteoarthritis patients: A prospective and cross-sectional study. Medicine (Baltimore). 2018;97:e10884
255. Allison MA, Cushman M, Callas PW, Denenberg JO, Jensky NE, Criqui MH. Adipokines are associated with lower extremity venous disease: the San Diego population study. J Thromb Haemost. 2010;8:1912-1918
256. Mrozinska S, Cieslik J, Broniatowska E, Undas A. Elevated leptin and decreased adiponectin independently predict the post-thrombotic syndrome in obese and non-obese patients. Sci Rep. 2018;8:6938
257. Eriksson BI, Eriksson E, Risberg B. Impaired fibrinolysis and postoperative thromboembolism in orthopedic patients. Thromb Res. 1991;62:55-64
258. Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Shen C, Newcomer LM, Goldhaber SZ, Hennekens CH. 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
259. 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
260. Bollen L, Peetermans M, Peeters M, Van Steen K, Hoylaerts MF, Declerck PJ, Verhamme P, Gils A. 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

261. 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
262. 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
263. 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
264. 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
265. Cefalu WT, Schneider DJ, Carlson HE, Migdal P, Gan Lim L, Izon MP, Kapoor A, Bell-Farrow A, Terry JG, Sobel BE. Effect of combination glipizide GITS/metformin on fibrinolytic and metabolic parameters in poorly controlled type 2 diabetic subjects. Diabetes Care. 2002;25:2123-2128
266. Fonseca VA, Theuma P, Mudaliar S, Leissinger CA, Clejan S, Henry RR. Diabetes treatments have differential effects on nontraditional cardiovascular risk factors. J Diabetes Complications. 2006;20:14-20
267. 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
268. Sillen M, Declerck PJ. Targeting PAI-1 in Cardiovascular Disease: Structural Insights Into PAI-1 Functionality and Inhibition. Front Cardiovasc Med. 2020;7:622473

Paper I

Paper II

Paper III

Paper IV

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.

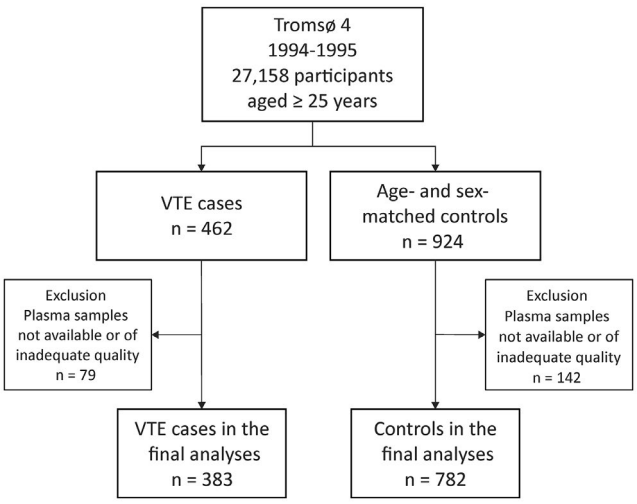

FIGURE 1 Flowchart of the study population. The flowchart illustrates the nested case-control study derived from the fourth survey of the Tromsø study (1994–1995). VTE, venous thromboembolism

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

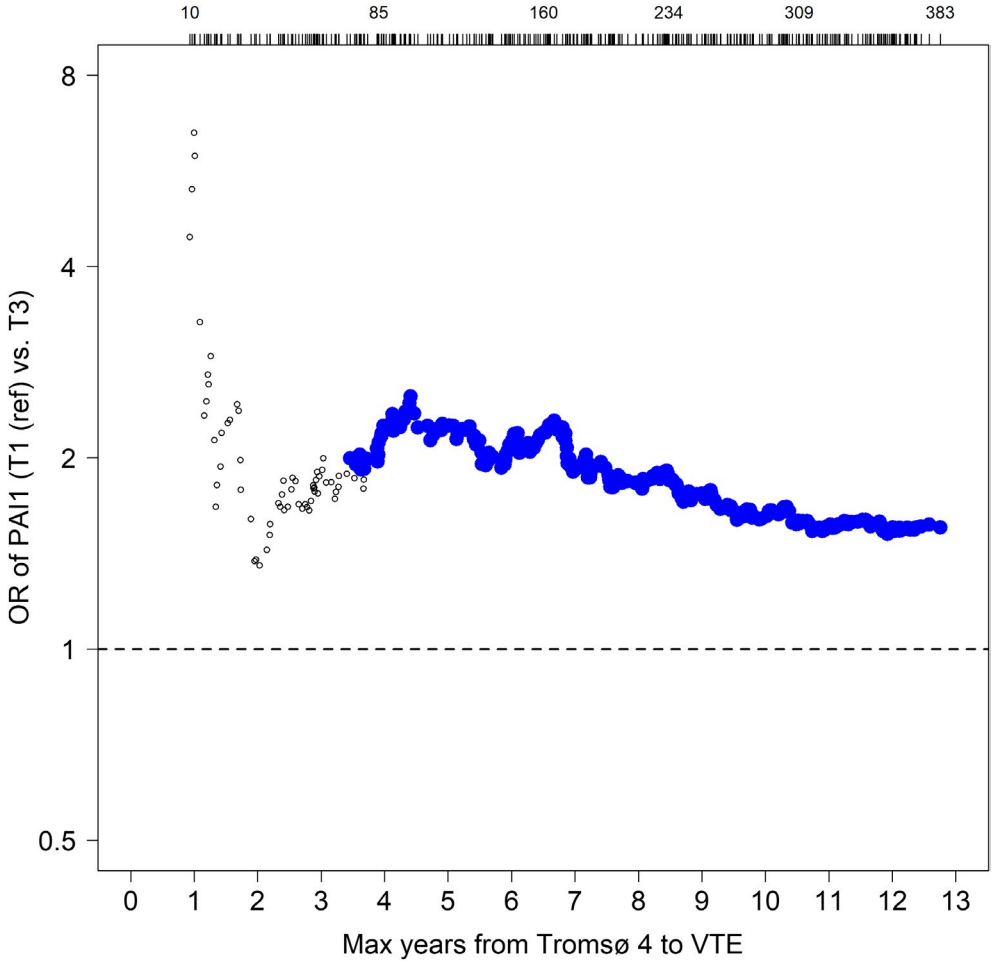

FIGURE 2 Plot of estimated odds ratios (ORs) for overall venous thromboembolism (VTE) as a function of time from blood sampling in Tromsø 4 (1994–1995) to VTE events. Participants with plasma levels of plasminogen activator inhibitor 1 (PAI-1) in the highest tertile (T3) were compared to those with levels 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;00:1–9. doi:10.1111/jth.15701

Appendix S1

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] and 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

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

Supplementary material

Supplementary Table 1 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism according to tertiles of plasma plasminogen activator inhibitor-1 (PAI-1) levels in subjects without self-reported arterial cardiovascular disease at baseline

Tertiles of PAI-1	**Controls**	**Cases**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
< 4.08 ng/mL	233	86	1(reference)	1(reference)	1(reference)
4.08-7.45 ng/mL	215	99	1.24 (0.88-1.75)	1.19 (0.84-1.68)	1.18 (0.83-1.67)
≥ 7.45 ng/mL	213	137	1.72 (1.24-2.39)	1.56 (1.12-2.19)	1.54 (1.10-2.17)
P for trend			0.001	0.008	0.01

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

Supplementary Table 2 Odds ratios (ORs) with 95% confidence intervals (CIs) for overall venous thromboembolism according to tertiles of plasma plasminogen activator inhibitor-1 (PAI-1) levels in subjects without self-reported cancer at baseline

Tertiles of PAI-1	**Controls**	**Cases**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**	**Model 3 OR (95% CI)**
< 4.08 ng/mL	249	90	1(reference)	1(reference)	1(reference)
4.08-7.45 ng/mL	253	111	1.20 (0.87-1.67)	1.16 (0.83-1.62)	1.14 (0.81-1.58)
≥ 7.45 ng/mL	253	157	1.70 (1.24-2.33)	1.53 (1.11-2.11)	1.47 (1.06-2.04)
P for trend			0.001	0.008	0.02

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

Supplementary Table 3 The Karlson, Holm and Breen (KHB) method for the mediation analysis of the association between obesity (≥ 30 kg/m^2) and venous thromboembolism (VTE)

Decomposition of logistic regression	Coefficient Model 1	Standard Error	Coefficient Model 2	Standard Error
Total effect	0.66	0.18	0.59	0.14
Direct effect	0.55	0.19	0.50	0.14
Indirect effect	0.11	0.04	0.09	0.03
Mediation through	%	(95% CI)	%	(95% CI)
PAI-1	16.8	(5.6-45.1)	14.9	(4.1-49.4)

PAI-1, plasminogen activator inhibitor-1. In the KHB method, the total effect of obesity on VTE risk is decomposed into the direct and indirect effects. The mediation through PAI-1 corresponds to the indirect effect. The 95% Confident intervals (CIs) were calculated by bootstrapping with 10,000 resamples.
Model 1: adjusted for age, sex. Model 2: adjusted for age, sex and C-reactive protein.