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Endocrine-related factors and risk of venous thromboembolism

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SUMMARY

Even though many environmental and inherited predisposing factors have been associated with venous thromboembolism (VTE), still 30–50% of the events have no obvious provoking factors. There is limited knowledge concerning the association between endocrine-related factors and VTE risk. Therefore we aimed to investigate the relation between hyperglycemia, thyroid function, vitamin D, calcium, parathyroid hormone (PTH), and risk of future VTE in a general population.

Our study participants were recruited from the Tromsø study (Tromsø 4; 1994-95, Tromsø 5; 2001-2, and/or Tromsø 6; 2007-8). Two of the papers are based on all three surveys, one paper is based on the fourth and fifth, and one on the fourth survey only. The fourth survey is the largest one, and a total of 27 158 men and women attended. A subset of participants (n=9 056) were further invited to a more extensive second visit. In Tromsø 5 and 6 only subgroups of the municipality of Tromsø were invited. The overall attendance rate went from 77% in Tromsø 4 to 66% in Tromsø 6. Incident VTE events were registered from the date of inclusion until the end of follow-up.

Our findings suggest that hyperglycemia does not play an important role in the pathogenesis of VTE, and that obesity is a more important contributor to VTE in subjects with hyperglycemia. TSH within the normal range were not associated with risk of VTE, whereas low and high TSH were associated with a moderately increased risk of VTE. However, the prevalence and the population attributable risk of thyroid dysfunction was low, suggesting that only a minor proportion of the VTE events in the population can be attributed to thyroid dysfunction. Vitamin D status was not associated with VTE risk. Finally, calcium and PTH alone were not associated with future risk of VTE. However, high levels of both calcium and PTH were associated with increased risk of VTE compared to subjects with normal calcium and PTH.

SAMMENDRAG

Flere erververde og arvelige tilstedeværende faktorer har blitt assosiert med venøs tromboembolisme (VTE), likevel forekommer 30-50% av VTE-hendelsene uten kjent årsak. Det foreligger lite forskning på sammenhengen mellom endokrin-relaterte faktorer og risiko for VTE. Målet med denne avhandlingen var å undersøke sammenhengen mellom hyperglykemi, thyroideafunksjonen, vitamin D, kalsium, parathyroideahormon (PTH), og risiko for VTE i en generell befolkning.

Studiepopulasjonen vår ble rekruttert fra Tromsø-undersøkelsen (Tromsø 4; 1994-95, Tromsø 5; 2001-2, og/eller Tromsø 6; 2007-8). To av artiklene er basert på alle tre undersøkelsene, én artikkel er basert på den femte og sjette, og én er kun basert på den fjerde undersøkelsen. Den fjerde undersøkelsen er størst, og totalt 27 158 menn og kvinner deltok. En undergruppe av deltagere (n=9 056) ble også invitert til en mer omfattende spesialundersøkelse. I Tromsø 5 og 6 ble kun deler av Tromsøs befolkning invitert til å delta. Responsraten varierte fra 77% i Tromsø 4 til 66% i Tromsø 6. Førstegangs VTE-hendelser ble registrert fra inklusjonsdato og ut oppfølgingstiden.

Våre funn tyder på at hyperglykemi ikke spiller en viktig rolle i sykdomsutviklingen av VTE, men at fedme er en vesentlig bidragsyter for VTE hos personer med hyperglykemi. Serumnivå av TSH innenfor normalområdet var ikke assosiert med risiko for VTE, men lav og høy TSH var assosiert med en moderat forhøyet risiko for VTE. Dog var prevalensen og den tilskrivbare risikoen av thyroideaforstyrrelser i befolkningen lav, noe som tilsier at bare en liten del av risikoen for VTE i befolkningen skyldes thyroideaforstyrrelser. Vitamin D status var ikke assosiert med risiko for VTE. Videre var kalsium og PTH alene ikke assosiert med risiko for VTE, mens høye verdier av både kalsium og PTH økte risikoen for VTE sammenlignet med normale verdier.

LIST OF PAPERS

The thesis is based on the following papers:

I. Hyperglycemia, assessed according to HbA1c, and future risk of venous thromboembolism: the Tromsø study.
Lerstad G, Brodin EE, Enga KF, Jorde R, Schirmer H, Njølstad I, Svartberg J, Brækkan SK, Hansen J-B.

J Thromb Haemost 2014; 12: 313–9.

II. Thyroid function, as assessed by TSH, and future risk of venous thromboembolism: the Tromsø study.
Gunhild Lerstad, Kristin F Enga, Rolf Jorde, Ellen E Brodin, Johan Svartberg, Sigrid K Brækkan and John-Bjarne Hansen.

Eur J Endocrinol 2015; 173, 83-90.

III. Serum levels of vitamin D are not associated with future risk of venous thromboembolism. The Tromsø Study.
Brodin E, Lerstad G, Grimnes G, Brækkan SK, Vik A, Brox J, Svartberg J, Jorde R, Hansen JB.

Thromb Haemost. 2013 May;109(5):885-90.

IV. Associations between serum levels of calcium, parathyroid hormone and future risk of venous thromboembolism -The Tromsø study
Gunhild Lerstad, Ellen E. Brodin, Johan Svartberg, Rolf Jorde, Jan Brox, Sigrid K. Brækkan and John-Bjarne Hansen

Manuscript.

ABBREVIATIONS

AA: Arachidonic acid

ADH: Alcohol dehydrogenase

APC: Activated protein C

ARIC: Atherosclerosis Risk in Community

BMI: Body mass index

CHS: Cardiovascular Health Study

COC: Combined oral contraceptives

CRP: C-reactive protein

CVD: Cardiovascular disease

DASH: Dietary to Stop Hypertension

DCH: Diet, Cancer and Health

DM: Diabetes Mellitus

DVT: Deep vein thrombosis

ECG: Electrocardiogram

EPA: Eicosapentaenoic acid

EPCR: Endothelial protein C receptor

Erg-1: Early growth response-1

FT_4: Free thyroxine

FVII: Factor VII

FVIII: Factor VIII

HbA1c: Glycosylated haemoglobin

HIF-1: Hypoxia induced factor-1

HR: Hazard ratio

HRT: Hormone replacement therapy

IWHS: Iowa women's health study

MI: Myocardial infarction

MISS: Melanoma Inquiry of Southern Sweden

n-3 LCPUFA: n-3 long chained polyunsaturated fatty acids

OC: Oral contraceptives

OR: Odds ratio

PAI-1: Plasminogen activator inhibitor-1

PE: Pulmonary embolism

PTH: Parathyroid hormone

PTS: Post thrombotic syndrome

RCT: Randomized controlled trial

RR: Relative risk

SIT: Seated immobility thromboembolism

SNPs: Single nucleotide polymorphisms

TF: Tissue factor

TFPI: Tissue factor pathway inhibitor

t-PA: tissue plasminogen activator

TSH: Thyroid stimulating hormone

U.S.: United States

VTE: Venous thromboembolism

vWF: von Willebrand Factor

WC: Waist circumference

WHO: Word Health Organization

25(OH)D: 25-hydroxyvitamin D

1,25(OH)$_2$D: 1,25-dihydroxyvitamin D

1. INTRODUCTION

Venous thromboembolism (VTE) is a collective term used to describe deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is the formation of a thrombus in the deep veins that prohibits normal venous blood flow back towards the heart. DVT primarily affects the large veins of the leg or thigh, but it can also occur in other parts of the body. The condition leads to pain, increased body temperature, discoloration and edema of the affected extremity. A PE is known as a potentially life-threatening complication of DVT, when an embolus dislodges from its original site and travels with the blood-stream to the arteries of the lungs. Where the vessel narrows, the clot is fixed and may obstruct the blood flow of the respective artery resulting in dyspnea, tachypnea and pleuritic chest pain[1]. However, without preexisting heart or lung disease, the signs and symptoms of a PE correlates with the extent of obstruction, and the clinical course ranges from asymptomatic to fatal circulatory collapse[2]. VTE is treated with anticoagulants, and the standard treatment has for many years consisted of an initial phase of concomitant low molecular weight heparin (LMWH) and a vitamin K antagonist (VKA), followed by VKA in monotherapy for the long-term treatment. However, direct oral anticoagulants (DOACs) are now implemented in the standard management of VTE, and these agents are recommended over VKA as long-term anticoagulant therapy in subjects with VTE and no cancer[3].

1.1 Epidemiology of venous thromboembolism

VTE is the third most common cause of cardiovascular death in the Western world following myocardial infarction (MI) and ischemic stroke[4]. The incidence of VTE is 1 to 2 per 1000 persons per year in Western countries, with a steep incline with age[5-7]. While the incidence of DVT and PE accounts for about two-thirds and one-third of the VTE cases, respectively[2], the

two conditions are often present at the same time. Of those presenting with an acute DVT, 50-80% have concurrent clinically silent PE[8]. Conversely, in about 50% of PE patients the source of the pulmonary emboli is unknown[9-11]. This could be explained by a complete evaporation or dislodging of the DVT. However, de novo pulmonary embolism should also be considered, possibly of cardiac origin[12] or in relation to trauma[13].

A VTE event is classified as provoked or unprovoked (idiopathic), based on the presence or absence of provoking factors at the time of diagnosis. Provoked VTE events occur in the presence of transient risk factors (e.g. hospitalization, acute medical illness, surgery, trauma, plaster-cast and long-haul travel) or in the presence of more long-lasting conditions (e.g. active cancer, paralysis and wheel-chair use). A VTE event with no apparent risk factors present is classified as unprovoked. This classification is important in terms of risk of recurrence and treatment duration as the presence of transient factors (e.g. surgery) is associated with lower recurrence rates[14-19] and justifies a shortened long-term treatment[20], whereas VTEs provoked by a persistent factor (e.g. active cancer) have a high risk of recurrence[15, 16, 18, 21, 22]. Patients with unprovoked VTE have an intermediate- to high risk of recurrence[14, 17-19, 23, 24]. Population-based studies find that 50-60% of the VTE events are associated with provoking factors[6, 25, 26].

VTE is associated with serious short- and long-term complications. In about 25% of PE patients, the initial clinical presentation is sudden death[5], and PE is estimated to be the leading preventable cause of death in hospitalized patients[4]. Furthermore, the overall mortality after a VTE event remains significantly increased up to eight years after the initial event[27]. Of patients presented with unprovoked VTE, 10-30% experience a recurrent VTE within 5 years despite adequate treatment[16, 28, 29]. Besides, patients initially diagnosed with DVT are more likely to develop a recurrent DVT rather than PE, and conversely, those who are initially diagnosed with PE are more likely to develop a recurrent PE[30, 31]. VTE is also associated with

the post-thrombotic syndrome (PTS) and pulmonary hypertension as a complication of PE[8, 32]. PTS occurs in 20-50% of the DVT patients, and the condition is characterized by chronic pain, edema, erythema, varicosities, paresthesias, and in more severe cases, leg ulcers and debilitating pain[33]. PTS is also associated with hampered quality of life and increased risk of disability pension[34]. Lastly, 3-5% among treated PE patients develop chronic pulmonary hypertension, which may result in disabling dyspnea, and shortened life expectancy due to progressive right ventricular failure[35-37].

Throughout the last decades, the diagnostics and treatment of VTE have been improved, but the incidence of VTE has not diminished[38]. In a cohort recruited from a general population, the incidence of VTE increased from 73 per 100,000 to 133 per 100,000 from 1985 to 2009, primarily due to an increase in pulmonary embolism[39]. While the increase in VTE incidence may partially be explained by enhanced sensitivity of diagnostic methods, it may also imply that current prevention and treatment strategies are not optimal. As the European population is becoming older and more people develops cancer, the incidence of VTE is expected to increase even more. Further research is therefore needed in order to improve risk stratification, prevention and management of VTE, and thereby reduce the health related and economic burden associated with VTE.

1.2 Pathophysiology of venous thromboembolism

The hemostatic system is faced with the intricate task of keeping the blood circulating, while simultaneously converting blood into an insoluble mass at sites of vascular injury. However, hemostasis may cause severe disease if a too large insoluble blood mass is formed at wrong places, a pathological course termed thrombosis. The development of venous thrombi is according to Virchow's triad (figure 1)[40] a result of (i) changes in the blood composition (e.g.

hypercoagulability), (ii) stasis of the blood flow (i.e. changes in blood flow) and (iii) alterations of the vessel wall (endothelial activation or damage), and nearly all risk factors for VTE falls into one or more of these three groups.

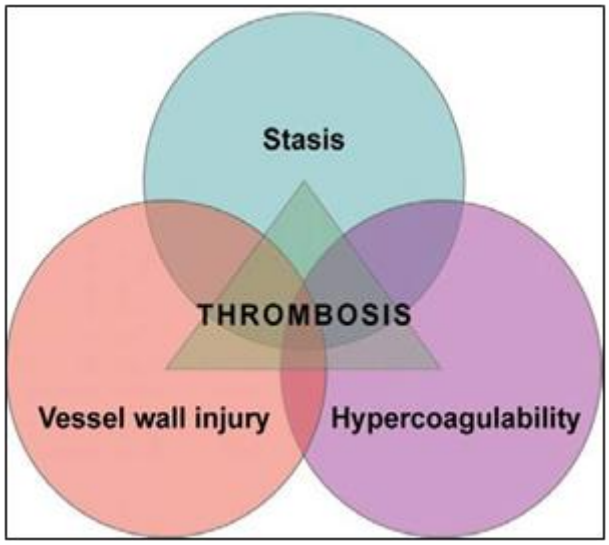

Figure 1. Virchow`s triad, illustrating the three most important factors contributing to venous thrombosis development.

Alterations in the blood composition are crucial in venous thrombus formation, and a *hypercoagulable* state may be acquired or inherited. For instance, cancer patients have been shown to have increased plasma clotting factors and higher levels of TF compared to cancer-free subjects[41-43], suggesting an acquired hypercoagulable state in cancer patients. A hypercoagulable state can also be observed in pregnant women, where there is an acquired decrease in protein S followed by a substantial increase in levels of FVIII and FV[44]. Inherited hypercoagulability can be exemplified by inherited deficiency of antithrombin, a potent inhibitor of the intrinsic coagulation pathway by neutralizing the enzymatic activity of serine proteases such as thrombin, factor X and IXa[45].

Non-trauma related venous thrombus formation has been found to be a localized process primarily originating close to the vein wall in the deepest recess of the venous valve sinuses in

the calf-veins[46]. This concept is supported by the direct correlation between the frequency of DVT and the number of valves in individuals[47]. In the venous valve sinuses blood tends to linger[48], assumingly due to *stasis* mediated through e.g. long-haul travel, advancing age, obesity and pregnancy. Severe hypoxia resulting from prolonged stasis has been documented at the level of the deepest recesses of the venous valve sinuses of dogs in the absence of calf muscle–driven pulsatile flow[49]. Furthermore, experimental studies have demonstrated a characteristic pattern of vortical blood flow within the valve sinuses during streamlined flow[46], potentially explaining the severe hypoxia and thrombus formation observed in the deepest recess of the valve sinuses. As the innermost layer of the vessel wall is supplied from the vessel lumen, low oxygen tension caused by blood stasis leads to localized hypoxia. Hypoxia-related activation of endothelial cells lining the valve sinuses are shown to cause procoagulant responses such as increased levels of PAI-1 and vWF, as well as exposure of P-selectin[46, 50, 51], rendering the venous valve sinus prone to thrombus formation.

Hemostasis is critical in the physiological management of *vessel wall* injury. However, the role of vascular injury have rarely been reported to cause venous thrombosis, apart from when it is associated with acute insults like surgery, trauma and the use of intravenous catheters[52]. In an autopsy study, Sevitt et al found no evidence of vein wall injury in 49 of 50 lower extremity thrombi, and the fibrin-rich regions attached the thrombi to the vessel wall, while the platelet-rich regions were localized further from the site of attachment[53]. These findings suggest that during the formation of a venous thrombi, activation of the coagulation system precedes the activation and aggregation of platelets[53]. This is in accordance to the greater effectiveness of anticoagulation as VTE prophylaxis than platelet inhibition. Finally, induced vessel wall injury is in experimental studies shown to be a poor stimulus to fibrin formation[54].

The surface of endothelial cells is covered by natural anticoagulants, such as endothelial protein C receptor (EPCR), tissue factor pathway inhibitor (TFPI), thrombomodulin and

heparin like proteoglycans[50, 51], normally leaving the endothelial cells resistant to thrombosis. The concentration of these components is significantly higher in the microcirculation as the concentration of these factors is determined by the ratio of the endothelial cell surface to the blood volume[55], and the efficacy of these natural anticoagulants strongly increases when the blood moves from the larger vessels to the microcirculation[56, 57]. Thus, the natural mechanisms controlling the coagulation in the large vessels are normally less effective. Moreover, both hypoxia caused by stasis, as well as vessel wall damage, may activate the venous endothelium in a procoagulant way. Granules containing vWF and membrane-bound P-selectin are released by activated endothelial cells and promote adhesion of leukocytes, platelets and TF- bearing microparticles (MP)[51, 52]. The tissue factor (TF)/factor VIIa complex is the primary physiological trigger of the so-called "extrinsic pathway" in the coagulation cascade[58], and MPs expressing TF has been suggested to be the key triggers of venous thrombosis[46, 50].

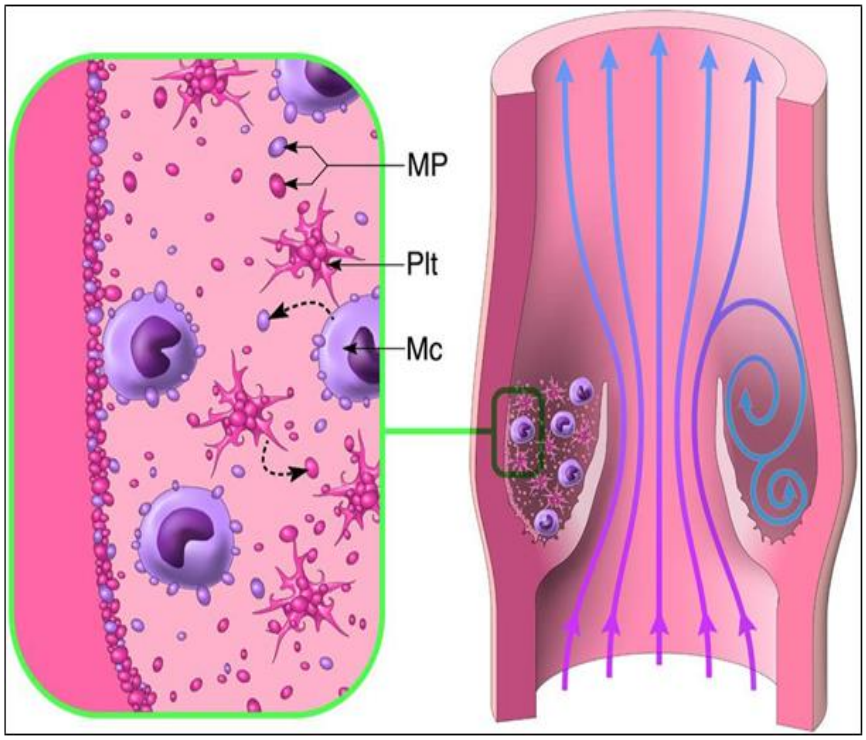

Figure 2. The figure depicts a venous segment near a venous valve. Oxygen tension may become particularly low in the pocket of the valve due to stasis and vortical flow pattern. This will result in activation of the venous endothelium, leading to the recruitment and binding of monocytes (Mc), platelets (Plt) and TF-positive microparticles (MP). Consequently, TF from activated monocytes and microparticles may activate the coagulation cascade and initiate thrombosis formation.

1.3 Risk factors of venous thromboembolism

In epidemiology, a risk factor is generally any characteristic that increases the likelihood of developing a disease. VTE is a multifactorial disease, implying that several risk factors need to be present at the same time in order to induce thrombus formation [59]. A dynamic, age-dependent explanatory model for thrombosis risk has been suggested, wherein both hereditary and acquired factors associated with VTE risk interact [59]. In this model, individual factors (e.g. high age and FV Leiden) alone may not be sufficient to cause VTE. However, by interacting with other provoking factors, such as surgery and immobilization, the joint effect of all risk factors may outweigh the natural anticoagulant properties, resulting in thrombosis (Figure 3).

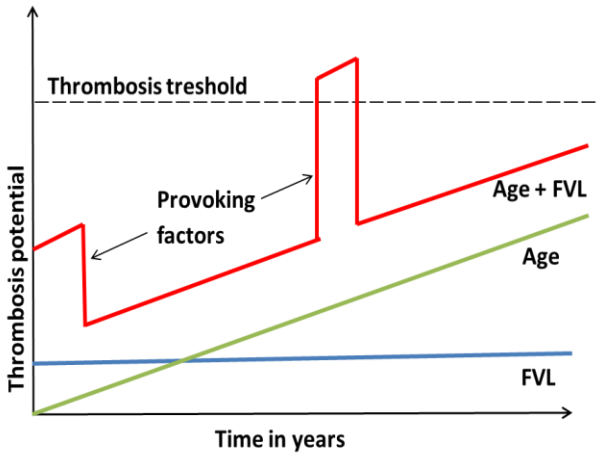

Figure 3. The thrombosis potential model. The blue line symbolizes a hereditary risk factor such as factor V Leiden (FVL), and the green line symbolizes the effect of age alone. The red line demonstrates the effect of both age and FVL, in combination with provoking factors early and late in life, respectively. The latter combination of both FVL, increased age and a provoking factor (e.g. surgery) is sufficient to exceed the thrombosis threshold.

1.3.1 Hereditary risk factors

Family history of VTE has been reported to provide a 2 to 3-fold higher risk of VTE[60-64], and family studies have estimated that approximately 60% of the variation in susceptibility to thrombosis can be attributed to genetic risk factors[65-67]. Inherited thrombophilia generally increases VTE risk by two mechanisms[59]. The first group comprises the "loss of function"

disorders including deficiency of antithrombin, protein C and protein S. The prevalence of these deficiencies is rare (<1%)[45, 68], nevertheless they increase VTE risk approximately 10-fold[45]. The second group covers the "gain of function" disorders such as factor V Leiden (FVL), prothrombin G20210A and non-O blood groups. These disorders are less thrombogenic. However, they are far more common than the deficiencies of group one[69]. The FVL mutation, yielding a 3-fold increased risk of VTE through resistance to the anticoagulant function of activated protein C[70], is present in about 5% of Caucasians in its heterozygous form[45]. Prothrombin G20210A is a polymorphism associated with increased prothrombin concentration. The prevalence is about 2% in the general population, and carriers with this polymorphism have 3-fold increased risk of VTE[45, 71]. However, the occurrence of FVL and Prothrombin G20210A varies with ethnicity and are more prevalent among whites than among patients with African or Asian descent[72]. The Non-O blood groups yields a 1.5-fold increased risk of VTE[70, 73], and the association is partially explained by increased von Willebrand Factor (vWF) and FVIII[74]. However, the Non-O blood groups remains significantly associated with VTE after adjusting for both factors[75, 76], implying that additional unknown pathways may contribute to the thrombotic risk among subjects with non-O blood.

Inherited risk factors may be modified by the presence of other genetic or environmental factors, and this is known as gene-gene or gene-environment interactions. For instance, the co-inheritance of FVL and prothrombin 20210A polymorphism has been reported to promote a 20-fold increase in VTE risk[77]. In regard to gene- environment interactions, there has been reported an additional risk of VTE in obese carriers of FVL compared to the non-obese carriers[78, 79], and the finding was described as an interaction on an additive scale.

Even though a significant amount of VTE events may be ascribed to genetic factors, a recent review reported that known thrombophilias identified so far only explain about 5% of

VTE heritability[70]. During the last decades the genome-wide association studies (GWAS) have identified an extensive amount of new genetic mutations that are frequent in the population (e.g. single nucleotide polymorphisms; SNPs), but they only have a modest effect on VTE risk[45, 70]. Ongoing and future whole-genome sequencing studies will hopefully identify novel genetic risk factors for VTE.

1.3.2 Non-hereditary risk factors

Advancing *age* is the strongest and most consistent risk factor for VTE, and the overall incidence of first symptomatic VTE is 1-2 per 1000 person years in the general population, increasing from 1 per 10 000 person years in the age-group 25-30 years to 5-8 per 1000 person years in those above 75 years[2, 6, 26, 80]. The steep incline in VTE risk by age may be explained by a larger increase in levels of procoagulant factors (e.g. FVII, FVIII, FX and fibrinogen) in the elderly than in anticoagulant factors[81]. The increased VTE risk could also be explained by age-specific risk factors (e.g. reduced muscle strength, endothelial dysfunction, venous insufficiency, and frailty)[46, 82]. For instance, altered venous blood flow caused by (age-related) changes in compliance in the vein wall has been reported[83], and consequently, altered venous blood could affect the frequency and duration of blood stasis in the microcirculation[46], potentially leading to thrombus formation.

The number of overweight and obese individuals in the population has increased dramatically in the past few decades, especially in Western countries, and the rising prevalence of *obesity* is a major public health concern. According to the WHO classification, more than a third of the world's population was in 2014 either overweight (defined as Body Mass Index (BMI) ≥25 kg/m²) or obese (BMI ≥30 kg/m²)[84]. Growing evidence has accrued for obesity as an important risk factor for VTE. A meta-analysis by Ageno and co-workers

reported an OR of 2.33 for VTE in obese subjects compared with normal weight subjects[85], and the association became stronger as the BMI increased[85, 86]. Although BMI has been the most commonly used anthropometric measure to assess the association between obesity and VTE, the composition of body fat and muscle mass can differ highly among subjects with the same BMI. The Danish Diet, Cancer and Health (DCH) study showed that all anthropometric measures (body weight, BMI, waist circumference (WC), hip circumference and total body fat) were associated with risk of VTE[87]. Investigators of the Tromsø study did also find all measures of obesity to be associated with VTE risk, though WC identified most subjects at risk and was the strongest predictor of VTE[88]. Some studies have assessed the joint effect of obesity with other genetic or environmental risk factors for VTE (e.g. factor V Leiden, body height), and showed that for some combinations there is a synergistic effect[79, 89]. A number of potential mechanisms by which obesity increases the risk of VTE have been suggested. Several studies have shown that high BMI is associated with increased plasminogen activator inhibitor-1 (PAI-1), TF, fibrinogen and FVIII[90-94], supporting a relationship between obesity and coagulation. Furthermore, leptin, a hormone produced mainly by adipocytes and consequently raised in obese subjects, is associated with increased thrombosis risk by promoting platelet aggregation and inducing TF expression[95]. Finally, abdominal obesity is associated with increased intra-abdominal pressure and reduced venous blood flow velocity due to resistance of venous backflow from the lower limbs[96, 97], potentially increasing the chance of thrombus formation.

Although the association between *cancer* and VTE was first noted by Bouillard in 1823[98], the most detailed early description was provided by Trousseau in 1865[99]. Cancer is today recognized as one of the most important risk factors for VTE. About 20% of all first VTE-events are associated with cancer[100, 101], and the overall VTE risk is estimated to be 4 to 7-fold higher in cancer patients compared to cancer-free subjects[16]. However, the VTE risk among

cancer patients varies highly according to cancer type, stage of disease, and treatment modality[102]. Brain and pancreas cancers are associated with the highest VTE risk, whereas prostate and breast cancers have generally been associated with a low VTE risk[102]. Several pathogenic mechanisms may promote thrombus formation in cancer patients including tumor induced platelet activation, enhanced expression of TF, reduced clearance of coagulation factors and decreased anticoagulant synthesis[103]. Furthermore, stasis induced by prolonged bed rest and/or vascular invasion by tumor may lead to thrombosis[104], and moreover, patients with active cancer are more exposed to major surgery, chemotherapy, infections and hospitalization which all increase VTE risk.

Hospitalization is another important risk factor for VTE[100, 105]. Hospitalized patients are often exposed to many risk factors for VTE (e.g. surgery, trauma, intravenous catheters, immobilization, pregnancy and chronic and acute medical conditions) at the same time[106, 107]. A nested case-control study showed that nearly 60% of all incident VTE events were attributed to institutionalization wherein hospitalization with surgery counted for 24%, other medical diseases for 22% and nursing home residence for 13%[100]. In a case-control study, recent hospitalization with and without surgery were associated with a 22 and 8-fold increased risk of VTE, respectively[105]. A recent meta-analysis has shown that anticoagulant prophylaxis reduces the risk of non-fatal PE and symptomatic DVT by 39% and 53% in non-surgical patients, respectively[108]. Nonetheless, according to a systematic review, only 11-19% of hospitalized patients received appropriate anticoagulant prophylaxis[109]. The Padua Prediction Score for Risk of VTE have been developed in order to identify patients with acute medical conditions that are admitted to hospital and whom may benefit from VTE prophylaxis[110]. The score encompasses almost every potential risk factor for VTE, and it provides a fairly simple score of 11 parameters, by which clinicians can stratify their patients according to a VTE prophylaxis threshold. Barbar et al found that 40% of consecutive patients admitted to an

internal medicine department were classified to have high risk of VTE (i.e. Padua Prediction Score ≥ 4), and the VTE risk was 87% lower in high risk patients who received anticoagulation than those who did not[110]. International guidelines strongly recommend that patients with high risk of VTE according to the PADUA Prediction Score receive anticoagulant thromboprophylaxis unless the risk of bleeding complications are too high[108]. Furthermore, the PADUA model provided the best available basis for judging the VTE risk in hospitalized medical patients[108].

Immobilization is an established risk factor for VTE. A meta-analysis has shown that immobilization among medical bedridden patients increases VTE risk by 2-fold[111]. However, the separate impact of immobilization among bed-rest patients could be difficult to estimate as this group could suffer from other underlying causes that may contribute to venous thrombosis. Nevertheless, an interrelation between immobilization and VTE is further supported by a meta-analysis where all-type travel was associated with a nearly 3-fold increased risk of VTE, and the VTE risk increased in a dose–response manner by 18% per each 2-hour increase in travel duration[112]. Several studies have also shown an increased risk for VTE in patients suffering from a stroke[113-115]. The predisposition to thrombosis in immobilized subjects may be caused by stasis due to a supine position that prohibits muscle pump activity. Additionally, muscle mass may decrease during bed-rest and could even after the period of prolonged immobility cause inadequate venous emptying.

Pregnancy is acknowledged as a risk factor for VTE, and pulmonary embolism is shown to be the leading cause of maternal death in the developed world[116]. In observational studies, pregnant women have a 4 to 5-fold higher risk of VTE compared to non-pregnant women, and in the post-partum period the VTE risk is 20 to 80-fold higher[38, 117]. Furthermore, the risk of pregnancy-associated VTE is increased by 52 and 31-fold in carriers of factor V Leiden and the prothrombin 20210A mutation, respectively, compared with non-pregnant

women without the mutation[117]. A hormone-induced shift in pregnant women increases levels of clotting factors and reduces fibrinolytic activity, probably to prevent fatal bleeding complications during delivery[118]. Furthermore, increased venous capacitance and reduced venous outflow in pregnant women, along with mechanical obstruction by the uterus, may contribute to pregnancy-associated thrombosis[119].

Growing evidence points to a bidirectional relationship between VTE and *arterial cardiovascular diseases* (CVD) (e.g. myocardial infarction (MI) and ischemic stroke)[120-128]. The association between arterial CVD and VTE could be attributed to shared risk factors, indirect causal factors, or a direct causal relationship[129]. Additionally, atherosclerosis may potentially initiate both venous and arterial thrombosis, however results concerning the association between atherosclerosis and VTE are diverging[130-133]. Prospective cohorts applying cause-specific regression models, have shown that of traditional atherosclerotic risk factors, only age, obesity and a family history of MI are shared risk factors for arterial CVD and VTE[26, 134-136]. Moreover, observational studies have demonstrated a transient association between MI, stroke and future risk of VTE in the general population[122-125], and the risk estimates were higher for provoked VTE, suggesting that indirect causal factors (e.g. hospitalization and subsequent immobilization) may contribute substantially to the observed association between MI, stroke and incident VTE[124, 125]. Nevertheless, direct causal mechanism(s) secondary to local disturbances in the cardiopulmonary circulation or electromechanical pathway (e.g. atrial fibrillation) may also contribute to the VTE risk observed in MI patients[124]. In the recent years, there have also been reported resemblances in the treatment between VTE and CVD. Rosuvastatin, a cholesterol-lowering drug, and acetylsalicylic acid, an antiplatelet drug, both used in the prevention of arterial CVD have been found to reduce the risk of VTE[137, 138].

Despite the current knowledge of hereditary and non-hereditary risk factors for VTE, still 30–50% of VTE events have no apparent provoking factor[2, 25]. Further research regarding risk factor management of VTE is therefore important.

1.3.3 Endocrine-related risk factors

Hormones regulate several organs and body functions. It is well known that current use of estrogens in terms of *combined oral contraceptives (COC)* and *hormone replacement therapy (HRT)* are associated with an increased risk of VTE[139], and COCs and HRT are both used widely in developed countries. The risk of VTE has been shown to vary according to type and amount of estrogen and combined progestogen in COCs, as well as in the duration of use, and a 2 to 6-fold increase in VTE risk has been reported[140]. The third generation COCs (i.e. containing desogestrel or gestodene) yields the highest risk, and the risk is at its uppermost the first year of use[140]. In a meta-analysis, current users of postmenopausal HRT were found to have a 2 to 3-fold increased risk of VTE compared to non-users, and as for COC users the risk was highest during the first year of use[141]. Usage of COCs induces an increase of clotting factors (e.g. fibrinogen, prothrombin, FVII, FVIII and FX) and a decrease of anticoagulants (e.g. antithrombin, protein S and TFPI)[142]. Similar changes take place in women taking HRT, but in HRT users there has been suggested a threshold effect as changes in hemostatic factors were higher in women taking conventional high dose HRT as compared with low-dose therapy[142]. Furthermore, supra-physiologic estrogen administration to men has also been reported to provoke VTE[143]. Several studies advocate that other hormones also influence the hemostatic balance[144], and some endocrine disorders (e.g. diabetes mellitus (DM), vitamin D deficiency, thyroid- and parathyroid dysfunction) have been associated with arterial cardiovascular events.

1.3.3.1 Hyperglycemia and subsequent diabetes mellitus

When blood glucose levels rise after a meal, insulin is secreted from islet of beta cells in the pancreas. Insulin activates uptake of glucose from the blood stream to insulin sensitive peripheral tissues. Insulin also stimulates the storage of glucose and suppresses the endogenous glucose production in the liver. In healthy individuals blood glucose levels are normalized within two hours after a meal, whereas in subjects with insufficient insulin secretion, hepatic or peripheral insulin resistance, blood glucose levels rise and may lead to hyperglycemia and subsequent DM[145, 146]. There are two distinct types of DM (i.e. DM type 1 and 2). While DM type 1 is an autoimmune disease with a sudden loss of beta cells leading to life-long insulin treatment, DM type 2 is a metabolic disorder associated with insulin resistance and characterized by chronic and slowly progressing hyperglycemia and altered lipid metabolism[146]. During the last decades the prevalence of hyperglycemia has increased substantially throughout the world, and hyperglycemia along with subsequent DM has become a major public health problem. Experimental studies have suggested that hyperglycemia in different ways may facilitate thrombosis through activation of the coagulation system[147], as well as by impaired fibrinolysis[148]. Both hyperglycemia and DM are well established risk factors for arterial CVD and all-cause mortality[149, 150], and a consistent relationship between glycated hemoglobin (HbA1c) and arterial CVD[151, 152] has been suggested. HbA1c is a marker of average plasma glucose in an individual over the preceding 8 to 12 weeks[153], and is recommended as a diagnostic test for DM with a cut-off at 6.5%[154]. Some observational studies have reported an increased risk of VTE in persons with hyperglycemia and/or DM[80, 155-159], while other studies have failed to find an association[135, 160-165]. A report from the Iowa Women`s Health Study[155] found a 2-fold increased risk of VTE in women with self-reported DM, and in a case-control study[158], hyperglycemia was associated with increased VTE risk independent of DM status. However, in a case-control

study by Heit et al[160], the observed link between DM and VTE was explained by more frequent hospitalizations of persons with DM, and thereby being predisposed for VTE. The observed inconsistencies regarding the impact of hyperglycemia and DM on VTE risk may also rely on differences in study design (e.g. reversed causation in case-control studies), study population, the definition of hyperglycemia and DM (e.g. non-fasting or fasting glucose levels, HbA1c, self-reported DM or previous discharge diagnosis of DM), number of VTE events (power issues) and failure in adjustment for important confounders such as obesity.

1.3.3.2 Thyroid function

The thyroid is a small butterfly-shaped endocrine gland localized below the larynx in front of the trachea. The thyroid gland produces two thyroid hormones (i.e. Thyroxin and Triiodothyronine). The main task of the thyroid hormones is to regulate our body's metabolism, but they may also influence various physiological and pathological processes in the body[166, 167]. The pituitary gland, located at the base of the brain, controls the thyroid gland by producing thyroid-stimulating hormone (TSH). TSH induces the thyroid gland to produce more thyroid hormones in situations when the body needs to increase the metabolism, and TSH is usually considered the most sensitive measure of thyroid function[168]. Imbalances in the production of thyroid hormones usually arises from dysfunction of the thyroid gland itself and can result in hypothyroidism or hyperthyroidism. Hypothyroidism occurs when the thyroid gland is less active than normal producing insufficient amounts of thyroid hormones. The TSH levels are then elevated since the body wants to increase the production of thyroid hormones. Contrary, in subjects with hyperthyroidism the thyroid is too active and produces more thyroid hormones than the body needs. In this situation, the levels of TSH are decreased. Subjects with normal levels of thyroid hormones, though slightly increased or decreased

levels of TSH, are said to have subclinical hypothyroidism or subclinical hyperthyroidism, respectively[169, 170]. The prevalence of thyroid disorders is clearly increasing in the general population, and the world faces a burden of thyroid disease that has reached epidemic proportions. A hypercoagulable state has been linked to both hyperthyroidism[171, 172] and subclinical- as well as overt moderate hypothyroidism[173-175]. Furthermore, a relationship between thyroid dysfunction and arterial CVD has been reported[176, 177]. Only four observational studies have investigated the association between thyroid function and risk of VTE[178-181]. A retrospective registry-based study reported a 1.6-fold increased risk of VTE in hypothyroid patients[178], whereas no association was found in hyperthyroid patients. In contrast, a case-cohort study found a 2-fold increased risk of PE in hyperthyroid patients[179]. Furthermore, a case-control and a nested case-control study have shown that even high normal levels of free thyroxin (FT4) increase the risk of VTE, whereas TSH levels are inversely and more moderately associated with VTE risk[180, 181].

1.3.3.3 Vitamin D

Vitamin D was discovered in 1922 and due to its effect on bone metabolism it has for decades been used in prevention and treatment of rickets in children and osteoporosis in adults[182, 183]. Vitamin D is a fat soluble vitamin that exists in two forms; ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D acts like a pre-hormone as it is not biologically active until it has been converted by the liver to 25-hydroxyvitamin D (25(OH)D)[183]. In the kidneys 25(OH)D is further converted to the more active form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and this step is regulated by serum ionized calcium and parathyroid hormone (PTH) levels[183]. However, serum levels of 25(OH)D is regarded as the biomarker of vitamin D status in individuals without kidney disease, as it is the substrate for the renal and non-renal

production of $1,25(OH)_2D$, has a longer biological half-life than $1,25(OH)_2D$ and circulates in much higher concentrations[184]. The two sources of vitamin D are diet and sun exposure[182], and serum 25(OH)D reflects the total production of vitamin D from both endogenous and exogenous sources, including sun exposure and intake of various dietary forms. Although the classic effect of vitamin D is regulation of calcium, phosphate and bone metabolism[183], vitamin D receptors have a broad tissue distribution[185], and serum 25(OH)D levels have been proposed to influence the risk of several common diseases that are not related to bone metabolism[182]. Several experimental and clinical studies have shown that vitamin D may promote antithrombotic effects[186-188]. Moreover, a prospective cohort study has reported an association between vitamin D insufficiency and risk of arterial CVD[189]. Regarding vitamin D and risk of VTE, only one study has been published reporting that women with active sun exposure habits have 30% lower risk of VTE compared to women with low sun exposure habits[190], presumably mediated by higher vitamin D levels[190].

1.3.3.4 Calcium and parathyroid hormones

Parathyroid disorders are among the most common endocrine disorders. Primary hyperparathyroidism, with an annual incidence of about 20 cases per 100 000, is the most frequent[191]. Primary hyperparathyroidism is caused by autonomous production of PTH by one or more of the four parathyroid glands localized on the back of the thyroid gland. PTH is a key hormone in calcium homeostasis with an inverse relation to ionized calcium under normal conditions. Low serum ionized calcium triggers the secretion of PTH from the parathyroid glands resulting in a rise in serum ionized calcium due to calcium mobilization from the bones, increased renal reabsorption and intestinal uptake of calcium via increased production of $1,25(OH)_2D$. In turn, an increase in serum ionized calcium inhibits PTH secretion[192].

Several experimental studies have shown a hypercoagulable state in patients with primary hyperparathyroidism and consequently chronic hypercalcemia[193-195]. Population based cohort studies have also demonstrated that serum calcium and PTH are independent risk factors for myocardial infarction in middle-aged men[196] and arterial cardiovascular mortality in elderly men[197], respectively. A randomized controlled trial (RCT) reported no effect on overall VTE risk by daily supplementation with calcium and vitamin D for 7 years in postmenopausal women, but they did observe an increased risk for unprovoked VTE[198]. To the best of our knowledge, no population-based study has investigated the associations between serum levels of calcium, PTH and future risk of VTE.

2. AIMS OF THE STUDY

The aims of the study were:

- To investigate the association between hyperglycemia, assessed according to HbA1c, and future risk of venous thromboembolism in a cohort recruited from a general population.

- To examine the association between thyroid function, assessed by TSH, and future risk of VTE in a general adult population with repeated measures of TSH.

- To assess whether serum levels of 25(OH)D were associated with risk of VTE in a large, prospective, population-based study.

- To examine the relationship between serum levels of calcium and PTH, and the future risk of VTE in a general adult population.

3. STUDY POPULATION AND METHODS

3.1 The Tromsø Study

The Tromsø study is a single-centre prospective, population-based study with repeated health surveys of the inhabitants of the municipality of Tromsø, Norway[199]. It was originally initiated in 1974 as the Tromsø heart study in order to investigate and prevent arterial CVD. At that point of time it was a high cardiovascular mortality in North-Norway. Today, the Tromsø study has developed into being a large epidemiological study including a broad spectrum of diseases, and seven surveys have been conducted so far. All four papers of this thesis are based on data from the Tromsø Study (Tromsø 4; 1994-95, Tromsø 5; 2001-2, and/or Tromsø 6; 2007-8). Two of the papers are based on all three surveys, one paper is based on the fourth and fifth, and one on the fourth survey only. The fourth Tromsø survey is the largest one, and consisted of two screening visits with an interval of 4-12 weeks. All inhabitants aged >24 years where invited to the first screening visit, and a total of 27 158 subjects took part. A subgroup (n=10 542) was further invited to the second screening visit that included blood samples for hormone analysis (e.g. vitamin D, TSH and PTH). In the fifth Tromsø survey, all men and women older than 29 years, living in the same area, and who participated in the second screening visit of the fourth Tromsø survey or became 30, 40, 45, 50, or 75 years old during 2001, were invited to participate. A total of 8 130 men and women aged 30-89 attended the fifth survey. In the sixth Tromsø survey the following groups of the municipality of Tromsø were invited to participate; subjects who took part in the fourth survey; a 10% random sample of subjects aged 30-39 years; all individuals aged 40-42 years and 60-87 years and a 40% random sample of subjects aged 43-59 years. A total of 12 984 men and women aged 30-87 attended the sixth survey. The overall attendance rate was high, ranging from 77% in the fourth Tromsø survey to 66% in the sixth Tromsø survey. The participants were followed from the date of enrollment through the end of the study period

(Paper 1 and 2; December 31, 2010, Paper 3; September 1, 2007, Paper 4; December 31, 2012).

3.1 Baseline measurements – (Tromsø IV, V and VI)

In all three surveys baseline information was collected by physical examinations, blood samples, and self-administered questionnaires. Information on self-reported DM, history of arterial CVD (i.e. angina pectoris, myocardial infarction and stroke), current daily smoking, and physical activity (≥ 1 hour per week) during leisure time was collected from the questionnaires. The self-reported data of DM were supplemented with data on confirmed diagnoses of DM from the MI registry of the Tromsø Study. Height and weight were measured, and BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was recorded with an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc., Tampa, FL, USA). Participants rested for 2 minutes in a sitting position before three readings were taken on the upper right arm at 2 minute intervals. The average of the two last readings was used in the analysis. Non-fasting blood samples were collected from an antecubital vein, serum prepared by centrifugation after 1 hour respite at room temperature, and further analyzed at the Department of Clinical Chemistry, University Hospital of North Norway. All samples were stored frozen at -70°C. Serum samples were analyzed for TSH with the AxSYM instrument (Abbott, IL, USA). In our laboratory, the reference range for serum TSH was 0.20–4.00 mIU/l. The Cobas Mira instrument was used to quantify HbA1c with an immunoturbidimetric method (Unimate 5 HbA1c, Hoffmann-La Roche). The reference range was 4.0% to 6.5%. Serum levels of 25(OH)D were measured in sera stored for a median of 13 years. $25(OH)D_3$ was determined by immunometry (ECLIA) using an automated clinical chemistry analyzer (Modular E170, Roche Diagnostics, Mannheim, Germany). The total analytical precision of the assay had a coefficient of variation

≤7.8% for any of three different concentrations (48.6, 73.8, and 177.0 nmol/l) according to the manufacturer. The manufacturer provides a population-based reference range of 27.7–107.0 nmol/l for serum concentrations of $25(OH)D_3$ in adults. We revealed that this particular assay artificially measured 15–20% higher serum 25(OH)D levels in smokers than in non-smokers (14). The Hitachi Model 917 analyzer was used to quantify serum concentrations of calcium and creatinine with reagents from Boehringer Mannheim (Mannheim, FRG). The respective reference ranges were for serum calcium 2.15-2.51 mmol/L, and for serum creatinine 60-105 μmol/L in men and 45-90 μmol/L in women. Creatinine values were used for estimation of the glomerular filtration rate (eGFR). eGFR was calculated using the recalibrated four-variable Modification of Diet in Renal Disease (MDRD) study equation; eGFR = 175 × (s-creatinine (μmol/l)/88.4)−1.154 × age-0.203 × (0.742 if female). Intact PTH was measured by an Immulite analyzer (Diagnostic Products, Los Angeles, CA, USA) on the basis of a two-site chemiluminescent immunometric assay. The reference range was 1.1-6.8 pmol/L for those below the age of 50, and 1.1-7.5 pmol/L for those 50 years and above. In the fourth Tromsø study serum calcium was analyzed within a week after sampling, whereas serum PTH was analyzed in 2001. Samples from the fifth Tromsø study were analyzed within 2 months for serum calcium and creatinine, and for serum PTH within 12 months. Serum total cholesterol and triglycerides were analyzed by enzymatic colorimetric methods and commercially available kits (CHOD-PAP for cholesterol and GPO-PAP for triglycerides: Boehringer-Mannheim, Mannheim, Germany). Serum HDL cholesterol was measured after precipitation of lower-density lipoproteins with heparin and manganese chloride.

3.3 Outcome measurements

3.3.1 Venous thromboembolism

All first-time events of VTE during follow-up were identified by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry of the University Hospital of North Norway. The University Hospital of North Norway is the only hospital in the Tromsø region, and all hospital care and relevant radiological procedures are offered here. The relevant discharge diagnosis codes were the International Classification of Diseases (ICD)-9 codes 325, 415.1, 451, 452, 453, 671.3, 671.4, 671.9 for the period 1994-1998 and the ICD-10 codes I26, I80, I82, I67.6, O22.3, O22.5, O87.1 and O87.3 for the period 1999-2012. The hospital discharge diagnosis registry included diagnoses from outpatient clinic visits and hospitalizations. The radiology procedure registry was searched in order to identify potential cases of objectively confirmed VTE that may have been missed due to coding errors in the hospital discharge diagnosis registry. The medical record for each potential VTE case was reviewed by trained personnel who were blinded with regard to the baseline variables. A VTE event was only verified and recorded when all four of the following criteria were fulfilled; (i) objectively confirmed by diagnostic procedures (compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan or autopsy), (ii) the medical record indicated that a physician had made a diagnosis of DVT or PE, (iii) sign and symptoms consistent with DVT or PE were present and (iv) the patient underwent therapy with anticoagulants (heparin, warfarin, or a similar agent), thrombolytics or vascular surgery unless contraindications were specified in the medical record. VTE events deriving from the autopsy registry were recorded as outcomes when the autopsy record indicated VTE as a cause of death or as a significant condition contributing to death. Concurrent DVT and PE were registered as PE, and verified VTE events were classified as unprovoked or provoked based on the presence of provoking factors at the time

of diagnosis. A VTE event was defined as provoked if one or more of the following factors were present: surgery or trauma within 8 weeks prior to the event, acute medical conditions (e.g. acute MI, ischemic stroke or major infectious disease), active cancer at the time of the event, marked immobilization (i.e. bed rest for >3 days, wheelchair use, or long-distance travels ≥4 h within the last 14 days) or any other factor described by a physician in the medical record (e.g. intravascular catheter).

3.3.2 Myocardial infarction, ischemic stroke and cancer

In paper 4, subjects who developed MI, ischemic stroke or cancer were censored at the date of event in the cause-specific model. Cases of first-time MI and incident ischemic stroke were identified by linkage to the hospital discharge diagnosis registry at the University Hospital of North Norway (outpatient diagnoses included) and by searching the National Causes of Death Registry at Statistics Norway. Validation of MI and ischemic stroke were performed by an independently endpoint committee[124, 125]. Information on incident cancer during follow-up was obtained from the Cancer Registry of Norway[200].

4. MAIN RESULTS

4.1 Paper I:

HYPERGLYCEMIA, ASSESSED ACCORDING TO HBA1C, AND FUTURE RISK OF VENOUS THROMBOEMBOLISM: THE TROMSØ STUDY

The aim of this study was to examine the association between hyperglycemia, assessed by HbA1c, and future risk of VTE in a general population. The Cobas Mira instrument was used to quantify HbA1c with an immunoturbidimetric method (Unimate 5 HbA1c, Hoffmann-La Roche). HbA1c was measured in 16 156 unique subjects (25-87 years) who participated in one or more surveys of the Tromsø study (Tromsø 4; 1994-95, Tromsø 5; 2001-2, and Tromsø 6; 2007-8). Incident VTE events were registered until December 31, 2010. Date of study enrollment for each individual was determined as the date of attendance in the first survey in which HbA1c measurements were available. Person-years were accrued from the date of enrollment through the date a VTE-event was first diagnosed, the date of migration or death or at the end of the study period. During a median of 7.1 years of follow-up, there were 333 validated first VTE events. There was no increased risk of VTE per 1 standard deviation (SD) (0.7%) increase in HbA1c after adjustment for potential confounders. In the categorized analysis adjusted for age and sex, subjects with HbA1c ≥6.5% had 67% higher risk of VTE than subjects with HbA1c <5.7% (95% CI 1.01-2.74), and there was a significant linear trend for increased VTE risk across categories of HbA1c (P for trend 0.04). However, after adjustment for BMI, the risk estimates were attenuated and no longer statistically significant (HR 1.27; 95% CI 0.72-2.26, P for trend 0.27). Furthermore, subgroup analyses revealed a tendency of higher risk estimates for provoked than for unprovoked events in subjects with HbA1c ≥6.5% than those with HbA1c <5.7%. In conclusion, our findings suggest that hyperglycemia does not play an important role in the pathogenesis of VTE, and that obesity is a more important contributor to VTE in subjects with hyperglycemia.

4.2 Paper II:

THYROID FUNCTION, ASSESSED BY THYROID STIMULATING HORMONE, AND FUTURE RISK OF VENOUS THROMBOEMBOLISM -THE TROMSØ STUDY

This study was undertaken to investigate the association between thyroid function, assessed by TSH, and future risk of VTE in a general population with repeated measures of TSH. TSH was measured in serum samples from 11 962 subjects, aged 25-89 years, who participated in Tromsø 4-6, starting in 1994-95. The reference range for serum TSH in our laboratory was 0.20–4.00 mIU/l. We used a time-varying analysis that allowed participants (n=3 035) who were re-measured in Tromsø 5 and Tromsø 6 to change (update) levels of TSH over time. Incident first-lifetime VTE were recorded through December 31, 2010. There were 289 validated VTE events during a median follow-up of 8.2 years. Serum levels of TSH within the normal range were not associated with risk of VTE. In categorized analyses, low (prevalence: 0.22%) and high (3.01%) TSH levels were associated with a moderate increase in VTE risk compared to normal TSH (multivariable HRs: 2.16, 95% CI 0.69-6.76 and 1.55, 95% CI 0.87-2.77, respectively). In subgroup analyses, the association between the lowest (<0.05 mIU/L) and highest (>5.00 mIU/L) category of TSH and VTE only applied to provoked events (multivariable HRs 2.51, 95% CI 0.62-10.19 and 1.99, 95% CI 1.01-3.90, respectively). Subjects with thyroid dysfunction (i.e. after merging the lower and upper categories of TSH) had a statistically significant increased risk of provoked VTE compared to euthyroid subjects (multivariable HR 1.67, 95% CI 1.06-2.64). The overall population attributable risk (PAR%) for VTE by thyroid dysfunction was 4.4% (95% CI 1.0%-9.1%). In conclusion, thyroid dysfunction may predispose for VTE through associated hospitalization or co-morbidities. At the same time, the low prevalence of thyroid dysfunction and the low PAR%, suggest that only a minor proportion of the VTE risk in the population can be attributed to thyroid dysfunction.

4.3 Paper III:

SERUM LEVELS OF VITAMIN D ARE NOT ASSOCIATED WITH FUTURE RISK OF VENOUS THROMBOEMBOLISM – THE TROMSØ STUDY

The purpose of this study was to investigate whether high levels of 25(OH)D were associated with decreased risk of VTE in a prospective population-based study. Serum levels of 25(OH)D were measured in 6 021 men and women, aged 25-84 years, who participated in the Tromsø Study in 1994-95. Incident VTE-events were registered from date of enrollment to the end of follow-up on September 1, 2007. During a median of 10.7 years of follow-up, there were 201 incident VTE events. The risk of VTE did not decrease per one SD (19.8nmol/L) increase in serum 25(OH)D (multivariable HR 1.02; 95% CI 0.91-1.22). Moreover, subjects with serum 25(OH)D ≥70 nmol/L (upper quartile) did not have decreased risk of VTE compared to those ≤44 nmol/L (lower quartile) in age- and sex-adjusted analysis (HR 0.91, 95% CI: 0.60-1.37) or multivariable analysis adjusted for age, sex, BMI, smoking, and physical activity (HR 0.76, 95% CI: 0.45-1.28). Similar risk estimates across quartiles of serum 25(OH)D were found for unprovoked and provoked VTE. In conclusion, our findings suggest that vitamin D status does not play an important role in the pathogenesis of VTE. However, our findings did not apply to subjects with vitamin D deficiency (<30 nmol/L) due to low statistical power in this subgroup.

4.4 Paper IV:

ASSOCIATIONS BETWEEN SERUM LEVELS OF CALCIUM, PARATHYROID HORMONE AND FUTURE RISK OF VENOUS THROMBOEMBOLISM -THE TROMSØ STUDY

The aim of this study was to examine the relationship between serum levels of calcium and PTH, and the future risk of VTE in a general population with repeated measures of calcium and PTH. A total of 27 712 subjects (25-87 years) who participated in Tromsø 4 (1994-95) and/or Tromsø 5 (2001-02) were included in the study, and calcium and PTH were measured in 27 685 and 8 547 subjects, respectively. The reference range for calcium was 2.15-2.51 mmol/L, and for PTH the reference range was 1.1-6.8 pmol/L and 1.1-7.5 pmol/L for those above and below the age of 50, respectively. A time-varying analysis was used allowing participants who were re-measured in Tromsø 5 (n=7 183) to change levels of calcium and PTH over time. Incident VTE was recorded through December 31, 2012. There were 712 validated incident VTE events during a median follow-up of 15.0 years. Calcium and PTH were not associated with future risk of overall VTE, neither by a continuous nor by a categorical approach. Furthermore, in subgroup analyses, calcium and PTH showed no apparent association with unprovoked or provoked VTE, DVT or PE. However, subjects with the highest levels of both calcium and PTH (calcium ≥2.45mmol/L and PTH ≥4.0pmol/L) had increased risk of VTE compared to subjects with normal calcium and PTH (multivariable HRs 1.78, 95% CI 1.12-2.84). In conclusion, our study showed that calcium and PTH were not associated with future risk of overall VTE. However, subjects with high levels of both calcium and PTH had increased risk of VTE.

5. GENEREAL DISCUSSION

5.1 Methodological considerations

5.1.1. Study design

The data used in the present papers are all based on the Tromsø study, a large prospective cohort study of a general adult population. In a cohort study design, subjects are followed from the date of attendance until the occurrence of an outcome (e.g. disease) or until end of follow-up, making it possible to compare the rates of outcome between the exposed and non-exposed subjects. In the present papers, incident VTE events during the study-period were registered, and absolute and relative risk estimates in terms of incidence rates and relative risks were obtained. In case-control studies individuals with an outcome of interest and matched controls without the outcome are included, and information about exposure is collected retrospectively in the same manner among cases and controls. Proportions of exposure among cases and controls are compared, providing only risk estimates in the form of odds ratios.

A cohort study has several advantages compared to a case-control study. First, the clear temporal sequence of exposure and outcome in cohort studies is one of the Bradford Hill criteria for establishing causality[201]. In comparison, the retrospective nature of case-control studies makes it more prone to temporal bias (or reversed causality) as it cannot be definitely established whether the exposure preceded the disease of interest. Furthermore, recall bias could be present in case-control studies as cases and controls may remember and report information about exposure or outcome differently, and control selection may introduce bias if the chosen controls are unrepresentative of the reference population, especially in terms of exposure distribution. This is a particular problem when cases and controls are recruited exclusively from hospitals. Hospital patients tend to have different characteristics than the

general population, and if these characteristics are related to the exposure under investigation, the estimate of the association between exposure and disease will be biased. Although a cohort study is more likely to obtain valid and unbiased information on the subjects` exposure compared to a retrospective study, the exposures are not randomly assigned and factors influencing both the exposure and the outcome may be poorly quantified, lacking or unknown, potentially leading to residual confounding. Furthermore, when the exposure is modifiable and time between exposure assessment and disease manifestation is long (which is the case in all papers included in this thesis), some participants` individuals risk profile may change during follow-up, leading to regression dilution bias and an underestimation of the associations (see Modifiable factors). Thus, one cannot definitely establish whether the observed difference in outcomes between the two comparison groups is attributed to the exposure rather than other factors (confounders). In RCTs participants are allocated to intervention or control groups by chance, minimizing confounding. However, this study-design has its disadvantages. Inclusion criteria's for RCTs are often strict, and may cause selection bias and consequently reduce the external validity, rendering the cohort study the best alternative in many cases. RCTs are also time-consuming, expensive and sometimes ethically unfeasible. For instance, inducing hyperglycemia over a long time-period in order to investigate VTE risk would be ethically immoral when we are well aware of the harmful side effects of having high blood sugar. Another way to establish causality within observational studies, is by performing a Mendelian randomization study[202]. In this study, common genetic polymorphisms (i.e. alleles) that are associated with the modifiable exposure of interest (e.g. calcium and PTH) are randomly assigned, thus the risk of confounding is minimized, making this study design an efficient approach when dealing with modifiable exposures that are measured poorly and/or considerably confounded [203]. However, the association between the genetic variant and the disease must be mediated through the exposure of interest, and suitable

genetic alleles to study particular exposures may not be available[202]. In paper 4, the Mendelian randomization approach could have been applied in order to investigate whether genetically raised serum levels of both calcium and PTH increases VTE risk, however no suitable genetic allele is available for this purpose.

Another advantage of population-based cohort studies is the typically large number of study participants, enhancing the external validity and generalization of the study findings to the background population. Furthermore, cohorts are well suited to investigate rare exposures as entire populations are often invited to participate. However, a cohort design is an inefficient approach for examining rare diseases with long latency periods, as this is both time-consuming and requires a great number of participants. The design of a case-control study, including only subjects with a certain disease and eligible controls, is therefore more proper for investigating rare diseases than cohort studies. Case-control studies are also less time-consuming and less expensive than cohorts.

5.1.2. Generalizability

In a cohort study, eligible inclusion and exclusion criteria, a high participation rate and minimal loss to follow-up are crucial factors in order to obtain high external validity or generalizability of the study findings. All papers of this thesis are based on data from the Tromsø study (Tromsø 4; 1994-95, Tromsø 5; 2001-2, and/or Tromsø 6; 2007-8). The fourth survey is the largest one, and all inhabitants of the Tromsø municipality aged above 24 years were invited. In Tromsø 5 and 6 only subgroups of the municipality of Tromsø were invited. The overall attendance rate was high, ranging from 77% in Tromsø 4 to 66% in Tromsø 6, and the age and gender distribution of the study population is similar to other Western populations in terms of the incidence and prevalence of risk factor distribution. The VTE

incidence found in our population is also comparable to other Western populations[6, 25, 163], contributing to the external validity of our findings. However, a lower attendance rate was noted for younger (<35 years) and older (≥80 years) subjects, as well as in men[199], threatening the generalizability toward these subgroups. Comparison of socioeconomic status and mortality rates between attendees and non-responders in population-based surveys have shown lower socioeconomic status and higher mortality among non-responders[204, 205]. According to Langhammer et al., the most important reason for non-participation in 30-year-old subjects is lack of time or an inconvenient time for appointment, whereas among 80-year-olds, one-fifth of the non-participation is explained by illness[205].

5.1.3. Confounding and potential mediation

In terms of epidemiology, a confounding factor is causally related to the outcome variable and correlated with the exposure in the study population, but it is not an intermediate or a result of either the exposure or the outcome[206]. In cohort studies, properties other than the exposure variable of interest may be unevenly distributed between the groups being compared and thereby confound the association between exposure and outcome. Confounding may bias the results in either directions; both over- and underestimate the actual effect, as well as change the apparent direction of an effect[206].

Restriction of study participants is known to be one of the most effective strategies to deal with confounding[207]. For example, by restricting the analysis to women or men only sex imbalances cannot confound the study findings. Unfortunately, such analyses will lower the external validity. Another simple method is to stratify for confounders by dividing the sample into subgroups or strata based on characteristics thought to confound the analysis. However, both restriction and stratification may result in unacceptably low statistical power. Matching

the comparison groups is also a way to deal with confounding. However, by selecting controls with characteristics homogenous to those of the cases, one may introduce selection bias if the controls are unrepresentative of the particular population. In all papers of this thesis, confounders were included as covariates in multivariable regression models. Multivariable regression analysis secures that each exposure is not confounded by the other exposures, and is the most common method for reducing confounding in observational studies[208]. The overall strongest confounders in the populations were age and gender. In paper 1 and 3, age was adjusted for in a multivariable model, whereas in paper 2 and 4 age was used as time-scale in the regression analyses in order to eliminate confounding by age in a more proper way than the standard adjustment in a multivariable model[209]. In paper 1, also BMI turned out to be a strong confounder, and when adjusting for BMI in the age-and gender adjusted analysis the association observed between HbA1c and risk of VTE (HR 1.67; 95% CI 1.01-2.74) was highly attenuated and lost statistical significance (HR 1.27; 95% CI 0.72-2.26). Abdominal obesity has previously been shown to be the main contributing risk factor for VTE among persons with metabolic syndrome[161], and the prevalence of insulin resistance is increased in obese individuals[210] and improves with weight loss[211-213]. Furthermore, Schouwenburg et al showed that insulin resistance was not associated with risk of VTE after adjustment for BMI in a population-based cohort[214]. High BMI in patients with DM may therefore have confounded the previous observed association between DM and VTE. We did also adjust for BMI in paper 2 as BMI is a well-established risk factor for VTE, and in non-smokers there is a positive association between serum TSH and BMI[215]. However, as the distribution of BMI is balanced between the comparison groups (i.e. subgroups of TSH), BMI will not have a confounding effect. This corroborate the no effect on risk estimates when adjusting for BMI in the multivariate analysis. Despite adjustment for potential confounders in prospective cohorts, one can never rule out the possibility of residual confounding. Unknown

confounders, imprecise definitions of potential confounders or insufficient data about potential confounders may all be a source of residual confounding. For instance, baseline information on inherited thrombophilic disorders were not available in the Tromsø study. However, information on inherited thrombophilias was collected at the time of VTE diagnosis. Only 16% of those with an unprovoked event had a known thrombophilic factor registered, suggesting that the majority of unprovoked events were caused by other risk factors. To the very best of our knowledge, we are not aware of any association between inherited thrombophilia and the exposures present in paper I-IV. Hence, we do not suspect inherited thrombophilias to be unrecognized confounders in our studies.

In paper 4, subjects with high levels of both calcium and PTH had increased risk of VTE. This association could potentially be explained by more frequent incident of MI, ischemic stroke or cancer among subjects with high PTH and calcium. Recent meta-analyses have demonstrated an increased risk of future arterial CVD in subjects with increased PTH and calcium[196, 197], and growing evidence support an association between MI, stroke and future VTE[122-125]. Furthermore, hypercalcemia is relatively common in patients with cancer[216], and cancer is a well-known risk factor for VTE. We therefore examined the effect of high PTH and calcium on VTE risk while eliminating the potential effect of MI, stroke and cancer on VTE risk by performing a cause specific analysis, and the association remained essentially unchanged. These findings suggest that intermediate development of arterial CVD and cancer had minor impact on the association between high levels of calcium and PTH and VTE risk.

5.1.4. Information bias and misclassification

Self-administered questionnaires are frequently used to collect information on large study populations, generally running a risk of information bias due to misclassification. This type of

bias takes place when the collection of information is faulty or variables are imperfectly defined, potentially leading to differential (related to the occurrence of the outcome variable) or non-differential (not related to the occurrence of the outcome variable) misclassification [217]. The first one will most likely either lead to over- or underestimation of the true association, while the latter one may attenuate the true association towards the no-effect value. In a prospective cohort study, exposure variables are measured before the disease actually occurs, and consequently exposure misclassification is typically non-differential. Nevertheless, there are many advantages in using self-administered questionnaires, especially when gathering data from large study populations. It is less expensive and time consuming than most other methods, and some studies have actually concluded that self-administered questionnaires have a higher degree of validity and accuracy[218, 219]. For instance, a questionnaire may be more accurate in collecting data on sensitive and embarrassing topics (e.g. mental health, sexuality and alcohol use) compared to interviewer-administered questionnaires.

In paper 1, misclassification in the variable of self-reported DM may have occurred. The prevalence of DM (type 2) is reported to be approximately 10% in western countries, and the prevalence is increasing[220]. The prevalence of self-reported DM ranging from 2% in Tromsø 4 to 5% in Tromsø 6 is lower than expected, and likely an underestimate of the true population prevalence. To deal with this issue, data on self-reported DM was supplemented with data on confirmed DM from the MI registry of the Tromsø Study. As fasting glucose levels were not available, subjects with non-fasting glucose levels equal to or above 11.1 mmol/L and subjects with HbA1c equal to or above 6.5% were classified as having DM in accordance with the WHO report[154]. In a systematic review of primary cross-sectional studies, no evidence was found for fasting plasma glucose to be superior to HbA1c in screening for DM or impaired glucose tolerance (IGT)[221].

Measurements of ionized calcium and albumin were not available in our study, and therefore we used total serum calcium (paper 4). Total serum calcium will not only reflect the calcium physiology, but also be a function of the serum albumin level. Finally, the serum samples from 1994 had been stored frozen at -70 degrees for many years before analysis and there appeared to have been some degradation of PTH during storage as described in a previous publication from the Tromsø study[222]. However, a strong correlation between serum PTH values from 1994 and 2001 do indicate that the degradation was similar in all samples[222], and if there was an uneven degradation, this would most likely mask rather than introduce an association between PTH and VTE.

Serum levels of 25(OH)D used in paper 3, were artificially measured 15-20% higher in smokers than in non-smokers[223]. However, separate analyses for smokers and non-smokers did not change the risk estimates.

5.1.5. Modifiable risk factors

Modifiable risk factors is a potential limitation of cohort studies, especially when information on exposure is only obtained once and time-lapse between exposure and disease manifestation is long. In the papers of this thesis, median follow-up time varied between 7.1 years and 15.0 years, and all exposures were modifiable. In paper 3, serum levels of vitamin D were only measured at baseline, and as the median follow-up was 10.7 years in this study, the participants' individual risk profile may have changed during follow-up, potentially leading to an underestimation of associations due to regression dilution bias[224]. Hence, the possibility of finding false positive associations (type I errors) decreases, whereas the risk of false negative associations (type II errors) increases[224]. In paper 2, it is likely to assume that subjects with low levels of TSH (<0.05 mU/ml) are detected and treated at an early stage of the disease

since overt hyperthyroidism most often have classical troublesome symptoms. This could also lead to regression dilution bias as the treatment of overt hyperthyroidism modifies the levels of TSH. In order to minimize a potential regression dilution effect, repeated measurements of participant characteristics during follow-up (paper 1, 2 and 4) allowed for changes in risk factors over time. In paper 1, subjects who attended more than one visit were included in a separate cox-regression model wherein HbA1c was entered as a time-varying covariate with multiple records per individual. If a subject had only two repeated measures, the last HbA1c value was carried forward until a new value was obtained. The results of this time-dependent analyses were similar to those using baseline measures only. In paper 2 and 4, we used a time-varying analysis that included all participants, and those individuals who were re-measured got their exposure variable updated over time by contributing with additional observational periods. For instance, a person participating in Tromsø 4 could potentially contribute with two or three observational periods if the exposure variable also was measured in Tromsø 5 or 6, respectively, and no VTE occurred between the respective surveys.

5.1.6. Missing values

Missing data is a matter of concern in most studies, especially in large cohorts. Study participants may not complete the entire questionnaire, they may be lost to follow-up, some laboratory samples may be missing because of equipment failure, or the samples may be lost in transit or are technically unsatisfactory. Several methods exists on how to deal with missing data. One option is to omit variables which have many missing values, or one could omit individuals who do not have complete data (list-wise deletion/available case analysis)[225]. One could also in statistical analyses exclude data on subjects where the missing information is needed (pair-wise deletion)[226]. List-wise deletion is probably the most common method. However, by simple deleting individuals the statistical power may get unacceptable low,

besides the results could become biased if the number of excluded participants is high and differ significantly from the ones included (i.e. the observation needs to be missing completely at random). Another way is to use imputation techniques to replace missing values[225]. Unfortunately, none of the methods are fully satisfactory.

The main exposures in this thesis were not self-reported (i.e. HbA1c, TSH, Vitamin D, calcium and PTH), and missing values were therefore assumed to be random. Furthermore, in respect of clinically relevant parameters, subjects with missing values were similar to the subjects included in the analyses. Thus, the missing status in all papers was most likely completely random, and subjects without complete data of interest were therefore omitted in all papers (list-wise deletion). For instance, in paper 1 and 2, subjects with missing HbA1c and TSH values, respectively, in all three surveys (n=1560 and 890, respectively), were excluded from the study population. As the study population of paper 1 and 2 is of 16 156 and 18 080 subjects, respectively, the deletion did not affect the results. However, in paper 3, the analytical sensitivity for serum 25(OH)D was 10 nmol/l, and five subjects had 25(OH)D below the detection limit. Their values were set to 5 nmol/l. Finally, subjects with missing values in other covariates were only omitted in the relevant statistical analyses (pair-wise deletion).

5.1.7. Study power

In regard to proper interpretation of risk estimates, sufficient statistical power is of critical importance. Power is directly related to effect size, number of outcomes and significance level, and an increase in either one of these measures will increase statistical power. If the power of a study is relatively high and a statistically significant effect is not observed, this implies that the effect, if any, is minor. In paper 2, our study provided 80% statistical power

for assessing a HR of 1.12 for VTE by increasing levels of TSH. However, we only had 5% statistical power to detect a 2.16 fold increased risk of VTE in subjects within the lowest category of TSH (<0.05mIU/L, i.e. overt hyperthyroidism) comprising only 41 VTE events. Consequently, there was a 95% probability of type II error (i.e. false negative finding). With a prevalence of overt hyperthyroidism by 0.2% in our study population we would have needed a cohort of approximately 550 000 subjects to detect a HR of 2.0 with 80% statistical power. Lack of statistical power was also a concern in paper 3, wherein our findings did not apply to subjects with vitamin D deficiency (<30 nmol/L) due to few VTE events among these subjects.

5.1.8. Detection and validation of outcome

All incident VTE events in our study were detected from the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry at the University Hospital of North Norway. By searching all three registries there was a higher chance of a complete VTE registry in case of coding errors in one of the registries. In addition, the hospital is the single provider of specialized health care services in the region, enhancing the completion of the VTE register even more. However, one cannot rule out that some VTEs may have been missed if they were diagnosed and treated elsewhere. In order to minimize the chance of misclassification, an episode of VTE was verified and recorded as a validated outcome when all the criteria listed in the section of methods were fulfilled.

Despite the use of strict validation criteria, misclassification in registration of VTEs cannot be completely ruled out. Retrospective registration of events depend on valid and complete information, therefore insufficient information in patient records may result in inaccuracy. Additionally, in the medical records the physician`s classification of events as

unprovoked or provoked were not based on any standardized instructions. However, the personnel registering the VTE events were blinded to the baseline characteristics, so any misclassification of VTEs was most likely non-differential. Unfortunately, information on VTE prior to baseline was not available for the study participants who did not experience VTE during follow-up. Thus, there is a possibility that subjects with former VTE, who should have been excluded from the analysis, might have been included and treated as healthy participants during follow-up. However, this would presumably have insignificant effects on the risk estimates as this concerns only a small fraction of the study population.

5.2 Discussion of main results

5.2.1. Hyperglycemia and subsequent diabetes mellitus and risk of venous thromboembolism

Among the studies examining the impact of hyperglycemia and subsequent DM on VTE risk, some studies have reported an increased risk[80, 155-159], while others have failed to find an association[135, 160-165]. In the present study (paper 1), we did not find HbA1c to be a risk factor for future development of VTE, neither by a continuous nor by a categorical approach. Furthermore, HbA1c showed no significant association with either unprovoked or provoked VTE in subgroup analyses.

Contrary to our findings, a case-control study[158] by Hermanides et al. reported a 2-fold increased risk of VTE in subjects with hyperglycemia, independently of known DM. However, as non-fasting glucose levels were measured at the time of a DVT diagnosis[158], inflammatory and counter-regulatory hormone action initiated by the VTE event itself may have caused increased glucose levels in DVT patients[165]. A case-control study [165] replicating the findings of Hermanides et al. reported no significant association between increased glucose levels and risk of VTE after adjusting for C-reactive protein (CRP). Nonetheless, in a retrospective population-based cohort by Petrauskiene et al.[157], diabetic patients had about two-fold higher risk of VTE than non-diabetic subjects. However, no adjustments for BMI were made. In a previous publication from our research group, abdominal obesity was found to be the main contributing risk factor for VTE in subjects with metabolic syndrome[161]. Furthermore, the prevalence of insulin resistance is raised in obese subjects[210] and decreases with weight loss[211-213], and a population-based cohort study reported that insulin resistance was not associated with risk of VTE after adjustment for BMI[214]. Thus, high BMI in patients with DM may have confounded the observed link regarding VTE risk. This notion corroborates our finding where an association between HbA1c and VTE fully disappeared after adjustment for BMI. In contrast to our findings, the Iowa Women`s Health Study[155]

reported an association between DM and VTE even after adjustment for BMI. BMI may have been underestimated in this study due to self-reported data on weight and height, thus the true confounding effect of BMI could be attenuated.

In the LITE study[80], which combined information from two prospective cohorts (the ARIC study and the CHS), DM (defined as fasting glucose >7 mmol/L or non-fasting glucose >11.1 mmol/L) was found to be associated with a 46% increased risk of provoked VTE (95% CI 1.03-2.05), but not with unprovoked VTE. A 2012 ARIC paper that updated DM status through follow-up[164] found no statistically significant association between DM and VTE. The discrepancy between these two studies could be due to differences in study populations, and the fact that Wattanakit et al[164] used a time-varying analysis and had longer follow-up time. Besides, the finding of an association between DM and provoked VTE only, do question whether the previous observed association between DM and VTE is explained by other provoking factors and not by DM itself. This is in accordance with our findings of a tendency of increased risk for provoked VTE in subjects with HbA1c levels of 6.5% or more. In a case-control study by Heit et al[160], the observed link between DM and VTE was explained by more frequent hospitalizations of persons with DM. Hence, hyperglycemia may predispose for VTE through provoking factors such as arterial cardiovascular events, immobilization or infections. In the retrospective cohort by Petrauskiene et al.[157], the frequency of coronary heart disease and congestive heart failure was significantly higher in the group of diabetic subjects compared to the group of non-diabetics, thus provoking factors such as arterial cardiovascular events and immobilization may also partly explain their positive finding between DM and VTE risk. In a more recent ARIC report from 2013[156], Bell et al suggests that a diagnosis of DM combined with high levels of HbA1c (≥7.00%) may increase the risk of unprovoked VTE a 3-fold. However, the result should be interpreted with caution as this subgroup had few VTE events. In persons diagnosed without DM no relationship between HbA1c and VTE risk was

found[156]. In accordance to our observation, adjustment for adiposity (BMI and waist-to-hip ratio) lowered the HRs, suggesting that obesity is a more important contributor to VTE risk in subjects with hyperglycemia. This notion is further supported by three recent meta-analyses demonstrating that the increased risk of VTE associated with DM mainly results from confounders such as BMI rather than an intrinsic effect of DM on venous thrombotic risk[227-229]. Finally, a recent retrospective cohort study found blood glucose as a marker of VTE in non-diabetic critically ill children[230]. However, there is no information on whether the glucose is measured in a fasting or non-fasting state and no adjustments for BMI were performed. Besides, the increased glucose levels may be caused by the VTE event itself.

5.2.2. Thyroid function and risk of venous thromboembolism

Thyroid hormones are the main regulators of metabolism, and TSH is known as the most sensitive measure of thyroid function. A hypercoagulable state has been linked to both hyperthyroidism[171, 172] and subclinical as well as overt moderate hypothyroidism[173-175]. Furthermore, a relationship between thyroid disease and CVD has been reported[176, 177]. A registry-based study[178] of 19 519 000 subjects reported a 1.6-fold increased risk of VTE in hypothyroid patients identified by diagnosis codes, but no association was found between hyperthyroidism and VTE. Unfortunately, information on time between the diagnosis of thyroid dysfunction and the VTE event was lacking, and no data was available on the degree of thyroid dysfunction, the number of patients on thyroid hormone replacement therapy and confounders such as BMI. In contrast, a case-cohort study[179] found a 2-fold increased risk of PE in hyperthyroid patients. However, adjustment for potential confounders such as BMI were lacking, and the diagnosis codes were identified from an administrative database and not directly from medical records. Nonetheless, a case-control study[180] and a nested case-control study[181] found an increased risk of VTE in persons with FT4 levels within the upper local

reference range, and in the nested case-control study[181] a non-significant OR of 1.3 was observed in the lower 2nd percentile of TSH (<0.37 mU/L). The analyses of the case-control study[180] were limited to a population with DVT, and as blood was drawn at the time of thrombosis, the FT4 levels could potentially have been influenced by the thrombotic event itself. In the nested case-control study[181] blood was sampled only once and the time between blood sampling and measurement of thyroid parameters was about 12 years. Thus, as thyroid parameters are modifiable they might not reflect the actual level at the time of thrombosis.

In order to minimize regression dilution effect, we performed a time-varying analysis (paper II), which allowed for changes in TSH over follow-up in subjects who were measured more than once. We found that TSH within the normal range was not associated with risk of VTE. However, we observed a moderately higher risk of VTE in subjects with low (prevalence: 0.22%) and high (2.58%) TSH levels compared to subjects with normal TSH, but the confidence intervals were wide due to low number of subjects in these subgroups. Furthermore, the share of events among the general population that can be explained by thyroid dysfunction (i.e. both increased and decreased TSH levels) was low (PAR 4%). Unfortunately, data on FT4 was not available in our study, and the potential relationship between levels of FT4 and VTE risk could therefore not be explored. However, third generation assays of serum TSH, as we have used in our study, is usually considered the most sensitive measure for diagnosing hyper- and hypothyroidism[168].

In agreement with previous findings linking both hyperthyroidism and subclinical- and overt hypothyroidism to a hypercoagulable state[171-173, 178, 231-234], we observed an apparently U-shaped association between TSH levels and risk of VTE. A 2-fold increased risk of VTE was observed in subjects with low TSH compared to subjects with normal TSH. This finding is in accordance with the previous case-control and nested case-cohort studies[180, 181], reporting an association between high normal levels of FT4 and VTE risk. In contrast, a recent

prospective multicenter cohort[235] of an elderly population found that subclinical hyperthyroidism (TSH<0.45 mU/L and normal FT4 levels) was associated with lower risk of VTE. However, they only investigated the risk of recurrent VTE the initial two years after incident VTE. Besides, due to few events among subjects with subclinical hyperthyroidism, this finding could be random. Another theory explaining the U-shaped association observed in our study is that thyroid dysfunction (i.e. both high and low TSH) may predispose for VTE through other factors such as associated hospitalization or co-morbidities. This is supported by the observed association between thyroid dysfunction and provoked VTE in our study, implying that factors other than TSH and thyroid hormones could play an important role in the risk assessment of VTE among subjects with thyroid dysfunction.

5.2.3. Vitamin D and risk of venous thromboembolism

Vitamin D status is most reliably determined by serum levels of 25(OH)D as it integrates vitamin D derived from endogenous production and dietary intake[184]. Previous experimental studies have suggested antithrombotic properties for vitamin D and observational data have supported the hypothesis of an inverse association between serum vitamin D concentration and VTE risk. In the present study, we found that serum levels of 25(OH)D were not associated with future risk of VTE (paper 3). Our findings were consistent regardless of whether vitamin D was investigated by using a categorical or a continuous approach. Furthermore, no associations were revealed between vitamin D and unprovoked or provoked VTE.

The Swedish MISS (Melanoma Inquiry of Southern Sweden) study, including 40 000 women aged 25-64 years with 11 years of follow-up, concluded that women with more active sun exposure habits had a 30% reduced risk of VTE and that the incidence of VTE was 50%

lower during the summer season[190]. They questioned whether both phenomena were mediated through higher vitamin D levels[190]. This is in conflict with our findings. However, the reduced risk of VTE among women with active sun exposure habits may be mediated by some unrecognized confounders. Furthermore, a seasonal variation of VTE incidence rates have been reported since 1939, and in a multicenter study on consecutive case series from Leiden, Milan and Tromsø[236] the lowest prevalence of VTE was noted in spring. However, the absolute difference in risk in spring as compared with the other seasons was small (2%) and without any clinical relevance[236]. Furthermore, levels of serum 25(OH)D are known to vary greatly with sun exposure. In Tromsø, one would therefore expect a significant seasonal variation of vitamin D status due to the tremendously seasonal variation in UV exposure. However, due to the generally high dietary intake of vitamin D provided by fish meals and fish oil supplementation in the Tromsø population, especially in winter, the seasonal variation in serum 25(OH)D status is diluted[237]. Nonetheless, in order to control for seasonal variation in our study, intra-monthly vitamin D quartiles were used in the analyses. Contrary to our finding, a report from the Copenhagen City Heart cohort and the Copenhagen General Population cohort[238] found a weak inverse association between 25(OH)D level and VTE after controlling for seasonal variation. However, in the samples from the Copenhagen City Heart Study there could have been some degradation of vitamin D during storage at –20°C. Furthermore, misclassification of VTE events may have occurred as some of the events were based on clinical suspicion only. Though, such misclassification would probably be non-differential, and therefore bias the results toward the null finding. In accordance to our findings, a population-based cohort study[239] and a recent case-control study in elderly patients[240] found no association between seasonally adjusted serum 25(OH)D and VTE risk.

Some prospective studies have reported that subjects with serum 25(OH)D levels below 30 nmol/l have increased risk of CVD[189, 241], and a recent case-control study[242] on an

Iranian population reported that low levels of 25(OH)D were associated with unprovoked DVT. In our study, we observed an increased risk of VTE in subjects with serum 25(OH)D deficiency (<30 nmol/l) compared to those with normal serum 25(OH)D levels, but the finding was not statistical significant. In the ARIC paper by Folsom and colleagues[239], a meta-analysis was performed of the HRs of 1.28, 1.32 and 1.14 for the highest vs. lowest 25 (OH)D categories in the Copenhagen[238], Tromsø (the present study) and ARIC[239] studies, resulting in a pooled HR of 1.25 (95% CI 1.07–1.45), and no evidence of between-study heterogeneity was observed. This finding suggests that low 25(OH)D levels could be associated with a modestly increased VTE risk. Finally, a post-hoc analysis from The Women's Health Initiative (WHI) Trial, reported nearly 40% reduced risk of unprovoked VTE in postmenopausal women randomized to calcium + vitamin D supplementation[198]. However, the unprovoked VTE events were limited and there was no association with overall risk of VTE. The inconsistency between the previous studies may to some extent rely on individual variations in outdoor exposure time, sun exposure, dietary vitamin D intake/supplements, and skin pigmentation[243, 244].

5.2.4. Calcium and parathyroid hormones and risk of venous thromboembolism

Calcium is an essential co-factor in the coagulation cascade, and several studies have shown associations between calcium, PTH and concentrations of hemostatic factors and coagulation activators[193-195], suggesting that high calcium and PTH causes an imbalance in the hemostatic system in an unfavorable prothrombotic direction. In the present study (paper 4), we found that neither increased serum levels of PTH nor total serum calcium was associated with future risk of total VTE or VTE subtypes. While our study is the first population-based study to investigate an impact of serum levels of calcium and PTH on VTE risk, supplementation with calcium and vitamin D have been explored as a possible risk factor for VTE in a RCT among

postmenopausal women[198] demonstrating that 7 years of daily supplementation with calcium and vitamin D had no effect on overall VTE risk. In contrast, recent meta-analyses of prospective studies have reported that serum calcium[245] and PTH[246] were significantly associated with risk of arterial CVD. Arterial CVD was associated with a HR of 1.08 by one standard deviation increase in serum calcium, and the risk estimate remained essentially unchanged after adjustment for atherosclerotic risk factors[245]. Furthermore, subjects in the highest quartile of serum PTH had 45% increased risk of arterial CVD than subjects within the lowest PTH quartile[246]. In our study, we found however that discordant high serum levels of both PTH and total calcium were associated with a 78% increase of VTE risk independent of age, sex and BMI. The effect could potentially be mediated by indirect (e.g. influence of atherosclerotic risk factors and arterial cardiovascular diseases) or direct prothrombotic mechanisms (e.g. prothrombotic imbalance of the hemostatic system).

In accordance with previous studies[197, 247, 248], we found that high levels of serum calcium and PTH was associated with an unfavorable profile of some atherosclerotic risk factors such as high blood pressure, and high total cholesterol and triglycerides. Results from prospective cohorts have revealed that of atherosclerotic risk factors only age, obesity and a family history of MI are shared risk factors for arterial CVD and VTE[26, 134-136, 229]. Thus, it is unlikely that atherosclerotic risk factors could explain the association between high levels of serum calcium, PTH and VTE risk found in our study.

Observational studies have found a transient association between MI, stroke and future risk of VTE in the general population[122-125]. The transient increase in VTE risk after MI and stroke may in the recovery phase partly be explained by indirect causal factors including hospitalization, immobilization and complications such as infections, which are strong predisposing factors for VTE[105]. Nonetheless, direct causal mechanisms secondary to local disturbances in the cardiopulmonary circulation or electromechanical pathway (e.g. atrial

fibrillation) may also contribute to the VTE risk observed in MI patients[124]. Additionally, hypercalcemia is relatively common in patients with cancer, and cancer is a well-known risk factor for VTE. However, the effect of discordant high levels of PTH and calcium on VTE risk in our study remained essentially unchanged after censoring for MI, stroke and cancer in a cause-specific model, implying that the apparent VTE risk by high levels of calcium and PTH is not mediated by arterial cardiovascular diseases (e.g. stroke and MI) or cancer.

Based on the limited existing literature and our present findings, serum levels of calcium and PTH alone do not appear to be independently associated with VTE. However, in our population-based study, discordant high serum levels of both PTH and calcium was associated with increased risk of VTE compared to normal PTH and calcium.

6. CONCLUSIONS

- In our study, serum levels of HbA1c were not significantly associated with future risk of VTE after adjustment for BMI. Our findings suggest that hyperglycemia does not play an important role in the pathogenesis of VTE, and that obesity is a more important contributor to VTE in subjects with hyperglycemia.

- We found no association between serum levels of TSH within the normal range and future risk of VTE. However, low and high TSH levels were rare and associated with a moderate higher risk of VTE. Nevertheless, the low prevalence of thyroid dysfunction and the low PAR%, imply that only a minor proportion of the VTE events in the general population may be attributed to thyroid dysfunction.

- Serum levels of 25(OH)D were not associated with future risk of VTE, suggesting that vitamin D status does not play an important role in the pathogenesis of VTE. However, our findings did not apply to subjects with vitamin D deficiency (< 30 nmol/L) due to lack of statistical power among these subjects.

- Serum levels of calcium and PTH were not associated with future risk of VTE. However, subjects with high levels of both calcium and PTH had increased risk of VTE compared to subjects with normal calcium and PTH.

7. FINAL REMARKS AND FUTURE PERSPECTIVES

The current knowledge concerning the association between endocrine-related factors (e.g. HbA1c, TSH, vitamin D, calcium and PTH) and risk of VTE is limited, and previous studies have reported diverging results. Based on the findings of our present studies, HbA1c, TSH, vitamin D, calcium and PTH do not appear to be individually associated with VTE risk. Previous studies investigating an association between these factors and VTE risk, ought to be carefully considered in respect of methodological differences (e.g. study design, study population, number of VTE events, provoking factors and unrecognized confounders).

In order to come closer to a conclusion on whether the incidence of VTE is related to blood glucose levels, the VTE incidence may be assessed as a secondary endpoint in diabetic subjects participating in RCTs aiming to improve blood glucose levels. Regarding thyroid function and VTE risk, it would be interesting to explore levels of FT4 as a risk factor for VTE within a population-based cohort as increased FT4 levels are associated with VTE risk in case-control studies. Furthermore, the incidence of VTE could be assessed as a secondary endpoint in hypo- or hyperthyroid patients participating in RCTs aiming to improve thyroid function. In order to reach a conclusion on the impact of vitamin D deficiency on risk of VTE, examination of VTE as an outcome in ongoing vitamin D supplementation trials is warranted.

Although serum levels of calcium and PTH alone did not appear to be individually associated with VTE risk in our study, we observed that high serum levels of both PTH and calcium were associated with increased VTE risk even after eliminating the potential effect of MI, stroke and cancer on VTE risk. As we are the first to investigate the associations between serum levels of calcium, PTH and future risk of VTE in a general population, further population-based studies are needed. Furthermore, the VTE incidence could be assessed as a secondary endpoint in RCTs studying treatment of subjects with primary hyperparathyroidism.

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Appendix