Risk factors for venous thromboembolism: Smoking, anthropometry, and genetic susceptibility

PhD thesis

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Preface

This PhD thesis is based on four studies carried out during my employments at the Department of Clinical Biochemistry, Aalborg Hospital, Aarhus University Hospital and at the Department of Clinical Epidemiology, Aarhus University Hospital.

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List of abbreviations

VTE	Venous thromboembolism
DVT	Deep venous thrombosis
PE	Pulmonary embolism
HRT	Hormone replacement therapy
PPV	Positive predictive value
CHD	Coronary heart disease
BMI	Body mass index
HR	Hazard ratio

This PhD thesis is based on the following papers:

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II. Severinsen MT, Kristensen SR, Johnsen SP, Dethlefsen C, Tjonneland A, Overvad K. Smoking and venous thromboembolism: a Danish follow-up study. *Journal of Thrombosis and Haemostasis 2009, 7:1297-1303.*

III. Severinsen MT, Kristensen SR, Johnsen SP, Dethlefsen C, Tjonneland A, Overvad K. Anthropometry, Body Fat, and Venous Thromboembolism: A Danish Follow-up Study. *Circulation 2009,120:1850-7.*

IV. Severinsen MT, Overvad K, Johnsen SP, Dethlefsen C, Madsen PH, Tjonneland A, Kristensen SR. Genetic susceptibility, Smoking, Obesity and risk of Venous Thromboembolism: A Danish Case-cohort Study.

Content

"Life is brief, art is long, opportunity is fleeting, experience is fallacious, judgment is difficult"
(Hippocrates 460-370 B.C.)

1. Introduction

1.1. Definition of venous thromboembolism (VTE)

Thrombosis is the formation of a clot or thrombus within the lumen of a blood vessel that obstructs the flow of blood through the circulatory system. The formation and growth of a thrombus are caused by local activation of the coagulation system, combined with an imbalance of procoagulant, anticoagulant, and fibrinolytic factors. A venous thromboembolism (VTE) is any thromboembolic event that occurs within the venous system. The majority of VTE start in the calf veins; from there, the thrombosis may progress to the proximal veins, and later, it may break free to lodge in the lungs, where it cause the potentially fatal condition, pulmonary embolism (PE)[1-4]. Thrombosis occurs in other veins, but not commonly. In this thesis, VTE is defined as deep venous thrombosis (DVT); i.e., thrombosis of a deep vein in an extremity (leg or arm); and PE which is considered to be the obliteration of the pulmonary arterial network by one or several blood clots.

1.2. The burden of VTE

Incidence rate

The incidence rate of VTE has varied between 1 and 2 per 1000 person-years in different study populations[5-14]. The incidence rate of VTE increases with age for both genders, and PE accounts for an increasing proportion of VTE events with increasing age. However, the true incidence rate of VTE is difficult to determine, due to the clinically silent nature of VTE and the fact that the first sign of the disease may be sudden fatal PE. Autopsy studies have shown that about one third of the people that died in hospital had VTE and 10% of all autopsies show signs of fatal PE[15-18]. A Danish autopsy study that included only forensic autopsies showed that 58 of 2,874 (2%) subjects had died of fatal PE. A review of the medical records, police reports, and interviews with relatives showed that PE was unexpected in all cases[19].

Consequences

VTE is a serious medical event associated with a substantial risk of adverse outcomes[6, 9, 20, 21]. The 30-day case fatality rate (i.e. proportion of patients who die) is about 5% for DVT and 10% for PE; the one-year case fatality rate is approximately 20% for both DVT and PE[6, 9, 20, 21]. The 10-year recurrence rate of VTE is 30%[21-24]. Predictors of VTE recurrence are male sex, idiopathic VTE, and persistent risk factors. The recurrence rates of DVT and PE are simi-

lar[25]. The initial clinical presentation of DVT or PE predicts the manifestation of a recurrence; hence, patients that had a previous PE event tend to have a recurrence of PE more frequently than patients that had a previous DVT event[26, 27]. Furthermore, VTE is associated with long-term complications. The post-thrombotic syndrome is a chronic, progressive condition that occurs despite optimal anticoagulant therapy. This syndrome occurs years after the VTE event, thus, it may not be interpreted as a result of thrombosis. The symptoms include pain, heaviness, swelling, and cramping in the leg; these symptoms are aggravated during standing or walking. In severe cases, a venous ulcer may develop. The post-thrombotic syndrome occurs in 20-50% of VTE patients within 10 years of the VTE, and severe post-thrombotic syndrome with an ulcer occurs in 3-7% of VTE patients[21, 24, 28]. Chronic pulmonary hypertension is a consequence of an unresolved thromboembolic occlusion in pulmonary vessels, and it is associated with considerable morbidity and mortality. Dyspnoea is the most common symptom of chronic pulmonary embolism. In advanced cases, signs of right heart failure are observed, including jugular venous distension, hepatomegaly, and peripheral oedema. Four percent of PE patients have been shown to develop symptomatic pulmonary hypertension and another 9% develop asymptomatic pulmonary hypertension[29, 30].

Prevention

Prevention of VTE is of particular importance, due to 1) the substantial proportion of sudden fatality and 2) the burden of long-term sequels that are mostly untreatable. Approaches for preventing VTE include eliminating risk factors or treating with anticoagulant medication. To improve the efficacy of VTE prevention and treatment approaches, it is important to gain a thorough understanding of the aetiologies of this potentially fatal condition.

1.3. Historical view

The mechanism of coagulation

Hippocrates (ca. 460–370 B.C.) is known as the father of medicine. He disagreed with the general belief of his time that diseases were a divine castigation. He claimed that disease was caused by disequilibrium and thought that coagulation of the blood was caused by low temperature[31]. Two thousand years later, the Scottish surgeon John Hunter (1728-1793) stated that air caused coagulation of the blood. However, a few years later, his student William Hewson (1739-1774) described fibrinogen and provided a means for others to find more co-

agulation factors[32, 33]. In 1905, Paul Morawitz described coagulation as an enzymatic cascade that included prothrombin, thrombin, fibrinogen, and fibrin[34]; this was the beginning of the modern concept of the coagulation cascade.

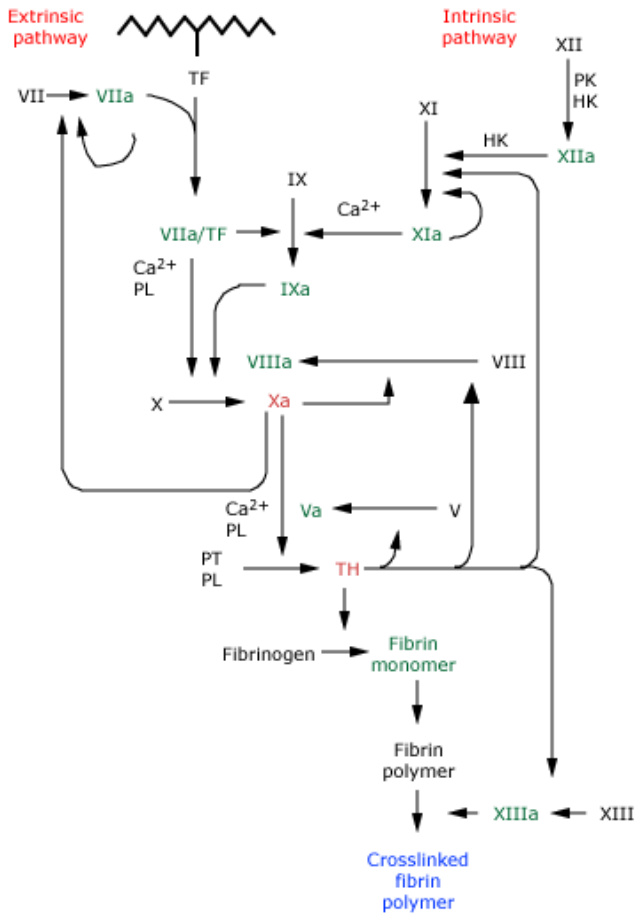

Figure 1.1. Coagulation cascade[35]
TF: Tissue factor, PT: Prothrombin, TH: Thrombin, PL: Phospholipid, PK: Prekallikrein

Clinical manifestation of venous thrombosis

Extensive research by medical historians has not revealed any descriptions that could be interpreted as venous thrombosis in the writings of Hippocrates, Galenus, Celius Aurelianus, or other ancient physicians [36, 37]. Furthermore, venous thrombosis is not among the numerous diseases described in the Bible[38]. The first known description of venous thrombosis was written in 1271. The document describes the case of a 20 year old man, Raoul, who developed unilateral oedema in the right foot, which subsequently extended up to the thigh with no symptoms in the contra-lateral leg. Raoul developed a septic ulcer and fistulae, but a surgeon

suggested that they should wait and see whether it would spontaneously resolve. He was advised to pray on the tomb of the blessed Saint Louis. After praying, he applied dust from the tomb to the fistulae and ulcers, and the oozing stopped - Raoul was cured. The manuscript was probably only retained for its religious implications[36, 37, 39].

The first known detailed description of DVT was written in England by Richard Wiseman in 1676[40]. He reported on the wife of a pharmacist, who after a difficult labour, developed swelling and pain in the right leg with no inflammation or discolouration of the skin. Wiseman deduced that a thrombus had formed, caused by changes in the blood; thus, he pioneered the concept of hypercoagulability. However, common understanding in the 17th century held that the clinical manifestation of DVT, known as phlegmasia dolens, was caused by an overabundance of breast milk during pregnancy and after delivery. It was thought that the stored milk had fallen into the leg, which led to inflammation of the leg. The condition was therefore called "milk leg"[41]. A famous French obstetrician, Nicolas Puzos (1686-1753), emphasised in 1769 that it was important for a new mother to nurse her infant; otherwise the milk would enter the blood vessels and cause swelling that could be fatal. In 1822, David D. Davis reported four cases of young women that had died after child birth and their autopsies demonstrated clots in the iliac veins. Davis did not examine the lungs, but one of the patients died suddenly and another died with severe chest pain. He reported that they all died of sepsis[41]. Davis showed for the first time that phlegmasia dolens was caused by venous thrombosis; however, he did not associate the lung symptoms with thrombosis.

The connection between DVT and PE was discovered by Rudolph Virchow (1821-1902). In Berlin, August 1846, Virchow performed 76 autopsies[42]. In 11 of these autopsies, he found clots in the pulmonary arteries, and in 10, he found additional clots in the deep veins of the legs. He hypothesised that the clots had formed in the veins and travelled in the circulation to the lungs; thus, they had not formed in the lung due to "phlebitis", which was the commonly accepted cause of clots in the lungs during that time. To prove his hypothesis, Virchow performed experiments in dogs. He inserted different types of foreign bodies into the jugular veins of dogs; often, he observed respiratory distress a few days later. Either the dogs died from the intervention or were killed for study. He then demonstrated that the foreign body had travelled from the jugular vein to the lung; this proved that the blood had the capacity to transport thrombi from the peripheral circulation to the lung[42]. The modern understanding of VTE arose from that precept. Based on the dog experiments, Virchow also suggested that

thrombus *propagation* was due to irritation of the vessel wall, components in the blood, or an interruption of blood flow. However, he did not describe these factors as *initiators* of thrombus formation[43]. Nevertheless, one hundred years later, Virchow was credited with the discovery of Virchow's triad; i.e., the pathophysiologic *initiators* of venous thrombosis, including endothelial injury, procoagulant status, and circulatory stasis. This misinterpretation of Virchow's description might be attributed to English-speaking authors who were denied a precise English translation. Despite this misnomer, Virchow's triad is currently used as model for the pathophysiologic mechanism of VTE.

Virchow believed that health and disease were products of the whole environment of a person, from the cells in the body to the social setting of the person. He was called "Hippocrates with the microscope" due to his finding that microscopic pathological entities gave rise to the clinical manifestations of diseases[44, 45]. On January 4, 1902, Virchow jumped off a streetcar in Berlin, stumbled, and sustained a femoral neck fracture that resulted in his hospitalisation for months. He never fully recovered, and shortly thereafter, he re-injured his hip and later developed pneumonia. Virchow died on September 5, 1902. He may have died from his "own" disease PE[46].

Factors that provoke VTE

Venous thrombosis was first known as "Milk leg" due to the association with pregnancy and delivery. Later, the condition now known as DVT was described in other patients. In 1810, Ferrier noted that some medical diseases were associated with DVT, particularly when combined with prolonged immobilisation in bed. He observed that the condition occurred during devastating infectious diseases, including typhus[38]. In 1860, a French internist, Armand Trousseau (1801-1867) discovered that malignancies were associated with the pathological phenomenon of clots forming, resolving, and then appearing again elsewhere in the body. Trousseau was the first to discover the association between cancer and VTE, and the phenomenon was called Trousseau´s syndrome. Unhappily, Trousseau later found the signs in himself; he was subsequently diagnosed with pancreatic cancer and died soon thereafter[38]. Spencer Wells was reported to be the first to discover the association between surgery and VTE in 1866. More recently, Gunnar Bauer (1942) called attention to the frequency of DVT in patients with leg fractures. Byrne (1955) investigated 748 cases of DVT and found that surgery was the second most common predisposing factor (the first was giving birth)[38, 47]. A

new research area was born in 1965, when Egeberg discovered antithrombin deficiency; this was the first description of a hereditary (genetic) risk factor for VTE[48]. This finding was followed by those from Griffin and Esmon in the early 1980s, who showed independently that protein S and protein C deficiencies were genetic risk factors for VTE[38]. Later, the Factor V Leiden (G1691A) and prothrombin (G20210A) mutations were identified as genetic risk factors for VTE[49]. These mutations are common in Caucasians, but absent in non-Caucasians. These single nucleotide mutations were found to occur approximately 22,000 years ago, after the evolutionary separation of non-African from African populations (100,000 years ago) and of Caucasian from Mongolian populations (40,000 years ago). The mutations are currently common in Caucasians, perhaps due to evolutionary advances of the mutations when bleeding from wounds and parturition was a major cause of mortality[50]. In the 1960s, the use of oral contraceptives and later, HRT, were recognised as risk factors for VTE[51]. Finally, in the 1980s, phospholipid antibodies were found to be associated with VTE[52].

Treatment of VTE

Before the discovery of the coagulation system, common treatments for VTE were bloodletting or the application of leeches. In 1935, the discovery of heparin prompted treatments with pure heparin given by injection; a few years later, dicoumarin, the first vitamin K antagonist, was on the market[40]. In 1960, Barrett and Jordan published the first randomized trial of a treatment for VTE[53]. Their study included 35 patients with symptoms of PE. The results showed that intravenous heparin given in combination with an oral vitamin K antagonist reduced death and recurrence compared with no anticoagulant therapy. The standard treatment became heparin during hospitalisation and a vitamin K antagonist after discharge. In the 1980s, it was found that low-molecular-weight heparin provided benefits over heparin. This led to the replacement of heparin with low-molecular-weight heparin in the standard treatment for VTE[54]. Today, anticoagulation is a cornerstone of VTE treatment.

1.4. Pathophysiology of VTE

The haemostatic balance

Haemostatic balance ensures that blood is constantly fluid in the vessels, and at the same time, is able to clot immediately when the vessel wall is injured. This balance is maintained with the complex interplay between procoagulant and anticoagulant factors. Initiation of a

thrombus appears to be different in arteries and veins. In arterial thrombosis, there is a clear relationship between vessel injury and the formation of a thrombus. Conversely, vessel injury does not appear to be involved in the majority of VTE events[55]. Blood stasis has been found to be an important feature for the initiation of venous thrombosis. Stasis causes local hypoxia, which leads to the activation of endothelial cells and synthesis of tissue factor from circulating monocytes. Likewise, the initiation of inflammation may involve the activation of endothelial cells[56]. It is suggested that small thrombi are formed constantly (more or less), and the balance between procoagulant, anticoagulant, and probably fibrinolytic factors, determine whether a thrombus is going to progress or dissolve.

Traditionally, the risk factors for VTE, as mentioned in the historical perspective, include: pregnancy, the use of oral contraceptives or HRT, cancer, fracture, surgery, medical disease, immobilisation, and thrombophilia (hereditary or acquired). Some of these factors contribute to stasis, and some contribute to hypercoagulability. Based on the presence of one or more of these risk factors, VTE events can be classified as provoked or idiopathic[6, 9].

Current research has focused on procoagulant and anticoagulant factors. Hypercoagulant factors have been identified that are associated with VTE. High plasma concentrations of Factor VIII[57], prothrombin[58, 59], and fibrinogen[60] have been established as risk factors for VTE. Further, high levels of Factor IX and Factor XI are potential risk factors for VTE[60]. In contrast, other studies have shown that plasma concentrations of Factor V, VII, X, and XII were either negative or inconclusive risk factors for VTE; thus, these are considered less important procoagulant factors[60]. Low plasma concentrations of the anticoagulant factors, antithrombin, Protein C, and Protein S, are also associated with hypercoagulability, because they are important down-regulators of coagulation[49]. Finally, it is not clear whether reduced fibrinolysis influences the development of VTE; however, some components of the fibrinolytic system appear to be associated with VTE[61, 62].

Age is one of the more potent risk factors for VTE, but the underlying mechanism is not fully understood. One explanation may be that older people are less mobile and have more provoking risk factors than commonly found in younger individuals. However, the incidence rate of idiopathic VTE also increases with increasing age; therefore, age-related changes in the vessel wall or blood composition may contribute to the higher risk of VTE with age. Aging may represent an imbalance of the coagulation system[63-66]. It was shown that the plasma concentra-

tions of Factor VIII and fibrinogen increased with age, and fibrinolytic activity decreased with age. Age-related endothelial damage may prevent the endothelium from inhibiting coagulation and initiating local fibrinolysis; this may contribute to the increased risk of VTE with age.

1.5. Studying risk factors for VTE

As previously suggested by Virchow, VTE is now known as a multifactorial disease that involves the combined actions of environmental and genetic risk factors[67]. Therefore, observational studies are suitable for studying the risk factors of VTE. When conducted properly, they can supply important knowledge about the disease [68].

1.6. Use of hospital discharge and other disease registries in research on VTE

The burden of VTE has been described in large cohort studies. To provide valid results in these cohort studies, it was important to have complete follow-ups or at least non-differential losses to follow-up. The use of administrative databases for the follow-up of study participants provides a valuable, cost-efficient, and complete way of collecting data on different diseases[69]. Routine data collection and often universal registration of the people in the target population can reduce the probability of biases in recall, diagnosis, and selection. However, an important disadvantage of registry data is the lack of investigator control over data collection and quality of data collection[69, 70]. The quality of registry data varies considerably among diseases, and poor data quality may invalidate the results of epidemiological studies. Thus, it is essential to validate the routinely collected registry data before use in research.

Few studies have examined the quality of the VTE diagnoses recorded in hospital discharge registries (Table 1.1.); most of these studies included diagnosis codes based on the International Classification of Diseases, 9th revision (ICD-9) or included selected groups of patients.

Table 1.1 Prior studies on the validity of VTE discharge diagnoses recorded in administrative registries.

Authors	Country	Study period	ICD number/ diagnose type	Study population	Number of VTE diagnoses	Methods used	Type of VTE	PPV
Larsen TB[113] 2005	Denmark/ Northern Jutland County	1980-2001	ICD-8/ ICD-10	Pregnant women	DVT 153 PE 22	Record review	DVT PE	86.3 % 81.8 %
White RH[114] 2004	US/California	1990-1998	ICD-9	Pregnant women	VTE 214	Record review	Different sub diagnoses	31-80 %
Cushman et al.[6] 2004	US	1987-1997	ICD-9	Participants in two cohorts: ARIC45-64 years old; CHS over 65 years old	756 VTE (incident and prevalent VTE diagnoses)	Record review	Different sub diagnoses	29-74%
Heckbert SR[115] 2004	US	1994-2000	ICD-9	Postmenopausal women participating in estrogen/progestin trial	DVT 139 PE 58	Record review	DVT PE	75 % 86 %
Birman-Deych E[116] 2005	US	1998-1999	ICD-9	Medicare patients with atrial fibrillation	DVT 332	Record review	DVT	72 %
Arnason T[117] 2006	Canada/Ottawa	1999-2000	ICD-9	Patients at a tertiary care hospital in Ottawa	VTE 86 (incident and prevalent VTE diagnoses)	Record review	VTE	74 %

1.7. Smoking, anthropometry, and genetic susceptibility

Life style factors, including smoking and anthropometry, have been shown to be associated with the risk of arterial thrombosis. A major component in these associations is the development of atherosclerosis, but other mechanisms may also play a role; for example, the level of procoagulants. A positive association was found between VTE and arterial thrombosis[71, 72]; hence, VTE was positively associated with myocardial infarction and patients with idiopathic VTE have atherosclerosis more frequently than patients with provoked VTE. However, it is not clear what mechanisms are involved in the associations between VTE and arterial thrombosis. Atherosclerosis may induce VTE and arterial thrombosis, or the two conditions may share common risk factors. Hypercoagulability may be the pathophysiologic link between arterial thrombosis and VTE[73-76].

1.7.1. Smoking and risk of VTE

Smoking may increase the risk of VTE through a number of mechanisms:

1) Smoking is a well established, potent risk factor for a number of diseases, including cancer and cardiovascular diseases (stroke and coronary heart diseases (CHD)); these, in turn, are associated with an increased risk of VTE. Therefore, smoking might be associated with the risk of provoked VTE.
2) Smoking is associated with a higher plasma concentration of fibrinogen[76-80] .
3) Smoking is associated with reduced fibrinolysis[77, 80].
4) Smoking is associated with inflammation[76, 80-84].
5) Smoking increases the viscosity of the blood[76, 77, 85].

A PubMed search was conducted for articles on VTE with the following MeSH terms or free text word-combinations: “venous thrombosis”, “venous thromboembolism”, “deep venous thrombosis”, “deep vein thrombosis”, or “pulmonary embolism”. We identified 67,561 articles on VTE. Another PubMed search for articles on smoking with the search word “smoking” provided 146,874 articles on smoking. When these two sets of articles were combined, 353 articles were identified that were related to both smoking and VTE (articles were limited to the English language, subjects included all adults over 19 years old, and species was limited to humans). Table 1.2 shows the most important observational studies that found this as-

sociation (criteria: population-based studies, follow-up studies, and large case-control studies).

It is clear that the data are inconsistent regarding the association between smoking and VTE. Only four follow-up studies were available[86-89]. Two included only men, one included only women, and one included both men and women. Five case control studies were found, and they included both genders. Of those nine studies, only three found a positive association between smoking and VTE; in all three studies different levels of tobacco smoking were considered. In two of the studies, the association was only found for the highest dose of tobacco consumption, and in one study, a positive association was also found between current smokers (all doses) and VTE; this study was a very large case-control study that included nearly 4,000 VTE events. Five studies found no association between smoking and VTE, and none of them included information regarding different doses of tobacco. One of these was a large case-control study that included 6,550 unvalidated VTE events; the data on smoking and the information on VTE outcome were obtained from the same database. Poor data quality may have invalidated the results in this study. Finally, a case-control study that included 636 VTE events found a protective effect of smoking on the risk of VTE. This finding was probably biased, because the reference group included patients with rhino pharyngeal infections, which may have been related to smoking.

An assessment of the association between smoking and VTE requires detailed data that includes smoking doses and validated VTE events. Furthermore, it may be valuable to classify the VTE events as provoked and unprovoked; this may elucidate the mechanisms that underlie the effects of smoking on VTE risk.

Table 1.2 Prior studies on smoking and risk of VTE

Authors	Country	Study period	Study design (no of cases)	Study population	Outcome identification	Exposure identification	Risk estimate	
Goldhaber et al.[87] 1997	US	1976-1992	Prospective cohort 280 PE	Nurses 30-55 years old Women	Self report/mailed questionnaires Incident VTE Validation (review of medical records) Classification	Self report/mailed questionnaires (tobacco dose in no. cigarettes)	Never Past Current 1-14 Current 15-24 Current 25-34 Current ≥ 34	1 0.9 (0.7-1.3) 0.8 (0.5-1.4) 1.1 (0.7-1.6) 1.8 (1.2-2.9) 2.1 (1.2-3.6)
Hanson et al.[88] 1999	Sweden	1963-1993	Prospective cohort 65 VTE	Men born in 1913 living in Göteborg Men	Validation (22 of 65 by autopsy) No classification Incident VTE	Examination, questionnaires (tobacco dose in no. cigarettes)	Never Past Current 1-14 Current ≥ 15	1 1.39 (0.60-3.22) 1.38 (0.63-3.06) 2.82 (1.30-6.13)
Samama et al.[118] 2000	France	1990-1991	Case-control (624 GPs) 636 DVT	Case: DVT patients GP Control: influenza/rhino pharyngeal symptoms	Validation (plethysmography allowed) Consecutive VTE, 21% recurrent VTE	Information from records	Regular smoking	OR:0.66 (p:0.04)
Heit et al.[119] 2000	Olmsted County, US	1976-1990	Nested, Case-control 626 VTE	Population based (38% acute fatal PE) Women and men	Validation Incident VTE	Information from medical records	None Current Former	1 1.30 (0.91-1.85) 1.07 (0.79-1.44)
Tsai et al.[89] 2002	US	1987-1998	Prospective cohort 215 VTE	ARIC 45-64 years old CHS over 65 years old Women and men	Telephone self-report followed by validation No classification	Self-report interview	Never Past Current	1 1.02 (0.75-1.40) 1.03 (0.71-1.49)
Glynn et al.[86] 2005	US	1982-2003	Prospective cohort 358 VTE	Physicians participating in trial Men	Self report/ questionnaires Validation Classification	Self-report	Never Past Current	1 0.91 (0.73-1.15) 0.86 (0.58-1.27)
Huerta et al.[120] 2007	UK	1994-2000	Nested, Case-control, 6550 VTE	Population based (1500 GPs) Women and men	VTE diagnosis and anticoagulation No validation	Information from general practice	Never Past Current	1 1.19 (1.05-1.35) 1.17 (1.05-1.28)
Pomp et al.[108] 2007	The Netherlands	1999-2004	Case-control 3989 VTE	Case: Participants in the MEGA study Control: partners of case or RDD Men and women	No validation but all cases from AK-clinics PPV DVT: 97%, PPV PE: 78% Classification, Non cancer VTE	Self report/ questionnaires	Never Current, 1-9 Current 10-19 Current ≥ 20	1 1.23 (1.00-1.50) 1.41 (1.21-1.64) 1.64 (1.41-1.90)
Lindqvist et al.[121] 2008	Sweden	1990-2002	Prospective cohort 312	Miss study (investigation on malignant melanoma)	No validation only claim data	Self report/ questionnaires Lifetime smoking dose	Never <100.000 ≥ 100.000	1 1.1 (0.8-1.5) 1.3 (1.0-1.9)

1.7.2. Anthropometry and risk of VTE

Obesity may increase the risk of VTE through a number of mechanisms:

1) Obesity, is a well-established, potent risk factor for a number of diseases, including cancer and cardiovascular diseases (stroke and CHD); thus, obesity might be associated with an increased risk for provoked VTE.
2) Obesity may be associated with a sedentary lifestyle and immobilisation.
3) Central obesity may be associated with increased intraabdominal pressure and reduced venous flow from the legs[90, 91].
4) Central obesity is a feature of the metabolic syndrome, which, in turn, is associated with VTE[92-95].
5) Obesity is associated with a procoagulant status (increased levels of fibrinogen, factor VIII, and factor IX)[73, 74, 96-98].
6) Obesity is associated with reduced fibrinolysis[73, 74, 97-101].
7) Obesity is associated with inflammation[102].

A PubMed search was conducted for articles on anthropometry with the following MeSH terms or free text word-combinations: “obesity”, “overweight”, “anthropometry”, “waist circumference”, “body fat”, “waist hip ratio”, or “hip circumference”. We identified 207,220 articles on anthropometry. This set of articles was combined with the set of articles on VTE, and 563 articles were identified that were related to both anthropometry and VTE (articles were limited to the English language; subjects included all adults over 19 years old; and species was limited to Humans). Table 1.3 shows the most important observational studies that found this association (criteria: population based studies, prospective studies, and large case-control studies).

These studies showed that obesity is an established risk factor for VTE. The majority of studies that assessed the association between obesity and VTE used the body mass index (BMI; i.e., the weight [kg] divided by the height squared [m^2]) as the measure of obesity. The BMI is a marker of excess body weight and correlates well with body fat content in adults; however, it fails to consider the distribution of body fat. Recently, waist circumference was shown to be associated with the risk of VTE[93, 95]. These studies did not evaluate whether different fat distribution had different effects on the risk of VTE. It was found that the distribution of body fat predicted the risk of arterial thrombotic events, including CHD. Central obesity, assessed as

either waist circumference or waist-to-hip ratio, was a better predictor of CHD than general obesity measured with the BMI. In contrast, peripheral obesity, assessed as hip circumference, was not a predictor of CHD[103, 104]. These findings indicated that central and peripheral obesity have different effects on CHD. However, no studies have tested whether central obesity might be a better predictor for VTE than peripheral obesity.

Table 1.3 Prior studies on anthropometry and risk of VTE

Authors	Country	Study period	Study design (no of cases)	Study population	Outcome identification	Exposure identification	Risk estimate	
Goldhaber et al.[87] 1997	US	1976-1992	Prospective cohort 280 PE	Nurses 30-55 years old Women	Self report/mailed questionnaires Validation	Self report/mailed questionnaires	BMI<21.0 BMI 21.0-22.9 BMI 23.0-24.9 BMI25.0-28.9 BMI ≥ 29.0	1 0.7 (0.4-1.1) 1.2 (0.7-1.9) 1.7 (1.1-2.6) 3.0 (2.0-4.7)
Hanson et al.[88] 1999	Sweden	1963-1993	Prospective cohort 65 VTE	Men born in 1913 living in Göteborg Men	Validation 22 of 65 by autopsy	Examination	Waist < 100 cm Waist ≥ 100 cm	1 3.92 (2.10-7.29)
Tsai et al.[89] 2002	US	1987-1998	Prospective cohort 215 VTE	ARIC 45-64 years old CHS over 65 years old Men and women	Telephone self-report followed by validation	Examination	BMI <25 BMI 25-30 BMI≥ 30	1 1.51 (1.06-2.14) 2.27 (1.57-3.28)
Glynn et al.[86] 2005	US	1982-2003	Prospective cohort 358 VTE	Physicians participating in trial Men	Self report/ questionnaires Validation	Examination	BMI per 1 kg/m^2	1.10 (1.07-1.14)
Huerta et al.[120] 2007	UK	1994-2000	Case-control, nested 6550 VTE	Population based Men and women	VTE diagnosis and anticoagulation No validation	Information from general practice (multicenter)	BMI 20-24 BMI <20 BMI 25-29.9 BMI≥ 30	1 0.89 (0.72-1.09) 1.27 (1.16-1.40) 2.11 (1.88-2.36)
Pomp et al.[107] 2007	The Netherlands	1999-2004	Case-control 3843 VTE/ 4683 control	Case: Participants in the MEGA study Control: partners of case or RDD Men and women	No validation but all cases from AK-clinics PPV DVT: 97% PPV PE: 78% Exclusion: Participants with malignancies 10 years before VTE	Self report/ questionnaires	Weight (kg) < 50 Weight 50-59 Weight 60-69 Weight 70-79 Weight 80-89 Weight 90-99 Weight 100-109 Weight ≥ 110	0.68 (0.40-1.16) 0.60 (0.49-0.73) 0.69 (0.60-0.78) 1 1.43 (1.26-1.62) 1.88 (1.63-2.17) 2.45 (2.01-2.99) 2.93 (2.28-3.77)
Steffen et al.[95] 2009	US	1987-1998	Prospective cohort 358 VTE	ARIC 45-64 years old CHS over 65 years old Men and women	Telephone self-report followed by validation	Examination	Waist < 88cm Waist ≥ 88 cm Waist < 102cm Waist ≥ 102 cm	1 1.57 (1.27-2.41) 1 2.14 (1.49-2.93)
Borch et al.[93] 2009	Norway	1995-2007	Prospective cohort 194 VTE	Citizens in Tromsø with metabolic syndrome Men and women	Validation	Examination	Waist < 88/102cm Waist ≥ 88/102 cm	1 2.03 (1.49-2.75)

1.7.3. Genetic susceptibility, smoking, obesity and the risk of VTE

Two genetic mutations that are common in Caucasians are well-established risk factors for VTE. One is the Factor V Leiden (G1691A) mutation, and the other is the prothrombin (G20210A) mutation[49]. The Factor V Leiden mutation leads to slower degradation of activated coagulation factor V and factor VIII[49, 105]. Consequently, individuals with this mutation have higher concentrations of these coagulation factors. The prothrombin mutation causes a higher plasma level of coagulation Factor II which at least partly may explain the procoagulant effect[59, 106]. Both mutations represent a persistent imbalance between procoagulant and anticoagulant factors due to higher baseline levels of procoagulant factors. Lifestyle factors, including smoking and obesity have been found to be positively associated with VTE [107-109]. The mechanisms underlying these associations are not fully understood, but it has been shown that obesity is associated with higher levels of factor II and factor VIII[73, 74, 96-98]; and smoking is associated with a higher level of fibrinogen[73, 74, 78, 110]. Procoagulant status may represent a mechanistic explanation of the associations between VTE and these lifestyle factors. A combination of lifestyle factors and the Factor V Leiden or prothrombin mutation may tip the balance of coagulation factors to favour thrombosis. Hypothetically, lifestyle factors may interact with a genetic risk factor and cause a larger (synergistic) effect that exceeds the sum of the separate effects[111].

A PubMed search was conducted for articles on the Factor V Leiden mutation with the following free text search word-combinations: “Factor V Leiden”, “G1691A”, “FV Leiden”, “FVL”, or “activated Protein C Resistance”; or with the MeSH term “genetic predisposition for disease”; or with the substance name “Factor V Leiden” . This set of articles was combined with the articles related to both VTE and smoking; the set was also combined with the articles related to both VTE and anthropometry. We found 38 articles that were related the combination of the Factor V Leiden mutation, VTE, and smoking. We found 45 articles related to the combination of the Factor V Leiden mutation, VTE, and anthropometry. Another PubMed search was conducted for articles on the prothrombin mutation with the following MeSH terms: “prothrombin mutation”, “G20210A”, “factor II gene mutation”, or “genetic predisposition for disease”. We found 18 articles related to the combination of the prothrombin mutation, VTE, and smoking. We found 21 articles related to the combination of the prothrombin mutation, VTE, and anthropometry (articles were limited to the English language; subjects were limited to all adults over 19 years old; and species was limited to Hu-

mans). Also we conducted an EMBASE search for articles, but found no further important articles on this topic. Table 1.4 shows the population-based observational studies that considered these combinations.

Few well-designed studies have examined the effects of a combination of lifestyle factors and genetic factors on the risk for VTE[107, 108, 112]. Only one follow-up study evaluated the combined effects of the Factor V Leiden mutation, smoking, and obesity. This study included only 216 unvalidated VTE events that were stratified by genotype (3 strata), smoking (2 strata), obesity (3 strata), and age (3 strata). A 10-year cumulative incidence of VTE was given for each stratum, but the number of cases in each stratum was omitted. The statistical precision may have been poor in some of the strata. Two large case-control studies examined the combinations of lifestyle and Factor V Leiden and prothrombin mutations; one evaluated the joint effects with smoking and the other with obesity. These studies indicated that combinations of lifestyle and genetic risk factors yielded a higher risk of thrombosis than could be explained by the sum of the separate effects.

Table 1.4 Studies on the combined effects of the Factor V Leiden mutation (FVL) or the Prothrombin mutation (PTM) with smoking or obesity on the risk of VTE								
Authors	Country	Study period	Study design (no of cases)	Study population	Outcome identification	Exposure identification	Risk estimate	
Juul et al.[112] 2004	Denmark	1976-1999	Prospective (216)	Participants in Copenhagen City Heart Study	The Danish National Patient Register and the Cause of Death Register. No validation	Examination and Questionnaires (smoking, BMI, FVL)	Combined effects of FVL and smoking or BMI resulted in 10 % higher risk in 10 years	
Pomp et al 2007[108]	The Netherlands	1999-2004	Case-control 3989 VTE/ 4900 control	Case: Participants in the MEGA study Control: partners of case or RDD Women and men	No validation but all cases from AK-clinics (PPV DVT: 97% PPV PE: 78%) Exclusion: Participants with malignancies 10 year before VTE	Self report/ questionnaires (BMI,	Never, FVL- Former, FVL- Current, FVL- Never, FVL+ Former, FVL+ Current, FVL+ Never, PTM- Former, PTM- Current, PTM- Never, PTM+ Former, PTM+ Current, PTM+	1 1.21 (1.06-1.39) 1.43 (1.26-1.63) 3.41 (2.53-4.58) 3.76 (2.58-5.49) 5.05 (3.46-7.38) 1 1.21-(1.06-1.37) 1.41 (1.25-1.60) 3.17 (1.94-5.18) 3.01 (1.60-5.68) 6.06 (2.67-13.76)
Pomp et al.[107] 2007	The Netherlands	1999-2004	Case-control 3843 VTE/ 4683 control	Case: Participants in the MEGA study Control: partners of case or RDD Women and men	No validation but all cases from AK-clinics (PPV DVT: 97% PPV PE: 78%) Exclusion: Participants with malignancies 10 year before VTE	Self report/ questionnaires	BMI<25, FVL- BMI25-30, FVL- BMI≥ 30, FVL- BMI<25, FVL+ BMI25-30, FVL+ BMI≥30, FVL+ BMI<25, PTM- BMI25-30, PTM- BMI≥30, PTM- BMI<25, PTM+ BMI25-30, PTM+ BMI≥30, PTM+	1 1.72 (1.54-1.93) 2.48 (2.13-2.88) 4.18 (3.12-5.61) 5.77 (4.20-7.93) 7.86 (4.70-13.15) 1 1.72 (1.54-1.91) 2.45 (2.12-2.82) 4.39 (2.56-7.51) 4.51 (2.64-7.72) 6.58 (2.31-18-69)

1.8. Aims of this thesis

The Aims of the thesis were:

1) To evaluate the predictive value of discharge diagnoses of VTE, including both DVT and PE, in the Danish National Patient Registry. (Study I)
2) To assess the association between smoking and VTE in middle-aged men and women. (Study II)
3) To assess the association between anthropometric variables and VTE in middle-aged men and women. (Study III)
4) To assess the effects of the factor V Leiden mutation combined with smoking or with obesity; and the effects of the prothrombin mutation combined with smoking or with obesity, on the risk of VTE in middle-aged men and women. (Study IV)

1.9. Designs and setting of the studies in this thesis:

Study I: Validation study
Studies II and III: Prospective cohort studies
Study IV: Case-cohort study

Setting: Diet, Cancer, and Health cohort, Denmark 1993-2006.

Participants: 26,674 men and 29,340 women, ages 50-64 years at entry into the Diet, Cancer, and Health study, with no history of cancer or VTE.

2. Data sources and definitions of outcomes

2.1. Data sources

The studies in this thesis were based on data from the Danish Diet, Cancer, and Health study[122, 123], the Civil Registration System[124], the Danish National Patient Registry[70], and the Cause of Death Registry[125]. These data sources were linked through the civil registration number, a unique, personal 10-digit identification number that has been issued to all Danish citizens at birth or immigration since 1968[124].

2.1.1. The Diet, Cancer, and Health study

The Diet, Cancer, and Health study was a prospective cohort study. Its primary objective was to investigate the etiological role of diet in the development of cancer. The study has been described in detail elsewhere[122, 123]. From December 1993 through May 1997, 80,996 men and 79,729 women aged 50 to 64 years were invited to participate in the study, and 27,178 men and 29,876 women accepted the invitation. Eligible cohort members were born in Denmark, living in the urban areas of Copenhagen and Aarhus, and at the time of the invitation, had not been registered with a previous diagnosis of cancer in the Danish Cancer Registry[126].

Participants completed detailed questionnaires regarding lifestyle factors, including diet, smoking habits, alcohol consumption, sports activities, education, work, and medication (for example, the use of HRT in women) at the time of enrolment into the Diet, Cancer, and Health study. The questionnaires were optically scanned into a computer, and in subsequent interviews performed by trained lab technicians, information was amended as necessary. In addition, physical examinations were performed. Data on blood pressure and anthropometry were obtained by trained laboratory technicians at two study clinics in Aarhus and Copenhagen at the time of enrolment. Immediately after the baseline interview, a blood sample (30 ml) was drawn from each participant. The samples were divided into separate tubes of serum, plasma, erythrocytes, and buffy coat and stored in liquid nitrogen vapour (max -150^{o}C). In 2000 – 2002, follow-up questionnaires were mailed to all surviving participants regarding diet and lifestyle changes.

2.1.2. The Danish National Patient Registry

From 1977, the Danish National Patient Registry has routinely collected nationwide data on all somatic hospitalisations. In 1995, visits to emergency departments and outpatient clinics were required to be reported to the registry. The registry has captured 99.4% of all somatic

admissions[70]. Recorded data included dates of admission and discharge, admission type (i.e. emergency or ward departments), a primary discharge diagnosis that reflected the main reason for investigation, and up to 20 secondary discharge diagnoses. The diagnoses were coded according to the Danish version of the International Classification of Diseases, 8th revision (ICD-8), for hospitalisations through the end of 1993, and according to the updated ICD-10 thereafter. All discharge diagnoses were determined exclusively by the physician that discharged the patient. It is not possible to alter these diagnoses once recorded, for example, for administrative or financial reasons.

2.1.3. The Cause of Death Registry and Death Certificates

The Cause of Death Registry was established in 1973[125]. It contains information from 1973 regarding the death of Danish citizens that died in Denmark. It includes the name, age, address, civil registration number, and municipality of residence of the deceased; it also includes information on the cause of death, determined by the physicians that signed the death certificate. For individuals that died outside the hospital, the death certificate is most often signed by the general practitioner. When an autopsy is performed, it is noted in the registry and the death diagnosis is corrected according to the results of the autopsy. Because the registry had not been updated regularly during the time period covered by this study, registry data was not available after 2002. Therefore, we obtained copies of death certificates from participants that died between 2003 and 2006. The death certificates included the primary results from an autopsy, when it had been performed.

2.1.4. The Civil Registration System

The Civil Registration System has maintained electronic records of all changes in civil and vital status for the entire Danish population since 1968, including changes in address, dates of emigration, and dates of death[124].

2.2. Definition of outcomes

Incident symptomatic VTE (DVT and PE) was the outcome in all studies included in this thesis. Potential VTE events among the participants in the Diet, Cancer, and Health study were identified with the nationwide Danish National Patient Registry and the Cause of Death Registry based on the civil registration numbers of the study participants.

A diagnosis of VTE was made when typical clinical symptoms, including unilateral swelling of the leg, sagittal leg pain, discolouration of the leg, dyspnoea, chest pain, hyperventilation, increased plasma D-dimer, or haemoptysis, were combined with a confirmatory diagnostic test result from ultrasonography, venography, echocardiography, a ventilation-perfusion lung scan (revealing two or more perfusion-ventilation defects), or a computed tomography (CT) scan, and other diagnoses were unlikely. The diagnosis was classified as probable when typical clinical symptoms were reported, other diagnoses were unlikely, but no confirmatory diagnostic test had been performed or the test results were inconclusive. VTE was ruled out when the results of diagnostic tests were negative, or when no test was performed because the symptoms disappeared, or were explained by another disease.

Information from the Cause of Death Registry was retrieved for all participants that died up to December 31, 2002; for participants that died between January 1, 2003 and June 30, 2006, death certificates were reviewed. The cause of death was only recorded as VTE when PE could be verified by autopsy.

Classification of VTE events

The location of the VTE was registered according to information in the medical records (concomitant DVT and PE in a patient was registered as PE). Verified VTE events were classified as provoked or idiopathic according to information in the medical records. Provoked VTE was indicated for patients that had cancer before or within three months after admission with VTE, and for patients that had one of the following conditions within three months of admission with VTE: surgery (general anaesthesia or spinal anaesthesia), trauma, an acute medical disease that lasted a minimum of three days (stroke, acute myocardial infarction, exacerbation of chronic lung disease, infection, active collagenous disease), immobilisation for a minimum of three days, or required a central vein catheter. In addition, the VTE event was classified as provoked for patients that had travelled by bus, train, or airplane for at least five consecutive hours in the three months before the VTE event. Alternatively, the VTE event was classified as idiopathic when the physician concluded that no factors associated with provoked VTE could be identified, or when the health of the patient was described as good, with no record that indicated provoked VTE. When the medical record contained insufficient information, the VTE event was un-classified.

3. Study I: Positive predictive value of DVT and PE discharge diagnoses

The purpose of this study was to evaluate the predictive value of discharge diagnoses of VTE, including both DVT and PE, in the Danish National Patient Registry.

3.1. Subjects and methods

In this study, hospital discharge diagnoses that were routinely coded in the Danish National Patient Registry were compared with diagnoses based on information obtained from medical records. The study population comprised all participants in the Diet, Cancer, and Health study. The patients in this cohort were linked to the Danish National Patient Registry by their civil registration numbers. We identified all participants that were registered with a discharge diagnosis of VTE (ICD-8: 450.99, 451.00, 451.08, 451.09, 451.99 and ICD-10: I26, I80.1 - I80.9) in the Danish National Patient Registry. We included primary and secondary discharge diagnoses from all types of departments (wards; outpatient clinics, emergency departments). We excluded participants that had been given a VTE discharge diagnosis before entry in the Diet, Cancer, and Health study. Thus, this study included patients that had been registered with a first time VTE diagnosis in the Danish National Patient Registry between the time of enrolment into the Diet, Cancer, and Health study and June 30, 2006.

The complete medical records for each patients were retrieved and reviewed by a physician familiar with VTE (specialist in haematology, ten years of seniority as physician with special interest in VTE) using the previously defined criteria for DVT and PE. When the complete medical record was unavailable, discharge letters, results of biochemical analyses, ultrasonography, venography, echocardiography, ventilation-perfusion lung scan, and CT-scan were retrieved, whenever possible. The review was based on all available information in the complete medical records, including the written radiology reports and results from laboratory tests (imaging films were not re-interpreted). The available information corresponded in most cases with that available for the physician who gave the discharge diagnosis.

3.2. Data analysis

The positive predictive value (PPV) of a VTE diagnosis registered in the Danish National Patient Registry was calculated as the proportion of patients with confirmed and probable events, based on a review of the medical records, compared to the total number of patients

with specific diagnoses registered in the Danish National Patient Registry. An overall PPV was calculated for all VTE diagnoses and separate PPVs were calculated for the sub-diagnoses of DVT and PE. Furthermore, the analyses were stratified by the type of hospital department (emergency room and hospital ward, or outpatient clinic); the type of diagnosis (primary, or secondary); and by the age and sex of the patients. The statistical analyses were performed with Stata version 9.2 (Stata Corporation, College Station, Texas, US).

3.3. Results

Of the 57,054 individuals that accepted the invitation to participate in the Diet, Cancer, and Health study, 56,014 met the inclusion criteria and were free of VTE before entry. During follow-up 1,135 first time VTE diagnoses were registered in the Danish National Patient Registry. Medical records were retrieved for 1,100 (96.9%) of these admissions.

Of the 1,100 VTE diagnoses, 646 were from wards and 454 were from emergency departments. Most of the patients diagnosed in an emergency department (418 patients) were subsequently discharged from a ward within a week. A diagnosis of VTE was given to 44% (184 of the 418) of these patients at discharge from the ward unit, and another (non-VTE) diagnosis was given to 56% (234 of the 418) of these patients. However, 13 of the 234 patients with non-VTE diagnoses were later (i.e., later than one week after the visit to the emergency department) discharged from a ward with a VTE diagnosis. Thus, the total number of first time VTE discharge diagnoses was 1,100; the number of first time VTE discharge diagnoses from wards was 843 (646 + 184 + 13); and the number of first time VTE discharge diagnoses from emergency departments was 454.

Based on the medical records of the 1,100 patients, the VTE diagnoses were confirmed in 626, and the diagnoses were considered probable in 17 patients. In 5 patients, the medical records did not include relevant information regarding a DVT or PE, and in 452 patients, a VTE diagnosis was ruled out. The PPV of the different types of VTE diagnoses are shown in Table 3.1. The overall PPV for a VTE discharge diagnosis in the Danish National Patients Registry was 58.5% [95% CI: 55.5-61.4]. This modest PPV was, in part, due to the fact that a substantial number of diagnoses given in emergency departments were based on clinical suspicion. When the diagnoses from emergency departments were excluded from the analysis, the PPV increased to 75.0% [95% CI: 71.9-77.9]. The PPVs were, in general, higher for PE than DVT. The PPV of a DVT diagnosis from a ward was 77.2% [95% CI: 72.2-81.6] in men, but only

63.2% [95% CI: 56.7-69.4] in women. Finally, the PPV of a primary VTE discharge diagnosis was 77.0% [95% CI: 73.7-80.1] and that for a secondary VTE discharge diagnosis was 66.5% [95% CI: 58.4-73.8].

Table 3.1.
PPV of VTE diagnoses in the Danish National Patient Registry (95% confidence intervals); the number of diagnoses is given at the right, in parentheses.

Stratified by type of department (VTE type)

	All departments (n=1,100) (353 PE / 742 DVT)	**Wards (n=843)** (285 PE / 558 DVT)	**Emergency departments (n=454)** (115 PE / 339 DVT)
VTE	58.5 (55.5-61.4) (643)	75.0 (71.9 – 77.9) (632)	31.3 (27.0 – 35.8) (142)
PE	66.5 (61.3-71.4) (238)	82.1 (77.2 – 86.4) (234)	29.6 (21.4 – 38.8) (34)
DVT	54.6 (50.9-58.2) (405)	71.3 (67.4 – 75.0) (398)	31.9 (26.9 – 37.1) (108)

Diagnoses from wards only (n=843); stratified by gender and type of diagnosis

	Men (n=455) (131 PE / 324 DVT)	**Women (n=388)** (154 PE / 234 DVT)	**Primary diagnoses (n=687)** (216PE/471DVT)	**Secondary diagnoses (n=155)** (68PE / 87DVT)
VTE	78.0 (73.9 – 81.7) (355)	71.4 (66.6 – 75.8) (277)	77.0 (73.7 – 80.1) (529)	66.5 (58.4 – 73.8) (103)
PE	80.2 (72.3 – 86.6) (105)	83.8 (77.0 – 89.2) (129)	87.0 (81.8 – 91.2) (188)	67.6 (55.2 – 78.5) (46)
DVT	77.2 (72.2 – 81.6) (250)	63.2 (56.7 – 69.4) (148)	72.4 (68.1 – 76.4) (341)	65.5 (54.6 – 75.4) (57)

The first time VTE discharge diagnosis in the Danish National Patient Registry did not always reflect the incident VTE events for the patient. In 29 of the 626 verified VTE events, the medical record contained information on a prior VTE that had not been registered in the Danish National Patient Registry. The majority of these had appeared before the Registry was established in 1977, and six VTE events occurred during the follow-up. Likewise for the participants among whom the VTE diagnosis was ruled out 18 had a prior VTE of which six occurred during follow-up; in addition, eight of the 452 patients had a verified VTE later in follow-up. In total 617 (597 + 6 + 8 + 6) incident VTE events were identified during follow-up (Figure 3.1). We found that the clinical information in the medical records in general were mostly complete. All patients with persistent symptoms of VTE received one or more objective tests. The descriptions of symptoms were quite detailed and considerations of provoking factors were well described in the medical records. Figure 3.2 shows the classifications of the 617 incident VTE events.

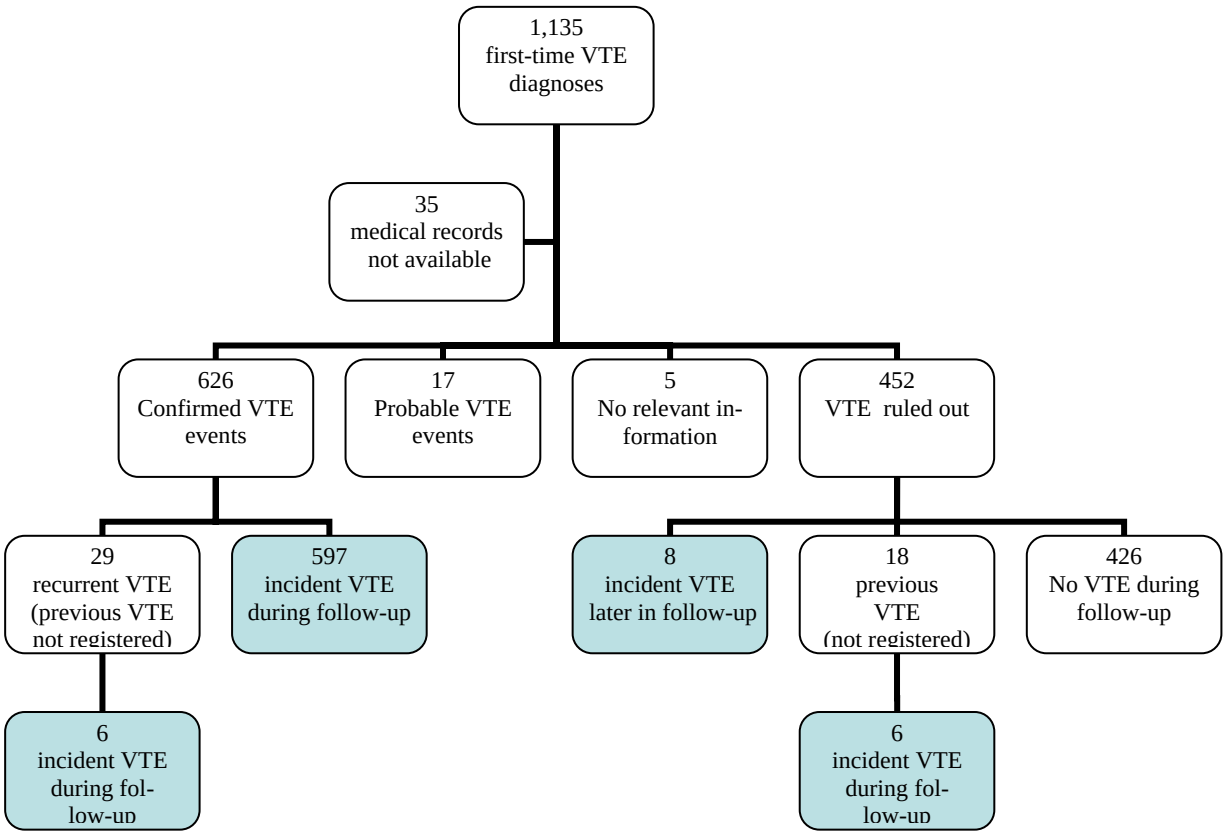

Figure 3.1. Results from the review of medical records

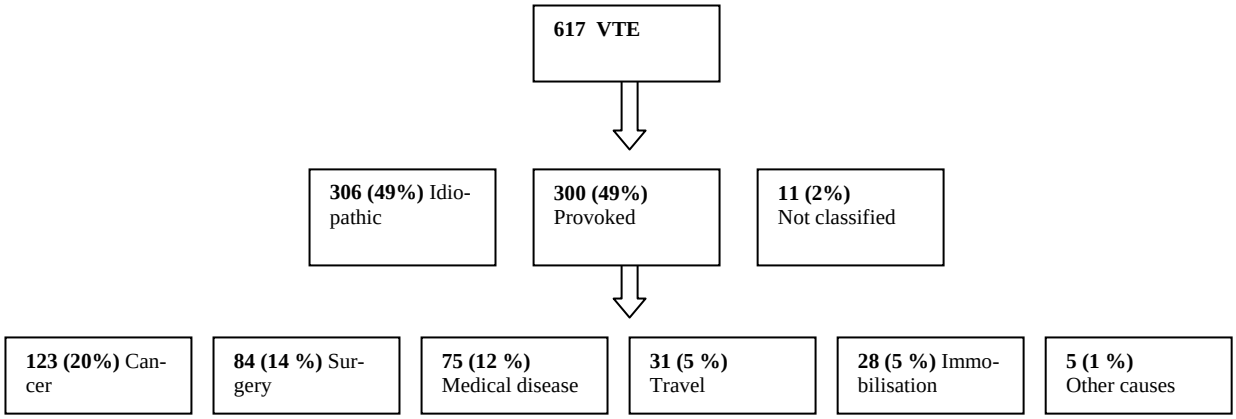

Figure 3.2. Classification of verified incident VTE events

3.4. Discussion

Strengths and weaknesses of the study

a) Selection problems

Selection into the study: The patients included in our study were participants in an ongoing cohort study and were living in urban areas at enrolment (the two largest cities of Denmark). The research participants tended to be healthier and better educated than the general Danish population; thus, the patients in this study might not represent the general population in terms of the incidence rate of VTE. However, because the Danish National Health Service provides free tax-supported health care for all inhabitants, there is no financial incentive for hospitals to deny optimal diagnostic work-ups based on the socioeconomic status of patients. The participants lived in large cities and were primarily admitted to large hospitals. The diagnostic work-up for VTE might be less thorough in minor Danish hospitals; this might lead to an over-estimate of the PPV of VTE discharge diagnoses. On the other hand, the provision of tax-supported health care and the relatively small area of Denmark improved the probability that exactly the same diagnostic work-up was provided for all patients in Denmark. The PPV is related to the incidence rate of VTE in the cohort studied; in this case, the 1,135 referred patients. The incidence rate of VTE in the subgroup of participants that underwent investigation based on a suspicion of VTE is probably similar to the incidence rate of VTE in the general population for individuals suspected of having VTE. Therefore, it is unlikely that the PPV of VTE diagnoses found in our study are substantially different from the PPV of VTE diagnoses given to the general Danish population. Nevertheless, we included only first time VTE diagnoses; thus, the PPV we found may not represent the PPV of subsequent VTE diagnoses.

b) Misclassification of VTE cases: In a few cases, it was not possible to decide whether the patient's condition was compatible with VTE (17 probable VTE cases). In the clinical situation, these events were characterised as VTE and the VTE discharge diagnoses appeared to be correct; therefore these events were included in the number of correctly diagnosed VTE when calculating the PPV of a VTE diagnosis. These cases were not included as VTE events in the association studies described in Chapter 4. Had these probable VTE events not been characterised as VTE, the overall PPV would have been 57% (643-17 of 1,100) instead of 58.5%; the PPV for the subgroup that comprised all wards would have been 72% (632-17 of 843) instead of 75%.

Comparison with other studies

Only a few studies have analysed the quality of VTE diagnoses in administrative databases (Table 1.1). Larsen et al. evaluated the PPV of VTE diagnoses among pregnant women within the Danish National Patient Registry[113]. In their study, the PPV for DVT diagnoses was 86.3%, and for PE diagnoses 81.8%. The data used in the study were from January 1, 1980, to December 31, 2001; i.e., the data were primarily recorded before the Registry included diagnoses given in emergency departments (in 1995). Furthermore, the study population was limited to pregnant women; this may have excluded discharge diagnoses from emergency departments, because most patients would have been admitted to a gynaecological department. Other studies evaluated the PPV of ICD-9 discharge diagnoses in different study populations[6, 114-117]. The results from those studies are comparable with our findings for the VTE-diagnoses given in wards.

General discussion

We evaluated the PPV of DVT and PE diagnoses in the Danish National Patient Registry. The magnitude of the PPV varied according to the subdiagnoses (DVT and PE), the types of diagnoses, the type of hospital department, and sex. The highest (up to 87%) PPVs were found for PE diagnoses, particularly those that were primary diagnoses, diagnoses coded at wards (in contrast to emergency departments), and diagnoses among men.

An accurate diagnosis of VTE can be challenging and requires both clinical assessment and objective testing[127-131]. Patients with VTE may have only minimal or atypical symptoms; conversely, the signs and symptoms of VTE may also be found in patients with non-thrombotic diseases. That is, VTE symptoms are not specific[127]. Furthermore, objective tests can give either false positive or false negative results[129]. A major challenge is to identify a DVT recurrence, because the symptoms of chronic venous insufficiency may mimic the symptoms of recurrent DVT and objective tests may be inconclusive. In clinical practice, it is important to treat all patients with VTE because it is a potentially fatal condition and treatment should be started immediately. However, it might not be possible to perform objective tests without delay; therefore, the general practice in most hospital departments was to start the treatment (anticoagulation with low-molecular heparin) when clinical suspicion was raised and afterwards perform objective tests to confirm or rule out the VTE diagnosis. That is, a number of patients were admitted to a hospital department (eventually from the emergency department), had anticoagulant treatment, and were evaluated with objective tests. Often, the VTE diagnosis was

ruled out, and sometimes there were no alternative diagnoses. However, the physician must provide a discharge diagnosis that justifies the admission and the use of medications; thus, sometimes a VTE diagnosis was recorded at discharge, despite the conclusion that VTE had been ruled out. This was the most common situation found when we could not confirm the VTE diagnosis. This general practice also explained the low PPV of diagnoses given in emergency departments. The diagnoses were based on clinical suspicion in the emergency department, the treatment was started, and the patient was subsequently referred to a ward unit.

This study showed a difference in the PPV of DVT diagnoses among men and women. The explanation could be that women are generally more likely than men to consult doctors. Thus, the scenario described above may have occurred more frequently in women than in men; where a hospital admission based on a suspicion of DVT was ruled out, but was recorded as DVT in the Registry.

The Danish National Patient Registry captures nearly complete information on nationwide admissions to all somatic hospital departments. The data includes routinely coded hospital discharge diagnoses by the physician that discharged the patient, and misclassification of cases is expected to be non-differential. The PPV is a measure of the misclassification of VTE cases. Our results suggest that using Registry data for the follow-up of participants would tend introduce a bias towards the null for a low PPV (assuming non-differential misclassification). Therefore, we recommend exclusion of data from emergency departments from the Registry data before use in research.

Conclusion

Data from the Danish National Patient Registry is suitable for the follow-up of participants in studies on VTE. However, diagnoses of VTE from emergency departments are not valid.

4. Studies II, III, and IV: Smoking, anthropometry, and genetic susceptibility

4.1. Subjects and methods

In study II, we assessed the association between smoking and VTE[109]; in study III, we assessed the association between anthropometry and VTE; and in study IV, we assessed the combined effects of genetics and environment on the risk of VTE. The genetic factors included Factor V Leiden and prothrombin mutations; the environmental factors included smoking and obesity. The study population in all three studies comprised the 56,014 participants in the Diet, Cancer, and Health study that did not have a prior diagnosis of VTE in the Danish National Patient Registry.

Outcomes for all three studies were objectively verified incident VTE events. Verified VTE events from the validation study (Study I, described in Chapter 3) were included in addition to participants that had died of VTE. Participants that died during follow-up were identified through the Civil Registration System. The VTE-related deaths were then identified by linking these participants with the Danish National Death Registry, which was updated until 2003; manual review of death certificates was performed for participants that died between 2003 and 2006. Only participants with autopsy-verified VTE were classified as VTE deaths. The end of follow-up was June 30, 2006.

Information on exposure and confounding variables was obtained from the questionnaires, interviews, and examinations performed at baseline and from the questionnaires performed during the follow-up of the Diet, Cancer, and Health study. DNA was extracted from the buffy coat of the blood samples from the baseline examinations in a subcohort of 1,841 participants and from all participants that developed VTE during follow-up. Genotyping was performed with the real-time PCR technique to determine the presence of Factor V Leiden (G1691A) and prothrombin (G20210A) mutations.

4.2. Data analysis

Cox regression was used for the analyses in the three studies. Age was used as the time axis to abrogate confounding by age, with the entry time defined as the subject's age at recruitment. The study exit time was determined as the age at VTE date, the age at death date, the age at

emigration date, or the age at 30th of June, 2006, whichever came first. Participants were excluded when information was missing on one or more confounder or exposure variables or, in the smoking study, when the smoking doses were unlikely (more than 100 g of tobacco/d).

4.2.1. Smoking and VTE

The association between smoking and risk of VTE was assessed separately for men and women. Smoking dosage was analyzed both as a categorical exposure variable, and as a continuous exposure variable in restricted cubic spline models. Alcohol consumption, anthropometry, physical activity, and women's use of HRT were considered potential confounders and adjustments were performed for these variables. In secondary analyses, we performed additional adjustments for years in primary school and educational status. We examined associations between smoking and VTE, idiopathic VTE, secondary VTE, secondary non-cancer related VTE, and PE. Incidence rates of VTE were computed for men and women according to different categories of tobacco consumption. The VTE incidence rate was evaluated as the number of verified incident VTE events divided by the sum of individual person-years at risk (follow-up time). The incidence rates were standardised according to the age distribution among never smokers and were expressed as the number of VTE events per 1,000 person-years at risk.

4.2.2. Anthropometry and VTE

We assessed associations between anthropometric variables and the risk of VTE separately for men and women. Weight, BMI, waist circumference, hip circumference, and body fat mass were analysed as continuous variables. Smoking, physical activity, height, hypertension, hypercholesterolemia, diabetes, and use of HRT (women only) were considered potential confounders and we performed adjustments for these factors. Furthermore, waist circumference was adjusted for the BMI, and waist circumference and hip circumference were adjusted mutually. Associations were assessed between anthropometry and VTE, PE, and subtypes of VTE; i.e. idiopathic and secondary VTE. The anthropometric variables were also grouped into quartiles, according to the exposure distribution, among cases with VTE and we assessed the risk of VTE in each quartile[132]. Additionally, the hazard ratios of VTE per one standard deviation were assessed. In secondary analyses, we assessed the association between VTE and categories of hip circumference and of waist circumference stratified according to quartiles of BMI. These analyses were stratified by sex.

4.2.3. Combined effects of genetic risk factors with obesity or smoking

We used a case-cohort design that included all the cases of VTE and a cohort sample (subcohort) of 1,841 participants that included 23 VTE cases. The subcohort was randomly selected from the whole cohort of 56,014 participants. A Cox proportional hazards model was used for the statistical analyses. Incidence rates were computed as if the full cohort were included, but modified with a weighting scheme, as described by Kalbfleisch and Lawless, and with a robust variance estimate[133].

The exposure was defined as the genotype, according to the Factor V Leiden and the prothrombin mutation. The genotypes were categorized into wild type, heterozygous, or homozygous profiles. Associations were analysed between VTE and the genotypes. The genotypes were further cross-tabulated according to categories of body weight and categories of tobacco consumption. We used the World Health Organization BMI cut off values for healthy-weight (<25 kg/m^2), overweight (25.0 to 29.9 kg/m^2), and obesity ($\geq$30 kg/m^2). For tobacco consumption, participants were categorised into non-smokers, moderate current smokers (1 to 24.9 g/d), and heavy current smokers ($\geq$25 g/d) based on results from a prior study that showed a substantially higher risk of VTE in heavy smokers compared to moderate smokers[109]. In a secondary analysis, participants were categorized into non-smokers and current smokers. All analyses were stratified by sex. Adjustments were performed for body height, for the use of HRT (for women), and either tobacco consumption (in the body weight analyses), or body weight (in the smoking analyses). The reference group was defined as the group of subjects that were not exposed for each combination of genotype and lifestyle; e.g., for the combination of smoking and the Factor V Leiden mutation, the reference group comprised participants without the Factor V Leiden mutation that were non-smokers.

The incidence rates of VTE were computed for each stratum of genotype and category of tobacco consumption and for each stratum of genotype and category of weight status. The crude effects of smoking and obesity were calculated as rate differences; i.e., the incidence rate of VTE in exposed participants minus the incidence rate of VTE in unexposed participants in individuals with and without the mutations. Interactions were evaluated on an additive scale[111].

We used Stata version 9.2 (Stata Corporation, College Station, Texas, US) for the statistical analyses in studies II, III, and IV.

4.3. Results

The median follow-up time of the 56,014 participants was 10.2 years with an interquartile range from 9.6 to 10.8 years. During follow-up, 617 incident VTE events were verified by review of medical records. Of the 56,014 participants, 4,084 died during the follow-up period. Of these, 24 participants had an autopsy that proved a PE diagnosis without a previously verified VTE. Thus, we identified a total of 641 objectively verified VTE events among participants during follow-up. The incidence rate of VTE was 1.15 [95% CI: 1.06-1.24] per 1,000 person years.

4.3.1. Smoking and VTE

Table 4.1 shows the association between tobacco consumption and VTE. As can be seen the hazard ratio (HR) among current smokers compared to never smokers were 1.52 [95% CI: 1.15-2.00] for women, and 1.32 [95% CI: 1.00-1.74] for men. A positive association between smoking and VTE was found among women at all doses of tobacco, and among men at doses above 15 g/d. The HR was highest for the highest doses of tobacco. Former smokers had the same risk as never smokers. For idiopathic VTE, a positive association was found with the highest dose of tobacco for both genders, with a HR of 4.34 [95% CI: 2.10-8.96] for women, and 1.89 [95% CI: 0.97-3.68] for men. There were no associations between idiopathic VTE and lower doses of tobacco. For secondary VTE, we found a statistically significant positive association with current smoking for both genders. The association was also present for non-cancer related secondary VTE. For PE, we found a less consistent positive association with current smoking for both genders. The HR of VTE according to daily tobacco consumption increased steeply for smoking more than 20 g of tobacco/d in women; whereas, the HR of VTE did not increase substantially before the dose exceeded 30 g/d in men.

After five years, a total of 45,271 participants answered a follow-up questionnaire. Data on smoking habits were complete for 44,379 participants. Of these, 1,560 (3.5%) smoked higher doses of tobacco at follow-up than at baseline, 6,612 (14.9%) smoked lower doses at follow-up than at baseline, and 36,307 (81.8%) had not changed their smoking habits.

Table 4.1

Incident rates and HRs of VTE [95% confidence interval] for different tobacco doses. Adjustments were made for BMI, alcohol consumption, physical activity, and use of HRT (women). The number of cases is shown in parentheses at the top of each cell. Incidence rates of VTE are expressed as the number per 1,000 person years, and was standardised to age in the non-smoking group.

Women	**Never smoked**	**Former smoker**	**Current smoker All doses**	**Current smoker <15 g/day**	**Current smoker 15-25 g/day**	**Current smoker >25 g/day**	
All VTE (262)	(100)	(56)	(106)	(48)	(44)	(14)	
Incidence rate	0.80 [0.64-0.95]	0.81 [0.60-1.01]	1.14 [0.93-1.35]	1.10 [0.80-1.39]	1.03 [0.72-1.33]	2.15 [1.01-3.29]	
Crude HR	1	0.96 [0.69-1.33]	1.40 [1.06-1.85]	1.28 [0.91-1.81]	1.33 [0.93-1.90]	2.78 [1.59-4.87]	
Adjusted	1	0.96 [0.69-1.33]	1.52 [1.15-2.00]	1.41 [1.00-2.00]	1.43 [1.00-2.04]	2.87 [1.63-5.05]	
Adjusted*	1	0.96 [0.69-1.33]	1.46 [1.11-1.94]	1.37 [0.97-1.94]	1.38 [0.97-1.99]	2.63 [1.46-4.74]	
Idiopathic VTE (110)	(45)	(26)	(39)	(16)	(14)	(9)	
Crude	1	1.00 [0.61-1.62]	1.15 [0.75-1.77]	0.96 [0.54-1.70]	0.94 [0.52-1.71]	3.90 [1.90-7.99]	
Adjusted	1	1.03 [0.63-1.66]	1.27 [0.83-1.96]	1.07 [0.60-1.90]	1.03 [0.56-1.88]	4.34 [2.10-8.96]	
Secondary VTE (135)	(51)	(26)	(58)	(28)	(25)	(5)	
Crude	1	0.86 [0.54-1.39]	1.51 [1.03-2.19]	1.46 [0.92-2.32]	1.48 [0.92-2.40]	1.97 [0.79-4.94]	
Adjusted	1	0.85 [0.53-1.37]	1.60 [1.10-2.35]	1.59 [1.00-2.53]	1.57 [0.97-2.55]	1.91 [0.75-4.85]	
Secondary-non-cancer VTE (80)	(32)	(17)	(31)	(14)	(14)	(3)	
Crude	1	0.90 [0.50-1.63]	1.29 [0.78-2.11]	1.17 [0.63-2.20]	1.32 [0.71-2.48]	1.86 [0.57-6.08]	
Adjusted	1	0.89 [0.49-1.61]	1.37 [0.83-2.26]	1.29 [0.69-2.43]	1.40 [0.74-2.65]	1.74 [0.52-5.79]	
PE (137)	(57)	(30)	(50)	(24)	(18)	(8)	
Crude	1	0.88 [0.57-1.38]	1.15 [0.79-1.69]	1.11 [0.69-1.79]	0.95 [0.56-1.62]	2.81 [1.34-5.89]	
Adjusted	1	0.90 [0.58-1.40]	1.28 [0.87-1.88]	1.25 [0.78-2.02]	1.06 [0.62-1.80]	2.95 [1.39-6.25]	
Men	**Never smoked**	**Former smoker**	**Current smoker All doses**	**Current smoker <15 g/day**	**Current smoker 15-25 g/day**	**Current smoker 25.1-35 g/day**	**Current smoker > 35 g/day**
All VTE (363)	(80)	(128)	(155)	(34)	(72)	(30)	(19)
Incidence rate	1.17 [0.92-1.43]	1.34 [1.10-1.58]	1.50 [1.26-1.73]	1.18 [0.78-1.58]	1.53 [1.18-1.89]	1.48 [0.96-2.00]	2.23 [1.26-3.23]
Crude	1	1.13 [0.85-1.49]	1.28 [0.97-1.67]	1.01 [0.67-1.51]	1.33 [0.97-1.83]	1.25 [0.82-1.91]	1.94 [1.18-3.20]
Adjusted	1	1.09 [0.82-1.44]	1.32 [1.00-1.74]	1.05 [0.70-1.57]	1.40 [1.02-1.93]	1.28 [0.83-1.95]	1.97 [1.19-3.26]
Adjusted*	1	1.09 [0.82-1.44]	1.30 [0.99-1.71]	1.04 [0.70-1.56]	1.39 [1.00-1.92]	1.27 [0.83-1.94]	1.85 [1.10-3.10]
Idiopathic VTE (186)	(46)	(60)	(80)	(22)	(33)	(14)	(11)
Crude	1	0.96 [0.65-1.40]	1.16 [0.81-1.67]	1.16 [0.70-1.93]	1.08 [0.69-1.69]	1.00 [0.55-1.83]	1.98 [1.02-3.82]
Adjusted	1	0.91 [0.62-1.33]	1.16 [0.80-1.68]	1.19 [0.71-1.98]	1.10 [0.70-1.74]	0.96 [0.52-1.77]	1.89 [0.97-3.68]
Secondary VTE (159)	(30)	(62)	(67)	(11)	(35)	(13)	(8)
Crude	1	1.40 [0.91-2.18]	1.45 [0.94-2.23]	0.85 [0.42-1.69]	1.69 [1.04-2.76]	1.47 [0.77-2.82]	2.15 [0.98-4.69]
Adjusted	1	1.38 [0.89-2.14]	1.58 [1.02-2.44]	0.91 [0.45-1.81]	1.87 [1.14-3.06]	1.61 [0.84-3.12]	2.33 [1.06-5.11]
Secondary-non-cancer VTE (96)	(17)	(37)	(42)	(6)	(21)	(10)	(5)
Crude	1	1.50 [0.84-2.66]	1.61 [0.92-2.83]	0.82 [0.32-2.08]	1.80 [0.95-3.41]	1.99 [0.91-4.34]	2.38 [0.88-6.45]
Adjusted	1	1.43 [0.80-2.55]	1.71 [0.97-3.03]	0.88 [0.35-2.23]	1.97 [1.03-3.75]	2.08 [0.94-4.59]	2.47 [0.90-6.73]
PE (138)	(34)	(46)	(58)	(15)	(29)	(9)	(5)
Crude	1	0.91 [0.57-1.46]	1.11 [0.73-1.70]	1.06 [0.56-1.98]	1.27 [0.76-2.13]	0.76 [0.33-1.73]	1.31 [0.51-3.36]
Adjusted	1	0.87[0.54-1.39]	1.16 [0.75-1.78]	1.12 [0.60-2.11]	1.38 [0.82-2.32]	0.78 [0.34-1.79]	1.33 [0.51-3.44]

4.3.2. Anthropometry and VTE

Table 4.2 shows the associations between the anthropometric variables and different types of VTE (total VTE, idiopathic VTE, secondary VTE, and PE). For each anthropometric variable, the crude and adjusted HRs were assessed comparing groups of participants that differed by one standard deviation. We found statistically significant positive associations between VTE and all measurements of body size, including body weight, BMI, total body fat mass, waist circumference, and hip circumference, among both men and women. Adjustments for potential confounding had no substantial impact on the risk estimates. Also, the associations persisted after adjustments for hypertension, cholesterol, and diabetes.

The mutually adjusted analysis of waist circumference and hip circumference showed a positive association between VTE and waist circumference in men, with an adjusted HR of 1.38 [95% CI: 1.17-1.64] per standard deviation, but no association in women. In contrast, the association between VTE and hip circumference adjusted for waist circumference showed a positive association in women, with an adjusted HR of 1.36 [95% CI: 1.15-1.59] per standard deviation, but no association in men. When waist circumference was adjusted for BMI, we found a HR of 1.36 [95% CI: 1.07-1.73] per standard deviation in men, but no association in women.

Furthermore, when stratified by quartiles of BMI, we observed a higher risk of VTE with higher hip circumference in normal-weight individuals. For example, in individuals with BMI in the lower-middle quartile (BMI 24.1-26.7), the HR was 1.40 [95% CI: 0.94-2.08] for hip circumferences of 97.5-102 cm, and 1.73 [95% CI: 1.12-2.67] for hip circumferences of 102.5-108 cm, compared to hip circumferences of 97 cm or less.

Table 4.2.
HRs for VTE [95% Confidence interval] in groups that were different by one standard deviation (SD) in anthropometric measures. Age was used for the time axis (thus, crude estimates are age-adjusted). Adjustments were made for physical activity, smoking categories, height, cholesterol, hypertension, diabetes, and use of HRT (women only).

	Women				**Men**			
	SD	**All VTE** (n=259)	**Idiopathic VTE** (n=109)	**PE** (n=126)	**SD**	**All VTE** (n=360)	**Idiopathic VTE** (n=182)	**PE** (124)
Weight, 1sd								
Crude	12.1 kg	1.40 [1.27-1.55]	1.47 [1.27-1.70]	1.58 [1.39-1.80]	12.3 kg	1.34 [1.22-1.46]	1.30 [1.14-1.48]	1.46 [1.25-1.69]
Adjusted		1.41 [1.27-1.57]	1.46 [1.24-1.73]	1.57 [1.36-1.81]		1.31 [1.18-1.46]	1.29 [1.11-1.50]	1.55 [1.31-1.85]
BMI, 1sd								
Crude	4.3 kg/m^2	1.33 [1.20-1.47]	1.37 [1.18-1.60]	1.48 [1.29-1.69]	3.6 kg/m^2	1.23 [1.12-1.35]	1.22 [1.07-1.39]	1.40 [1.20-1.62]
Adjusted		1.40 [1.26-1.55]	1.45 [1.24-1.71]	1.56 [1.36-1.80]		1.28 [1.16-1.41]	1.27 [1.11-1.46]	1.50 [1.28-1.75]
Body fat, 1sd								
Crude	8.8 kg	1.36 [1.23-1.51]	1.39 [1.19-1.62]	1.54 [1.35-1.76]	7.5 kg	1.30 [1.18-1.42]	1.29 [1.13-1.47]	1.48 [1.28-1.71]
Adjusted		1.38 [1.24-1.53]	1.40 [1.19-1.65]	1.55 [1.34-1.78]		1.29 [1.17-1.43]	1.30 [1.13-1.49]	1.55 [1.32-1.81]
Waist, 1sd								
Crude	11 cm	1.33 [1.20-1.48]	1.32 [1.11-1.56]	1.52 [1.32-1.76]	9.9 cm	1.33 [1.21-1.47]	1.33 [1.16-1.51]	1.48 [1.26-1.72]
Adjusted		1.33 [1.19-1.50]	1.31 [1.09-1.56]	1.54 [1.31-1.79]		1.33 [1.20-1.47]	1.34 [1.16-1.54]	1.55 [1.31-1.83]
Hip, 1sd								
Crude	8.8 cm	1.37 [1.25-1.51]	1.45 [1.26-1.66]	1.51 [1.33-1.70]	6.7 cm	1.27 [1.15-1.39]	1.22 [1.07-1.40]	1.38 [1.19-1.61]
Adjusted		1.40 [1.26-1.54]	1.48 [1.27-1.72]	1.53 [1.34-1.75]		1.24 [1.12-1.37]	1.20 [1.04-1.39]	1.43 [1.21-1.69]

4.3.3. Combined effects of genetic risk factors with obesity or smoking

The Factor V Leiden mutation (heterozygous) was found in 139 (7.7 %) of the subcohort members, and the prothrombin mutation was found in only 25 (1.4 %) of the subcohort members. Three (0.17%) of the subcohort members had both mutations. No participants of the subcohort were homozygous for the genetic risk factors; therefore, risk estimates for homozygous strata could not be computed. The Factor V Leiden mutation was associated with an adjusted HR of 2.60 [95% CI: 1.94-3.50] and the prothrombin mutation was associated with an adjusted HR of 2.79 [95% CI: 1.46-5.33].

Table 4.3 shows the combined effects of the genetic risk factors and smoking. The effect of heavy smoking compared with non-smoking on the VTE rate was evaluated for groups with the different genetic mutations. Compared to non-smokers, individuals that smoked heavily without the Factor V Leiden mutation had 59 additional VTE events per 100,000 person years; those with the mutation had 128 additional VTE events per 100,000 person years. Similarly, compared to non-smokers, individuals that smoked heavily without the prothrombin mutation had 58 additional VTE events per 100,000 person years; those with the mutation appeared to have substantially more VTE events than non-smokers with the mutation, but this observation was based on a small number of study participants. Thus, the effect of smoking on the risk of VTE (rate difference) was higher in participants with a mutation than in participants without a mutation.

Table 4.4 shows the combined effects of genetic risk factors and obesity. Compared with healthy-weight individuals, obese individuals without the Factor V Leiden mutation had 103 additional VTE events per 100,000 person years; those with the mutation had 222 additional VTE events per 100,000 person years. However, compared with healthy-weight individuals, obese individuals without the prothrombin mutation had 107 additional VTE events per 100,000 person years; those with the prothrombin mutation had 705 additional VTE events per 100,000 person years. Thus, the effect of obesity on the risk of VTE was higher in participants with a mutation than in participants without a mutation.

Table 4.3.
Combined effects of genetic risk factors for VTE and smoking on the risk of VTE. [95% confidence intervals]. Hazard ratios (HR) were adjusted for BMI, Factor V Leiden/prothrombin genotype, body height, and use of HRT among women. (stratified for sex). Incidence rates are expressed per 100,000 person years.

Smoking status	Factor V genotype		Prothrombin genotype	
	Wild type	G1691A	Wild type	G20210A
Non smoker				
• Number of VTEs	(287)	(61)	(334)	(14)
• Incidence rate	90 [80-103]	277 [197-389]	100 [88-113]	249 [124-509]
• Crude HR	1 (reference)	2.91 [2.00-4.25]	1 (reference)	2.75 [1.31-5.79]
• Adjusted HR	1 (reference)	2.74 [1.84-4.08]	1 (reference)	2.49 [1.06-5.88]
Current, < 25 g/day				
• Number of VTEs	(152)	(36)	(183)	(5)
• Incidence rate	101 [84-122]	239 [156-370]	112 [95-133]	220 [68-759]
• Crude HR	1.13 [0.90-1.43]	2.68 [1.68-4.28]	1.15 [0.93-1.42]	1.87 [0.54-6.44]
• Adjusted HR	1.18 [0.93-1.49]	2.81 [1.73-4.56]	1.17 [0.94-1.46]	1.65 [0.48-5.69]
Current, ≥25 g/day				
• Number of VTEs	(51)	(11)	(58)	(4)
• Incidence rate	149 [109-210]	405 [165-975]	158 [116-216]	17741 [9551-30286]
• Crude HR	1.55 [1.07-2.23]	3.87 [1.60-9.37]	1.47 [1.04-2.08]	175.3 [99-310]
• Adjusted HR	1.63 [1.12-2.37]	4.46 [1.83-10.88]	1.53 [1.07-2.19]	76.8 [29.2-201.7]

Table 4.4.
Combined effects of genetic risk factors for VTE and weight on the risk of VTE. [95% confidence intervals]. Hazard ratios (HR) were adjusted for smoking, Factor V Leiden/prothrombin genotype, body height, and use of HRT among women. (stratified for sex). Incidence rates are expressed per 100,000 person years.

Weight status	Factor V genotype		Prothrombin genotype	
	Wild type	G1691A	Wild type	G20210A
Healthy weight				
• Number of VTEs	(165)	(34)	(194)	(5)
• Incidence rate	73 [62-87]	193 [127-298]	81 [69-95]	143 [50-463]
• Crude HR	1 (reference)	2.60 [1.62-4.16]	1 (reference)	1.77 [0.59-5.34]
• Adjusted HR	1 (reference)	2.63 [1.62-4.28]	1 (reference)	1.43 [0.44-4.63]
Over weight				
• Number of VTEs	(207)	(50)	(245)	(12)
• Incidence rate	100 [86-116]	305 [208-447]	111 [96-129]	326 [145-733]
• Crude HR	1.20 [0.94-1.52]	3.53 [2.29-5.45]	1.20 [0.96-1.50]	3.76 [1.56-9.06]
• Adjusted HR	1.28 [1.00-1.64]	3.60 [2.31-5.63]	1.26 [1.00-1.59]	4.06 [1.62-10.14]
Obese				
• Number of VTEs	(118)	(24)	(136)	(6)
• Incidence rate	176 [141-220]	415 [224-757]	188 [153-233]	848 [106-4353]
• Crude HR	2.15 [1.61-2.88]	4.87 [2.53-9.37]	2.08 [1.59-2.73]	8.24 [1.64-41.49]
• Adjusted HR	2.34 [1.73-3.16]	5.27 [2.74-10.14]	2.22 [1.67-2.94]	6.89 [1.18-40.22]

4.4. Strengths and weaknesses of studies II, III, and IV

There may be alternatives to a causal interpretation of the study findings. Factors that can influence the interpretation include the selection of the study participants, collection of data on exposure, outcomes, potential confounding factors, and statistical precision (Figure 4.1).

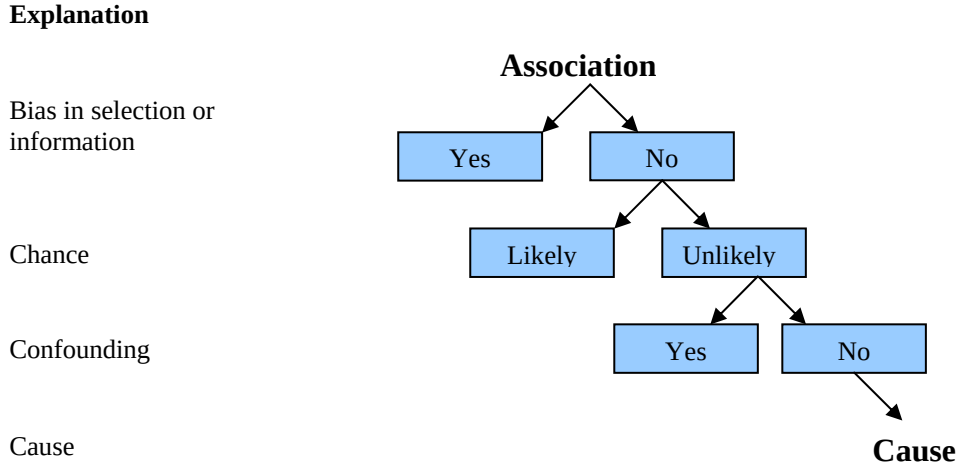

Figure 4.1 Association and cause from Fletcher "Clinical Epidemiology *The Essentials*"[68]

Selection problems in studies II, III, and IV

Internal validity: The complete follow-up of the study population through record linkage with population-based nationwide registries made this type of bias less likely because there was limited loss to follow-up. However, in the analyses, we assumed that individuals that died had the same risk for VTE as those that survived. If the participants had died of other diseases than VTE that were also related to smoking or obesity and risk of VTE our assumption would tend to underestimate the effect of smoking or obesity.

External validity: The patients included in our study were participants in an ongoing cohort study. Research participants generally tend to be healthier and better educated than the background population; this was also true in this study. Thus, the participants in this study were not representative of the general Danish population. This might have limited the variation in smoking dose or anthropometric measurements in our population. However, variation did not

appear to be limited, because a substantial number of the participants in our study were heavy smokers or obese.

Information problems

a) Exposure

Erroneous measurements of an exposure would probably be independent of a later diagnosis of VTE, and thus would constitute non-differential misclassification. This would tend to dilute apparent associations. The data on smoking habits were self-reported, but were very detailed; e.g., the type of tobacco and dose were specified. Smokers may tend to underreport the dose of tobacco consumption, but this would probably be independent of a later diagnosis of VTE. Furthermore, participants may have stopped smoking or reduced the dose of tobacco consumption during follow-up. The data from a follow-up examination after five years showed a high degree of concordance with the baseline information. The changes generally tended toward a lower consumption of tobacco at follow up, which may have resulted in an underestimation of the effect of smoking on the risk of VTE.

Self-reported data on obesity have been shown to be inaccurate, because people tend to underreport weight and overreport height[134]. In our study, the information on anthropometric variables was measured by trained laboratory technicians at baseline, thus the underreporting of weight measurements was unlikely. However, participants may have changed weight during follow-up. In conditions where the overweight and obese participants gained more weight than the lean participants (reference group), the association between obesity and risk of VTE would have been overestimated.

In addition, misclassification of the confounders could influence the results. For example, the data on the use of HRT were recorded at baseline. Some women may have discontinued HRT and others may have started HRT after the baseline examination. This would bias the effect of HRT and result in an incomplete adjustment for HRT.

b) Outcome:

In most situations, misclassification of VTE cases would dilute the actual association between an exposure and VTE, particularly when the misclassification is not related to exposure. The information on exposure was not available for the reviewer during the validation process (the reviewers were blinded to the exposure); therefore, any misclassification of cases by the re-

viewer would have been unrelated to exposure and, consequently, non-differential. However, smoking may result in a lack of PE diagnoses, because the symptoms of PE (dyspnoea) may be common in individuals that smoke. Thus, dyspnoea may be interpreted as a symptom of an exacerbated chronic obstructive lung disease, and PE might not be suspected or diagnosed. This would tend to underestimate the effect of smoking on VTE risk. Likewise, obesity may result in a lack of DVT diagnoses, because the symptoms of DVT (a swollen leg) may go unnoticed in individuals with obese legs and it may be impossible to perform a conclusive ultrasound examination. This would thus also tend to underestimate the effects of obesity.

Confounding factors

Detailed information on a range of potential confounding factors was available for these studies. However, adjusting for these factors in the statistical analyses had only a minor impact on the estimated hazard ratios. This may indicate that residual confounding is not a likely explanation for the observed associations. However, as in all observational studies, we cannot completely rule out the possibility that our findings may have been influenced by unknown confounding factors.

Precision

The precision in the studies was reflected by the width of the 95% confidence interval. In study II, the confidence intervals were generally narrow and in the main analysis including all VTE events the confidence intervals did not include one in a number of analyses. In study III, the confidence intervals were narrow, and did not include “one” in any of the analyses of the associations between anthropometric measurements and VTE. In study IV, the statistical precision was low in some of the strata.

Conclusion regarding strength and weaknesses of studies II, III, and IV

Selection or information problems and confounding factors appear to be unlikely explanations of our results in studies II, III, and IV. The statistical precision was high in studies II and III. However, although our study was one of the largest prospective studies on VTE, the statistical precision was low in some of the analyses in study IV. Therefore, some caution is required when interpreting the findings from this study.

4.5. Discussion

4.5.1. Smoking and VTE

In this large prospective study of men and women aged 50-64 at baseline, current smoking was associated with a higher risk of VTE in both sexes. Former smokers had essentially the same risk of VTE as never smokers. Further, the results indicated that smoking more than 20 g/d among women and 30 g/d among men was associated with a 150-300% higher risk of VTE. Lower rates of tobacco consumption were associated with only a 10% to 40% higher risk of VTE compared to never smokers.

Comparison with other studies: Our findings are consistent with the results from other large-scale epidemiological studies[87, 88, 108]. Most previous studies did not include tobacco dose, but used simpler categories of smoking status (never, former, or current smoker)[86, 89, 118-120]. However, the studies that did include tobacco dose also found the strongest associations with the highest doses of tobacco, consistent with our results. Our data suggest that the thresholds for the effect of smoking are different for men and women. No previous studies have reported sex-stratified data on the association between smoking dose and VTE.

Plausibility: A causal relation between VTE and smoking may be mediated by different mechanisms. Our results suggested that an acute mechanism may mediate the effect of smoking on the risk of VTE, because former smokers had risks similar to never-smokers. Moreover, the effects were not solely due to secondary smoking-related diseases, because we found a positive association between smoking and both idiopathic and secondary VTE. Smoking is associated with a procoagulant status, due to a higher level of fibrinogen in the plasma[12, 77, 78, 80, 118]. It was shown that the fibrinogen concentration decreased quickly after cessation of smoking; thus, smoking had an acute effect on the fibrinogen level[135]. Yarnell et al. found variations in haemostatic variables associated with smoking in men[110]. The fibrinogen concentration was nearly equal in former smokers and never smokers, while the concentration was higher in smokers but did not depends on the dose of tobacco consumption. A related mechanism may be that smoking reduces fibrinolysis[77]. The plasma concentration of plasminogen activator inhibitor-1 was found to be higher in smokers than never smokers. Yarnell et al. found that, in contrast to fibrinogen, the plasminogen activator inhibitor-1 concentration seemed to increase steeply when the tobacco dose exceeded 25 g/day[80]. This might explain

the threshold we found for the effect of smoking on VTE. Because women are generally smaller than men, a given dose of tobacco may contribute to a larger effect in women than men. Smoking was also found to be associated with activation of the inflammation system[76, 82, 83, 136]. Yarnell et al. showed that the white blood cell count was nearly equal in never smokers and former smokers, but increased in a dose dependent manner according to tobacco consumption [86]. Bain et al. showed that the blood count decreased quickly after cessation of smoking[137]. Finally, the effect of smoking on VTE risk could be caused by increased viscosity of the blood[76, 77, 80]. It has been shown that the viscosity of the blood decreases soon after smoking cessation[137]. In conclusion, these studies suggest different mechanisms that could explain an acute causal association between VTE and smoking. However, our study was not designed to evaluate the validity of these proposed mechanisms.

Conclusion: We found a positive association between smoking and VTE. The hazard ratio of VTE was especially high at tobacco doses above 20 g/d of tobacco for women and 30 g/d for men. The effects of smoking appeared to be mediated by an acute mechanism, because former smokers had the same risk as individuals that never smoked.

4.5.2. Anthropometry and VTE

In this large prospective study, we found statistically significant positive associations between VTE and all measurements of obesity for both men and women. We tested the hypothesis that central obesity might be a better predictor for VTE than peripheral obesity, measured as hip circumference. This was shown to be a valid hypothesis in CHD, but our results showed that peripheral and central obesity were both strong risk factors for VTE. These results indicated that there is a distinction between VTE and CHD, because no associations have been found between hip circumference and CHD. Moreover, our results indicated that the effect of obesity on the risk of VTE was not mediated purely by hypertension, cholesterol level, or diabetes.

Comparison with other studies: Few studies have evaluated the association between VTE and measures of obesity other than BMI. Our findings are in accordance with the results from large-scale epidemiological studies on BMI and VTE[86, 87, 89, 107]. We also found a statistically significant positive association between waist circumference and VTE in both men and women. This finding is in agreement with a small study by Hansson et al. in men born in

1913. They found that men with waist circumferences above 100 cm had a higher risk of VTE than those with waist circumferences below 100 cm[88]. Lately, studies on the association between VTE and the metabolic syndrome have shown that, among the features of the metabolic syndrome, central obesity is the pivotal risk factor for VTE[93, 95]. Other studies have shown inconsistent data on the associations between VTE and diabetes mellitus, hypertension, and dyslipidemia. In most studies, no associations between these factors and VTE were found after adjusting for BMI[86, 89, 93]. The associations between obesity persisted in our study after adjustment for hypertension, diabetes, and cholesterol levels; this indicated that the effect of obesity was not purely mediated by these factors.

This study also showed a statistically significant positive association between VTE and hip circumference in both men and women. However, this effect of peripheral obesity could be caused indirectly by central obesity, because when people become very obese, fat accumulates all over the body; thus, individuals with a large hip circumference may also have a large waist circumference. On the other hand, no studies have reported an association between hip circumference and CHD[103, 104]. To eliminate the effect of waist circumference in the analysis of hip circumference, and the effect of hip circumference on the analysis of waist circumference, we performed mutually adjusted analyses. When hip circumference was adjusted for waist circumference, the association between hip circumference and VTE was eliminated for men, but was still significant for women. In contrast, when waist circumference was adjusted for hip circumference, the association between waist circumference and VTE was eliminated for women, but was still significant for men. These differences may be explained by gender specific fat distributions. In general, men accumulate fat around the abdomen and women accumulate fat on the hips[102]. Therefore, in women, hip circumference is more informative than waist circumference when predicting the risk of VTE, because the variation in hip circumference is highest; conversely, waist circumference is more informative than hip circumference in men because the variation in waist circumference is highest. However, the correlation coefficients between waist and hip circumference was 0.80 in men and 0.75 in women; thus, the results might reflect colinearity and not biology. In an analysis of the combined measurements of hip circumference and BMI, we confirmed that a high hip circumference was associated with a higher risk of VTE, even in normal-weight individuals; this result contrasted with findings in CHD[103, 104]. Furthermore, it has been shown that waist circumference adjusted for BMI is an adequate approximation of intraabdominal fat content[138]. We therefore computed the HR of waist circumference adjusted for BMI as a model to assess the effect of intraabdominal fat.

We found a statistically significant positive association with VTE in men, but no association in women. This suggested that intraabdominal fat was only a risk factor in men. However, this model assumes that, in groups of individuals with equal BMI, a higher waist circumference would be concomitant with smaller compartments in other parts of the body. In fact, the BMI does not account for different ratios of fat: muscle mass; thus, individuals with the same BMI may not have equal fat content. Consequently, the HR we found with the model (higher waist circumference and equal BMI) may have indicated the effects of more intraabdominal fat, diminished hip circumference, less muscle mass, or a combination of these factors. Finally, we found statistically significant positive associations between all measurements of obesity and idiopathic VTE. These data underscore the notion that the effect of obesity on VTE risk was not mediated purely by obesity-related diseases.

Plausibility: The mechanisms responsible for the association between VTE and obesity are unknown. We showed that the association could not be completely explained by hypertension, diabetes mellitus, cholesterol levels, or secondary obesity-related diseases. Furthermore, our study suggested that fat mass, independent of the fat distribution in the body, is positively associated with VTE. One mechanism underlying this association could be related to the biologically active substances that are secreted by adipose tissue. A number of these substances are associated with procoagulant activity or reduced fibrinolysis[73, 74, 101, 102]. Enlarged fat cells produce a higher amount of these substances than normal-sized fat cells[74, 99, 100]. It is therefore plausible that peripheral and central obesity are risk factors for VTE. However, our study is not designed to evaluate the validity of this proposed mechanism. Obesity might also be associated with venous stasis, which would promote venous thrombosis; however, this association is hypothetical and not well-established.

In conclusion, we found positive associations between VTE and body weight, BMI, waist circumference, hip circumference, and total fat mass in both sexes. The results indicate a distinction between VTE and CHD, because no associations have been found between hip circumference and CHD.

4.5.3. Effects of genetic risk factors combined with obesity or smoking

As expected, Factor V Leiden and the prothrombin mutation were positively associated with the risk of VTE. Further, we found that the effects of obesity and of heavy smoking were more potent in individuals with the Factor V Leiden and the prothrombin mutations.

Comparison with other studies: The prevalence of the Factor V Leiden mutation (7.7%) and of the prothrombin mutation (1.4%) found in our study are similar to those that others have found in the Danish population[112, 139]. We found a HR of about 2.5 for both the Factor V Leiden mutation and the prothrombin mutation, which is in accord with the findings from other studies[59, 112, 140]. However, Weischer et al. recently found no association between the prothrombin mutation and VTE[139]. Their results were based on data from the Danish National Patient Registry and the Danish National Death Registry without any described restrictions (for example, there was no exclusion of diagnoses from emergency departments) and no validation. Misclassification of VTE cases may have biased their results towards unity, because we found that the Danish National Patient Registry had a positive predictive value of only 58.5% for VTE diagnosis but restricting the analyses to diagnoses given in wards or outpatients clinics yielded a positive predictive value of a VTE diagnosis of 75.0%[141]. From an additive point of view our study indicated interactions between lifestyle factors and genetic risk factors, because at an absolute scale the effects of obesity and smoking were higher in individuals with a mutation than in those without a mutation. Few studies have evaluated the combined effects of lifestyle factors with the Factor V Leiden or prothrombin mutations[107, 108, 112]. In one Danish follow-up study, 216 unvalidated VTE events were divided into 18 strata of weight status, smoking status, age groups, and status of Factor V Leiden. They found that the simultaneous presence of smoking, obesity, and old age resulted in a 10-year absolute 10% risk of VTE in individuals with heterozygous Factor V Leiden[112]. In a large case-control study, Pomp et al. found that the effect of smoking combined with the Factor V Leiden mutation and the prothrombin mutation was higher than that expected from the sum of the separate effects[108]. Our results showed similar synergistic effects. However, we assessed the HR according to different doses of tobacco consumption in addition to the smoker/non-smoker/former smoker categories. In our study, only 23 VTE events occurred in individuals with the prothrombin mutation; of these, 4 were heavy smokers (17%). This indicated that the combination of the prothrombin mutation with heavy smoking was associated with a high risk of VTE. Nevertheless, the small number of individuals in the group with this mutation may have led to an overestimation of the risk of VTE. Pomp et al. found an odds ratio of 6.06

[95% CI: 2.67-13.76] for the combined effect of current smoking and the prothrombin mutation on the risk of VTE[108]. In secondary analyses, we found a hazard ratio of 3.75 [95% CI: 1.27-11.04] for the effect of the combination of current smoking (all doses of tobacco) and the prothrombin mutation on the risk of VTE.

Also, the effects of obesity combined with the Factor V Leiden or with the prothrombin mutation on the risk of VTE were previously investigated in a case-control study[107]. As in our study, they found that the combined effects of obesity with the Factor V Leiden mutation and the prothrombin mutation were higher than expected for the sum of the separate effects. We measured these effects by comparing the incidence rates in the different strata and we evaluated the rate differences between exposed and unexposed groups. However, the crude incidence rates might have been influenced by confounding factors. In the Cox regression, we adjusted for a number of confounding factors, but this did not essentially change the estimate in strata with high statistical power. In other strata, including doubly exposed participants, the statistical power was not sufficient for adjustments, but we assumed that adjustments in these strata would not have essentially changed the estimate. Therefore, our study indicated that there was an additive interaction between Factor V Leiden and the lifestyle factors of heavy smoking and obesity. The effects of life-style factors on the risk of VTE in individuals with the prothrombin mutation were limited by low statistical precision. Nevertheless, we found an interesting indication that heavy smoking was associated with a relatively high risk of VTE in individuals with the prothrombin mutation. This result should be confirmed in future studies.

Plausibility: The Factor V Leiden mutation leads to a slower degradation of activated coagulation factors V and VIII[105]. A high factor VIII concentration may comprise the underlying mechanism for the effect of the Factor V Leiden mutation on VTE risk. The prothrombin mutation causes an elevation of plasma coagulation factor II and thus, a procoagulant status, although the mechanism is not clear[59]. Therefore, it is plausible that a combination of these factors added to the procoagulant status from the lifestyle factors may cause an imbalance in haemostasis that tips towards thrombosis.

In conclusion, we found that lifestyle factors had a substantial impact on the risk of VTE in individuals with the common genetic risk factors, Factor V Leiden and the prothrombin mutation. Individuals with these genetic risk factors seemed to be more susceptible to the unfa-

vourable effects of smoking and obesity. Although genetic risk factors cannot be modified, the risk of VTE might be reduced in these individuals by maintaining a healthy weight and refraining from smoking tobacco.

5. Main conclusions of this thesis

Based on the results from the studies described above and an evaluation of potential biases, confounding factors, and chance, we drew the following conclusions:

Study I

Data from the Danish National Patient Registry is suitable for the follow-up of participants in studies on VTE. However, diagnoses of VTE from emergency departments are not valid.

Study II

Smoking is positively associated with the risk of VTE. The hazard ratios for VTE were particularly high at tobacco doses above 20 g/d for women and 30 g/d for men. The smoking effect appears to be acutely regulated, because former smokers had the same risk as never-smokers.

Study III

Body weight is positively associated with the risk of VTE. Overweight and obesity effects are independent of body fat distribution; all anthropomorphic measurements of body weight are positively associated with the risk of VTE. These results indicate a distinction between VTE and CHD, because no associations have been found between hip circumference and CHD.

Study IV

Lifestyle factors, including smoking habits and body weight status had substantial impacts on the risk of VTE in individuals with genetic mutations in coagulation factors. Individuals with the Factor V Leiden and prothrombin mutations appeared to be more susceptible to the unfavourable effects of smoking and obesity. Clearly, genetic risk factors cannot be modified; however, our results suggest that the risk of VTE would be significantly lower if these individuals maintained a healthy weight and did not smoke tobacco.

6. Perspectives

Venous thromboembolism is a common, potentially fatal disease that recurs frequently and causes serious long-term complications. It is, therefore, important to reduce the incidence of VTE and, secondarily, to reduce recurrence after the first event. Improved individual risk stratification is needed in order to modify exposure and to target primary and secondary prophylaxis for individualised treatment and advice.

The present work represents a first step towards improving clinical practice. Scientifically, our results have extended the understanding of the importance of life style factors, genetic risk factors, and their interactions in the incidence rate of VTE.

This thesis showed that lifestyle factors are important contributors to the risk of incident VTE and that these factors interact with common genetic risk factors, namely the Factor V Leiden mutation and the prothrombin mutation. Clinically, these lifestyle factors should be taken into account when evaluating the risk for thrombosis in individuals with and without these mutations. These studies were not designed to evaluate whether lifestyle factors were also important risk factors for VTE recurrence, and we did not evaluate the effect of weight loss on the risk of VTE. Therefore, it is not clear whether weight reduction would benefit the risk of VTE. However, study II showed that former smokers and never-smokers had the same risk for VTE; this underscored the benefit of smoking cessation.

In the future, other lifestyle factors should be evaluated with respect to the risk of VTE. Moderate alcohol consumption has been shown to reduce the risk of CHD, but the association between VTE and alcohol consumption is not well established. In addition, it is not known whether physical activity might influence the risk of VTE.

In addition, other combinations of lifestyle factors and genetic risk factors may be important contributors to the VTE incidence. Several other common genetic risk factors have been found to be “weak” risk factors for VTE. The ABO blood type has consistently been shown to predict the risk of VTE; e.g., type A blood is associated with a higher risk of VTE compared to type O blood. However, the A-type is very common, therefore, it is important to identify factors that contribute to the risk of VTE in these individuals. Other common polymorphisms

have been identified among patients with VTE, but it is not yet clear whether these genotypes affect the risk of VTE. The appearances of VTE in individuals with “weak” risk factors may depend on environmental factors or a combination of factors. Identification of high risk combinations will improve risk stratification and, thus, reduce the frequency of VTE events. Life style factors are largely modifiable. Consequently, more knowledge of these effects extends the clinician‘s repertoire for providing advice and potentially reducing the frequency of this disease.

The hope is that the findings in this thesis will contribute to continuous improvements in our ability to predict, understand, and reduce the risk for VTE in the population.

7. Summary

Venous thromboembolism (VTE), including deep venous thromboses (DVT) and pulmonary emboli (PE), is a common disease with substantial personal, clinical, and social implications. Venous thromboembolism is thought to start in the calf veins, can progress to the proximal veins, and may break free to cause the potentially fatal condition of PE. VTE is associated with acute symptoms and long-term complications, including post-thrombotic syndrome and chronic pulmonary hypertension. Despite the serious consequences of this disease, 25-50% of VTE events occur in individuals that have no known risk factors. The associations between VTE lifestyle factors like smoking and obesity described by various anthropometric variables have not been established. Furthermore, it is not clear whether there are interactions between lifestyle factors and common genetic risk factors for VTE.

The aims of this thesis were: (1) to evaluate the validity of discharge diagnoses of DVT and PE in the Danish National Patient Registry (study I); (2) to asses the association between smoking and VTE (study II); (3) to assess the association between anthropometry and VTE (study III); and (4) to assess the interactions between genetic risk factors (the Factor V Leiden G1691A mutation and the prothrombin G20210A mutation) and lifestyle factors (smoking and obesity) (study IV).

Study I was a descriptive study that included 1,100 individuals from the Diet, Cancer, and Health cohort that had a record of a VTE discharge diagnosis in the Danish National Patient Registry during a 10 year follow-up period. Medical records and discharge letters were retrieved and reviewed according to a standardised format. We found that VTE discharge diagnoses from wards were reasonably valid, but diagnoses given in emergency departments were largely based on the suspicion of VTE and, therefore, had a low positive predictive value. We also found significant differences between the positive predictive values for DVT and PE, for primary and secondary diagnoses, and for men and women.

In study II, we found that smoking was a risk factor for VTE. The risk of VTE was especially high among women that smoked more than 20 grams of tobacco per day and men that smoked more than 30 grams of tobacco per day. Former smokers had the same risk as never-smokers for VTE. This underscored the benefits of smoking cessation.

Obesity, measured as body mass index (BMI), was previously found to be associated with VTE. The BMI is a marker of excess weight and correlates well with body fat content in adults; however, it fails to consider the distribution of body fat. In study III, we tested the hypothesis that central obesity might be a better predictor for VTE than peripheral obesity, measured as hip circumference. This hypothesis was valid for the risk of coronary heart disease, but our results showed that both peripheral and central obesity were strong risk factors for VTE. The results indicated a distinction between VTE and coronary heart disease, because no associations have been found between hip circumference and coronary heart disease. Furthermore, this study indicated that the effect of obesity on the risk of VTE was not mediated purely by hypertension, cholesterol levels, or diabetes.

The incidence rate of VTE is highly variable in individuals with the common genetic risk factors, Factor V Leiden (G1691A) and the prothrombin (G20210A) mutation. We evaluated whether the genetic susceptibility to venous thromboses was influenced by obesity and smoking. We found that individuals with one of these mutations seemed to be more susceptible to the unfavourable effects of smoking and obesity. Although genetic risk factors cannot be modified, our study indicated that the risk of VTE might be reduced in genetically disposed individuals by maintaining a healthy body weight and refraining from smoking tobacco.

8. Dansk resumé

Venøs tromboemboli (VTE) dvs. dyb venøs trombose (DVT) og lunge emboli (LE) udgør et betydeligt klinisk og samfundsmæssigt problem. Sygdommen er hyppig idet 1-2 per 1000 personer hvert år rammes af førstegangs VTE. Sygdommen debuterer oftest i venerne distalt på underekstremiteterne, herfra kan tromben brede sig op i bækkenvenerne og vena cava. Trombemasser kan rive sig løs fra underekstremiteterne og med blodstrømmen blive ført til lungerne og dermed give anledning til den potentielt livstruende tilstand LE. Såvel DVT som LE har desuden betydelige invaliderende senfølger som posttrombotisk syndrom og kronisk pulmonal hypertension. For 25-50 % af VTE tilfældene er der ikke en sikker forklarende årsag. Betydningen af livsstilsfaktorer som rygning og kropsbygning er ikke afklaret i forhold til risiko for VTE. Samspillet mellem livsstilsfaktorerne og hyppige arvelige dispositioner til VTE er heller ikke afklaret.

Formålet med denne afhandling var: (1) at vurdere kvaliteten af udskrivningsdiagnoserne for DVT og LE i det danske Landspatientregister (studie I), (2) at vurdere sammenhængen mellem rygning og VTE (studie II), (3) at vurdere sammenhængen mellem kropsbygning og VTE (studie III) samt (4) at vurdere samspillet mellem arvelige risikofaktorer (Faktor V Leiden (G1691A) og protrombin (G20210A) mutation) og livsstilsfaktorer (rygning og kropsbygning) i forhold til risiko for VTE (studie IV).

Studie I var et deskriptivt studie omfattende 1100 personer fra Kost, kræft og helbred kohorten som i gennem en 10-årig periode havde fået en udskrivnings diagnose for DVT eller LE i det nationale Landspatientregister. Diagnoserne blev valideret vha. en standardiseret gennemgang af journaler, samt evt. udskrivnings breve og skadekort. Vi fandt at VTE udskrivningsdiagnoserne som ikke var givet på skadestuer havde en acceptabel positiv prædiktiv værdi, hvorimod diagnoser givet på skadestuer overvejende grad byggede på mistanke om sygdommen og derfor havde en lav positiv prædiktiv værdi. Vi fandt desuden betydelige forskelle i de positive prædiktive værdi mellem DVT og LE, mellem primære og sekundære diagnoser, og mellem mænd og kvinder.

I studie II fandt vi, at rygning er en risikofaktor for VTE. Risikoen for VTE var særlig høj hos personer, der røg mere end 20 g tobak per dag for kvinder og mere end 30 g tobak per dag for

mænd. Tidligere rygere havde den samme risiko for VTE som aldrig rygere, hvilket bekræfter fordelen ved rygeophør.

Body mass index (BMI) er et indirekte mål for kropsbygning og det er fundet at korrelere godt med kroppens fedtindhold hos voksne. BMI fortæller dog ikke noget om fedtfordelingen i kroppen. I forhold til blodpropper i hjertet er det vist, at abdominal fedme (æble form) er farlig hvorimod perifer fedme (pæreform) beskytter mod blodprop i hjertet. Dette tyder på at fedtet har forskellig effekt i kroppen afhængig af hvor det er lokaliseret. I studie III undersøgte vi sammenhængen mellem de forskellige kropsmål og risikoen for VTE, samt hvorvidt pæreform beskyttede imod VTE. Vi fandt at alle former for overvægt var en stærk risikofaktor for VTE og at pæreform ikke beskytter mod VTE. Resultaterne indikerer altså at det er forskellige mekanismer, der ligger til grund for VTE (vene blodpropper) og arterielle blodpropper. Yderligere viste studiet, at effekten af fedme på risikoen for VTE ikke kunne forklares af forhøjet blodtryk, forhøjet kolesterol eller sukkersyge som en følge af fedmen.

Forekomsten af Faktor V Leiden (G1691A) mutationen og protrombin (G20210A) mutationen er hyppig blandt kaukasier, og begge er velkendte arvelige risikofaktorer for VTE. Forekomsten af VTE hos personer med disse mutationer er dog stærkt varierende. I studie IV undersøgte vi samspillet mellem livsstilsfaktorerne rygning og kropsbygning og disse arvelige mutationer. Vi fandt, at personer med arvelig disposition var betydelig mere modtagelige for de ugunstige virkninger af rygning og fedme. Dvs. at vores studie tyder på, at personer med disse arvelige risikofaktorer kan reducere deres risiko betydelig ved at have en sund vægt samt ved at undlade rygning.

Afhandlingen viser således, at livsstilsfaktorerne rygning og overvægt er risikofaktorer for VTE. Resultaterne tyder også på, at personer med de to hyppigste genetiske risikofaktorer er mere følsomme for de ugunstige effekter af livsstilsfaktorerne. Således viser afhandlingen, at en bedre livsstil i form af rygeophør samt forebyggelse af overvægt kan reducere forekomsten af VTE.

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ORIGINAL ARTICLE

Venous thromboembolism discharge diagnoses in the Danish National Patient Registry should be used with caution

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Abstract

Objective: We validated discharge diagnoses of venous thromboembolism (VTE) in the Danish National Patient Registry.

Study Design and Setting: We identified all first-time VTE discharge diagnoses in the Danish National Patient Registry among participants of the Danish cohort study ''Diet, Cancer, and Health'', in the period from 1994 to 2006. Medical records were retrieved and VTE diagnoses were verified by one of the authors using a standard protocol. The positive predictive value (PPV) of a discharge diagnosis of VTE was calculated as percent of registry diagnoses with the corresponding true diagnosis in the chart among all registry diagnoses.

Results: We retrieved medical records from 1,100 of 1,135 participants (96.9%) registered with a discharge diagnosis of VTE; 626 diagnoses were confirmed and 17 were considered probable. The PPV of diagnoses coded at wards was 75.0% (95% confidence interval: 71.9, 77.9). Diagnoses from emergency departments were not valid. The PPV varied by type of VTE (deep venous thrombosis and pulmonary embolism), type of diagnosis (primary or secondary), and sex.

Conclusion: Data on VTE obtained from administrative registries are a valuable source of information but should be used with caution in medical research.

Keywords: Venous thromboembolism (VTE); Deep venous thrombosis (DVT); Pulmonary embolism (PE); Positive predictive value (PPV); Cohort study; Discharge diagnosis

1. Introduction

Venous thromboembolism (VTE), that is, deep venous thrombosis (DVT) and pulmonary embolism (PE) is a common disease, with substantial clinical implications. Patients with VTE have a mortality of 16−21% the first year after diagnosis; the cumulative incidence of recurrent VTE is 30% and of postthrombotic syndrome is 29% after 8 years of follow-up [1−4]. Possible risk factors for VTE, identified in recent cohort studies [2,5−11], include both genetic predispositions and acquired factors. However, 25−50% of VTE events remain idiopathic, underscoring the need for well-designed studies elucidating the causes of this disease.

Hospital discharge registries are a potentially valuable source of data on VTE [12], with data readily available at low cost. Routine data collection and often universal registration of people in the target population help avoid biases of recall, diagnosis, or selection. However, lack of investigator's control over data collection and quality are important disadvantages of registry data [12,13]. Quality of registry data varies considerably from disease to disease and poor data quality may invalidate results of epidemiologic studies. Thus, it is essential to validate routinely collected registry data before using them for research. Few studies have examined the data quality of VTE diagnoses in registries in selected patient groups [14−17]. Validation was done only by secondary diagnoses to determine the frequencies of in hospital-acquired VTE by others [18,19]. We aimed to determine the positive predictive value (PPV) of VTE diagnoses in the Danish National Patient Registry, a nationwide hospital discharge register.

2. Materials and methods

2.1. The Diet, Cancer, and Health study

Diet, Cancer, and Health is a prospective cohort study, with the primary objective to investigate the etiologic role

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What is new?

This study provide the positive predictive value (PPV) of discharge diagnoses of Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE) in the nationwide Danish National Patient Registry. The study shows

1. The PPV of diagnoses given in wards units and Emergency Departments
2. The PPV of primary and secondary types of diagnoses
3. The PPV of diagnoses among men and women

of diet in the development of cancer. The study has been described in detail elsewhere [20,21]. Briefly, between December 1993 and May 1997, 80,996 men and 79,729 women, aged 50−64 years, were invited to participate in the study; 27,178 men and 29,876 women accepted the invitation. Eligible cohort members were born in Denmark, were living in the urban areas of Copenhagen and Aarhus and were not, at the time of invitation, registered with a previous diagnosis of cancer in the Danish Cancer Registry.

Both Diet, Cancer, and Health and the present substudy were approved by the Regional Ethical Committees in Copenhagen and Aarhus and by the Danish Data Protection Agency.

2.2. The Danish National Patient Registry

Since 1977, the Danish National Patient Registry routinely collects nationwide data on all somatic hospitalizations. In 1995, visits to emergency departments and outpatient clinics have also become reportable to the registry. The registry captures 99.4% of all somatic admissions [13]. Recorded data include dates of admission and discharge, patient type (i.e., emergency or wards department), primary discharge diagnosis, reflecting the main reason for investigation, and up to 20 secondary discharge diagnoses. The diagnoses are coded according to the Danish version of the *International Classification of Diseases, 8th revision* (ICD-8) for hospitalizations through the end of 1993, and according to the corresponding international version of ICD-10, thereafter. All discharge diagnoses are determined exclusively by the physician who discharges the patient. These diagnoses cannot later be altered, for example, for administrative or financial reasons. We linked the Diet, Cancer, and Health cohort with the Danish National Patient Registry automatically using the civil registration number of the study participants. The civil registration number, assigned at birth to all Danish citizens, is a unique personal identifier that is used in all public registries in Denmark and enables unambiguous record linkage [22].

2.3. Identification of possible cases of DVT and PE

We identified all participants in the Diet, Cancer, and Health cohort who were registered with a discharge diagnosis of VTE (ICD-8: 450.99, 451.00, 451.08, 451.09, 451.99 and ICD-10: I26, I80.1−I80.9) in the Danish National Patient Registry from the time of enrolment into the Diet, Cancer, and Health study until June 30, 2006. We considered only first-time VTE diagnoses and excluded participants with a discharge diagnosis of VTE registered before enrolment into the Diet, Cancer, and Health study. Diagnosis indicating VTE suspicion was not included.

2.4. Review of medical records

Medical records were retrieved and reviewed by one of the authors (MTS), who is a hematologist familiar with VTE. If the complete medical record was unavailable, discharge letters, results of biochemical analyses, ultrasonography, venography, echocardiography, ventilation-perfusion lung scan, and computer tomography (CT) scan were retrieved, whenever possible.

A VTE diagnosis was considered confirmed when typical clinical symptoms (unilateral swelling of leg, sagittal leg pain, miscoloured leg, dyspnoea, chest pain, coughing, hyperventilation, increased plasma D-dimer, and hemoptysis) were combined with a confirmatory diagnostic test result (ultrasonography, venography, echocardiography, ventilation-perfusion lung scan, or CT-scan). The diagnosis was classified as probable when typical clinical symptoms were reported, other diagnoses were unlikely, but no confirmatory diagnostic test had been performed. VTE was ruled out when results of diagnostic tests were negative, or if no test was performed because symptoms disappeared, or were explained by another disease.

Whenever a diagnosis of VTE in the Danish National Patient Registry could not be confirmed, we performed a new search of the registry to identify additional VTE diagnoses registered later than the diagnosis that were ruled out, and likewise reviewed records from these admissions. Information in medical records about prior VTE events not registered in the Danish National Patient Registry was also noted.

A leg thrombosis was classified as distal when the thrombus was located in the calf only; as proximal when the thrombus was located in or above vena poplitea; and as pelvic when the thrombus was above ligamentum inguinale. Upper-extremity thrombosis included thromboses of the arm, axillary, or subclavian veins. We registered concurrent DVT and PE as PE.

An event was regarded as provoked (secondary) when any of the following criteria was registered in the medical record: a cancer diagnosis before or within 3 months after admission with VTE, or within 3 months before VTE event: surgery, trauma, travel (at least 5 hours long), acute medical disease with a duration of minimum 3 day (stroke, acute myocardial

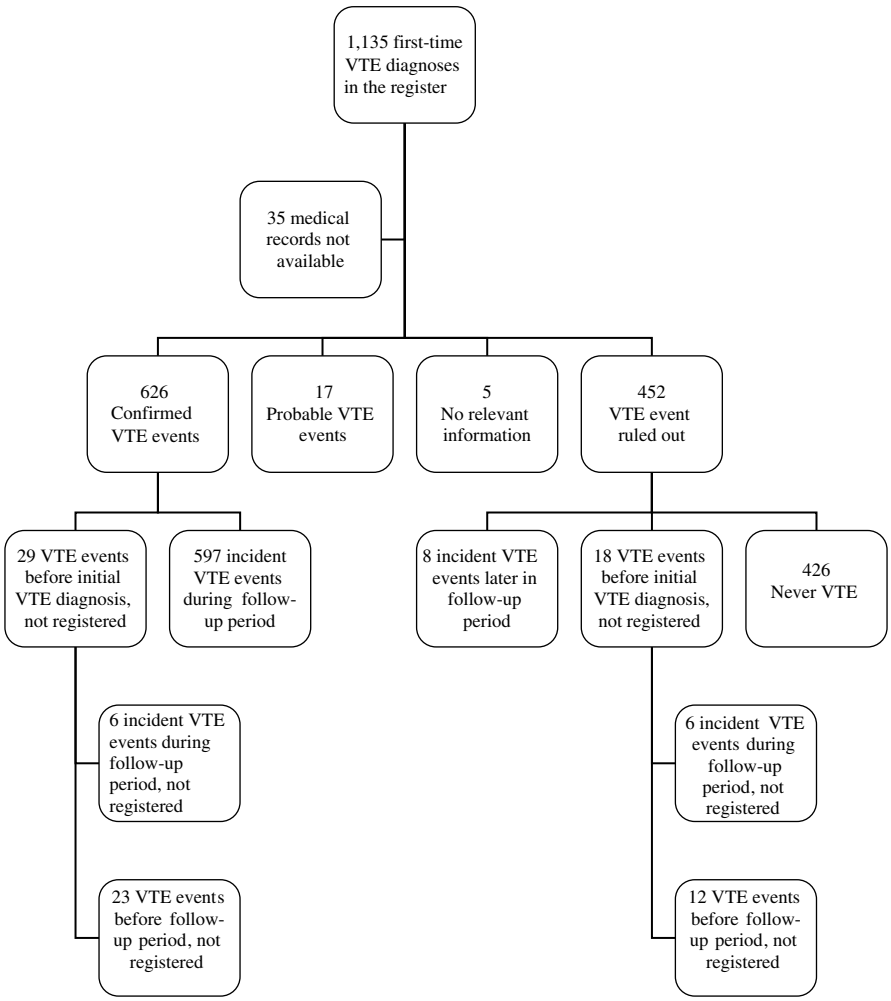

Fig. 1. Results of review of medical records.

infarction, exacerbation of chronic lung disease, infection, and activity in collagenous disease), immobilization in a minimum of 3 days, or central vein catheter, or other provoking factor (vein obstruction, vessel anomaly). An event was regarded as unprovoked (idiopathic) when the physician concluded that no provoking factors could be identified or when the health of the patient was described as good, without information indicating provoked VTE.

2.5. Data analysis

PPVs of a diagnosis of VTE registered in the Danish National Patient Registry were calculated as proportions, that is, the numerator containing the number of patients with confirmed and probable events after reviewing medical records, and the denominator containing the total number of patients registered in the Danish National Patient Registry with the specific diagnoses. The PPV was calculated for diagnoses of VTE overall and separately for the subdiagnoses DVT and PE. Furthermore, analyses were stratified by type of hospital department (emergency room or ward including outpatient clinic), type of diagnosis (primary or secondary), and by age and gender. We analyzed data using Stata version 9.2 (Stata Corporation, College Station, TX).

3. Results

3.1. Validation of VTE diagnoses in the Danish National Patient Registry

Figure 1 shows the results of the review of medical records. We identified 1,135 participants in the Diet, Cancer, and Health cohort study with a first-time VTE diagnosis recorded in the Danish National Patient Registry during the follow-up period. We were able to retrieve the medical records from 1,100 (96.9%) of these admissions. Of the 1,100 VTE diagnoses, 454 were given in emergency departments (Fig. 2). All but 36 patients were discharged into a ward unit within 1 week ($n = 418$). Of these, 184 were later discharged with a VTE diagnosis and 234 were discharged without a VTE diagnosis from the ward units.

Among the 626 patients with confirmed VTE events, we identified 29 with information about a prior VTE not registered in the Danish National Patient Registry; six of these

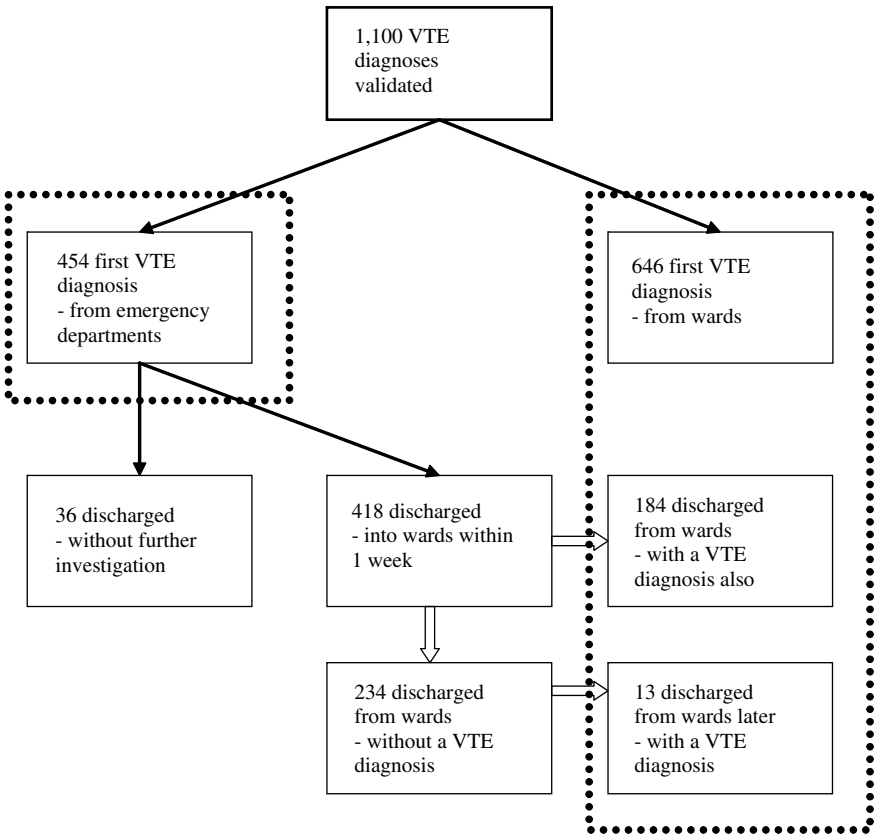

Fig. 2. Diagnoses from emergency departments ($n = 454$) and wards ($n = 843$).

VTE events occurred in the follow-up period, whereas most of the remaining events had occurred before the Danish National Patient Registry was established (1977). Among the 452 participants by whom VTE diagnosis was ruled out we identified 18 patients with information in medical records of a prior VTE, not registered in the Danish National Patient Registry. Six of these 18 events were in the follow-up period. In addition, we identified VTE-diagnoses registered after the initial diagnosis but in the follow-up period. Hereby, we confirmed eight additional incident VTE events.

Thus, we identified 617 ($597 + 6 + 6 + 8$) confirmed incident VTE events in the follow-up period among the study participants and 17 probable VTE events (Fig. 1).

The 617 confirmed VTE events were classified—49% as idiopathic ($n = 306$) and 49% ($n = 300$) as provoked events. Eleven VTE events could not be classified because of sparse information. A total of 123 (19.9%) patients with confirmed VTE had a diagnosis of cancer and 24 of them had undergone surgery. In all, 84 (13.6%) patients had undergone surgery, including the 24 cancer patients. An active medical disease was registered in 75 (12.0%) patients, including cancer ($n = 8$), surgery ($n = 9$), or both ($n = 2$). Immobilization without cancer, surgery, or medical disease was registered as a cause of the VTE event in 28 (4.5%) patients. Traveling was registered in another 31 (5.0%) patients without cancer, surgery, or medical disease. Five patients had other risk factors.

Among the confirmed VTE events, 12.3% ($n = 76$) were distal DVT, 36.0% ($n = 222$) were proximal DVT, 7.1% ($n = 44$) were pelvic DVT, 2.6% ($n = 16$) were upper-extremity thrombosis, and 42.0% ($n = 259$) were PE.

3.2. Analyses of PPV

Table 1 shows the various PPVs of VTE diagnoses in the Danish National Patient Registry. The PPV of a VTE diagnosis made in wards was 75.0% (95% confidence interval [CI]: 71.9, 77.9), that is, excluding those made by emergency departments in which the PPV was significantly lower. The PPV of the primary diagnoses from wards was 77.0% (95% CI: 73.7, 80.1). Furthermore, among patients discharged from wards, the PPV was higher for PE (82.1%, 95% CI: 77.2, 86.4) than for DVT (71.3%, 95% CI: 67.4, 75.0). The PPV of DVT diagnoses was higher among men than among women: 77.2% (95% CI: 72.2, 81.6) vs. 63.2% (95% CI: 56.7, 69.4), whereas PPVs for PE were similar. Finally, we found no difference between the PPV of VTE diagnoses according to age (data not presented).

5. Discussion

The PPV varied according to the subdiagnoses (DVT and PE), types of diagnoses (primary or secondary), type

Table 1
Positive predictive value of discharge diagnoses of venous thromboembolism including deep venous thrombosis and pulmonary embolism in the Danish National Patient Registry

Stratified by type of hospital department									
	All departments ($N = 1{,}100$)[a] 353 PE/742 DVT			Wards[b] ($N = 843$)[a] 285 PE/558 DVT			Emergency departments ($N = 454$)[a] 115 PE/339 DVT		
	PPV%	95% CI[c]	n[d]	PPV%	95% CI[c]	n[d]	PPV%	95% CI[c]	n[d]
VTE	58.5	55.5–61.4	643	75.0	71.9–77.9	632	31.3	27.0–35.8	142
PE	66.5	61.3–71.4	238	82.1	77.2–86.4	234	29.6	21.4–38.8	34
DVT	54.6	50.9–58.2	405	71.3	67.4–75.0	398	31.9	26.9–37.1	108

Diagnoses from wards.[b] Stratified by gender and type of diagnosis												
	Men ($N = 455$)[a] 131 PE/324 DVT			Women ($N = 388$)[a] 154 PE/234 DVT			Primary diagnosis ($N = 687$)[a] 216 PE/471 DVT			Secondary diagnosis ($N = 155$)[a] 68 PE/87 DVT		
	PPV%	95% CI[c]	n[d]	PPV%	95% CI[c]	n[d]	PPV%	95% CI[c]	n[d]	PPV%	95% CI[c]	n[d]
VTE	78.0	73.9–81.7	355	71.4	66.6–75.8	277	77.0	73.7–80.1	529	66.5	58.4–73.8	103
PE	80.2	72.3–86.6	105	83.8	77.0–89.2	129	87.0	81.8–91.2	188	67.6	55.2–78.5	46
DVT	77.2	72.2–81.6	250	63.2	56.7–69.4	148	72.4	68.1–76.4	341	65.5	54.6–75.4	57

Abbreviations: PPV, positive predictive value; VTE, venous thromboembolism; DVT, deep venous thrombosis; PE, pulmonary embolism; CI, confidence interval.

[a] *N*: number of diagnoses.

[b] Wards VTE diagnoses including participants with additional VTE diagnosis from emergency department.

[c] 95% confidence interval.

[d] *n*: number of confirmed diagnoses (incl. probable cases).

of hospital department, and gender. The highest (up to 87%) PPVs were found for PE diagnoses, in particular if it was a primary diagnosis; for diagnoses coded at wards (in contrast to emergency departments); and for diagnoses among men.

A number of other studies have examined the quality of VTE diagnoses in administrative databases [14–19]. Two studies estimated the PPV of VTE diagnoses among pregnant women to be 80% [15,17]. Also the validity of VTE diagnoses has been validated in the UK General Practice Research Database in a selected group of women who was exposed to combined oral contraceptive at the time of the VTE event and who was treated with anticoagulation. In this study, a PPV of 84% was found [16]. Other researchers have analyzed the validity of secondary VTE diagnoses and reported contradictory results (PPV: 24–90%) [18,19]. In a single center study the validity of VTE discharge diagnoses was validated [14]. They confirmed 64 of 86 VTE diagnoses finding a PPV of 74%. In our study, inclusion of diagnoses from emergency departments in the Danish National Patient Registry, in 1995, seems to have affected the validity of the discharge diagnoses of selected diseases, particularly those of VTE. Restricting the analysis to VTE diagnoses given on the wards yielded a PPV estimate comparable with the high values reported by others. We found the PPV for DVT diagnoses to be significantly higher among men compared with women. To our knowledge no prior studies have evaluated the validity of VTE diagnoses separately among men and women. If confirmed, this difference should be considered before using registry data in epidemiologic studies on VTE. Our finding is in accordance with the pattern found in another recent study from our group on the PPVs of acute coronary syndrome discharge diagnoses [23]. Furthermore, a study on the PPV of primary diagnoses in the Danish National Patient Registry performed by the Danish National Board of Health also reported an overall higher PPV of discharge diagnoses among men than among women in general. The study included 1,094 medical records from 1,990 with different diagnoses and from different hospital departments [24]. The explanation for these differences is unclear.

The Danish National Patient Registry was established for administrative rather than research purposes. For administrative reasons, the primary diagnosis must reflect the main reason for the diagnostic work-up or treatment to display the cost of health services. In many cases, the treating physicians entered a diagnostic code for VTE when no other diagnosis was confirmed, even though the VTE was ruled out. In such situations, a code indicating ''observation for VTE'' is supposed to be used but was not. Even though the interrater reliability of the tools used for diagnosing VTE may be low, there were only few disagreements between the diagnosis made by the treating physician in the medical record and the conclusion of our standardized record review. Incorrect coding of discharge diagnoses rather than incorrect diagnostic work-up or interpretation of tests was therefore the major source of misclassification.

In emergency departments the VTE diagnosis was in general based on clinical suspicion only. Patients from emergency departments suspected with VTE were medicated as having VTE and discharged to a ward unit where objective tests were performed (418 patients). In 184 of these patients, the discharge diagnosis from the ward was also VTE, however now based on findings from appropriate diagnostic tests, but in other 234 cases the ward diagnosis differed from the emergency room diagnosis. The divergent diagnoses made on the wards included, for example, Baker cyst, superficial thrombophlebitis, as well as chronic venous insufficiency, arthritis, rupture of muscle fiber, and erysipelas. Dyspnoea was often explained by heart or lung diseases other than PE.

The patients included in our study were participants in an ongoing cohort study and at entry they were living in urban areas (the two largest cities of Denmark). Because research participants generally tend to be healthier and better educated than the background population [20], patients in this study might not be representative of the Danish National Patient Registry as a whole. However, because the Danish National Health Service provides free universal tax-supported health care for all inhabitants, hospitals have no financial incentive to deny optimal diagnostic work-up to patients of low socioeconomic position. Furthermore, the overall incidence rate of confirmed VTE in our cohort was 1.15 per thousand person years, which is similar to the reported incidence in the general population in this age-group in a recent systematic review [4]. Thus, even though our cohort were not necessarily representative of the general population with regarding socioeconomic factors, it appears unlikely that the PPV of VTE diagnoses found in our study differ substantially from the predictive value for the general Danish population.

In conclusion, data on VTE from administrative registries may be useful but should be used with caution in medical research on VTE.

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ORIGINAL ARTICLE

Smoking and venous thromboembolism: a Danish follow-up study

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Summary. *Background:* Large-scale prospective studies are needed to assess whether smoking is associated with venous thromboembolism (VTE) (i.e. deep venous thrombosis and pulmonary embolism) independently of established risk factors. *Objective:* To investigate the association between smoking and the risk of VTE among middle-aged men and women. *Methods:* From 1993 to 1997, 27 178 men and 29 875 women, aged 50–64 years and born in Denmark, were recruited into the Danish prospective study 'Diet, Cancer and Health'. During follow-up, VTE cases were identified in the Danish National Patient Registry. Medical records were reviewed and only verified VTE cases were included in the study. Baseline data on smoking and potential confounders were included in gender stratified Cox proportional hazard models to asses the association between smoking and the risk of VTE. The analyses were adjusted for alcohol intake, body mass index, physical activity, and in women also for use of hormone replacement therapy. *Results:* During follow-up, 641 incident cases of VTE were verified. We found a positive association between current smoking and VTE, with a hazard ratio of 1.52 (95% CI, 1.15–2.00) for smoking women and 1.32 (95% CI, 1.00–1.74) for smoking men, and a positive dose-response relationship. Former smokers had the same hazard as never smokers. *Conclusions:* Smoking was an independent risk factor for VTE among middle-aged men and women. Former smokers have the same risk of VTE as never smokers, indicating acute effects of smoking, and underscoring the potential benefits of smoking cessation.

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Keywords: deep venous thrombosis (DVT), prospective cohort study, pulmonary embolism (PE), smoking, tobacco dose, venous thromboembolism (VTE).

Introduction

Venous thromboembolism (VTE) (i.e. deep venous thromboses, DVT, and pulmonary emboli, PE), is a common disease with substantial clinical implications [1–4].

Smoking is an established risk factor for arterial thrombosis, but data on its association with VTE are inconsistent. Recently a meta-analysis found a statistically insignificant odds ratio for VTE of 1.15 (95% CI, 0.92–1.44) for smokers compared with non-smokers [5]. Subsequently, a large case-control study found a positive association between smoking and VTE, with an odds ratio of 1.43 (95% CI, 1.28–1.60) [6]. Four prospective studies are available, two of which reported a positive association between smoking and VTE [7,8]. Further clarification of the effect of smoking on VTE risk requires large-scale epidemiological studies based on detailed prospective data on smoking, including dose, possible confounders, and verified VTE events.

The Danish Diet, Cancer and Health study is a prospective cohort study started in 1993 with the primary objective of investigating the etiological role of diet in the development of cancer. This prospective study includes very detailed data on smoking habits and relevant confounders according to VTE. In this follow-up we analyzed the effect of smoking, including dose, on the risk of VTE adjusted for potential confounders (i.e. obesity, physical activity, alcohol intake, and women's use of hormone replacement therapy, HRT). We aimed to assess the association of smoking with both idiopathic and secondary VTE, as well as PE.

Methods

Study population

From December 1993 through to May 1997, 80 996 men and 79 729 women aged 50–64 years were invited to participate in

the Danish prospective study 'Diet, Cancer and Health'. The study has been described in detail elsewhere [9,10]. In short, eligible cohort members were born in Denmark, were living in the urban areas of Copenhagen and Aarhus, and were not, at the time of invitation to join the study, registered with a previous diagnosis of cancer in the Danish Cancer Registry. Participants were identified from computerized records of the Civil Registration System in Denmark, which has included all Danish inhabitants since 1968. The information includes a unique personal identification number in addition to name, address and vital status [11]. Diet, Cancer and Health and the present sub-study were approved by the regional ethics committees in Copenhagen and Aarhus, and by the Danish Data Protection Agency.

Outcomes and follow-up

We linked the Diet, Cancer and Health cohort with the Danish National Patient Registry, using the participants' civil registration numbers. Based on the available hospital discharge history of each participant, we identified those who were registered with a discharge diagnosis of VTE (ICD-8: 450.99, 451.00, 451.08, 451.09, 451.99 and ICD-10: I26, I80.1–I80.9). Participants with a discharge diagnosis of VTE before enrollment into the Diet, Cancer and Health cohort were excluded. We reviewed medical records from participants with a first time VTE diagnosis in the Danish National Patient Registry from the time of enrollment into Diet, Cancer and Health until 30 June 2006. Information was obtained regarding symptoms, results of biochemical analyses, and diagnostic tests including duplex ultrasound, Doppler ultrasound, venography, echocardiography, ventilation-perfusion lung scan and CT scan. A VTE diagnosis was considered to be verified when typical clinical symptoms (unilateral swelling of leg, sagittal leg pain, discolored leg, dyspnea, chest pain, hyperventilation, increased plasma D-dimer, and hemoptysis) were combined with a confirmatory diagnostic test result (ultrasound, venography, echocardiography, ventilation-perfusion lung scan, or CT-scan). A leg thrombosis was classified as distal when the thrombus was located in the calf only, proximal when the thrombus was located in or above the popliteal vein, and pelvic when the thrombus was above the inguinal ligament. Upper extremity thromboses included thrombosis of the arm, axillary or subclavian veins. Concurrent DVT and PE were registered as PE.

Verified VTE events were classified as idiopathic or secondary (provoked) according to information in the medical records. The medical records include information regarding visits to outpatient clinics and inpatient clinics at the hospital. The complete medical record was reviewed carefully in every case. An event was regarded as secondary when any of the following criteria were registered in the medical record: a cancer diagnosis prior to, or within 3 months after, admission with VTE, surgery, trauma, travel (at least 5 h), acute medical disease with bed rest of at least 3 days (stroke, acute myocardial infarction, exacerbation of chronic lung disease, infection, activity in collagenous disease), immobilization, central vein catheter, or other provoking factors (vein obstruction, vessel anomaly) 3 months or less before the VTE. An event was regarded as idiopathic when the physician who examined the patient concluded that no provoking factors could be identified, or when the health of the patient was described as good without information indicating secondary VTE. The event was registered as 'unclassified' when information in medical records was sparse.

In the present study, we also included participants who died of VTE. We identified VTE deaths by linkage with the Danish National Death Registry (until 2003), and by review of death certificates from participants who died between 2003 and 2006. Only participants whose autopsy verified VTE were classified as VTE deaths.

Data on smoking and other lifestyle factors

Participants filled in detailed questionnaires about lifestyle factors at the time of enrollment into the Diet, Cancer and Health cohort. Data on smoking included smoking status (never, former and current smoker), tobacco doses, smoking duration, and time since cessation. Current tobacco consumption was calculated in grams per day using the following conversion factors: 1 cigarette equals 1 g, 1 cigar equals 4.5 g, 1 cheroot equals 3 g, and 1 pipe stop equals 3 g of tobacco. In addition, information on medication, education, work, diet, sports activities and alcohol consumption was obtained. The questionnaires were optically scanned. During the following interviews performed by trained laboratory technicians, information was amended. Technicians obtained anthropometric measurements in a standardized way. In 2000–2002, follow-up questionnaires were mailed to all surviving participants. Questions about diet and lifestyle changes were asked.

Participants for whom information was missing on one or more smoking or confounding variables and participants with unlikely smoking doses (more than 100 g day^{-1} tobacco) were excluded from analyses.

Statistical analysis

We assessed the association between smoking and risk of VTE separately for men and women, using Cox regression analyses. Age was used as the time axis to prevent confounding by age, with entry time defined as the subject's age at recruitment. Study exit time was determined as age at VTE, date of death or emigration, or as 30 June 2006, whichever came first. Smoking doses were analyzed separately as a categorical exposure variable, and as a continuous exposure variable in restricted cubic spline models. Alcohol consumption, obesity, physical activity and women's use of hormone replacement therapy (HRT) were considered as potential confounders. Therefore, all models included adjustment for these variables. In a supplementary model, we included education and performed analysis adjusted for categories of years in primary school and education after primary school in addition to the other

adjustments. Model adequacy was assessed graphically and found appropriate in all analyses. Adjustment for physical activity was performed including activity as a dichotomous variable (i.e. above or below 30 min day^{-1} of activity, including bicycling). Body mass index (BMI) and alcohol consumption were included as continuous variables. Information on use of HRT by women was included as a dichotomous variable. The associations between smoking and VTE, idiopathic VTE, secondary VTE, secondary non-cancer VTE and PE were assessed.

Incidence rate of VTE was calculated for men and women according to tobacco consumption. We used the number of verified incident VTE events as the numerator and the sum of the individual person time at risk (follow-up time) as denominator. The incidence rates were standardized according to the age distribution among never smokers and were given as number of VTE events per thousand person-years at risk. We used STATA version 9.2 (Stata Corporation, College Station, Texas, USA) for the statistical analyses.

Results

Study population and case ascertainment

In total, 27 178 men and 29 876 women joined the study (Fig.1). All but 43 participants answered the questionnaire and participated in the following interview. We excluded 564 participants who were later identified in the Danish Cancer Registry as having a cancer diagnosis before the invitation, or in the weeks between invitation and the baseline examination. We also excluded 433 participants with a diagnosis of VTE before enrollment into the Diet, Cancer and Health study, which left 56 014 participants for analysis. Table 1 provides baseline characteristics of the participants.

The median follow-up time was 10.2 years with an interquartile range from 9.6 to 10.8 years. During follow-up we verified 617 VTE events in the Danish National Patient Registry. Of these, 58% (n = 358) were DVT, and 42% (n = 259) were PE. Confirmed VTE events were characterized as idiopathic in 49% (n = 306) and secondary in 49% (n = 300) of cases. The remaining 2% (n = 11) could not be classified due to sparse information. Of the 56 014 participants, 4084 died during follow-up. We found 57 participants with an autopsy-proven PE diagnosis. However, 33 of these were already known from the Danish National Patient Registry with a prior verified VTE event. Our study, therefore, included 617 VTE events identified by review of medical records and another 24 PE events identified by autopsy. Thus in total, 641 confirmed VTE events were identified. The incidence rate of VTE was 1.15 (95% CI, 1.06–1.24) per 1000 person years.

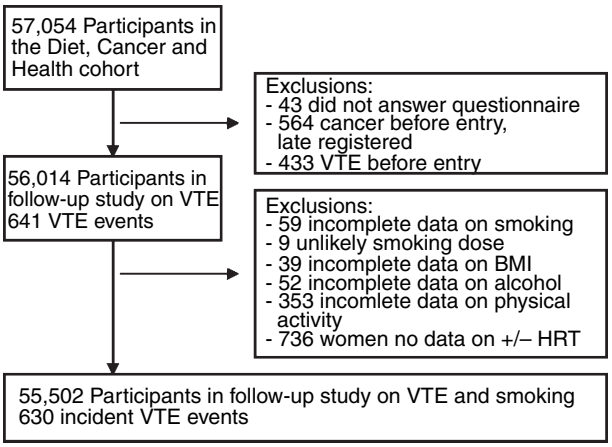

Fig. 1. Inclusions and exclusions before analysis of the association between smoking and VTE.

Table 1 Baseline characteristics of the participants in the Diet, Cancer and Health cohort

	Women	Men
Number of participants	29 340	26 674
Age[1], years	56 (51–64)	56 (51–64)
Smoking status, %		
Never smokers	43.6	25.7
Former smokers	23.5	34.6
Current smokers, < 15 g day^{-1}	15.4	10.7
Current smokers, 15–25 g day^{-1}	14.9	17.5
Current smokers, 25.1–35 g day^{-1}	2.6	8.2
Current smokers, > 35+ g day^{-1}	–	3.4
Smoking duration[1], if ever a smoker, years	31 (5–45)	33 (7–47)
Alcohol consumption[1], g day^{-1}	9 (0–42)	19 (2–80)
Exercise > ½ h day^{-1}, %	41	38
Hormone replacement therapy, %	31	Not relevant
Education[2], %		
7 years	31.4	34.8
8–10 years	50.2	41.5
> 10 years	18.5	23.7
Higher education[3], %		
No	19.3	10.0
Short	31.7	13.8
Middle	37.7	42.0
Long	11.3	34.1
Weight[1], kg	67 (53–91)	82 (65–105)
Height[1], cm	164 (155–174)	177 (166–188)
Body mass index[1], kg m^{-2}	25 (20–34)	26 (21–33)

[1]Median (5–95 percentiles).
[2]Education, three categories of years in primary school: 7 years, 8–10 years, 11+ years.
[3]Education after primary school: no education after primary school, short, middle, long.

Analyses

Table 2 shows the crude and adjusted hazard ratios of VTE for former smokers, current smokers, and for various smoking doses. The reference group is never smokers. The hazard ratio among current smokers was 1.52 (95% CI, 1.15–2.00) for women and 1.32 (95% CI, 1.00–1.74) for men. A positive association between smoking and VTE was found among women at all doses of tobacco, and among men at doses above 15 g day^{-1}. The hazard ratio was highest for the highest doses of tobacco. Former smokers had the same risk as never smokers.

Table 2 Hazard ratios (HR) of venous thromboembolism (VTE) and pulmonary embolism (PE), crude and adjusted for BMI, alcohol intake, recreational physical activity, and women's use of HRT. Reference group is never smokers. Hazard ratios with 95% confidence interval are given. Number of cases in brackets. Incidence rates of VTE in number per 1000 person years according to tobacco dose standardized to age in never smoking group with 95% confidence interval

Women	Never smoked	Former smoker	Current smoker. All doses	Current smoker < 15 g day^{-1}	Current smoker 15–25 g day^{-1}	Current smoker > 25 g day^{-1}
All VTE (262)	(100)	(56)	(106)	(48)	(44)	(14)
Incidence rate	0.80 [0.64–0.95]	0.81 [0.60–1.01]	1.14 [0.93–1.35]	1.10 [0.80–1.39]	1.03 [0.72–1.33]	2.15 [1.01–3.29]
Crude HR	1	0.96 [0.69–1.33]	1.40 [1.06–1.85]	1.28 [0.91–1.81]	1.33 [0.93–1.90]	2.78 [1.59–4.87]
Adjusted	1	0.96 [0.69–1.33]	1.52 [1.15–2.00]	1.41 [1.00–2.00]	1.43 [1.00–2.04]	2.87 [1.63–5.05]
Adjusted*	1	0.96 [0.69–1.33]	1.46 [1.11–1.94]	1.37 [0.97–1.94]	1.38 [0.97–1.99]	2.63 [1.46–4.74]
Idiopathic VTE (110)	(45)	(26)	(39)	(16)	(14)	(9)
Crude	1	1.00 [0.61–1.62]	1.15 [0.75–1.77]	0.96 [0.54–1.70]	0.94 [0.52–1.71]	3.90 [1.90–7.99]
Adjusted	1	1.03 [0.63–1.66]	1.27 [0.83–1.96]	1.07 [0.60–1.90]	1.03 [0.56–1.88]	4.34 [2.10–8.96]
Secondary VTE (135)	(51)	(26)	(58)	(28)	(25)	(5)
Crude	1	0.86 [0.54–1.39]	1.51 [1.03–2.19]	1.46 [0.92–2.32]	1.48 [0.92–2.40]	1.97 [0.79–4.94]
Adjusted	1	0.85 [0.53–1.37]	1.60 [1.10–2.35]	1.59 [1.00–2.53]	1.57 [0.97–2.55]	1.91 [0.75–4.85]
Secondary, non-cancer VTE (80)	(32)	(17)	(31)	(14)	(14)	(3)
Crude	1	0.90 [0.50–1.63]	1.29 [0.78–2.11]	1.17 [0.63–2.20]	1.32 [0.71–2.48]	1.86 [0.57–6.08]
Adjusted	1	0.89 [0.49–1.61]	1.37 [0.83–2.26]	1.29 [0.69–2.43]	1.40 [0.74–2.65]	1.74 [0.52–5.79]
PE (137)	(57)	(30)	(50)	(24)	(18)	(8)
Crude	1	0.88 [0.57–1.38]	1.15 [0.79–1.69]	1.11 [0.69–1.79]	0.95 [0.56–1.62]	2.81 [1.34–5.89]
Adjusted	1	0.90 [0.58–1.40]	1.28 [0.87–1.88]	1.25 [0.78–2.02]	1.06 [0.62–1.80]	2.95 [1.39–6.25]

Men	Never smoked	Former smoker	Current smoker. All doses	Current smoker < 15 g day^{-1}	Current smoker 15–25 g day^{-1}	Current smoker 25.1–35 g day^{-1}	Current smoker > 35 g day^{-1}
All VTE (363)	(80)	(128)	(155)	(34)	(72)	(30)	(19)
Incidence rate	1.17 [0.92–1.43]	1.34 [1.10–1.58]	1.50 [1.26–1.73]	1.18 [0.78–1.58]	1.53 [1.18–1.89]	1.48 [0.96–2.00]	2.23 [1.26–3.23]
Crude	1	1.13 [0.85–1.49]	1.28 [0.97–1.67]	1.01 [0.67–1.51]	1.33 [0.97–1.83]	1.25 [0.82–1.91]	1.94 [1.18–3.20]
Adjusted	1	1.09 [0.82–1.44]	1.32 [1.00–1.74]	1.05 [0.70–1.57]	1.40 [1.02–1.93]	1.28 [0.83–1.95]	1.97 [1.19–3.26]
Adjusted*	1	1.09 [0.82–1.44]	1.30 [0.99–1.71]	1.04 [0.70–1.56]	1.39 [1.00–1.92]	1.27 [0.83–1.94]	1.85 [1.10–3.10]
Idiopathic VTE (186)	(46)	(60)	(80)	(22)	(33)	(14)	(11)
Crude	1	0.96 [0.65–1.40]	1.16 [0.81–1.67]	1.16 [0.70–1.93]	1.08 [0.69–1.69]	1.00 [0.55–1.83]	1.98 [1.02–3.82]
Adjusted	1	0.91 [0.62–1.33]	1.16 [0.80–1.68]	1.19 [0.71–1.98]	1.10 [0.70–1.74]	0.96 [0.52–1.77]	1.89 [0.97–3.68]
Secondary VTE (159)	(30)	(62)	(67)	(11)	(35)	(13)	(8)
Crude	1	1.40 [0.91–2.18]	1.45 [0.94–2.23]	0.85 [0.42–1.69]	1.69 [1.04–2.76]	1.47 [0.77–2.82]	2.15 [0.98–4.69]
Adjusted	1	1.38 [0.89–2.14]	1.58 [1.02–2.44]	0.91 [0.45–1.81]	1.87 [1.14–3.06]	1.61 [0.84–3.12]	2.33 [1.06–5.11]
Secondary, non-cancer VTE (96)	(17)	(37)	(42)	(6)	(21)	(10)	(5)
Crude	1	1.50 [0.84–2.66]	1.61 [0.92–2.83]	0.82 [0.32–2.08]	1.80 [0.95–3.41]	1.99 [0.91–4.34]	2.38 [0.88–6.45]
Adjusted	1	1.43 [0.80–2.55]	1.71 [0.97–3.03]	0.88 [0.35–2.23]	1.97 [1.03–3.75]	2.08 [0.94–4.59]	2.47 [0.90–6.73]
PE (138)	(34)	(46)	(58)	(15)	(29)	(9)	(5)
Crude	1	0.91 [0.57–1.46]	1.11 [0.73–1.70]	1.06 [0.56–1.98]	1.27 [0.76–2.13]	0.76 [0.33–1.73]	1.31 [0.51–3.36]
Adjusted	1	0.87 [0.54–1.39]	1.16 [0.75–1.78]	1.12 [0.60–2.11]	1.38 [0.82–2.32]	0.78 [0.34–1.79]	1.33 [0.51–3.44]

*Adjusted for primary school education (years in primary school) and higher education.

For idiopathic VTE, we found a positive association with the highest dose of tobacco in both genders, with a hazard ratio of 4.34 (95% CI, 2.10–8.96) for women and 1.89 (95% CI, 0.97–3.68) for men. There was no association at lower doses of tobacco. For secondary VTE, we found a statistically significant positive association with current smoking for both genders. The association was also present for non-cancer-related secondary VTE. For PE, we found a less consistent positive association with current smoking for both genders. The incidence rates of VTE according to tobacco dose are given.

Figure 2 shows the hazard ratios of VTE according to daily tobacco consumption for each gender. The reference groups are non-smoking women and men. The hazard ratio of VTE among women increased steeply for smoking more tobacco than 20 g day^{-1}. Among men, the hazard ratio of VTE did not increase substantially before the dose exceeded 30 g day^{-1}.

A total of 45 271 participants answered a follow-up questionnaire after 5 years. Data on smoking habits were complete for 44 379 participants, out of whom 1560 (3.5%) smoked more tobacco at follow-up than at baseline, 6612 (14.9%) smoked less at follow-up than at baseline, and 36 307 (81.8%) had not changed their smoking habits.

Discussion

In this large prospective study, we found current smoking to be positively associated with VTE, among both men and women. Former smokers had essentially the same risk of VTE as never smokers, indicating that the mechanism of smoking's effect on VTE is acute. We found that smoking more than 20 g day^{-1} among women and 30 g day^{-1} among men was associated with a 150–300% higher hazard of VTE. Lower rates of tobacco consumption were associated with only a 10% to 40% higher hazard of VTE compared with never smokers, indicating a threshold difference for both men and women (Fig. 2).

Strengths and limitations of our study

This prospective study included 641 VTE events, and was one of the largest prospective studies on VTE. Data on smoking habits were detailed, and data from follow-up after 5 years showed a high degree of concordance with the baseline information. The changes were generally toward decreased consumption of tobacco at the follow-up, which may have resulted in an underestimation of risk. All VTE events were validated by review of medical records, and only objectively verified VTE events were included. The hospital system in Denmark is financed by taxation and almost everybody with VTE symptoms will be admitted to and examined in a hospital. The Danish National Patient Registry has collected nationwide data on all somatic hospital admissions since 1977. Since 1995, discharges from emergency departments and out-patient clinics have also been included in the registry [12]. The medical record includes information on visits to outpatient clinics as well as inpatients clinics. Obviously, there could be VTE events among participants that we missed. For example, because of a very low frequency of autopsy in Denmark (6% of all deaths in 2001) an unknown number of the participants died of PE. However, because smoking habits were unlikely to be associated with the chance of autopsy, any misclassification of fatal PE events was unlikely.

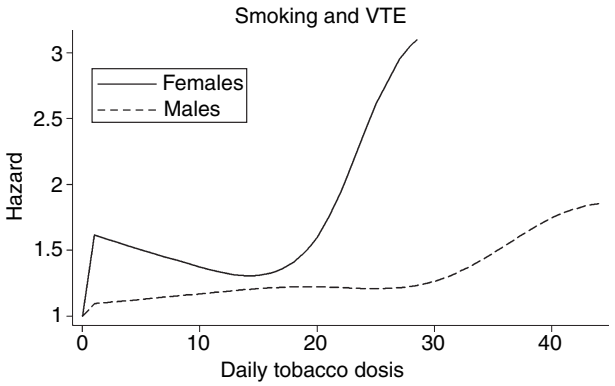

Fig. 2. Hazard ratio of VTE according to daily tobacco dose in g/day modelled as a restricted cubic spline. Non-smokers are used as reference. Adjusted for BMI, alcohol and HRT (women only).

Smoking is an established risk factor for chronic obstructive lung diseases. Diagnosing PE is challenging, especially among patients with chronic obstructive lung disease, because perfusion/ventilation scintegraphy may not be conclusive in these patients. In addition, the most prominent symptom of PE, dyspnea, is normal for these patients. Dyspnea may be interpreted as a result of an exacerbation of the chronic obstructive lung disease, or as a result of pneumonia, which also occurs very often among these patients. The effect of smoking on PE may therefore be underestimated due to information bias.

Detailed information on a range of potential confounding factors was available for this study. However, adjusting for these factors in the statistical analyses had only a minor impact on the estimated hazard ratios. This indicates that residual confounding is not a likely explanation for the observed associations. However, Rosengren *et al.* [13] found a statistically significant positive association between PE and occupational class in men. Therefore we performed secondary analysis including adjustment for educational status and for years in primary school. The adjustments slightly weakened the association between smoking and VTE. Socioeconomic status may, however, directly influence smoking habits. Inclusion of socioeconomic status reduced the variation in smoking habits but also took into account potential confounding due to an association between socioeconomic status and smoking habits. However, as in all observational studies, we cannot completely rule out possible confounding by unknown factors.

Comparison with other studies

Our findings accord with the results from a number of other large-scale epidemiological studies. In a recent large case-control study, Pomp *et al.* [6] also found higher risk of VTE among smokers. Two prospective studies have found a positive

association between smoking and venous thrombosis. Goldhaber *et al.* [7] investigated the association between smoking and PE among nurses. They reported 280 PE events during 16 years of follow-up. They found a significant positive association between smoking and PE among women who smoked more than 25 g day^{-1}, and no association with lower tobacco doses, exactly as we did. Hansson *et al.* [8] also found a positive association between smoking more than 15 g day^{-1} of tobacco and the occurrence of VTE in a study of 851 men, followed for 30 years. The study included 65 VTE cases, with a substantial proportion, 21 of 65, diagnosed by autopsy, explaining why as many as 36 had PE. Also a substantial proportion (69%) was secondary VTE cases, of which 23 (35.5%) had cancer. After including comorbidity in a multivariate analysis, Hansson *et al.* were still able to find the association between smoking and VTE, in accordance with our findings.

In contrast, other prospective studies have not found an association between smoking and VTE. Tsai *et al.* combined data from two cohorts (the Atherosclerosis Risk in Communities study, ARIC, and the Cardiovascular Health study, CHS) in the Longitudinal Investigation of Venous Thromboembolism study (LITE). The ARIC cohort included men and women, 45–64 years of age, in which 130 VTE events occurred during follow-up. The CHS cohort included men and women above 65 years of age, in whom 85 VTE events occurred. Data in the LITE study included smoking status (never, former or current smoker). No association between VTE and smoking status was found. They also analyzed pack-years of smoking and found no association with VTE. The ARIC cohort data included current doses of tobacco. Analysis of this cohort showed a positive association between VTE and smoking more than 25 g day^{-1} of tobacco, compared with never smokers with adjusted hazard ratio of 1.68 (95% CI, 0.91–3.1) [14]. Glynn *et al.* also found no association between smoking and VTE in the prospective Physicians' Health study. This study included American male physicians and 358 VTE events. The information on smoking included data on smoking status (current, former or never smoker), but not on tobacco dose [15].

We analyzed the dose-response relationship in a spline model that included current smoking dose as a continuous variable. The hazard ratio of VTE among women increased steeply for smoking more tobacco than 20 g day^{-1}. Among men, the hazard ratio of VTE did not increase substantially before the tobacco dose exceeded 30 g day^{-1} (Fig. 2). Most previous studies did not include tobacco dose, only smoking status (never, former or current smoker). However, studies that did include tobacco dose also found the strongest association for the highest doses of tobacco, in line with our results. Our data suggest thresholds for the effect of smoking, at different levels for men and women. This could be explained, in part, by the differences between genders in body volume or liver metabolism. Further studies are needed to confirm these findings.

In both genders, we found a strong positive association between the highest doses of tobacco and idiopathic VTE (i.e. 4.34 [95% CI, 2.10–8.96] in women and 1.89 [95% CI, 0.97–3.68] in men), underscoring that the effect of smoking on the risk for VTE may not only be mediated through secondary diseases caused by smoking. We reviewed the updated discharge history from the Danish National Patient Registry for the 20 heavy smokers who suffered from idiopathic VTE. Of the 20 patients, one woman had a diagnosis of lung cancer 1 year later than the date of VTE, another woman had a diagnosis of breast cancer 6 years later and a man had a diagnosis of lung cancer 6 years later. The remaining 17 patients did not have diagnosis of cancer before April 2008 (mean of follow-up, 6 years from the date of VTE diagnosis). In secondary analysis we excluded the woman with a cancer diagnosis 1 year later than the VTE event because she might have had the cancer at the date of VTE (1 year before the cancer diagnosis) and found a hazard ratio of 3.85 (95% CI, 1.80–8.25) for idiopathic VTE in women smoking more than 25 g of tobacco per day. Our findings suggest a direct effect of heavy smoking on the risk of VTE. These findings are biologically plausible because smoking increases the level of coagulation factors in the blood and it also promotes activation of the inflammatory system, both of which are found to be associated with venous thrombosis [16,17]. We can not rule out possible confounding by unknown factors; however, it is difficult to imagine a strong confounder that only occurs among the heaviest smokers and that can explain our findings. Steffen *et al.* [18] showed that a diet including more plant food and fish and less meat is associated with lower incidence of VTE and there might be an association between smoking habits and diet; however, we think it is unlikely that poor diet explains our findings. Further studies are needed on this topic.

Among women the highest doses of tobacco (more than 25 g day^{-1}) were positively associated with PE (i.e. hazard ratio of 3.07 [95% CI, 1.45–6.54]), whereas among men an insignificant positive association with PE was found (i.e. hazard ratio of 1.33 [95% CI, 0.51–3.44]) with the highest doses of tobacco (more than 35 g day^{-1}). This may indicate that smoking promotes coagulation but is not involved in the process of embolization.

In conclusion, we found a positive association between smoking and VTE. The hazard ratio of VTE was especially high at tobacco doses above thresholds of 20 g day^{-1} of tobacco for women and 30 g day^{-1} for men. The smoking effect seems to be mediated by an acute effect because former smokers have the same risk as those who never smoked.

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Disclosure and Conflict of Interests

The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The authors state that they have no conflict of interests.

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Anthropometry, Body Fat, and Venous Thromboembolism: A Danish Follow-Up Study

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Anthropometry, Body Fat, and Venous Thromboembolism
A Danish Follow-Up Study

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Background—Obesity, measured as body mass index, is associated with venous thromboembolism (VTE). Body mass index is a marker of excess weight and correlates well with body fat content in adults; however, it fails to consider the distribution of body fat. We assessed the association between anthropometric variables and VTE.

Methods and Results—From 1993 to 1997, 27 178 men and 29 876 women 50 to 64 years of age were recruited into a Danish prospective study (Diet, Cancer, and Health). During 10 years of follow-up, the outcome of VTE events was identified in the Danish National Patient Registry and verified by review of medical records. Body weight, body mass index, waist circumference, hip circumference, and total body fat were measured at baseline. We used Cox proportional hazard models to assess the association between anthropometry and VTE. Age was used as a time axis, with further adjustment for smoking, physical activity, height, hypertension, diabetes mellitus, cholesterol, and, among women, use of hormone replacement therapy. We verified 641 incident VTE events and found monotonic dose-response relationships between VTE and all anthropometric measurements in both sexes. In mutually adjusted analyses of waist and hip circumference, we found that hip circumference was positively associated with VTE in women but not in men, whereas waist circumference was positively associated with VTE in men but not in women.

Conclusions—All measurements of obesity are predictors of the risk for VTE. Positive associations were found between VTE and body weight, body mass index, waist circumference, hip circumference, and total body fat mass. **(*Circulation*. 2009;120:1850-1857.)**

Key Words: venous thromboembolism ■ anthropometry ■ obesity ■ follow-up studies

Obesity is an established risk factor for venous thromboembolism (VTE), ie, deep venous thrombosis (DVT) and pulmonary embolism (PE).[1–6] The body mass index (BMI; ie, weight in kilograms divided by the square of height in meters) is commonly used as a measurement of obesity, and the majority of previous studies of the association between obesity and VTE used BMI as the exposure. BMI is a marker of excess body weight and correlates well with body fat content in adults; however, it fails to consider the distribution of body fat.

Clinical Perspective on p 1857

The distribution of body fat predicts the risk of arterial thrombotic events, such as coronary heart disease (CHD). Central obesity, measured as waist circumference or waist-to-hip ratio, is a better predictor of CHD than general obesity as measured with BMI, whereas peripheral obesity, measured as hip circumference, is not a predictor of CHD[7,8]; however, only a few studies have evaluated the association between VTE and anthropometric measures other than BMI. One study evaluated the association between VTE and central obesity in men and study found that a waist circumference >100 cm was associated with a higher risk of VTE than a waist circumference <100 cm.[4] Recently, Steffen et al[9] studied the association between VTE and the metabolic syndrome, which is defined by the presence of 3 or more of the following factors: Central obesity (ie, waist circumference >102 cm for men and >88 cm for women), dyslipidemia, hypertension, and diabetes mellitus. They evaluated the different features of the metabolic syndrome and found a positive association between central obesity and risk of VTE in both sexes.

In a follow-up study, we aimed to assess the associations between anthropometric variables (body weight, BMI, waist circumference, and hip circumference) and VTE among middle-aged men and women. We also included measurement of bioelectrical impedance, from which total body fat mass can be calculated.[10–12]

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Methods

The Diet, Cancer, and Health Cohort

From December 1993 to May 1997, 80 996 men and 79 729 women 50 to 64 years of age were invited to participate in the Danish prospective study entitled Diet, Cancer, and Health. The study has been described in detail elsewhere.[13] Eligible cohort members were born in Denmark, were living in the urban areas of Copenhagen and Aarhus, and were not, at the time of invitation, registered with a previous diagnosis of cancer in the Danish Cancer Registry. Participants were identified from computerized records of the Civil Registration System in Denmark, which since 1968 has included all Danish residents.[14] The information includes a unique personal identification number in addition to name, address, and vital status. The Diet, Cancer, and Health study and the present substudy were approved by the regional ethics committees in Copenhagen and Aarhus and by the Danish Data Protection Agency.

Identification of Outcome

Incident VTE events among participants were identified by linking the Diet, Cancer, and Health cohort with the Danish National Patient Registry by use of the civil registration number of the study participants. The Danish National Patient Registry has collected nationwide data on all nonpsychiatric hospital admissions since 1977. Since 1995, discharges from emergency departments and outpatient clinics have also been included in the registry.[15] On the basis of the entire available hospital discharge history of each participant, we identified those who were registered with a discharge diagnosis of VTE (International Classification of Diseases, Revision 8 codes 450.99, 451.00, 451.08, 451.09, and 451.99, and International Classification of Diseases, Revision 10 codes I26 and I80.1 through I80.9; revision 9 was never used in Denmark). Participants with a discharge diagnosis of VTE before enrollment into the Diet, Cancer, and Health cohort were excluded. We reviewed medical records from participants with a first-time VTE diagnosis in the Danish National Patient Registry from the time of enrollment into the Diet, Cancer, and Health study until June 30, 2006. Information about symptoms, results of biochemical analyses, and diagnostic tests, including duplex ultrasound, Doppler ultrasound, venography, echocardiography, ventilation-perfusion lung scan, and computed tomography scan, were obtained. A VTE diagnosis was considered verified when typical clinical symptoms (unilateral swelling of leg, sagittal leg pain, discoloration of the leg, dyspnea, chest pain, hyperventilation, increased plasma D-dimer, or hemoptysis) were combined with confirmatory diagnostic test results (ultrasound, venography, echocardiography, ventilation-perfusion lung scan [Prospective Investigation of Pulmonary Embolism Diagnosis criteria], or computed tomography scan). Only objectively verified VTE events were included in the present study. Concurrent DVT and PE were registered as PE. In addition, events were classified as idiopathic or secondary (provoked) according to information from medical records. An event was regarded as secondary when any of the following criteria were registered in the medical record: A cancer diagnosis before or within 3 months after admission with VTE, surgery, trauma, travel (at least 5 hours' duration), acute medical disease with bed rest for at least 3 days (stroke, acute myocardial infarction, exacerbation of chronic lung disease, infection, or activity in collagenous disease), immobilization, use of a central vein catheter, or other provoking factors that were present within the 3 months before the VTE. An event was regarded as idiopathic when the physician concluded that no provoking factors could be identified or when the health of the patient was described as good without information that indicated secondary VTE. The event was registered as unclassified when information in medical records was sparse.

In the present study, we also included participants who died of VTE. We identified VTE deaths by linkage with the Danish National Death Registry (until 2003) and by review of death certificates from participants who died between 2003 and 2006. Only participants with autopsy-verified VTE were included as VTE deaths.

Data on Anthropometry and Lifestyle Factors

Data on anthropometry were obtained by trained laboratory technicians at 2 study clinics in Aarhus and Copenhagen at the time of enrollment into the Diet, Cancer, and Health study. All measurements were performed in a standardized manner. Measurement of bioelectrical impedance was obtained with a BIA 101-F device (Akern/RJL, Florence, Italy) at a single frequency (50 Hz) with the participant lying relaxed on a couch; arms and leg were not in contact with any other body part. The legs were 45° apart, and arms were 30° from the torso. Sensing electrodes were placed over the right wrist and ankle; current electrodes were placed over the metacarpals and metatarsals. The reliability and validity of the impedance method have been investigated in a Danish population (35 to 65 years of age) by use of a 4-compartment model with counting of potassium and total body water (dilutometry).[10] The equation obtained from that study was used to calculate total body fat mass. Blood pressure was measured in the right arm with automatic devices (Takeda UA-751 and UA-743, Takeda Pharmaceutical Co Ltd, Osaka, Japan) with participants in a supine position after a minimum of 5 minutes of rest.

Information on sociodemographic and lifestyle characteristics (tobacco consumption and physical activity) and medication use (use of hormone replacement therapy) was obtained from standardized detailed questionnaires at study entry. The questionnaires were optically scanned into a computer, and in subsequent interviews performed by trained laboratory technicians, information was amended as necessary. Immediately after the baseline interview, a blood sample was drawn from each participant, and total cholesterol was determined. Participants for whom information on 1 or more confounder or exposure variables was missing were excluded from the analysis.

Statistical Analysis

We assessed the association between the anthropometric variables and the risk of VTE separately for men and women using Cox proportional hazards regression. Age was used as the time axis to prevent confounding by age, with entry time defined as the subject's age at recruitment and exit time defined as age at VTE or censoring because of death, emigration, or June 30, 2006, whichever came first. Weight, BMI, waist circumference, hip circumference, and body fat mass were analyzed as continuous variables. Smoking, physical activity, height, hypertension, hypercholesterolemia, diabetes mellitus, and use of hormone replacement therapy (women only) were considered as potential confounders. Therefore, in addition to the crude models, we included adjustment for these variables. The following exposures were included as dichotomous covariates: Use of hormone replacement therapy (user, nonuser), physical activity (above or below 30 minutes of sport per day, including cycling), hypertension (systolic blood pressure $\geq$140 mm Hg, diastolic blood pressure $\geq$90 mm Hg, or self-reported use of antihypertensive medication), diabetes mellitus (self-reported), height, and cholesterol (in mmol/L) as continuous variables, along with smoking habit (in categories of nonsmoker, former smoker, smoker $<$15 g/d, smoker 15 to 25 g/d, and smoker $>$25 g/d). In addition, waist circumference was analyzed after adjustment for BMI, and waist circumference and hip circumference were analyzed with mutually adjusted models. Model adequacy was assessed graphically with a log-log plot and by use of a log-rank test based on Schoenfeld residuals and was found to be appropriate in all analyses. The associations between anthropometry and subtypes of VTE (ie, idiopathic and secondary VTE and PE) were assessed similarly. We used Stata version 9.2 (Stata Corp, College Station, Tex) for the statistical analyses.

All anthropometric variables were categorized in quartiles according to the exposure distribution among cases with VTE.[16] Hazard ratios of VTE were assessed by Cox regression, with the lowest quartile used as the reference level. We further computed the hazard ratios of VTE per 1 SD of the anthropometric variables.

In secondary analyses, we assessed the association between VTE and categories of hip circumference and waist circumference stratified according to quartiles of BMI, to evaluate the hazard ratio of

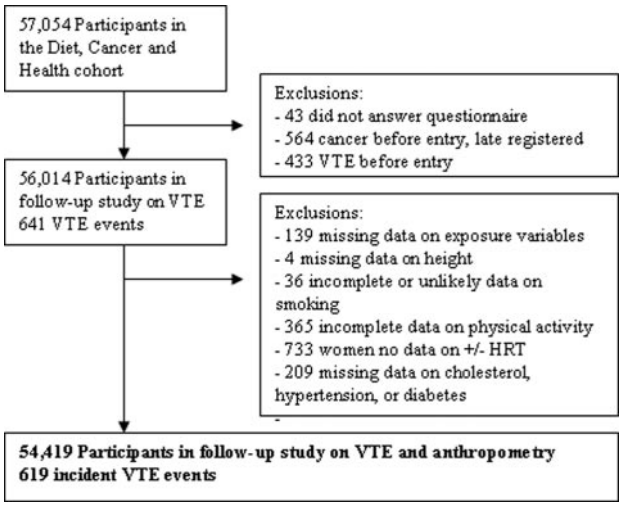

Figure. Inclusions and exclusions before analysis of the association between anthropometric variables and VTE. HRT indicates hormone replacement therapy.

VTE according to differences in waist and hip circumference in normal-weight persons. These analyses were stratified by sex and adjusted for age (with age as the time axis).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Population and Case Ascertainment

In total, 27 178 men and 29 876 women accepted the invitation to participate in the study (Figure). All but 43 individuals answered the questionnaire and participated in the subsequent interview. We excluded 564 participants who were later identified in the Danish Cancer Registry as having a cancer diagnosis before the study invitation or in the weeks between invitation and the baseline examination. We also excluded 433 participants with a diagnosis of VTE before enrollment into the Diet, Cancer, and Health study, which left 56 014 participants for the follow-up study on VTE. Table 1 provides baseline characteristics of the participants.

The median follow-up time was 10.2 years, with an interquartile range from 9.6 to 10.8 years. During follow-up, we identified and verified 617 incident VTE events by review of medical records. Of these, 58% (n=358) were DVT and 42% (n=259) were PE. Confirmed VTE events were characterized as idiopathic in 49% of cases (n=306) and secondary in 49% (n=300). The remaining 2% (n=11) could not be classified owing to sparse information. Of the 56 014 participants, 4084 died during the follow-up period. We found 24 participants with an autopsy-proven PE diagnosis without a prior verified VTE event during follow-up. The autopsy-verified events were not classified. Thus, in total, 641 confirmed VTE events were identified among participants during follow-up. The incidence rate of VTE was 1.15 (95% confidence interval 1.06 to 1.24) per 1000 person-years.

Analyses

The analyses were based on the 54 737 participants for whom we had complete information on all exposure and confounder variables. Table 2 shows the crude and adjusted hazard ratios for the anthropometric variables according to the different types of VTE (total VTE, idiopathic VTE, secondary VTE, and PE). We found statistically significant positive associations between VTE and all measurements of body size, including body weight, BMI, total body fat mass, waist circumference, and hip circumference, among both men and women. The associations were the same according to different types of VTE. Adjustment for potential confounding had no substantial implications. The mutually adjusted analysis of waist circumference and hip circumference showed a statistically significant positive association between VTE and waist circumference in men but not in women. Conversely, the association between VTE and hip circumference showed a statistically significant association in women but not in men. In the analysis of waist circumference adjusted for BMI, we found a statistically significant positive association in men but no association in women (Table 2).

Table 1. Baseline Characteristics of Participants in the Follow-Up Study on VTE and Anthropometry

	Women	Men
No. of participants	29 340	26 674
Age, y	56 (51–64)	56 (51–64)
Weight, kg	67 (53–91)	82 (65–105)
Height, cm	164 (155–174)	177 (166–188)
Body mass index, kg/m^2	24.8 (19.9–33.7)	26.2 (21.5–33)
Total body fat, kg	23 (13–41)	22 (12–36)
Waist circumference, cm	80 (67–103)	95 (81–114)
Hip circumference, cm	101 (89–118)	100 (90–112)
Total cholesterol, mmol/L	6.2 (4.5–8.4)	5.9 (4.3–7.9)
Systolic blood pressure, mm Hg	136 (106–175)	140 (114–177)
Education, basic school, %*		
7 y	31.4	34.8
8–10 y	50.2	41.5
≥11 y	18.5	23.7
Education after basic school, %		
None	19.3	10.0
<3 y	31.7	13.8
3–4 y	37.7	42.0
≥4 y	11.3	34.1
Smoking status, %		
Never smoked	43.7	25.7
Former smoker	23.5	34.6
Current smoker, <15 g/d	15.4	10.7
Current smoker, 15–25 g/d	14.9	17.5
Current smoker, >25 g/d	2.6	11.6
Activity >0.5 h/d, %	41	38
Hormone replacement therapy, %	31	Not applicable

Values are median (5th to 95th percentiles) unless otherwise stated.

*Education based on 3 categories of duration of basic school (starting at age of 6 to 7 years): 7 years, 8–10 years, ≥11 years.

Table 2. Anthropometry and VTE

	Women, HR (95% CI)				Men, HR (95% CI)			
	All VTE (n=259)	Idiopathic VTE (n=109)	Secondary VT (n=133)	PE (n=126)	All VTE (n=360)	Idiopathic VTE (n=182)	Secondary VTE (n=160)	PE (n=124)
Weight (kg)								
Crude	1.03 (1.02–1.04)	1.03 (1.02–1.05)	1.02 (1.01–1.04)	1.04 (1.03–1.05)	1.02 (1.02–1.03)	1.02 (1.01–1.03)	1.03 (1.02–1.04)	1.03 (1.02–1.04)
Adjustment 1	1.03 (1.02–1.04)	1.03 (1.02–1.05)	1.03 (1.01–1.04)	1.04 (1.02–1.05)	1.02 (1.01–1.03)	1.02 (1.00–1.03)	1.02 (1.01–1.04)	1.03 (1.02–1.05)
Adjustment 2	1.03 (1.02–1.04)	1.03 (1.02–1.05)	1.03 (1.01–1.04)	1.04 (1.03–1.05)	1.02 (1.01–1.03)	1.02 (1.01–1.03)	1.03 (1.01–1.04)	1.04 (1.02–1.05)
BMI (kg/m^2)								
Crude	1.07 (1.04–1.09)	1.08 (1.04–1.12)	1.06 (1.02–1.09)	1.09 (1.06–1.13)	1.06 (1.03–1.09)	1.05 (1.02–1.09)	1.07 (1.03–1.11)	1.10 (1.05–1.14)
Adjustment 1	1.08 (1.06–1.11)	1.09 (1.05–1.13)	1.07 (1.04–1.11)	1.10 (1.07–1.14)	1.07 (1.04–1.10)	1.06 (1.03–1.10)	1.08 (1.04–1.12)	1.11 (1.06–1.15)
Adjustment 2	1.08 (1.05–1.11)	1.09 (1.05–1.13)	1.07 (1.04–1.11)	1.11 (1.07–1.14)	1.07 (1.04–1.10)	1.07 (1.03–1.11)	1.08 (1.04–1.13)	1.12 (1.07–1.17)
Body fat (kg)								
Crude	1.04 (1.02–1.05)	1.04 (1.02–1.06)	1.03 (1.02–1.05)	1.05 (1.03–1.07)	1.03 (1.02–1.05)	1.03 (1.02–1.05)	1.04 (1.02–1.06)	1.05 (1.03–1.07)
Adjustment 1	1.04 (1.03–1.05)	1.04 (1.02–1.06)	1.04 (1.02–1.05)	1.05 (1.03–1.07)	1.03 (1.02–1.05)	1.03 (1.01–1.05)	1.04 (1.02–1.06)	1.05 (1.03–1.08)
Adjustment 2	1.04 (1.02–1.05)	1.04 (1.02–1.06)	1.04 (1.02–1.05)	1.05 (1.03–1.07)	1.03 (1.02–1.05)	1.04 (1.02–1.05)	1.04 (1.02–1.06)	1.06 (1.04–1.08)
Waist (5 cm)								
Crude	1.14 (1.09–1.20)	1.14 (1.06–1.23)	1.12 (1.05–1.20)	1.21 (1.13–1.29)	1.15 (1.10–1.21)	1.15 (1.07–1.22)	1.18 (1.10–1.26)	1.21 (1.12–1.31)
Adjustment 1	1.14 (1.09–1.20)	1.14 (1.06–1.23)	1.13 (1.06–1.21)	1.21 (1.13–1.29)	1.14 (1.09–1.20)	1.15 (1.07–1.23)	1.17 (1.09–1.26)	1.22 (1.12–1.32)
Adjustment 2	1.14 (1.08–1.20)	1.13 (1.04–1.22)	1.14 (1.06–1.22)	1.21 (1.13–1.30)	1.15 (1.10–1.21)	1.16 (1.08–1.24)	1.18 (1.09–1.27)	1.25 (1.15–1.36)
Adjustment 2+hip	1.02 (0.94–1.10)	0.95 (0.84–1.07)	1.02 (0.91–1.14)	1.09 (0.97–1.22)	1.18 (1.08–1.28)	1.23 (1.09–1.38)	1.15 (1.00–1.31)	1.25 (1.08–1.44)
Adjustment 2+BMI	0.97 (0.88–1.08)	0.87 (0.73–1.02)	1.00 (0.86–1.17)	1.03 (0.89–1.19)	1.17 (1.03–1.32)	1.22 (1.03–1.45)	1.17 (0.98–1.40)	1.18 (0.96–1.45)
Hip (5 cm)								
Crude	1.20 (1.14–1.27)	1.25 (1.15–1.34)	1.18 (1.09–1.28)	1.26 (1.18–1.35)	1.19 (1.11–1.28)	1.16 (1.05–1.28)	1.25 (1.13–1.38)	1.28 (1.14–1.43)
Adjustment 1	1.21 (1.15–1.28)	1.25 (1.16–1.36)	1.20 (1.11–1.30)	1.26 (1.17–1.36)	1.17 (1.08–1.26)	1.14 (1.02–1.27)	1.23 (1.11–1.38)	1.28 (1.14–1.44)
Adjustment 2	1.21 (1.14–1.28)	1.25 (1.15–1.36)	1.20 (1.11–1.31)	1.27 (1.18–1.37)	1.17 (1.09–1.27)	1.15 (1.03–1.28)	1.23 (1.11–1.38)	1.30 (1.15–1.48)
Adjustment 2+waist	1.19 (1.08–1.30)	1.30 (1.14–1.50)	1.18 (1.04–1.35)	1.18 (1.04–1.34)	0.96 (0.85–1.10)	0.90 (0.75–1.07)	1.05 (0.87–1.27)	1.00 (0.81–1.24)

Hazard ratios (HRs) with 95% confidence intervals (CIs). Age as time axis (crude: age adjusted).

Adjustment 1: physical activity, smoking categories, height, and use of hormone replacement therapy (women only). Adjustment 2: Adjustment 1 plus hypertension, diabetes mellitus, and cholesterol.

Table 3 shows the hazard ratios for VTE according to quartiles of the anthropometric variables. The analyses show a statistically significantly higher hazard ratio for VTE in the highest quartile than the lowest quartile for all anthropometric variables in both sexes. The analyses showed monotonic dose-response relationships. Table 4 shows the hazard ratios for VTE according to categories of hip circumference and waist circumferences stratified for quartiles of BMI. It appeared that the risk of VTE was higher in normal-weight persons with high hip circumference than in persons with low hip circumference. Sex-specific hazard ratios of VTE according to 1 SD showed no preferable anthropometric measure of obesity for assessing the risk of VTE (online-only Data Supplement Table I).

Discussion

In this large prospective study, we found statistically significant positive associations between VTE and all measurements of obesity for both men and women. The hypothesis tested was that central obesity might be a better predictor of VTE than peripheral obesity measured as hip circumference (as has been shown in CHD), but the results showed that both peripheral and central obesity were strong risk factors for VTE. However, the anthropometric variables were only surrogate measures for the actual regional fat deposits and not independent measurements. Therefore, these results do not exclude differences between different types of fat tissues, but they do indicate a distinction between VTE and CHD, because no association has been found between hip circumference and CHD.

Strengths and Limitations of the Present Study

This prospective study included 641 incident VTE events and was one of the largest prospective studies on VTE. Data on anthropometry were collected prospectively by trained laboratorial technicians. All VTE events were validated by review of medical records by a physician familiar with VTE (MTS), and we included only objectively verified incident VTE events. The hospital system in Denmark is financed by taxation, and almost every Danish inhabitant with VTE symptoms is admitted to and examined in a hospital. The medical record included information on visits from both outpatient and inpatient clinics. Obviously, there will be VTE events among participants that we missed. For example, because of a very low frequency of autopsy in Denmark (6% of all deaths in 2001), an unknown number of the participants died of PE. However, obesity is unlikely to influence the chance of an autopsy being performed, and thus, selection bias because of autopsy performance is not a likely explanation of the present findings.

The data on anthropometry were not available during review of medical records, and therefore, information bias is

Table 3. Hazard Ratio (95% Confidence Interval) of VTE by Quartiles of Anthropometric Variables

	Women	Level, Women	Men	Level, Men
Weight (kg)				
Lowest	1	<64	1	<77
Lower-middle	1.24 (0.88–1.76)	64–72	1.15 (0.85–1.55)	77–85
Upper-middle	1.91 (1.34–2.74)	72–80	1.58 (1.16–2.15)	85–94
Highest	2.27 (1.57–3.28)	>80	1.88 (1.35–2.62)	>94
BMI (kg/m^2)				
Lowest	1	<23.7	1	<24.4
Lower-middle	1.45 (1.03–2.05)	23.7–26.3	0.98 (0.73–1.31)	24.4–26.8
Upper-middle	1.81 (1.27–2.56)	26.3–29.9	1.32 (0.98–1.79)	26.8–29.4
Highest	2.82 (1.96–4.04)	>29.9	1.72 (1.27–2.33)	>29.4
Waist (cm)				
Lowest	1	<77	1	<91
Lower-middle	1.34 (0.96–1.88)	77–84	1.04 (0.78–1.39)	91–98
Upper-middle	1.58 (1.11–2.25)	84–92	1.36 (1.00–1.83)	98–105
Highest	1.92 (1.35–2.73)	>92	2.00 (1.47–2.72)	>105
Hip (cm)				
Lowest	1	<98	1	<97
Lower-middle	1.45 (1.02–2.04)	98–103	0.98 (0.73–1.31)	97–102
Upper-middle	1.67 (1.18–2.37)	103–110	1.34 (1.00–1.80)	102–105
Highest	2.54 (1.78–3.64)	>110	1.43 (1.05–1.93)	>105
Fat weight (kg)				
Lowest	1	<20	1	<19
Lower-middle	1.15 (0.81–1.63)	20–26	1.18 (0.88–1.59)	19–23
Upper-middle	1.62 (1.14–2.30)	26–33	1.34 (0.99–1.81)	23–29
Highest	2.33 (1.62–3.34)	>33	1.89 (1.39–2.57)	>29

Adjusted for age, physical activity, smoking categories, height, cholesterol, hypertension, diabetes mellitus, and use of hormone replacement therapy (women only).

not a likely explanation of our findings, because the reviewer was blinded with regard to exposure. However, it can be a challenge to diagnose DVT in persons with obese legs, in whom it may be impossible to make a conclusive ultrasound examination; therefore, obesity might cause a lack of verification of DVT diagnosis. This would tend to underestimate the effects of obesity. Nevertheless, the association between anthropometric measurements and PE was similar to the association for VTE. Using Cox regression, we assumed that those people who died had the same risk of VTE as those who did not die. If those who died had a higher risk of VTE, our assumption would tend to underestimate the effect of obesity. Detailed information on a range of potential confounding factors was available in the present study. Adjustment for these factors in the statistical analyses had only a minor impact on the risk estimates, which indicates that residual confounding was not a likely explanation of the observed associations.

Comparison With Other Studies

Few studies have evaluated the association between VTE and measures of obesity other than BMI. The findings of the present study are in accordance with the results from large-scaled epidemiological studies of BMI and VTE.[1–3,6] In a recent large case-control study, Pomp et al[5] also found a positive association between VTE and body weight in addition to BMI. They found a monotonic dose-response relationship, as we identified in the present study.[5]

We also found a statistically significant positive association between waist circumference and VTE in both men and women. This finding is in agreement with a small study of men born in 1913, in which Hansson et al[4] found a higher risk of VTE in men with waist circumference >100 cm than in men with waist circumference <100 cm. Steffen et al[9] assessed the association between VTE and the metabolic syndrome. They found a positive association between VTE and central obesity in both sexes. In addition, they found that the metabolic syndrome and its other features did not appear important in the cause of VTE. This finding is in agreement with a study by Ray et al,[17] who analyzed the association between VTE and the different features of the metabolic syndrome and found that central obesity was associated with VTE, whereas no association existed between VTE and diabetes mellitus, hypertension, or dyslipidemia. Recently, Borch et al[18] confirmed the findings that obesity is the pivotal risk factor for VTE among the features of the metabolic syndrome. Other studies evaluated the associations between VTE and dia-

Table 4. Hazard Ratio (95% Confidence Interval) of VTE According to Categories of Hip Circumference and Waist Circumference Stratified for Quartiles of BMI

	Lowest BMI (≤24 kg/m^2)	Lower-Middle BMI (24.1–26.7 kg/m^2)	Upper-Middle BMI (26.71–29.5 kg/m^2)	Highest BMI (>29.5 kg/m^2)
Hip circumference ≤97 cm				
No. of cases	113	37	14	0
HR (95% CI)	1.27 (0.88–1.85)	1 (Reference)	1.52 (0.82–2.82)	…
Hip circumference 97.5–102 cm				
No. of cases	37	73	51	8
HR (95% CI)	1.18 (0.75–1.87)	1.40 (0.94–2.08)	1.70 (1.11–2.59)	1.28 (0.60–2.75)
Hip circumference 102.5–108 cm				
No. of cases	9	46	66	36
HR (95% CI)	1.51 (0.72–3.14)	1.73 (1.12–2.67)	1.65 (1.11–2.47)	1.66 (1.05–2.62)
Hip circumference ≥108.5 cm				
No. of cases	1	3	28	115
HR (95% CI)	…	…	2.22 (1.35–3.65)	2.89 (1.99–4.20)
Waist circumference ≤80 cm				
No. of cases	77	27	13	1
HR (95% CI)	0.84 (0.54–1.30)	1 (Reference)	2.47 (1.28–4.79)	…
Waist circumference 80–88 cm				
No. of cases	49	37	23	8
HR (95% CI)	1.26 (0.77–2.06)	0.99 (0.60–1.64)	1.22 (0.70–2.12)	1.78 (0.81–3.93)
Waist circumference 88–102 cm				
No. of cases	33	89	88	38
HR (95% CI)	1.51 (0.87–2.60)	1.30 (0.81–2.08)	1.43 (0.91–2.27)	1.35 (0.82–2.21)
Waist circumference ≥102 cm				
No. of cases	1	6	35	112
HR (95% CI)	…	2.34 (0.94–5.81)	1.95 (1.13–3.35)	2.42 (1.54–3.78)

HR indicates hazard ratio; CI, confidence interval.

Age as time axis, with stratification for sex. There were 637 cases with information on BMI, waist circumference, and hip circumference.

betes mellitus, hypertension, and dyslipidemia. Goldhaber et al[3] investigated the risk of PE in women and found no association with diabetes or dyslipidemia; a positive association with hypertension was identified. Tsai et al[6] found no association with hypertension or dyslipidemia, and after adjustment for BMI, there was no association with diabetes. Glynn et al[2] analyzed VTE in men and found no association with hypertension, dyslipidemia, or diabetes. No data on waist circumference were included in these studies, but the results indicate that the effect of the metabolic syndrome on VTE risk may be entirely due to obesity. We performed adjustment for hypertension, diabetes mellitus, and cholesterol, but the addition of these variables to the model did not change our estimates, which indicates that the mechanism of obesity is not mediated by these covariates.

To the best of our knowledge, no studies have investigated the association between peripheral obesity, measured as hip circumference, and VTE. We found a statistically significant positive association between VTE and hip circumference both in men and in women; however, this effect of peripheral obesity could be caused indirectly by central obesity, because when people become very obese, fat accumulates all over the body, and thus, persons with a large hip circumference also have a large waist circumference.[19] On the other hand, no association has been found between hip circumference and CHD.[7,8] To eliminate the effect of waist circumference in the analysis of hip circumference, as well as the effect of hip circumference in the analysis of waist circumference, we performed mutually adjusted analyses. When hip circumference was adjusted for waist circumference, the association between hip circumference and VTE was eliminated for men but was still significant for women. In contrast, when waist circumference was adjusted for hip circumference, the association between waist circumference and VTE was eliminated for women but was still significant for men. These differences may be explained by the sex-specific distribution of fat. In general, men accumulate fat around the abdomen, and women accumulate fat on the hips. Therefore, in women, hip circumference is more informative than waist circumference when predicting the risk of VTE because the variation in hip circumference is highest, whereas waist circumference is more informative than hip circumference in men because the variation in waist circumference is highest. However, the correlation coeffi-

cients between waist and hip circumference were 0.80 in men and 0.75 in women, and thus, the results might reflect collinearity and not biology. In secondary analysis of combined measurements of hip circumference and BMI, we confirmed that a high hip circumference was associated with a higher risk of VTE even in normal-weight persons, in contrast to studies of CHD.[7,8] Waist circumference adjusted for BMI has been shown to be an approximation of intra-abdominal fat content.[20] We therefore analyzed the hazard ratio of waist circumference adjusted for BMI as a model to assess the effect of intra-abdominal fat. We found a statistically significant positive association in men but no association in women, which indicates that intra-abdominal fat is only a risk factor in males. However, this model includes a redistribution of masses, because when we investigate the hazard ratio of higher waist circumferences with equal BMI, other body compartments must concomitantly be smaller. The hazard ratio we found using this model may indicate the effect of more intra-abdominal fat, diminished hip circumference, less muscle mass, or a combination of these factors.

We also found statistically significant positive associations between idiopathic VTE and all measurements of obesity. These data underscore the fact that the effect of obesity on VTE risk was not mediated solely through diseases caused by obesity.

The mechanisms responsible for the association between VTE and obesity are unknown. The association between arterial thrombosis and obesity is explained in part by the strong association between central obesity and hypertension, type 2 diabetes mellitus, and dyslipidemia, all of which are major risk factors for atherosclerosis and arterial thrombosis[2,7,8]; however, these factors are not established risk factors for VTE.[2–4,6] Therefore, the effect of obesity on VTE risk may be mediated by other mechanisms. The present data suggest that fat mass, independent of its distribution in the body, is positively associated with VTE. This is biologically plausible, because adipose tissue is more than an energy-storage organ; it is also metabolically active, secreting several biologically active substances. A number of these substances are associated with procoagulant activity or inhibition of fibrinolysis, ie, interleukin-6, tumor necrosis factor-α, tissue factor, and plasminogen activator inhibitor-1.[19,21–23] Enlarged fat cells produce a higher amount of these substances than normal-sized fat cells.[22,24,25] It is therefore plausible that both peripheral and central obesity are risk factors for VTE due to fat-cell biosynthesis; however, the present study was not designed to evaluate the validity of this proposed mechanism. Estrogen produced by fat cells might also be a potential mediator of VTE risk, because the plasma estrogen level is positively associated with obesity.[26] In addition, obesity might be associated with venous stasis, which promotes venous thrombosis; however, this association is hypothetical and not well established. Further studies are warranted on this topic.

In conclusion, we found statistically significant positive associations between weight, BMI, waist circumference, hip circumference, and total fat mass and VTE in both sexes. Further studies are needed to explain the mechanism underlying the associations.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The distribution of body fat predicts the risk of coronary heart disease, and central obesity consistently has been shown to be a risk factor for coronary heart disease, whereas peripheral obesity (measured as high hip circumference) appears to protect against coronary heart disease. The importance of fat distribution with regard to the risk of venous thromboembolism (VTE), ie, deep venous thrombosis and pulmonary embolism, has not been evaluated. In a 10-year follow-up study of 56 014 middle-aged men and women, which included 641 verified incident events of VTE, we evaluated the risk of VTE according to different measurements of fat distribution in the body. Our results show that all measurements of obesity are positively associated with VTE in both sexes. We also showed that a higher hip circumference in normal-weight persons was associated with a higher risk for VTE, which is in contrast to studies on coronary heart disease. We found statistically significant positive associations between idiopathic (unprovoked) VTE and all measurements of obesity. The associations between VTE and the anthropometric measurements persisted after adjustment for hypertension, diabetes mellitus, or hypercholesterolemia, which shows that the effect of obesity was not mediated solely by these factors.

Supplementary table 1. Hazard ratios [95% Confidence interval] for venous thromboembolism (VTE) according to one standard deviation of the anthropometric measure. Age was used as time axis (crude estimate age adjusted). Adjusted for physical activity, smoking categories, height, cholesterol, hypertension, diabetes and use of hormone replacement therapy (women only).

	Women				***Men***			
	All VTE (n=259)	**Idiopathic VTE** (n=109)	**Secondary VTE** (n=133)	**PE** (n=126)	**All VTE** (n=360)	**Idiopathic VTE** (n=182)	**Secondary VTE** (n=160)	**PE** (124)
	HR	HR	HR	HR	HR	HR	HR	HR
Weight, 1sd								
Crude	1.40 [1.27-1.55]	1.47 [1.27-1.70]	1.35 [1.17-1.55]	1.58 [1.39-1.80]	1.34 [1.22-1.46]	1.30 [1.14-1.48]	1.39 [1.21-1.59]	1.46 [1.25-1.69]
Adjusted	1.41 [1.27-1.57]	1.46 [1.24-1.73]	1.37 [1.17-1.60]	1.57 [1.36-1.81]	1.31 [1.18-1.46]	1.29 [1.11-1.50]	1.37 [1.17-1.60]	1.55 [1.31-1.85]
BMI, 1sd								
Crude	1.33 [1.20-1.47]	1.37 [1.18-1.60]	1.29 [1.12-1.49]	1.48 [1.29-1.69]	1.23 [1.12-1.35]	1.22 [1.07-1.39]	1.28 [1.11-1.47]	1.40 [1.20-1.62]
Adjusted	1.40 [1.26-1.55]	1.45 [1.24-1.71]	1.36 [1.17-1.58]	1.56 [1.36-1.80]	1.28 [1.16-1.41]	1.27 [1.11-1.46]	1.34 [1.16-1.55]	1.50 [1.28-1.75]
Body fat, 1sd								
Crude	1.36 [1.23-1.51]	1.39 [1.19-1.62]	1.33 [1.16-1.54]	1.54 [1.35-1.76]	1.30 [1.18-1.42]	1.29 [1.13-1.47]	1.34 [1.17-1.54]	1.48 [1.28-1.71]
Adjusted	1.38 [1.24-1.53]	1.40 [1.19-1.65]	1.37 [1.18-1.59]	1.55 [1.34-1.78]	1.29 [1.17-1.43]	1.30 [1.13-1.49]	1.34 [1.16-1.55]	1.55 [1.32-1.81]
Waist, 1sd								
Crude	1.33 [1.20-1.48]	1.32 [1.11-1.56]	1.31 [1.12-1.52]	1.52 [1.32-1.76]	1.33 [1.21-1.47]	1.33 [1.16-1.51]	1.39 [1.20-1.59]	1.48 [1.26-1.72]
Adjusted	1.33 [1.19-1.50]	1.31 [1.09-1.56]	1.33 [1.13-1.56]	1.54 [1.31-1.79]	1.33 [1.20-1.47]	1.34 [1.16-1.54]	1.39 [1.19-1.61]	1.55 [1.31-1.83]
Hip, 1sd								
Crude	1.37 [1.25-1.51]	1.45 [1.26-1.66]	1.35 [1.18-1.55]	1.51 [1.33-1.70]	1.27 [1.15-1.39]	1.22 [1.07-1.40]	1.35 [1.17-1.54]	1.38 [1.19-1.61]
Adjusted	1.40 [1.26-1.54]	1.48 [1.27-1.72]	1.39 [1.20-1.60]	1.53 [1.34-1.75]	1.24 [1.12-1.37]	1.20 [1.04-1.39]	1.33 [1.15-1.55]	1.43 [1.21-1.69]

Genetic susceptibility, Smoking, Obesity and risk of Venous Thromboembolism

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Abstract

Background: The Factor V Leiden and prothrombin mutations are linked to an increase in the incidence rate of venous thromboembolism (VTE), but their effects are highly variable. We investigated whether the effects of smoking and obesity might explain this variability.

Methods: We analysed verified VTE events that occurred in the 10-year follow-up of 57,054 Danes (50 to 64 years old) enrolled in the prospective Danish Diet, Cancer, and Health study (1993 to 1997). Baseline information included detailed data on lifestyle, body weight, height, and blood analysis. In this case-cohort study, we computed incidence rates and Cox proportional hazards ratios for VTE in individuals with and without the genetic mutations, categorised by weight and tobacco consumption.

Results: Compared to non-smoking individuals, heavy smoking increased the incidence rate with 128 and 59 additional VTE events per 100,000 person years in individuals with and without the Factor V Leiden mutation, respectively. Individuals with the prothrombin mutation that smoked heavily also appeared to have an increased risk for VTE. Compared to normal-weight individuals, obese individuals with and without Factor V Leiden had 222 and 103 additional VTE events per 100,000 person years, respectively; obese individuals with and without the prothrombin mutation had 705 and 107 additional VTE events per 100,000 person years, respectively.

Conclusion: The Factor V Leiden and prothrombin mutations conferred increased susceptibility to the unfavourable effects of smoking and obesity on the risk for VTE. Thus, individuals with genetic risk factors for VTE might benefit from maintaining a healthy lifestyle.

Key Words: Venous thromboembolism (VTE), Factor V Leiden, prothrombin mutation, smoking, obesity, follow-up study

Introduction

Venous thromboembolism (VTE), i.e. deep venous thromboses (DVT) and pulmonary emboli (PE), is a common disease with substantial clinical implications. Patients with VTE have a high risk of death, recurrence of VTE, post-thrombotic syndrome and chronic thromboembolic pulmonary hypertension [1-4].

Several acquired and genetic risk factors for VTE have been identified [5]. Well established genetic risk factors include the Factor V Leiden (G1691A) and the prothrombin mutation (G20210A) [6]. Both mutations are common in Caucasians [7-9]. However, the incidence rate of VTE in individuals with Factor V Leiden or the prothrombin mutation is highly variable; many individuals with these mutations never experience VTE, even under predisposing conditions of surgery or pregnancy [10]. Therefore, it is important to understand whether the effects of the Factor V Leiden (G1691A) and prothrombin (G20210A) mutations are dependent on the presence of other risk factors. It is well-known that smoking and obesity are positively associated with VTE [11]. Biological mechanisms underlying these associations are not fully understood, but it has been shown that smoking is associated with a higher level of fibrinogen [12-15] and obesity is associated with a higher level of factor II and factor VIII [16-21]. Thus, procoagulant status may be the underlying mechanism for the associations between VTE and these lifestyle factors. A combination of these factors with the Factor V Leiden or the prothrombin mutation may cause an imbalance the coagulation system to levels where the coagulation runs out of balance. One previous prospective study evaluated the effect of Factor V Leiden in combination with obesity and smoking on the incidence of VTE. The study included only 216 non- validated VTE events in 23 years of follow-up [22]. One previous case control study evaluated the effects of smoking in combination with Factor V Leiden and the prothrombin mutation; another previous case control study evaluated the effect of obesity in combination with Factor V Leiden and the prothrombin mutation [23, 24]. These studies

reported that combinations of lifestyle and polymorphisms conferred higher risks for VTE than expected for the sum of the separate effects.

The aims of our study were to explore the potential interactions between genetics (Factor V Leiden and the prothrombin mutation) and lifestyle factors (obesity and smoking) and their associations with the risk of VTE. The study included 641 objectively verified VTE events and prospectively collected detailed information on lifestyle factors.

Material and methods

The study population

The Danish prospective Diet, Cancer, and Health study was conducted from December 1993 to May 1997. A total of 80,996 men and 79,729 women, aged 50 to 64 years old, were invited to participate. The study has been described in detail elsewhere [25, 26]. In short, eligible cohort members were born in Denmark, living in the urban areas of Copenhagen and Aarhus, and, at the time of invitation, were not registered with a previous diagnosis of cancer in the Danish Cancer Registry. Participants were excluded from the study if they did not participate in the baseline examination or were later identified in the Danish Cancer Registry with a previous cancer diagnosis, either before the invitation, or in the weeks between the invitation and the baseline examination. In addition, participants were excluded that had experienced a VTE before enrolment into the Diet, Cancer, and Health study. After the exclusions, 56,014 participants were included in this VTE cohort study (26,674 men and 29,340 women). Both the Diet, Cancer and Health study and the present sub-study were approved by the regional ethics committees in Copenhagen and Aarhus, and by The Danish Data Protection Agency.

Data on smoking dose, weight status, and genotypes

Baseline information was collected at two study clinics in Aarhus and Copenhagen at the time of enrolment into the Diet, Cancer, and Health study. Information on lifestyle characteristics (tobacco consumption, medications, physical activity) was obtained from standardised questionnaires that were optically scanned into a computer. In subsequent interviews performed by trained lab technicians, information was amended as necessary. Data on body height and weight were measured by laboratory technicians. Blood samples were drawn from each participant that included a citrate sample for preparing buffy coats. The samples were stored in liquid nitrogen vapour (max -150^{o}C). DNA was extracted from frozen lymphocytes as described by Miller et al [27]. Generally, 100µg DNA was obtained from 10^{7} lymphocytes. Genotyping for the presence of the Factor V Leiden (G1691A) and the prothrombin mutation (G20210A) was performed with real-time PCR analysis of samples from a subcohort of 1,841 participants and from all participants that developed VTE during follow-up. Controls were included in each run, and yielded 100% identical genotypes. In 2000 – 2002, follow-up questionnaires were mailed to all surviving participants. Questions regarded diet and lifestyle changes.

Outcome

The outcome was a verified first-time VTE event. Objectively verified incident VTE events were identified among the participants in the Diet, Cancer, and Health study; these have been described in detail in a prior study [28]. In short, VTE events were identified by linking the civil registration numbers of the study participants in the Diet, Cancer, and Health cohort with the Danish National Patient Registry and the Danish National Death Registry. Medical records (including reports from visits to emergency departments and out-patient clinics) were retrieved and reviewed from all participants that had a first time discharge diagnosis of VTE during the follow-up period. The

review of the medical records was conducted by a physician familiar with VTE (MTS). Data was collected from the time of enrolment into the Diet, Cancer, and Health study (1993-1997) until the 30th of June, 2006. In addition, death certificates from participants that died during follow-up were reviewed. Thus, the present study included only objectively verified VTE events and only participants with autopsy-verified VTE were identified as VTE deaths.

Statistical methods

We used a case-cohort design that included all cases of VTE in a cohort of 56,014 participants and a cohort sample (subcohort) of 1,841 participants (including 23 VTE cases), randomly selected from the whole cohort. A Cox proportional hazards model was used for the statistical analyses. Incidence rates were computed as if the full cohort were included, then modified with a weighting scheme, as described by Kalbfleisch and Lawless, with a robust variance estimate [29]. In the case-cohort design, subjects were assigned a weight of one for VTE cases and N/n for non-VTE cases in the subcohort, where N was the number of non-VTE cases in the cohort and n was the number of non-VTE cases in the subcohort.

Age was used for the time axis in the Cox analyses for the efficient prevention of confounding by age. Entry time was defined as the subject's age at recruitment, and exit time was defined as the age at VTE or at censoring due to death, emigration, or 30th of June 2006, whichever came first. The exposure variables were the genotype according to the presence of the Factor V Leiden or the prothrombin mutation. Both genotypes were classified as wild type, heterozygous, or homozygous profiles. The separate and the combined effect of the genotypes were computed. The genotypes were further cross-tabulated according to categories of weight and tobacco consumption. We used the World Health Organisation BMI cut off values for the categories of healthy-weight (<25 kg/m^2), overweight (25.0 to 29.9 kg/m^2), and obesity (≥ 30 kg/m^2). For tobacco consumption,

participants were categorised into non-smokers, moderate current smokers (1 to 24.9 g/d), and heavy current smokers (≥25 g/d), based on a prior study that showed a substantially higher risk of VTE in heavy smokers compared to moderate smokers [11]. In a secondary analysis, participants were categorised into non-smokers and current smokers. All analyses were stratified by sex. Adjustments were performed for body height, use of hormone replacement therapy (HRT; women only), and either tobacco consumption (in the body weight analyses) or weight status (in the smoking analyses). The reference group was defined as the group of individuals that were not exposed for each combination of genotype and lifestyle; e.g., for the combination of smoking and Factor V Leiden, the reference group comprised non-smoking participants without the Factor V Leiden mutation. Participants were excluded from the analysis when information was incomplete on one or more confounder or exposure variables.

The incidence rates of VTE were computed for each genotype according to categories of tobacco consumption and for each genotype according to weight status. The crude effects of smoking and obesity were calculated as the rate differences; i.e., the incidence rate of VTE in exposed participants minus the incidence rate of VTE in unexposed participants. Interactions were evaluated on an additive scale [30]. Thus, we evaluated if the rate difference according to smoking or obesity differed in individuals with and without the Factor V Leiden mutation and the prothrombin mutation. Model adequacy was assessed graphically with a log rank test based on Schoenfeld residuals. We used Stata version 9.2 (Stata Corporation, College Station, Texas, US) for the statistical analyses.

Results

Study population, case ascertainment, and genotypes

Figure 1 shows the inclusion and exclusion of study participants, and the subcohort sampling. Table 1 shows the baseline characteristics of the participants in the subcohort. Buffy coats were available for 614 of the 641 patients with VTE and for 1803 of the 1841 subcohort members. In the subcohort, 139 (7.7 %) were heterozygous for Factor V Leiden and 25 (1.4 %) were heterozygous for the prothrombin mutation; only 3 individuals had a combination of both mutations.

Analyses

The Factor V Leiden mutation (heterozygous) was associated with an adjusted hazard ratio (HR) of 2.60 [95% CI: 1.94-3.50]. The prothrombin mutation (heterozygous) was associated with an adjusted HR of 2.79 [95% CI: 1.46-5.33] (data not shown). The combined effects of the Factor V Leiden and the prothrombin mutation taking the other into account are shown in Table 2. The crude incidence rate of VTE for the combination of the two mutations was 411 VTE events per 100,000 person years. Seven patients with VTE, but no subcohort members, were homozygous for Factor V Leiden, and no patients with VTE or subcohort members were homozygous for the prothrombin mutation; therefore, we were not able to estimate the HRs or the incidence rates for homozygous individuals.

Table 3 shows the combined effects of the genetic risk factors and smoking. For the combination of heavy smoking and Factor V Leiden, the adjusted HR was 4.46 [95% CI: 1.83-10.88] compared with non-smokers without the Factor V Leiden mutation. The incidence rate was 405 [95% CI: 165-975] VTE events per 100,000 person years. The effect of heavy smoking (rate difference) compared to no smoking was 59 additional VTE events per 100,000 person years in individuals without the Factor V Leiden mutation and 128 additional VTE events per 100,000

person years in individuals with the mutation. For the combination of heavy smoking and the prothrombin mutation, the adjusted HR was 76.8 [95% CI: 29.2-201.7] compared with no smoking without the prothrombin mutation. The incidence rate was 17,741 [95% CI: 9551-30286] VTE events per 100,000 person years. The effect of heavy smoking (rate difference) compared to no smoking was 58 additional VTE events per 100,000 person years in individuals without the prothrombin mutation; the effect of heavy smoking was apparently much higher among participants that carried the mutation.

Table 4 shows the combined effects of the genetic risk factors and obesity. For the combination of obesity and the Factor V Leiden mutation, the adjusted HR was 5.27 [95% CI: 2.74-10.14] compared with healthy-weight individuals without the mutation. The crude incidence rate was 415 [95% CI: 224-757] VTE events per 100,000 person years. The effect of obesity (rate difference) compared to healthy-weight was 103 additional VTE events per 100,000 person years in individuals without the Factor V Leiden mutation and 222 additional VTE events per 100,000 person years in individuals with the mutation. For the combination of obesity and the prothrombin mutation, the adjusted HR was 6.89 [95% CI: 1.18-40.22] compared to healthy-weight individuals without the mutation. The incidence rate was 848 [95% CI: 106-4353] VTE events per 100,000 person years. The effect of obesity (rate difference) compared to healthy-weight was 107 additional VTE events per 100,000 person years in individuals without the prothrombin mutation and 705 additional VTE events per 100,000 person years in individuals with the prothrombin mutation.

Discussion

As expected, we found that the Factor V Leiden and the prothrombin mutation were positively associated with risk of VTE. Furthermore, we found that the effects of obesity and heavy smoking were more potent in individuals with the Factor V Leiden mutation and the prothrombin mutation

than in individuals without these genotypes. Our results indicated an additive interaction between the lifestyle factors and the genetic risk factors analysed in this study.

Strengths and limitations of the study

This study was one of the largest prospective studies on VTE. The complete follow-up of participants was possible with the nationwide Danish National Patient Registry and minimised the risk of selection bias. All VTE events were validated by a review of the medical records by a physician familiar with VTE (MTS); thus, the study included only objectively verified, incident VTE events. All data were collected prospectively. Data on weight and height were measured by trained laboratorial technicians. Data on smoking were self-reported in the questionnaire and, during subsequent interviews with the participants, the information was amended as necessary. Although under-reporting of the smoking dose might have occurred, it probably would have been independent of later diagnoses of VTE; therefore, under-reporting would have resulted in non-differential misclassifications. In addition, the smoking dose might have changed during follow-up; however, data from the five-year follow-up questionnaires showed that 80% of smokers maintained the same doses of tobacco. Changes were generally towards a lower dose of tobacco; this would tend to cause an underestimation of the association between smoking and VTE. Also, the BMI might have changed during follow-up. If the participants that were obese at baseline experienced larger weight gains than the healthy-weight participants, the association between baseline obesity and the risk of VTE would have been overestimated. In this study, we had detailed information on potential confounding factors. We found that adjustments for these factors did not essentially change the results; therefore, residual confounding was not a likely explanation for our findings. The main limitation of our study was the low statistical precision in some strata; in particular, a small number of participants comprised the strata for the prothrombin mutation.

Comparison with other studies

The prevalence of the Factor V Leiden mutation (7.7%) and of the prothrombin mutation (1.4%) in our study cohort was equivalent to those from other reports on the Danish population [22, 31]. The magnitude of the relative risk associated with the genetic risk factors differed between studies because the relative estimate is dependent on the risk in the reference group. Therefore, a lower relative risk will appear in a population of middle-aged participants than in a younger population because the baseline risk is higher in the middle-aged population. However, despite the lower relative risk, the absolute number of VTE events "caused" by the mutation in an older cohort may be much larger than in a younger population. We found HRs of approximately 2.5 for both Factor V Leiden and the prothrombin mutation. This was in accordance with findings from other studies [22, 32, 33]. However, Weischer et al. recently found no association between the prothrombin mutation and VTE [31]. Their outcome was based on registry data (the Danish National Patient Registry and the Danish National Death Registry). It is possible that a misclassification of cases may have biased their results towards unity. In a prior study, we found that the positive predictive value of a VTE diagnosis in the Danish National Patient Registry was only 58.5% [28].

We found additive interactions between the genetic risk factors and the lifestyle factors analysed in this study. The effects of obesity and smoking were higher in individuals with than in those without the genetic mutations considered here. Few studies have evaluated the combined effects of lifestyle factors with Factor V Leiden or the prothrombin mutation [22-24]. Juul et al. included 216 unvalidated VTE events divided into 18 strata of weight status, smoking status, age groups, and status of Factor V Leiden. They found that the simultaneous presence of smoking, obesity, and old age resulted in a 10% absolute 10-year risk of VTE in individuals that were heterozygous for Factor V Leiden [22]. Consistent with our findings, Pomp et al. found in a large

case-control study that the joint effects of smoking, Factor V Leiden, and the prothrombin mutation were higher than that expected from the sum of the separate effects. However, in our study, we assessed the HRs according to different doses of tobacco consumption in addition to the category that included all doses of tobacco. In our study, only 23 VTE events occurred in individuals with the prothrombin mutation; of these, 4 were heavy smokers (17%). This indicated that the combination of the prothrombin mutation with heavy smoking was associated with a high risk of VTE. However, the small number of participants with this mutation may have led to an overestimation of the risk of VTE. Pomp et al. found an odds ratio of 6.06 [95% CI: 2.67-13.76] for the joint effects of current smoking and the prothrombin mutation [24]. In secondary analyses, we found a hazard ratio of 3.75 [95% CI: 1.27-11.04] for the combination of current smoking (all doses of tobacco) and the prothrombin mutation. Also, the effects of obesity combined with Factor V Leiden or the prothrombin mutation were previously investigated in a case-control study [23]. Again, as in our study, they found that the effects of obesity combined with Factor V Leiden or with the prothrombin mutation were higher than expected from the sum of the separate effects.

We computed the incidence rates in the different strata and evaluated the rate difference between exposed and unexposed groups as a measure of effect. However, the crude incidence rates were likely to be confounded. In the Cox regression, we adjusted for a number of confounding factors, but this did not essentially change the estimates for strata with good statistical power. In the strata that included double mutations, the statistical power was insufficient for adjustment, but we assumed that adjustments in these strata would have resulted in similar estimates. Therefore, our study indicated additive interactions between Factor V Leiden and the lifestyle factors of heavy smoking and obesity. Our study had low statistical precision for evaluating the effects of life-style factors on the risk of VTE in individuals with the prothrombin mutation;

however, we did observe an indication that heavy smoking increased the risk of VTE in these individuals. This result should be confirmed in future studies.

The Factor V Leiden mutation leads to reduced degradation of activated coagulation factor V and factor VIII [34]. The mechanism underlying the Factor V Leiden effect on the VTE risk may involve the elevated factor VIII concentration [35-38]. The prothrombin mutation causes an elevated level of plasma coagulation factor II. Thus, a procoagulant status may be involved with the effect of the prothrombin mutation on the risk of VTE; however, the mechanism is not clear [32, 35, 39, 40]. Therefore, it is plausible that these genetic factors might be additive with the procoagulant status caused by lifestyle factors and cause an imbalance in haemostasis that favours thrombosis.

In conclusion, we found that lifestyles that included smoking and obesity had a substantial impact on the risk of VTE in individuals with the Factor V Leiden and the prothrombin mutation. Individuals with these genetic risk factors appeared to be more susceptible to the unfavourable effects of smoking and obesity on the incidence rate of VTE. The genetic risk factors cannot be modified, but the risk of VTE might be reduced in these individuals if they maintained a healthy weight and refrained from smoking tobacco.

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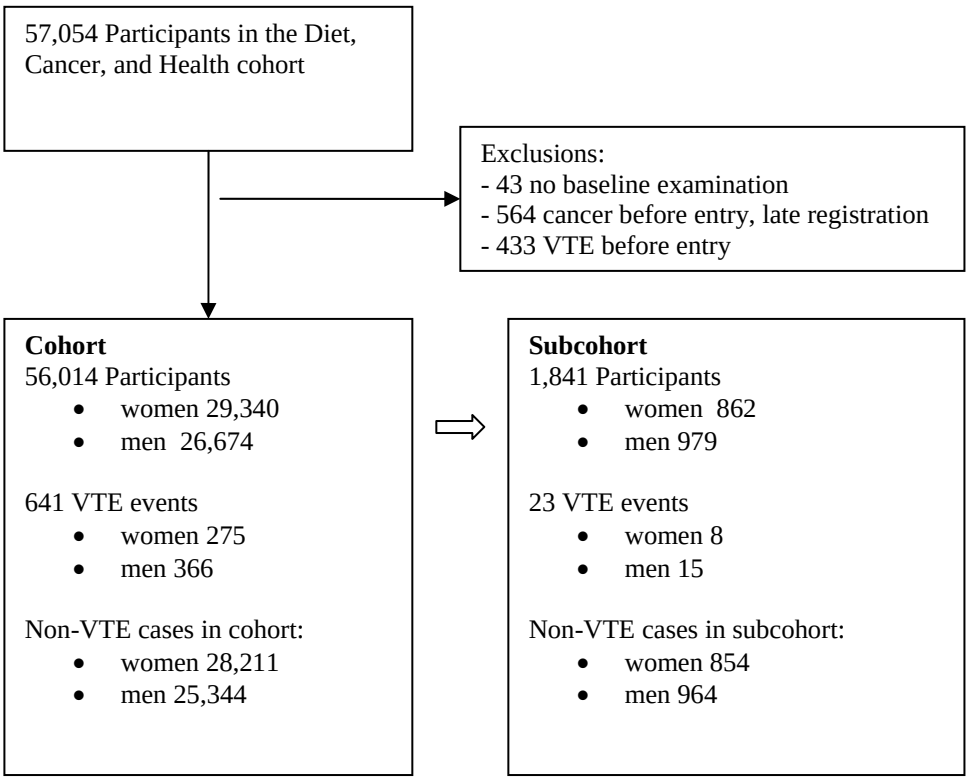

Figure 1. Participant inclusions and exclusions and subcohort sampling (randomly selected from cohort).

Table 1. Baseline characteristics of the 1841 participants in the subcohort.		
	Women	**Men**
Number of participant	862	979
Age, years[*]	56 (51-64)	56 (51-64)
Factor V Leiden[#]		
• Wild type	777 (92.4%)	887 (91.9 %)
• Heterozygous	64 (7.6%)	75 (7.9 %)
• Homozygous	0	0
• No test	21	17
Prothrombin mutation[#]		
• Wild type	830 (96.3%)	948 (96.8 %)
• Heterozygous	11 (1.3%)	14 (1.4 %)
• Homozygous	0	0
• No test	21	17
Smoking status, (%)		
• Never smokers	42.1	25.9
• Former smokers	20.8	36.3
• Current smokers, < 25 g/day	34.3	26.1
• Current smokers, ≥ 25 g/day	2.8	11.6
Body mass index (kg/m^2)[*]	24.6 (19.8-33.7)	26.4 (21.7-32.5)
Height, (cm)[*]	164 (154-174)	176 (166-188)
Hormone replacement therapy, (%)	32	Not relevant

[*]Median (5 - 95 percentiles)

[#] Percent of tested persons

[†]Education based on 3 categories on duration of basis school (starting in age of 6-7 years): 7 years, 8–10 years, 11+ years

Table 2. Combined effects of Factor V Leiden (FVL) and the prothrombin mutation (PTM) on the risk of venous thromboembolism (VTE) in a subcohort of 1803 individuals (50 to 64 years old) randomly selected from the Danish Diet, Cancer, and Health study (1993 to 1997) that were followed until June, 2006. [95% confidence intervals].

	Wild type prothrombin gene	PTM polymorphism
Wild type Factor V gene		
• Number of VTE events	471	19
• VTE incidence rate	95 [86-106]	274 [149-510]
• crude HR for VTE	1 (reference)	2.89 [1.49- 5.59]
• adjusted HR for VTE	1 (reference)	2.61 [1.25- 5.46]
FVL polymorphism		
• Number of VTE events	104	4
• VTE incidence rate	268 [207-347]	411 [78-1973]
• Crude HR for VTE	2.71 [2.04-3.62]	4.80 [1.01-22.85]
• Adjusted HR for VTE	2.63 [1.95-3.55]	5.66 [1.23-26.09]

HR: Hazard ratio. Adjusted HR: HR adjusted for body mass index, smoking, height, and use of hormone replacement therapy among women. Incidence rates are expressed in units of 100,000 person years.

Table 3.

Combined effects of genetic risk factors for VTE and smoking on the risk of VTE in a subcohort of 1803 individuals (50 to 64 years old) randomly selected from the Danish Diet, Cancer, and Health study (1993 to 1997) that were followed until June, 2006. [95% confidence intervals].

Smoking status	**Factor V gene**		**Prothrombin gene**	
	Wild type	**G1691A**	**Wild type**	**G20210A**
Non-smoker				
• Number of VTEs	287	61	334	14
• Incidence rate	90 [80-103]	277 [197-389]	100 [88-113]	249 [124-509]
• Crude HR	1 (reference)	2.91 [2.00-4.25]	1 (reference)	2.75 [1.31-5.79]
• Adjusted HR	1 (reference)	2.74 [1.84-4.08]	1 (reference)	2.49 [1.06-5.88]
Current, < 25 g/day				
• Number of VTEs	152	36	183	5
• Incidence rate	101 [84-122]	239 [156-370]	112 [95-133]	220 [68-759]
• Crude HR	1.13 [0.90-1.43]	2.68 [1.68-4.28]	1.15 [0.93-1.42]	1.87 [0.54-6.44]
• Adjusted HR	1.18 [0.93-1.49]	2.81 [1.73-4.56]	1.17 [0.94-1.46]	1.65 [0.48-5.69]
Current, ≥25 g/day				
• Number of VTEs	51	11	58	4
• Incidence rate	149 [109-210]	405 [165-975]	158 [116-216]	17741 [9551-30286]
• Crude HR	1.55 [1.07-2.23]	3.87 [1.60-9.37]	1.47 [1.04-2.08]	175.3 [99-310]
• Adjusted HR	1.63 [1.12-2.37]	4.46 [1.83-10.88]	1.53 [1.07-2.19]	76.8 [29.2-201.7]

Hazard ratios (HR) were adjusted for BMI, Factor V Leiden/prothrombin genotype, body height, and use of HRT among women. Incidence rates are expressed in units of 100,000 person years.

Table 4.

Combined effect of genetic risk factors for VTE and weight on the risk of VTE in a subcohort of 1803 individuals (50 to 64 years old) randomly selected from the Danish Diet, Cancer, and Health study (1993 to 1997) that were followed until June, 2006. [95% confidence intervals].

Weight status	**Factor V genotype**		**Prothrombin genotype**	
	Wild type	**G1691A**	**Wild type**	**G20210A**
Healthy weight				
• Number of VTEs	165	34	194	5
• Incidence rate	73 [62-87]	193 [127-298]	81 [69-95]	143 [50-463]
• Crude HR	1 (reference)	2.60 [1.62-4.16]	1 (reference)	1.77 [0.59-5.34]
• Adjusted HR	1 (reference)	2.63 [1.62-4.28]	1 (reference)	1.43 [0.44-4.63]
Over weight				
• Number of VTEs	207	50	245	12
• Incidence rate	100 [86-116]	305 [208-447]	111 [96-129]	326 [145-733]
• Crude HR	1.20 [0.94-1.52]	3.53 [2.29-5.45]	1.20 [0.96-1.50]	3.76 [1.56-9.06]
• Adjusted HR	1.28 [1.00-1.64]	3.60 [2.31-5.63]	1.26 [1.00-1.59]	4.06 [1.62-10.14]
Obese				
• Number of VTEs	118	24	136	6
• Incidence rate	176 [141-220]	415 [224-757]	188 [153-233]	848 [106-4353]
• Crude HR	2.15 [1.61-2.88]	4.87 [2.53-9.37]	2.08 [1.59-2.73]	8.24 [1.64-41.49]
• Adjusted HR	2.34 [1.73-3.16]	5.27 [2.74-10.14]	2.22 [1.67-2.94]	6.89 [1.18-40.22]

Hazard ratios (HR) were adjusted for smoking, Factor V Leiden/prothrombin genotype, body height, and use of HRT among women. Incidence rates are expressed in units of 100,000 person years.

Validation scheme for Venous Thromboembolism (VTE)

VTE diagnosis and date confirmed:

No yes, date _____

Prior VTE confirmed

No yes, date _____

If no VTE, are there later confirmed VTE

No yes, date _____

Clinical information

☐ No suspicion of VTE (no further registration)

Swelling	☐ Yes	☐ No information	☐ No
Redness	☐ Yes	☐ No information	☐ No
Pain	☐ Yes	☐ No information	☐ No
Coughing	☐ Yes	☐ No information	☐ No
Chest pain	☐ Yes	☐ No information	☐ No
Dyspnoea	☐ Yes	☐ No information	☐ No

Other symptoms _____

Diagnostic work out

X- If performed

☐ Ultra sound ……confirming VTE ….☐Yes …☐No……. ☐ No information

☐ Flexography……confirming VTE …☐Yes …☐No……. ☐ No information

☐ CT ……..confirming VTE …☐Yes …☐No……. ☐ No information

☐ P/V……..……confirming VTE …☐Yes …☐No……. ☐ No information

☐ ECCO……..……indicating VTE ….☐Yes …☐No……. ☐ No information

☐ D-dimer …….increased……... ☐Yes …☐No……. ☐ No information

☐ A-puncture ……hyperventilatin ….. ☐Yes …☐No……. ☐ No information

Location of VTE

☐ ...Crus DVT ☐ ..Proximal DVT ☐ ..Pelvic DVT ☐ ...Upper extremity

☐ Dexter ☐ Sinister

☐ PE without haemodynamic ☐ PE with haemodynamic

Classification

Cancer…….☐ Yes……☐No……..... ☐ No information

Surgery ..…....☐ Yes……☐No……..☐No information

Trauma ..…....☐ Yes……☐No……..☐No information

Travel …….....☐ Yes……☐No……..☐No information

Immobilisation.☐Yes…..☐ No……..... ☐No information

CVC…….....☐Yes…….☐No…….....☐No information

Medical disease☐Yes…...☐No……..☐No information

1. Myocardial infarction < 3 month
2. Apoplexia cerebri < 3 month
3. Respiratoric distres duration > 3 days, < 3 month
4. Decompensatio cordis > 3 days, < 3 month
5. Infection > 3 days, < 3 month
6. Connective disease active > 3 days, < 3 month
7. Myeloproliferative diseases/other

Thrombofilia……☐AT deficiency ☐ Protein S deficiency ☐ Protein C deficiency

☐ Antiphosphorlipid syndrome

Suggestions about cause of VTE ☐ Yes ☐ No

Idiopathic VTE **Provoked VTE** **Unclassified**

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1. Ane Marie Thulstrup: Mortality, infections and operative risk in patients with liver cirrhosis in Denmark. Clinical epidemiological studies. *2000.*

2. Nana Thrane: Prescription of systemic antibiotics for Danish children. *2000.*

3. Charlotte Søndergaard. Follow-up studies of prenatal, perinatal and postnatal risk factors in infantile colic. *2001*.

4. Charlotte Olesen: Use of the North Jutland Prescription Database in epidemiological studies of drug use and drug safety during pregnancy. *2001*.

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11. Andrea Floyd: Drug-associated acute pancreatitis. Clinical epidemiological studies of selected drugs. *2004*.

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14. Reimar W. Thomsen: Diabetes mellitus and community-acquired bacteremia: risk and prognosis. *2004*.

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18. Kort- og langtidsoverlevelse efter indlæggelse for udvalgte kræftsygdomme i Nordjyllands, Viborg, Ringkøbing og Århus amter 1995-2005. *2005*.

19. Mette Nørgaard: Haematological malignancies: Risk and prognosis. *2006*.

20. Alma Becic Pedersen: Studies based on the Danish Hip Arthroplastry Registry. *2006*.

    Særtryk: Klinisk Epidemiologisk Afdeling - De første 5 år. *2006*.

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22. Andre sygdommes betydning for overlevelse efter indlæggelse for seks kræftsygdomme i Nordjyllands, Viborg, Ringkjøbing og Århus amter 1995-2005. *2006*.

23. Ambulante besøg og indlæggelser for udvalgte kroniske sygdomme på somatiske hospitaler i Århus, Ringkjøbing, Viborg, og Nordjyllands amter. *2006*.

24. Ellen M Mikkelsen: Impact of genetic counseling for hereditary breast and ovarian cancer disposition on psychosocial outcomes and risk perception: A population-based follow-up study. *2006.*

25. Forbruget af lægemidler mod kroniske sygdomme i Århus, Viborg og Nordjyllands amter 2004-2005. *2006.*

26. Tilbagelægning af kolostomi og ileostomi i Vejle, Ringkjøbing, Viborg, Nordjyllands og Århus Amter. *2006.*

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32. Mette Skytte Tetsche: Prognosis for ovarian cancer in Denmark 1980-2005: Studies of use of hospital discharge data to monitor and study prognosis and impact of comorbidity and venous thromboembolism on survival. *2007.*

33. Estrid Muff Munk: Clinical epidemiological studies in patients with unexplained chest and/or epigastric pain. *2007.*

34. Sygehuskontakter og lægemiddelforbrug for udvalgte kroniske sygdomme i Region Nordjylland. *2007*

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49. Operativ fjernelse af galdeblæren. Region Midtjylland & Region Nordjylland. 1998-2008. 2009.

50. Mette Søgaard: Diagnosis and prognosis of patients with community-acquired bacteremia. *2009*