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"Coffee Consumption and Risk of First and Recurrent Venous Thromboembolism (VTE) and All-cause Mortality After VTE"

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Abstract

Coffee is one of the most consumed beverages in the world and it has been reported to be associated with the risk of cardiovascular disease. Venous Thromboembolism (VTE) is a major public health burden, although its association to dietary habits including coffee consumption is still underinvestigated. The aim of this thesis was to investigate the association between daily coffee intake and the risk of incident VTE, as well as recurrence and all-cause mortality after a first VTE event.

The study included 30,236 participants aged 25-97 from the fourth (1994-95), fifth (2001-02) and sixth (2007-08) surveys of the Tromsø study. Information about daily coffee consumption was obtained from questionnaires at the time of enrollment and updated at each of the subsequent surveys. All cases of incident VTE, recurrent VTE and death were registered during the follow-up period (end of follow up: 31st Dec 2012).

A total of 491 incident VTEs occurred during 312,688 person-years (overall IR 1.57, 95% CI 1.44-1.72). Consuming at least one cup of coffee per day was inversely associated with the risk of incident VTE (hazard ratio (HR) 0.63, 95% CI 0.44-0.89) and the risk estimates were particularly strong for moderate coffee consumers (3-4 cups: HR 0.58, 95% CI 0.39-0.84, 5-6 cups: HR 0.59, 95% CI 0.4-0.88) compared to coffee abstainers. Among 491 VTE cases, 76 experienced recurrent VTE (recurrent rate: 35.0, 95% CI 28.0-43.8) and 240 died (mortality rate: 99.1 95% CI, 79.4-115.8) during the mean follow-up of 4.9 years. Coffee drinking was associated with a 66% reduced risk of recurrence (HR 0.34, 95% CI 0.17-0.67)

and 18 % lower risk of death (HR 0.82, 95% CI 0.50-1.34). The inverse association persisted for all coffee consumption categories.

In conclusion, coffee drinking was inversely associated with the risk of incident VTE, recurrence and death. A possible U-shape association was revealed, indicating that moderate coffee intake might reduce the risk of VTE, recurrence event and death. However, further epidemiological studies are necessary in order to elucidate the true association.

List of Abbreviations

BMI – Body Mass Index

CGA – Chlorogenic Acid

CI – Confidence Intervals

CVD – Cardiovascular Disease

DVT – Deep Vein Thrombosis

EU – European Union

FFQ – Food Frequency Questionnaire

FMD – Flow-Mediated Dilation

FVIII – Factor VIII

HDL – High-Density Cholesterol

HR – Hazard Ratio

ICD – International Classification of Disease

IR – Incidence Rate

ISM – The Department of Community Medicine

IWHS – Iowa Women's Health Study

MEGA study – Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study

MR – Mortality Rate

NHDS – National Hospital Discharge Survey

OR – Odds Ratio

PE – Pulmonary Embolism

PTS – Post-thrombotic Syndrome

RR – Relative Risk

UNN – University Hospital of Northern Norway

VTE – Venous Thromboembolism

vWF – von Willebrand factor

1 INTRODUCTION

1.1 Venous Thromboembolism (VTE)

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). Blood clots occurring in the deep veins, most commonly in the lower limbs are called DVT. PE occurs when a part of the blood clot breaks off, is transported in the circulation system and obstructs the lung arteries, resulting in interruption of blood flow to the lung (Figure 1) [1]. The typical symptoms of DVT are leg pain, swelling, itchiness, and dropsy or eczema in severe cases [2], while symptoms for PE are respiratory distress, dyspnea and chest pain [3].

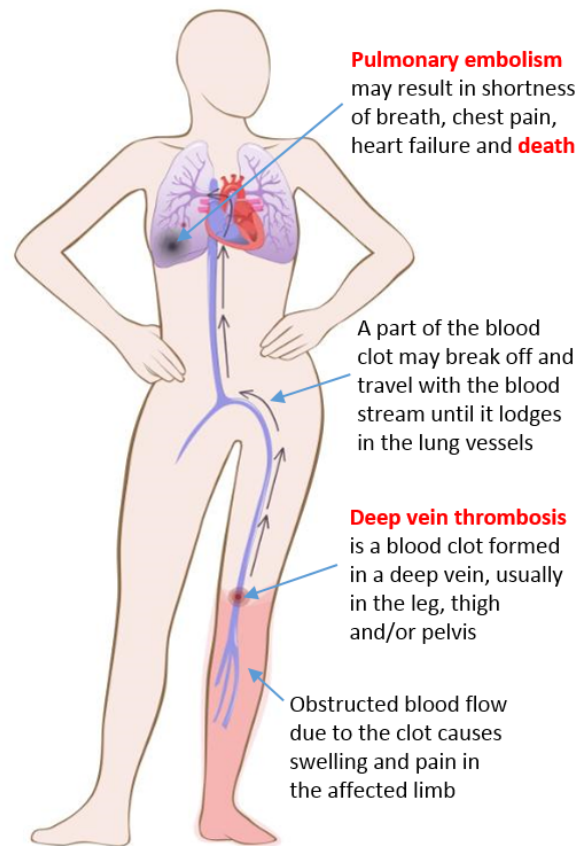


Figure 1: Venous thromboembolism (VTE)

1.2 Pathophysiology

The mechanism of haemostasis is vital for survival in case of vascular injury. In the event of blood vessel injury, endothelial cells signal the platelets in the blood vessels to form a platelet plug at the site of vessel injury [4]. This process is called primary haemostasis. Once the platelet aggregation completes, the coagulation cascade is triggered by exposure to tissue factor/thromboplastin in the sub-endothelial space to create fibrin from fibrinogen [1,

4, 5]. Fibrin generated from the coagulation cascade links to the plug and stabilizes the platelet clot. This process is called secondary haemostasis [1, 4]. The clots formed through primary and secondary haemostasis prevent blood from flowing out of the blood vessels, allowing the vessel injury to heal. However, excessive haemostatic activity in off-target places leads to thrombus formation and thromboembolic diseases. Venous thrombi often occur in venous valvular sinuses where blood is especially vulnerable to hypoxia and

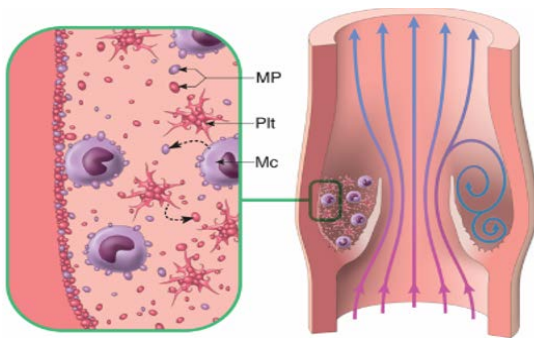


Figure 2: Venous thrombi formation

Thrombi most often originate in the venous valve pockets characterized by vortex flow and hypoxia. The mechanisms that leads to initiation of thrombus formation here remains unsettled.

hemoglobin desaturation (Figure 2) [6]. In contrast to platelet-rich white thrombi which occur in arteries where shear stress is high, thrombi which form in veins where shear stress is low are referred to as red thrombi due to their richness in fibrin and red blood cells [1, 7, 8].

1.3 Epidemiology

VTE is the third most common cardiovascular disease in the Western world [9, 10], and represents a major public health burden. The incidence rate of VTE is 1-2 per 1,000 person-years in the adult population, [10-13], thus corresponding to an estimated total number of 1.1 million VTE events each year in the European Union (EU) [14]. The incidence rate of DVT in the general population is 0.5-1.2 per 1,000 person-years [12, 15], while the incidence rate of PE with or without concurrent DVT is 0.3-0.8 per 1000 person-years [13]. The burden of VTE might additionally be slightly underestimated due to asymptomatic cases and sudden deaths caused by undiagnosed PE [14].

Most of the fatal cases in VTE are associated with PE. The short-term mortality rate (at 30 days) after the acute onset of PE ranges from 3 to 11% [10, 12], though some studies reported increased long-term mortality rate (>3 months from onset). An Australian registry showed 31.5% cumulative mortality rate after 5 years from an acute PE event, which was 2.5 times higher compared to the age-and sex- matched general population mortality rate [16]. The case-fatality rate was especially high among patients with cancer-related thrombosis (25%) [17]. Patients with a first DVT or PE often experience one or more recurrences [10]. The recurrence rate after one year from the first VTE is about 7-13% [2, 17-19], and the rate is higher among patients with idiopathic VTE [20, 21].

Pulmonary hypertension and post-thrombotic syndrome (PTS) are two chronic complications that can emerge after an VTE event. Two to four percent of the patients develop chronic pulmonary hypertension after acute PE, and the condition contributes largely to the elevated long-term mortality rate [10]. PTS is a chronic condition that may evolve after a DVT. The symptoms include pain, heaviness, and swelling in the limbs and lipodermatosclerosis in severe cases [2]. PTS occur in up to 50% of the patients who experienced DVT [2, 22], and 5-10% of the patients who had symptomatic DVT can develop severe PTS [23]. Advanced age, unprovoked VTE and poorly controlled anticoagulation therapy after the incident are strong risk factors for PTS [2, 22].

1.4 General Risk Factors and Triggers of VTE

There are three notable contributors to VTE according to Rudolph Virchow; (i) blood flow stasis, (ii) blood composition and (iii) injury in the blood vessel wall [24]. Most risk factors for VTE fall into the first two categories (stasis and hypercoagulability) although recent studies

have suggested that inflammatory response at the level of the vein wall also is involved in thrombogenesis [4]. Known risk factors for VTE include advanced age, obesity, use of contraceptive pills, pregnancy, cancer and institutionalization [25, 26]. The risk factors can be classified into two categories; hereditary and non-hereditary factors. Among the non-hereditary factors, there are conditions that last for short periods of time and trigger VTE (provoking factors), and chronic factors which persist for a long time (clinical risk factors). VTE is a multifactorial disease, therefore co-existence of multiple factors significantly increases the risk of developing VTE [27, 28]. For example, a synergistic effect was highlighted in a study when carriers of Leiden mutation taking oral contraceptives had a 35-fold higher risk of VTE compared with non-carriers, which was higher than the sum of risk estimations by each risk factor (taking oral contraceptives: Relative Risk (RR) = 3.7, Factor V Leiden: RR = 6.9) [27].

Hereditary risk factors

Recently the significance of genetic factors, especially coagulation abnormality has been discovered and initiatively researched [27-29]. A meta-analysis involving 126,525 cases and 184,068 controls revealed that factor V (G1691A, A4070G), Prothrombin G20210A and Plasminogen-activator inhibitor I were significantly associated with VTE in the Caucasian population [30]. Factor V Leiden is responsible for activated protein C resistance [31] while the Factor II G20210A mutation is associated with exceeding plasma prothrombin concentration [28]. The risk of VTE increases by three to eight times for carriers of Factor V Leiden [32], and the mutation is found in 1-15% of the Caucasian population and in 10-50% of VTE patients [27, 32]. Deficiencies of protein C, protein S and antithrombin are also strong

genetic risk factors. The risk of VTE increases by approximately 10-fold for carriers of protein C or protein S deficiencies, and 20-fold for antithrombin deficient subjects [27, 31, 33]. Yet, the prevalence is rare compared to Factor V Leiden (0.02-0.4% among the healthy population) [31]. ABO blood group is associated with the levels of von Willebrand factor (vWF) and factor VIII (FVIII). Several studies have revealed that subjects with non-O blood type have an about two times higher risk of VTE compared to those with blood type O [34-37]. Elevated concentrations of FVIII (>150 IU per deciliter) are also partly due to heredity [38], and are reported to increase the risk of first and recurrent VTE by 5-fold and 7-fold, respectively [39, 40].

Non-hereditary risk factors

Advanced age is a significant risk factor of VTE. VTE occurs in 1 per 10,000 in the young population (<40) while the incidence in the elderly population (>80) is 50-80 per 10,000 [41-43]. The frequency of PE is especially high in the elderly, as it increases from 12 to 70 per 10,000 as age increases from 65-69 to over 85 years old [41]. Therefore morbidity and case fatality rate are high among the elderly [43]. The reasons for the high incidence rate in the elderly are still unclear, although accumulation of multiple risk factors and biological change due to aging might play an important part.

Obesity, defined as BMI ≥ 30 is another clinical risk factor for VTE [9, 44-46]. The relative risk of VTE in obese patients was 2.51 (95% confidence interval (CI), 2.49-2.51) compared to non-obese patients in the data from National Hospital Discharge Survey (NHDS) [45]. Also obese patients had a 60% increased risk of recurrent VTE compared to non-obese patients (HR 1.6, 95% CI 1.1-2.4) [44].

The use of contraceptive pills, estrogen replacement therapy, pregnancy and the postpartum period are all associated with increased risk of VTE in females. The use of contraceptive pills increases the risk of developing VTE by two to four times [47-50], especially for contraceptives which contain a high dosage of estrogen ($\geq 50 \mu\text{g}$) [49]. Due to hemostatic changes during pregnancy [51], pregnant women have a four to five times higher risk of VTE compared to non-pregnant women [52-54] and a 22-fold increased risk of VTE was revealed during the first six weeks after delivery (the postpartum period) [54].

Chronic diseases such as congestive heart failure, chronic kidney disease, and chronic inflammatory diseases are also known clinical risk factors of VTE. A case-control study revealed a 2.6-fold increase in risk of VTE for patients with congestive heart failure [55]. End-stage chronic kidney disease was shown to be associated with 2.3-fold higher risk of VTE compared to the general population [56]. Also, patients with chronic inflammatory disease had 3.5 times greater risk of developing VTE compared to the controls, and the risk was particularly high during flare-up periods (HR = 8.4) [57].

Cancer is a major risk factor for VTE, associated with a four to seven times higher risk of VTE compared to non-cancer subjects [58-60]. Malignant disorders are associated with a hypercoagulable state, and often show abnormalities in coagulation tests [61]. The highest risk of VTE was found among patients with pancreas, brain, stomach and lung cancer [59, 60]. A population based nested case-control study revealed that almost one fifth of all VTE cases in the community could be attributed to malignant neoplasms [62].

Triggers of VTE

Provoking factors are transient conditions that trigger VTE, and often those are more strongly associated with the risk of VTE compared to chronic risk factors [63]. For example, surgery and trauma are well-known triggers of VTE [26, 63]. The risk of asymptomatic VTE increased 21 times after surgery and 12 times after trauma in a nested case-control study (odds ratio (OR) 21.7 and 12.7 respectively) [63]. Furthermore, the association between immobilization and the risk of VTE is well established [26, 63, 64]. For example, in stroke patients with hemiplegia, 60% developed DVT in the paralyzed leg while only 7% developed it in the other leg [65]. Bed rest for more than three days is also associated with higher risk of VTE. An early study found VTE at autopsy in 15% of the patients who were on bed rest for more than three days before death [66]. The percentage rose to 80% for those who were in bed for 1-2 weeks before death [66]. Travel by air, car, bus or train for more than four hours increased the risk of VTE by 2-fold compared to non-travelers [67]. Also a recent case-control study reported that work-related seated immobility (minimum two hours of seating continuously for at least ten hours during a 24-hour period) increased the risk of VTE almost 3-fold (OR 2.8) [68]. Acute medical conditions and institutionalization also contribute to increased risk of VTE since the patients are exposed to multiple risk factors such as surgery, immobility, infection and cancer [62, 69]. A retrospective review of a population-based cohort revealed a more than 100 times higher risk of VTE among hospitalized patients compared to community residents [69].

Unprovoked VTE

Despite all the known risk factors, 30-50% of VTE cases occur without any predisposing factor(s) [13, 33]. These events are classified as unprovoked VTE and this classification has clinical importance since the five-year recurrence rate in patients with unprovoked VTE is 2-10 times higher than for those who had provoked VTE. Extended anticoagulation treatment is recommended for patients with unprovoked VTE [70]. Further research is vital in order to elucidate new risk factors and triggers of VTE, in order to prevent future events and reduce the public health burden caused by VTE.

1.5 Lifestyle Factors and VTE

Although a number of studies examined lifestyle factors such as smoking, physical activity, dietary habits and alcohol consumption in relation to the risk of VTE, the findings are diverse. Cigarette smoking is an independent risk factor of arterial cardiovascular diseases [9, 71], but the association to the risk of VTE is still debatable. A systematic review with 21 observational studies revealed a 1.2-fold increased risk of VTE for current smokers compared to never smokers, and the association followed a dose-response pattern [72]. Some studies demonstrated increased risk of VTE only among heavy smokers [73-75] while many revealed no association between cigarette smoking and VTE [9, 73, 76, 77], particularly when analysis was restricted to non-cancer patients or intermediate development of cancer was taken into account [73].

Muscle activity is known to decrease venous pressure and increase blood flow [78, 79] which could lead to a decrease in VTE. Yet, the findings of studies that investigated the relation between physical activity and VTE are not consistent. A large cohort study in Sweden with 40,000 female participants revealed a 50% decreased risk of VTE among women who engaged in regular physical activity [74]. On the other hand, the Physician's Health Study in the U.S. observed 10% increase in the risk of VTE, if participants increased frequency of physical exercise [9]. A prospective study investigating the cardiovascular risk factors in relation to VTE revealed no association between physical inactivity and the risk of VTE [77].

Diet and alcohol consumption might also have an effect on VTE risk. The Longitudinal Investigation of Thromboembolism Etiology (LITE) discovered a lower risk of VTE for participants who consumed more than four servings of fruit and vegetables per week (HR = 0.47) and ate fish one or more times a week (HR = 0.58) [80]. Yet, not all papers confirmed the association between fish intake and the risk of VTE [81, 82]. Moderate alcohol consumption was reported to be beneficial against VTE in several papers [74, 81, 83], while some yielded no association [9, 84].

1.6 Coffee Consumption and Disease Risk

Coffee is one of the most popular beverages worldwide and therefore its association to health has gained considerable attention. Inverse associations have been reported between coffee consumption and several diseases like diabetes type II [85], Parkinson's disease [86,

87], Alzheimer's disease [88] and some types of cancer [89, 90]. Moreover some reports indicated that coffee intake was inversely associated with overall mortality [91, 92].

There were diverse results reported for the associations between coffee intake and cardiovascular diseases. Recently, a large German prospective cohort study revealed no association between coffee intake and myocardial infarction (MI) or stroke [93] while meta-analysis of 21 cohort studies showed a lower risk of coronary heart disease for moderate coffee drinkers (1-4 cups per day) [94]. Most studies which assessed the risk association between coffee intake and stroke found a decreased risk of fatal and nonfatal stroke for coffee consumers [95-97]. Several studies were conducted on coffee consumption and heart failure, and the most recent meta-analysis showed a significant J-shaped association between coffee consumption and the risk of heart failure [98].

1.7 Coffee Consumption and VTE

The association between coffee consumption and the risk of VTE has not been extensively studied compared to other cardiovascular diseases. Currently, three observational studies [81, 99, 100], and one meta-analysis [101] investigating the relation between coffee consumption and the risk of developing incident VTE have been published. In the Iowa Women's Health Study (IWHS), 37,393 women aged 55-69 years were followed for more than 19 years and 1,950 VTE events occurred [81]. The study revealed an inverse association between moderate coffee consumption and the risk of incident VTE, when the model was adjusted for age, energy intake, education, smoking status and physical activity (28 to <42 servings per week, HR 0.85, 95% CI 0.71-1.01). However, the association was attenuated when BMI and diabetes were added to the analytical model (HR 0.88, 95% CI 0.74-1.05) [81].

Enga et al. investigated the association between coffee intake and the risk of VTE using the fourth survey of the Tromsø study, a prospective cohort study of the inhabitants of the municipality of Tromsø, Norway. Among 26,755 participants, 462 developed VTE during 13 years of follow-up. The subjects who consumed 3-4 cups of coffee per day had a 30% lower risk of VTE (HR 0.70, 95% CI 0.48-1.02), and the subjects who consumed 5-6 cups per day had a 33% lower risk of VTE, compared to coffee abstainers (HR 0.67, 95% CI 0.45-0.97) [99]. The risk estimates weakened for excessive coffee drinkers (>6 cups per day), indicating a possible U-shaped association.

Findings from a large case-control study, the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, are in line with the above studies. The study included 1,803 VTE cases matched with partner controls with the information of coffee drinking habits and risk of VTE obtained from the anticoagulation clinics or questionnaires. Regardless of the amount of coffee consumed per day, the daily coffee consumption was correlated with a 25% decreased risk of VTE (OR 0.75, 95% CI 0.55-1.04) compared to the coffee abstainers [100].

A meta-analysis that pooled all the three studies described above (inter-study heterogeneity: 78%, $P < 0.001$) indicated a pooled RR of 0.97 (95% CI 0.88-1.08) for coffee consumers against coffee abstainers. Consuming 1-4 cups per day yielded a RR of 1.11, (95% CI 1.0-1.22), however, a significant inverse association was revealed for the group with ≥ 5 cups of coffee intake per day (RR 0.75, 95% CI, 0.67-0.85) compared to the participants who did not drink coffee [101].

1.8 Regression Dilution Bias and Time-Varying Analysis

The risk factors in a traditional cohort study are usually measured at the time of the study enrollment. However, the status of the exposure might change over time especially when the study has a long follow-up period (called time-varying risk factors) [102]. Such unpredictable fluctuation of the exposure variables can occur by random measurement error, such as imprecise measurement methods or true biological variability [103], or change of contexts of the study subjects during the follow-up period. For example, if a study participant changes their coffee consumption habit during the study period, the subject will be misclassified from the day he/she changed the habit. The transient fluctuations and/or true change of exposure occurring during follow-up period could then bias the regression slope towards null, resulting in an underestimation of the true association between risk factors and outcome [104]. The phenomenon is known as attenuation or regression dilution bias [103] and could become a major limitation in conventional prospective cohort studies with long follow-up periods. When serial measurements of the exposure variables are available in a study, time-varying analysis can be used to correct for the regression dilution bias. In time-varying analysis, the follow-up time of each participant is divided into separate time-windows. Hazard ratios (HRs) are obtained for each time-window separately by Cox analysis, and then, a weighted average of all HRs is calculated (Figure 3) [102]. Since coffee consumption habits may vary over time and could influence the risk estimation of the outcome, time-varying analysis could be a useful method to overcome the possible regression dilution bias.

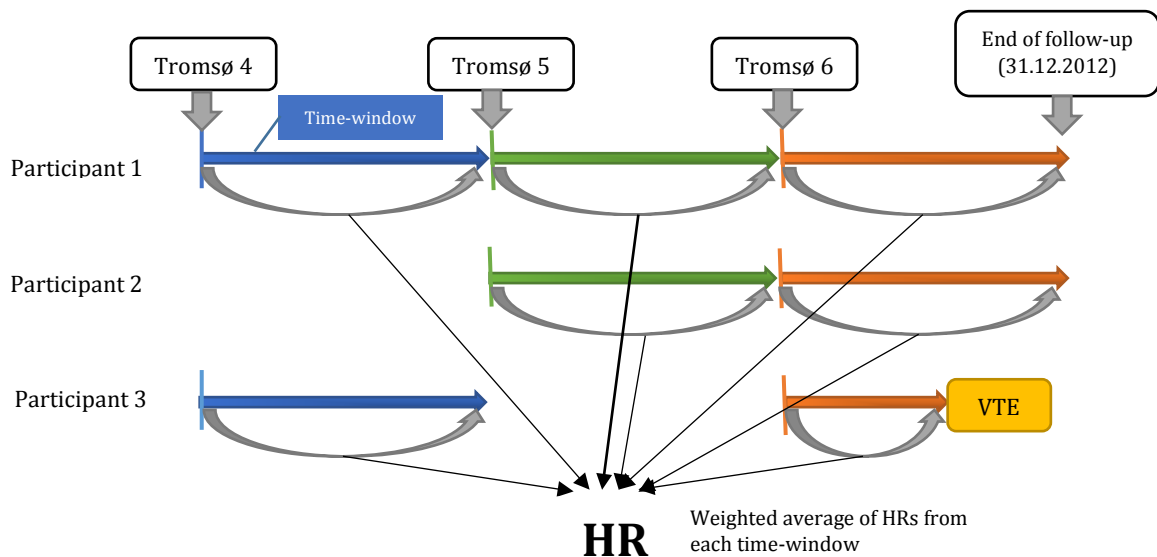


Figure 3: Time-varying Analysis (example)

The previous study by Enga et al had a median follow-up time of 12.5 years, and the coffee consumption habits were only measured once at the time of study enrollment [99]. For that reason, there is a possibility that the study was affected by the regression dilution bias, and thus, the association between coffee and VTE could potentially be stronger than estimated in their study. The present study uses a time-varying analysis with repeated measurements of the coffee consumption variable to increase the accuracy of the risk estimation between coffee intake and VTE.

2 OBJECTIVES

2.1 Research Problem and Aims

All three previous investigations have reported an inverse association between coffee intake and the risk of incident VTE [81, 99, 100]. However, the effect of regression dilution has not been addressed in two of the cohort studies. No previous study has investigated the association between coffee consumption and risk of recurrent VTE and all-cause mortality after VTE. Therefore, the aims of the present study were:

- 1) To investigate the association between coffee consumption and the risk of incident VTE by using a time-varying analysis to minimize regression dilution bias
- 2) To investigate the association between coffee consumption and risk of recurrence and all-cause mortality after a first event of VTE

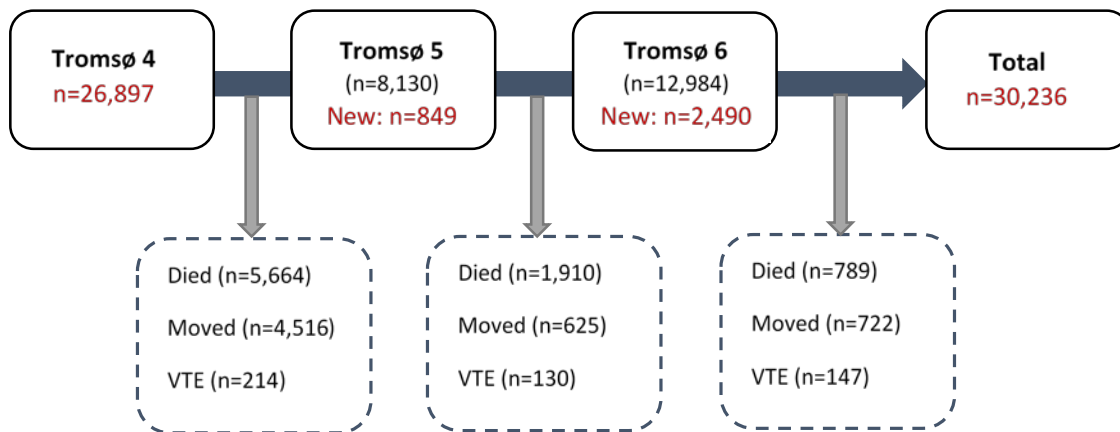
3 METHODS

3.1 Study Population

The study population was recruited from the fourth (1994-1995), fifth (2001-2002) and sixth (2007-2008) surveys of the Tromsø study. The Tromsø study is a single centered, population-based cohort study initially aimed at investigating the reason for high mortality rate due to cardiovascular incidences in the Tromsø region. Seven surveys have been conducted until the present; in 1974, 1979-1980, 1986-1987, 1994-1995, 2001-2002, 2007-2008 and 2015-2016. In total, 53,731 residents of the Tromsø municipality were invited and more than 45,000 unique individuals participated in at least one of the surveys [105]. The Department of Community Medicine (ISM) is administrating the study in association with the University Hospital of Northern Norway (UNN), the Norwegian Institute of Public Health and the Tromsø City Council.

The fourth Tromsø study invited men and women aged over 25 years for a first examination (n=27,158, 77% attendance rate). Then, men aged 55-74, women aged 50-74 and a small subgroup of residents in different age groups were invited for the extensive second examination (n=7,965). The participants of the second examination in Tromsø 4 and parts of the inhabitants from particular age groups were invited to Tromsø 5 (n=8,130, 79% attendance rate) and Tromsø 6 (n=12,984, 66% attendance rate). In total, 30,586 unique individuals attended one or more surveys. Participants who had a VTE event prior to the study enrollment (n=78), who did not answer the questionnaires regarding coffee consumption in any of the surveys (n=54), who did not give consent to the study (n=181), or who moved before the screening (n=37) were excluded from the study analysis. In total, 30,236 subjects were included in the present study and followed until 31st December 2012

(Figure 4). Coffee consumption habits and confounding factors were measured repeatedly and updated at each survey. All participants gave written informed consent and the study was approved by the Regional Committee of Medical Health Research Ethics North Norway.



Recruited from Tromsø 4 (1994-1995), Tromsø 5 (2001-2002), and Tromsø 6 (2007-2008).

Figure 4: Study population

3.2 Measurements

The information of baseline characteristics was collected at each survey using questionnaires, physical examination and blood sampling. Self-administered questionnaires covering a wide range of lifestyle aspects, dietary habits, diseases and symptoms were completed on the date of inclusion. The question regarding coffee consumption in the Tromsø 4 questionnaires was “How many cups of coffee do you usually drink daily?” with the categories of “coarsely ground coffee for brewing” or “other coffee”. In the Tromsø 5 and 6 questionnaires, the categories for the same question were “filtered coffee”, “boiled coffee/coarsely ground coffee for brewing” or “other types of coffee”. The answers for each

category were combined into a single variable; “total coffee consumption” and categorized into “non-coffee drinkers” (0 cups per day) and “coffee drinkers” (>1 cups per day) to obtain overall results. Then the variable was classified into five categories; ‘0 cups per day’, ‘1-2 cups per day’, ‘3-4 cups per day’, ‘5-6 cups per day’ and ‘>6 cups per day’. This variable was the main exposure of the present study, and the group who did not drink coffee (0 cups per day) was set as reference in the analysis. Moreover, the total coffee consumption variable was divided into “Boiled coffee” (coarsely ground coffee for brewing) and “Other coffee” (filtered coffee and other types of coffee) variables for separate analysis. The information of smoking status was drawn from the question “Do you/did you smoke daily?--Never/ Yes, previously/ Yes, now”. The baseline data of diabetes, the level of physical activities, history of cardiovascular diseases (heart attack/angina pectoris/stroke), dietary habits and illnesses in the family were also obtained from the Tromsø 4, 5, and 6 questionnaires.

At the physical examination, body weight was measured in kilograms with participants wearing light clothes and no shoes and height was measured to the nearest centimeter. Body Mass Index (BMI) was calculated as weight divided by the square of height in meters (kg m^2). Non-fasting blood samples were collected from an antecubital vein to measure serum total cholesterol, serum high-density cholesterol (HDL), and triglyceride. Blood samples were respite in room temperature for an hour, and then further measurements were conducted at the UNN, Department of Clinical Chemistry. Enzymatic colorimetric methods and commercially available reagents were used to determine cholesterol and triglyceride levels (cholesterol: CHOD-PAP and triglycerides: GPO-PAP, Boeringer Mannheim). HDL cholesterol was computed after heparin-manganese chloride precipitation of low-density lipoproteins.

Information on cancer before study start and during follow-up was derived from the Cancer Registry of Norway, which has over 98% registration completeness (estimation for period 2001-2005) [106].

3.3 Measurements (Outcome)

Part I –Incident Venous Thromboembolism (VTE)–

All incident VTE events during the follow-up period (1994-1995 to 31st December 2012) were searched and derived from the hospital discharge diagnosis registry, the radiology procedure registry and the autopsy registry at the UNN. This hospital exclusively provides all the outpatient consultation, hospital care and relevant diagnostic radiology for VTE since UNN is the only hospital in the municipality. Outpatient clinic visits and hospitalization were included in the hospital discharge diagnosis registry. Not all VTE events were recorded in the hospital discharge diagnosis registry due to coding errors, therefore the radiology procedure registry was conducted to find potential cases of VTE events, which were missed from the diagnosis registry. The relevant *International Classification of Disease* (ICD) codes used in the search were 325, 415.1, 451, 452, 453, 671.3, 671.4, 671.9 for version 9 (1994-1998) and I26, I80, I81, I82, I67.6, O22.3, O22.5, O87.1, O87.3 for version 10 (1999-2012) [107]. Trained personnel reviewed the diagnostic procedures of VTE performed at the Department of Radiology during the follow-up period and objectively confirmed the cases of VTE which were missing from the registry. Moreover, the autopsy diagnosis registry was searched through to identify the events of VTE.

The review of the medical records was undertaken by trained personnel, who were blinded to baseline characteristics of the patients, including coffee consumption habits. Each potential VTE event derived from the hospital discharge diagnosis registry or the radiology procedure registry was investigated and confirmed as a validated event if the following four conditions were fulfilled; 1) thrombosis confirmed through objective diagnostic procedure such as compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography or autopsy; 2) the medical record indicated that a physician had diagnosed DVT or PE; 3) symptoms or clinical signs consistent with DVT or PE were evident; 4) the medical record indicated that the patient underwent treatment with anticoagulants (heparin, warfarin, or non-vitamin K anticoagulants), thrombolytic therapy or vascular surgery except when specific contradictions were noted. Unclear cases were taken to a discussion with a senior consultant with experience on VTE diagnosis (J. B. Hansen). Also if the death certificate of the cases collected from the autopsy registry indicated VTE as cause of death or the incidence of VTE had significantly contributed to death, the case was added as validated VTE event.

All incident VTEs were categorized into either “provoked VTE” or “unprovoked VTE” for separate analysis. If one or more of the following factors were present at the time of diagnosis, the event was considered as “provoked VTE”: previous surgery or trauma within eight weeks, acute medical conditions like acute MI, ischemic stroke or major infectious disease, active cancer, immobilization (bed rest for three or more days, use of wheelchair, or more than four hours duration travel by car, train, boat or airplane within the previous 14 days), or other possible provoking factors specified by a physician such as intravascular catheter. The cases with no present provoking factor were classified as “unprovoked VTE”.

Part II –Recurrent VTE and Mortality–

The information on recurrent VTE events and all-cause mortality for the study participants during the follow-up period was also recorded. The recurrent VTE events were collected and validated through the same procedures and criteria as used for incident VTE cases described earlier. The deaths of the participants were identified by using the unique national person identification number and derived from the Norwegian Population Registry.

3.4 Statistical Analysis

Statistical analyses were performed with STATA version 14.0 (Stata Corporation, College Station, TX, USA) and the significance level was set to 0.05. Baseline patient characteristics were calculated at the point when the participants attended the study survey for the first time (Tromsø 4 (1994-1995): 26,897 participants, Tromsø 5 (2001-2002): 849 participants, Tromsø6 (2007-2008): 2,490 participants). The subjects who died (n=5,744) or moved (n=4,993) during the follow-up period were censored at the date of death or migration (Figure 4). The baseline data were given either in crude numbers with percentages or mean with standard deviation, and the distribution across coffee consumption categories is shown in Table 1. There were some variations in the number of subjects for each risk factor due to missing data, although the differences were not substantial in all covariates (<1% missing: except for physical activity: 5.7% missing).

In order to avoid possible regression dilution bias [103], time-varying Cox proportional hazards regression analysis was used in the present study. The person-years at

risk for each participant were calculated in three separate time-windows; the period from Tromsø 4 to 5, from Tromsø 5 to 6, and from Tromsø 6 until the end of follow-up (Figure 3). Person-years were accrued from the date the subjects attended the survey to the date of VTE, death, migration or end of the time-window period. Crude incidence rates (IRs) were calculated as number of events divided by the person-years at risk, and given per 1,000 person-years with 95% CIs. HRs were computed for each time-window and a weighted average of the separate time-windows was given as overall HR. Age was set as time scale so all the HRs were automatically adjusted for age. Furthermore, HRs were either adjusted for sex (Model 1) or multivariable (sex, BMI, smoking status (current smoker/ previous smoker/ never), physical activity (hard physical exercise for less than 1h/ 1-2h/ 3-4h/ none), self-reported diabetes, history of CVD event, and cancer (cancer diagnosis within 10 years prior to enrollment) (Model 2).

Separate analyses were carried out for provoked and unprovoked VTE outcomes. Analysis stratified by sex, age group under 70 years and type of consumed coffee (boiled or other) was also undertaken. Age stratification was conducted under the hypothesis of greater effects for young participants since age is a strong risk factor of VTE. Among participants who answered questions for both coffee types (n=27,707), 4,958 subjects answered that they consumed both boiled and other types of coffee daily. Those were excluded from the subgroup analysis separated by the different types of coffee intakes. The parallelism of log-log survivor function was evaluated to test the proportional hazard assumption. In addition, Schoenfeld residuals were tested for all the risk factors and the assumption was fulfilled for all the relevant variables when age was set as timescale.

As for the Part II analysis, risk of recurrent VTE events and all-cause mortality were analyzed. The subjects were followed from the date of VTE until the end of the study period, death (n=240), migration (n=13) or the date of recurrent VTE (n=77) for the recurrence analysis, whichever came first. Crude recurrence/mortality rates and HRs were calculated. Recurrence/mortality rates were obtained as number of events/deaths divided by the total person-years at risk and given in per 1,000 person-years with 95% CIs. HRs were computed using the same method as in the first analysis. HRs for the second VTE analysis were adjusted for the confounders in Model 1 and 2, and HRs for all-cause mortality were also adjusted for the same confounders except for cancer. The participants who did not have active cancer at the time of VTE events were analyzed separately for all-cause mortality analysis.

4 RESULTS

4.1 Part I: Coffee Consumption and Risk of Incident VTE

Among 30,236 participants, there were 491 validated incident VTEs during 312,688 person-years (median follow up time for the time-windows: 6.57 years). The baseline characteristics of the study participants are shown in Table 1. More than 89% of the participants (n=26,892) indicated that they drink at least one cup of coffee per day. The subjects who did not drink coffee (0 cups per day) were relatively younger (mean age 38.4 ± 13.7) and experienced less cardiovascular events (2.5%) compared to the coffee drinkers. The level of cholesterol and number of current smokers significantly increased along with increase in daily coffee consumption. On the other hand, the level of hard physical activity decreased as coffee intake amount increased. The proportion of males and current smokers were high among excessive coffee drinkers (>6 cups per day) (59.7% and 58.6% respectively). In general, the participants who only drank boiled coffee (n=8,856) were older, had higher cholesterol, experienced more CVD events and had a higher proportion of current smokers compared to the participants who consumed other types of coffee (n=10,814) (S1 Table).

Table 1: Baseline characteristics of the subjects measured at the time of enrollment* (n=30,236)

	0 cups per day	1-2 cups per day	3-4 cups per day	5-6 cups per day	>6 cups per day	P for trend
Subjects	3106	3430	7498	7922	8042	-
Male	1218 (38.9)	1345 (38.8)	3178 (42.0)	3796 (47.6)	4834 (59.7)	<0.001
Age (years)	38.4 ± 13.7	46.4 ± 16.6	49.3 ± 15.8	48.1 ± 14.2	45.8 ± 12.4	<0.001
BMI (kg m ⁻²)	24.9 ± 4.2	25.1 ± 4.0	25.3 ± 3.9	25.4 ± 3.9	25.5 ± 3.9	<0.001
Cholesterol (mM)	5.4 ± 1.2	5.8 ± 1.3	6.0 ± 1.3	6.1 ± 1.3	6.2 ± 1.3	<0.001
Diabetes**	37 (1.2)	83 (2.4)	169 (2.2)	165 (2.1)	123 (1.5)	0.45
History of CVD**	79 (2.5)	208 (6.0)	494 (6.5)	452 (5.7)	363 (4.5)	0.32
Current smoker	557 (17.8)	574 (16.6)	1798 (23.8)	3120 (39.2)	3051 (58.6)	<0.001
Physical activity§**	1172 (37.4)	1095 (31.6)	2228 (29.5)	2281 (28.6)	2243 (27.7)	<0.001
Cancer‡	38 (1.2)	73 (2.1)	159 (2.1)	157 (2.0)	121 (1.5)	0.69

*Crude number (%) or means ± crude standard deviation **Self-reported. §Includes weekly average of hard physical activity (sweating/out of breath) for more than one hour. ‡Cancer diagnosis within 10 years from the study enrolment.

The overall IR of incident VTE was 1.57 (95% CI, 1.44-1.72) per 1,000 person-years. In total, 193 cases (39.3%) were PE with or without concurrent DVT and 298 cases (60.7%) were DVT alone (Table 2). Among total VTE events, 202 cases (41.1%) occurred without any provoking factor(s) (unprovoked VTE). The most frequent risk factors among provoked VTE were active cancer (25.1%), immobility (20%), and acute medical conditions such as MI, stroke or major infectious disease within eight weeks prior to the event (13.4%). There were no differences in proportions of DVT, PE, unprovoked or provoked VTEs between sexes. The prevalence of risk factors were mostly comparable between gender groups though females had a slightly higher rate of surgery (16.1%) and trauma (8.9%) than male participants (14.3% and 6.7% respectively) (data not shown).

Table 2: Characteristics of VTE events (n=491)

	N (%)
Male	224 (45.62)
Deep vein thrombosis	298 (60.69)
Pulmonary embolism	193 (39.31)
Unprovoked VTE	202 (41.14)
Clinical risk factors	
Estrogens*	26 (5.3)
Pregnancy*	4 (0.81)
Heredity†	15 (3.06)
Other medical conditions‡	110 (22.4)
Provoking factors	
Surgery	83 (16.9)
Trauma	35 (7.13)
Acute medical conditions	66 (13.44)
Cancer‡	123 (25.05)
Immobility§	98 (19.96)
Other¶	26 (5.3)

*Analysis only includes female. †Known/reported incidence of VTE in first-degree relative before the age of 60 years.

‡Comorbid condition such as COPD, MI, asthma, chronic infection, chronic obstructive pulmonary bowel disease or myeloproliferative disorders within the previous year. §Active cancer at the time of VTE event. §Includes bed rest for 3 or more days, more than 4h duration travel by car, train, boat or airplane within last 14 days, or other immobilization. ¶Other possible provoking factors indicated in the medical record (e.g. intravascular catheter)

Table 3 contains IRs and HRs for total VTE events, provoked and unprovoked VTE, and analysis restricted to participants under the age of 70 (n=27,667). The coffee drinkers (>1 cups per day) were associated with 37% decreased risk of incident VTE compared to non-coffee drinkers (Model 2 HR 0.63, 95% CI 0.44-0.89). The crude IR was highest in the group who took 1-2 cups of coffee per day (2.15, 95% CI 1.73-2.66), however, the IRs were not adjusted for age or other confounding factors. For total VTE events, drinking 3-4 cups per day and 5-6 cups per day were associated with 42% and 41% reduced risk of the outcome events respectively (Model 2 HR 0.58, 95% CI 0.39-0.84 and HR 0.59, 95% CI 0.4-0.88) compared to the reference group (0 cups per day). Also drinking 1-2 cups per day was borderline associated with VTE events (Model 2 HR 0.67, 95% CI 0.44-1.01). The inverse

association to the risk of VTE slightly weakened for excessive coffee drinkers (>6 cups per day) (Model 2 HR 0.72, 95% CI 0.49-1.07).

Table 3: Incidence rates (IRs) and hazard ratios (HRs) for VTE events by coffee consumption categories

	Person-years	Events	IR¶(95% CI)	M1 HR* (95% CI)	M2 HR** (95% CI)
Total VTE					
Coffee yes/no	312688.56	491	1.57 (1.44-1.72)	0.69 (0.49-0.97)	0.63 (0.44-0.89)
0 cups per day	27382.74	36	1.31 (0.95-1.82)	REF.	REF.
1-2 cups per day	39100.97	84	2.15 (1.73-2.66)	0.78 (0.52-1.15)	0.67 (0.44-1.01)
3-4 cups per day	84570.55	151	1.79 (1.52-2.09)	0.65 (0.45-0.94)	0.58 (0.39-0.84)
5-6 cups per day	83834.35	123	1.47 (1.23-1.75)	0.65 (0.45-0.95)	0.59 (0.40-0.88)
>6 cups per day	77799.95	97	1.25 (1.02-1.52)	0.75 (0.51-1.1)	0.72 (0.49-1.07)
P for trend				0.22	0.43
Provoked VTE					
Coffee yes/no	312688.56	289	0.92 (0.82-1.04)	0.69 (0.80-1.08)	0.61 (0.39-0.96)
0 cups per day	27382.74	21	0.77 (0.5-1.18)	REF.	REF.
1-2 cups per day	39100.97	34	0.87 (0.62-1.22)	0.52 (0.30-0.90)	0.44 (0.25-0.79)
3-4 cups per day	84570.55	97	1.15 (0.94-1.4)	0.70 (0.43-1.13)	0.59 (0.36-0.95)
5-6 cups per day	83834.35	77	0.92 (0.73-1.15)	0.69 (0.42-1.12)	0.63 (0.38-1.03)
>6 cups per day	77799.95	60	0.77 (0.6-0.99)	0.8 (0.48-1.33)	0.77 (0.46-1.28)
P for trend				0.56	0.42
Unprovoked VTE					
Coffee yes/no	312688.56	202	0.65 (0.56-0.74)	0.70 (0.41-1.19)	0.65 (0.37-1.13)
0 cups per day	27382.74	15	0.55 (0.33-0.91)	REF.	REF.
1-2 cups per day	39100.97	50	1.28 (1.97-1.69)	1.16 (0.64-2.10)	1.04 (0.56-1.93)
3-4 cups per day	84570.55	54	0.64 (0.49-0.83)	0.58 (0.33-1.04)	0.56 (0.31-1.03)
5-6 cups per day	83834.35	46	0.55 (0.41-0.73)	0.60 (0.33-1.07)	0.55 (0.29-1.01)
>6 cups per day	77799.95	37	0.48 (0.34-0.66)	0.67 (0.37-1.23)	0.66 (0.35-1.25)
P for trend				0.01	0.03
Total VTE § (aged <70)					
Coffee yes/no	278,372.13	292	1.05 (0.94-1.18)	0.62 (0.41-0.93)	0.64 (0.42-0.98)
0 cups per day	25863.4	26	1.01 (0.68-1.48)	REF.	REF.
1-2 cups per day	32310.71	35	1.08 (0.78-1.51)	0.61 (0.37-1.02)	0.63 (0.37-1.07)
3-4 cups per day	70979.84	72	1.01 (0.81-1.28)	0.54 (0.34-0.85)	0.55 (0.32-0.88)
5-6 cups per day	75385.81	80	1.06 (0.85-1.32)	0.60 (0.38-0.94)	0.62 (0.39-0.98)
>6 cups per day	73832.36	79	1.07 (0.86-1.33)	0.73 (0.47-1.15)	0.79 (0.50-1.25)
P for trend				0.91	0.88

¶ Incidence rates (IRs) per 1000 person-years. *Model1 (M1): adjusted for age and sex. **Model 2 (M2): adjusted for age, sex, body mass index (BMI), smoking status, physical activity, diabetes, history of cardiovascular disease (CVD) and cancer. § Includes only participants aged <70 years (n=27,667).

For separate analysis on provoked and unprovoked VTE, coffee consumption was inversely associated with the outcome in both analyses (provoked: Model 2 HR 0.69, 95% CI 0.39-0.96), unprovoked: Model 2 HR 0.65, 95% CI 0.37-1.13). For provoked VTE analysis, 1-2 cups per day (Model 2 HR 0.44, 95% CI 0.25-0.79) and 3-4 cups per day (Model 2 HR 0.59, 95% CI 0.36-0.95) were significantly associated with decreased VTE. As for analysis on unprovoked VTE, none of the categories revealed significance, however, overlapping 95% CIs indicated that the results were in line with the trend for provoked VTE results.

The risk estimates for the analysis restricted to participants under 70 years of age were almost identical to the overall results (Table 3). Analysis adjusted by the use of estrogen also revealed a similar risk estimation trend (data not shown). Sex-stratified analysis revealed stronger inverse association to total VTE events for female participants (3-4 cups: Model 2 HR 0.5, 95% CI 0.3-0.81 and 5-6 cups: Model 2 HR 0.53, 95% CI 0.32-0.88), nevertheless the differences between gender were not significant (S2 Table). The analysis according to different types of coffee (boiled/other) showed similar risk estimation to overall results, both for drinkers of boiled coffee and drinkers of other types of coffee. The associations were stronger in subjects who consumed other types of coffee (1-2 cups per day: Model 2 HR 0.49, 95% CI 0.29-0.84, 3-4 cups per day: Model 2 HR 0.57, 95% CI 0.37-0.88, 5-6 cups per day: Model 2 HR 0.62, 95% CI 0.39-0.97) (S3 Table). Moreover, a separate analysis was conducted for participants aged under 70, although risk estimates were not altered (data not shown).

The results from Model 2 of our analysis were compared to the findings from Enga et al [99] (Table 4). Overall, the present study revealed a stronger association between coffee

consumption and VTE, and the risk estimates were lower in all categories compared to the previous study. Especially the HRs for 1-2 cups per day and 3-4 cups per day were notably lower in the time-varying analyses (1-2 cups: HR 0.78, 95% CI, 0.51-1.21 versus HR 0.67, 95% CI 0.44-1.01; 3-4 cups: HR 0.70, 95% CI 0.48-1.02 versus HR 0.58, 95% CI 0.39-0.84) (Table 4).

Table 4: Comparison of results from the present study and Enga et al. 2011

	Enga et al. 2011*	Present study*
0 cups per day	REF.	REF.
1-2 cups per day	0.78 (0.51-1.21)	0.67 (0.44-1.01)
3-4 cups per day	0.70 (0.48-1.02)	0.58 (0.39-0.84)
5-6 cups per day	0.67 (0.45-0.97)	0.59 (0.40-0.88)
>6 cups per day	0.85 (0.58-1.24)	0.72 (0.49-1.07)
P for trend	0.71	0.43

* HRs adjusted for age, sex, body mass index (BMI), smoking status, physical activity, diabetes, history of cardiovascular disease (CVD) and cancer.

The table is an adaptation of the results from the paper by Enga et al⁸⁴

4.2 Part II: Risk of VTE Recurrence

Of the 491 participants who experienced VTE, 12 died on the day of the VTE (total subjects n=479, total person-years=2,270) and 76 developed VTE for the second time during the follow-up period (mean time from the first VTE incident: 5.4 ± 4.6 years). The characteristics of recurrent VTEs are given in Table 5. Mean age at the time of recurrence was 65 ± 12.2 years but the range varied from 27 to 85 years. There were 46 cases (60%) of DVT and 30 cases (40%) had concurrent PE. Out of these, 45% (34 cases) were unprovoked VTE and 21% (16 cases) had active cancer at the time of the recurrence event.

Table 5: Characteristics of recurrent events (n=76)

	N (%)
Male	39 (51)
Age*	65 ± 12.2
Deep vein thrombosis	46 (60)
Pulmonary embolism	30 (40)
Unprovoked VTE	34 (45)
Cancer**	16 (21)
Time from 1 st VTE*	5.4 ± 4.6

*Mean (year) ± crude standard deviation. **Active cancer at the time of VTE recurrence

Overall recurrence rate was 35.0 (95% CI 28.0-43.8) per 1,000 person-years. The highest recurrence rate was 69.6 (95% CI 38.6-125.7) for the coffee abstainers (0 cups per day). The HRs adjusted for possible confounders (age, sex, BMI, smoking status, physical activity, diabetes, CVD and cancer) for coffee drinkers was 0.34 (95% CI 0.17-0.67), indicating a 66% lower risk of recurrent VTE for participants who drink more than one cup of coffee daily. All four categories (1-2 cups, 3-4 cups, 5-6 cups and >6 cups per day) were significantly associated with the decreased risk of recurrent VTE; 1-2 cups per day (Model 2 HR 0.08, 95% CI 0.02-0.38), 3-4 cups per day (Model 2 HR 0.38, 95% CI 0.17-0.84), 5-6 cups per day (Model 2 HR 0.36, 95% CI 0.16-0.82) and >6 cups per day (Model 2 HR 0.43, 95% CI 0.19-0.94) (Table 6). Further analysis was conducted excluding the cancer-related VTE patients (n=123), however the risk estimates did not change largely (data not shown).

Table 6: Incidence rates (IRs) and hazard ratios (HRs) of recurrent VTE (n=479)

	Person-years	Events	IR (95% CI) ¶	M1 HR* (95% CI)	M2 HR** (95% CI)
Total 2nd VTE					
Coffee yes/no	2171.0	76	35.0 (28.0-43.8)	0.38 (0.20-0.75)	0.34 (0.17-0.67)
0 cups per day	158.0	11	69.6 (38.6-125.7)	REF.	REF.
1-2 cups per day	341.8	3	8.8 (2.8-27.2)	0.10 (0.27-0.37)	0.08 (0.02-0.38)
3-4 cups per day	603.3	25	41.4 (28.0-61.3)	0.50 (0.23-1.00)	0.38 (0.17-0.84)
5-6 cups per day	481.7	18	37.4 (23.5-59.3)	0.43 (0.20-0.94)	0.36 (0.16-0.82)
>6 cups per day	586.2	19	32.4 (20.6-50.8)	0.44 (0.20-0.95)	0.43 (0.19-0.94)

¶Crude IRs per 1,000 person-years. *Model1 (M1): adjusted for age and sex. **Model 2 (M2): adjusted for age, sex, body mass index (BMI), smoking status, physical activity, diabetes, history of cardiovascular disease (CVD) and cancer.

4.3 Part II: All-cause Mortality after VTE

During a mean follow-up of 4.9 years (range from 1 day to 18.1 years), 240 participants died. Twelve patients who died on the day of the VTE event were given one day of follow-up period and included in the analysis. The overall crude mortality rate was 99.1 (95% CI 87.3-112.4) per 1,000 person-years and the highest was among participants who drink 1-2 cups of coffee per day (125.5, 95% CI 93.3-168.6) (Table 7). The mortality rate was slightly higher for female (101.8, 95% CI 85.8-120.8) than male participants (95.9, 95% CI 79.4-115.8) (data not shown). The proportional HRs decreased as coffee consumption increased and the estimated risk was smallest for the excessive coffee drinkers (>6 cups per day) (Model 2 HR 0.63, 95% CI 0.35-1.13). However, the risk estimations were not significant in all categories. The subgroup analysis only including the non-cancer related VTE cases (n=368) revealed a similar trend to overall mortality analysis (Table 7).

Table 7: All-cause mortality rate (MR) and HRs after first VTE incident (n=491)

	Person-years	Events	MR (95% CI) ¶	M1 HR* (95% CI)	M2 HR§ (95% CI)
All-cause mortality					
Coffee yes/no	2422.8	240	99.1 (87.3-112.4)	0.88 (0.74-1.25)	0.82 (0.50-1.34)
0 cups per day	198.5	18	90.7 (57.1-143.9)	REF.	REF.
1-2 cups per day	350.7	44	125.5 (93.3-168.6)	1.01 (0.57-1.78)	1.00 (0.56-1.80)
3-4 cups per day	686.5	78	113.6 (91.0-141.8)	0.92 (0.54-1.56)	0.80 (0.46-1.38)
5-6 cups per day	559.6	60	107.2 (83.2-138.1)	0.89 (0.52-1.53)	0.92 (0.53-1.58)
>6 cups per day	627.5	40	63.7 (46.8-86.9)	0.71 (0.40-1.26)	0.63 (0.35-1.13)
All-cause mortality ✕ (non-cancer related VTE)					
Coffee yes/no	2251.6	129	61.7 (52.3-72.9)	0.82 (0.43-1.60)	0.73 (0.38-1.43)
0 cups per day	175.7	10	56.9 (30.6-105.7)	REF.	REF.
1-2 cups per day	316.0	32	101.2 (71.6-143.2)	1.14 (0.57-2.39)	1.10 (0.51-2.35)
3-4 cups per day	628.1	49	78.0 (59.0-103.2)	0.89 (0.44-1.80)	0.79 (0.39-1.63)
5-6 cups per day	532.6	27	50.7 (34.8-73.9)	0.65 (0.31-1.37)	0.55 (0.25-1.18)
>6 cups per day	599.2	21	35.0 (22.8-53.7)	0.74 (0.34-1.61)	0.63 (0.29-1.39)

¶Crude all-cause mortality rate (MR) per 1,000 person-years. *Model 1 (M1): Adjusted for age and sex. § Adjusted for age, sex, body mass index (BMI), smoking status, physical activity, diabetes, and history of cardiovascular disease (CVD)

✕Includes only non-cancer related VTE (no active cancer at the time of VTE event) cases (n=368).

5 DISCUSSION

5.1 Overall Results

The results of the present study indicated an inverse association between coffee consumption and the risk of incident VTE, recurrent VTE and all-cause mortality after a VTE event. Daily intake of coffee was associated with a 37% lower risk of incident VTE compared to coffee abstainers after adjusting with multiple confounding factors. The association was particularly strong for the group who consumed 3-4 cups and 5-6 cups of coffee daily. The inverse association was slightly weakened for the excessive coffee drinkers (>6 cups per day), suggesting a possible U-shaped association between VTE and coffee drinking habits. Subgroup analyses of unprovoked/provoked VTE, different types of coffee, gender and age (>70) were all corresponding to the main results. Among those with a first VTE, the recurrence rate was 66% lower in those who consumed at least one cup of coffee daily. The recurrence rate was lowest among the participants with low coffee consumption (1-2 cups), but the risk estimations were low across all coffee intake categories. Moreover, the coffee drinkers had an 18% reduced risk of all-cause death following a first VTE. After stratifying for non-cancer related VTE patients, the inverse association was strongest for the subjects who drank 5-6 cups per day (45% reduction), revealing a possible J-shaped association.

5.2 Comparison to Other Studies

Incident VTE

The findings of the Part I analysis are in agreement with the results of three previous observational studies and a meta-analysis pooling the three [81, 99-101]. A large case-control study (MEGA study) conducted to explore the risk factors of VTE showed a 25% lower risk of VTE for coffee consumers when matched against the partner controls (OR 0.75, 95% CI 0.55-1.04), regardless of the amount of coffee consumed daily [100]. Also the prospective cohort study (IWHS) with over 37,000 female participants revealed an inverse association between VTE and consumption of 28 to <42 cups of coffee per week (corresponding to 4-6 cups per day) (HR 0.85, 95% CI 0.71-1.01), which is in accordance with our findings [81]. However, the authors of IWHS reported the attenuated risk estimates after further adjustment with BMI and diabetes. The present study included both genders and the analysis were adjusted for multiple factors including BMI and self-reported diabetes status. Yet the inverse association with VTE remained strong for moderate coffee consumption (3-6 cups daily). A meta-analysis including all three observational studies demonstrated no significant association between overall coffee intake and VTE (cumulative RR 0.97, 95% CI 0.88-1.08), while a 25% risk reduction was revealed for high-amount coffee drinkers (≥ 5 cups daily: RR 0.75, 95% CI 0.67-0.85). The inter-study heterogeneity was high (I-squared 78%, $P < 0.001$), thus the pooled results need to be assessed with caution, nonetheless the overall trends were still in line with our risk estimations.

The previous study based on the participants of the Tromsø 4 survey reported a 30% and 33% decreased risk of VTE for participants who consumed 3-4 cups (HR 0.70, 95% CI, 0.48-1.02) and 5-6 cups of coffee daily (HR 0.67, 95%CI 0.45-0.97) [99]. As mentioned in the introduction, the present study used time-varying analysis to minimize the influence of possible regression dilution bias which was not addressed in the previous Tromsø 4 study

[99] and IWHS [81]. In our study, the information about the coffee consumption habit was measured at each survey and the classification was updated a maximum of three times during the follow-up period. Also, the present study used the same confounding variables as the Tromsø 4 study [99] to adjust the models for comparison purposes.

The number of total subjects increased from 26,755 in the previous study to 30,236, due to the inclusion of participants from Tromsø 5 and 6. The prevalence of baseline characteristics across the coffee consumption categories was almost identical in both studies, indicating that the results can be compared. The present study demonstrated lower risk estimations across all coffee intake categories and the associations were stronger compared to the previous study. Especially the multivariable adjusted HRs dropped noticeably for the groups with 1-2 cups and 3-4 cups of daily coffee consumption (1-2 cups: HR 0.78 to 0.67, 3-4 cups: HR 0.70 to 0.58), which indicates that the results of the previous study were affected by regression dilution bias to some extent. Hence, the misclassification might have altered the findings of the previous study slightly. However, the 95% CIs of the risk estimations were overlapping in both studies, thus the differences are not significant. It can be assumed that the exposure variable remained relatively consistent throughout the study period, meaning one does not change one's coffee consumption habit predominantly over time. The findings of the present study suggest that the regression dilution bias was not a crucial limitation in the previous observational studies.

Recurrent VTE

To the best of our knowledge, no studies have investigated the association between coffee intake and the risk of VTE recurrence. Our study revealed a 66% lower risk of recurrence (HR 0.34, 95% CI 0.17-0.67) for coffee drinkers compared to patients who do not drink coffee.

The index event bias can be a limitation in recurrence studies when the population was selected on the basis of the occurrence of the index event [108-110]. However, the recurrent analysis of the present study indicated extensive decrease in risk of VTE recurrence among coffee drinkers, suggesting a limited chance of the findings being affected by the index event bias.

The strong association could potentially be explained by uneven prevalence of other diseases among coffee abstainers. We conducted a further analysis excluding cancer-related VTE patients since cancer is a strong predicting factor of VTE recurrence [18, 20, 111]. However, the risk estimates were not greatly altered, indicating that the high prevalence of cancer among non-coffee drinkers was not the explanation of the inverse association between coffee consumption and the risk of recurrent VTE. There is a possibility that the study was affected by unmeasured confounding factors which are still unidentified and yet have strong influence on risk of VTE recurrence, or coffee drinking truly has a protective effect against recurrent VTE.

All-cause Mortality

The analysis revealed an inverse association between coffee consumption and all-cause mortality after a VTE event (HR 0.82, 95% CI 0.5-1.34 for habitual coffee drinkers), with very

high-amount coffee consumers (>6 cups per day) having the lowest risk estimates (HR 0.63, 95% CI 0.35-1.13). While our study is believed to be the first to investigate the relation between coffee consumption and mortality after a incident VTE, a large number of studies can be found on coffee intake and total mortality on other populations. Most of these studies revealed a U-shaped association between coffee intake and mortality [112-117]. A recent paper from the HAPIEE study (Health, Alcohol and Psychological factors in Eastern Europe) reported that consuming 3-4 cups of coffee per day was associated with 17% and 37% lower risk of death for men and women respectively (men: HR 0.83, 95 % CI 0.71-0.99, women: HR 0.63, 95 % CI 0.47-0.84), although further intake was not significantly associated with all-cause mortality [112]. Similarly, a large Danish observational study with a total of 95,366 individuals and 5,422 deaths during a median of six years study period revealed the lowest mortality rate for subjects with a moderate coffee intake compared to coffee abstainers, and the overall association was U-shaped [114]. A meta-analysis of 20 prospective cohorts also concluded that there was an inverse association between coffee intake and the risk of death (pooled RR 0.86, 95 % CI 0.80-0.92), but not necessarily a dose-response relationship, since high coffee consumption (\leq 5-9 cups/day) did not reduce the risk of death further compared to moderate consumption [116]. Our study also illustrated the U-shaped risk estimates when cancer-related VTE patients were excluded from the analysis.

5.3 Underlying Mechanisms

Coffee is a complex mixture of active components such as caffeine, polyphenols, aminoacids and minerals, which can affect human health [117, 118]. The amount of intake and

metabolites of these components depend on the coffee type, methods of brewing, and serving size, which all influence the beneficial effect of coffee on health [117]. The complete mechanisms of how coffee acts beneficially in reducing the risk of VTE are still unclear, but several hypotheses can be outlined from previous studies [100, 119].

Hemostatic Factors and Fibrinolytic Activity

Plasma levels of vWF and FVIII are associated with the risk of VTE [120, 121]. Roach et al suggested in their study that the inverse association between coffee consumption and VTE might be mediated through levels of vWF and FVIII which were found to be lower among coffee drinkers compared to non-coffee drinkers [100]. Moreover, the inverse association of coffee and VTE was curtailed when procoagulant factors (fibrinogen, vWF, and FVIII) were added to the multivariable model [100]. No association was shown between coffee intake and levels of protein C, protein S, and antithrombin. However, another study showed that coffee extracts had an anti-thrombin activity in humans independent from caffeine, particularly in some types of coffee beans (Blue Mountain, Yunnan and Kilimanjaro) [122]. On the other hand, a small randomized controlled trial conducted in the Netherlands revealed no difference in the levels of fibrinogen and FVIII activity after nine weeks of coffee consumption [123].

Decreased fibrinolytic activity might be associated with the increased risk of developing VTE [124-126]. There are several studies which investigated the effect of coffee on fibrinolytic activity, yet the findings are diverse. An experiment on the short-term effect of coffee demonstrated increased fibrinolytic activity and shortened clot lysis time for ten

out of 12 coffee drinkers [127], indicating possible benefits of coffee consumption on the fibrinolytic system. On the other hand, Roach et al found no difference in fibrinolytic clot lysis time for coffee drinkers and coffee abstainers [100]. In contrast to the above two, a Greek study found an increased level of plasminogen activator inhibitor 1 (PAI-1) and an impaired fibrinolysis system among heavy coffee drinkers with hypertension and a smoking habit [128]. The findings of our study also demonstrated attenuated association to VTE events among excessive coffee drinkers, and therefore the results are not necessarily conflicting. Overall, the amount of evidence on this topic is limited, and thus further investigations are necessary to confirm the effect of coffee on plasma hemostatic levels and fibrinolytic activity, as well as its relation to the risk of VTE.

Platelet Aggregation

Platelets are involved in the formation of both arterial and venous thrombosis [129], thus platelet hyperaggregability could be related to increased risk of VTE [130]. The potential influence of coffee on platelet aggregation might play another role in explaining the association between coffee intake and the risk of VTE. A crossover study conducted on ten healthy subjects showed decreased platelet aggregation and increased phenolic acid platelet concentration in coffee drinking subjects [131]. Similar findings were reported from older experiments on humans and rabbits, showing that intravenous administration of coffee extracts had anti-aggregatory effects in vitro [132]. Natella et al suggested that the interaction of coffee polyphenols with intracellular networks relating to platelet aggregation might play a role [131]. Nardini et al demonstrated in their systematic review that the

supplementation of phenolic-rich foods such as coffee, grape juice or green tea had an anti-platelet aggregation effect in the majority of cases [133]. However, not all studies agreed with the findings above, for example, a report from Polaguruto et al revealed no influence on clotting time or plasma prostacyclin concentrations for coffee consumers [134]. Moreover, an older study from 1988 showed a significant increase in platelet reactivity one hour after administration of 100 mg of caffeine, which corresponds to one cup of coffee [135].

Polyphenols and Endothelial Function

Another possible explanation could be the favorable effect of polyphenols on endothelial functions. The most abundant polyphenol in coffee is 5-caffeoylquinic acid, also called chlorogenic acid (CGA) [136], which was proven to have an acute improvement effect on endothelial function in a recent crossover study with 16 healthy men and women. CGA was given in two doses, 450 mg and 900 mg per day (equivalent to four and eight cups of coffee), and both groups were associated with ameliorated continuous mean flow-mediated dilation (FMD) response [137]. The findings are in accordance with several studies that investigated the effect of coffee polyphenols on endothelial function in healthy male participants [138-140]. Another study conducted on healthy and diabetic women reported that both caffeinated and decaffeinated coffee was inversely associated with inflammation markers and endothelial dysfunction [141]. On the other hand, two studies indicated that caffeinated coffee induced an unfavorable effects on endothelial function in healthy male subjects while decaffeinated coffee had no such effect [142, 143]. The discussion is still ongoing whether

the unfavorable effect was induced by caffeine or antioxidants [144]. Nonetheless, as Roach et al commented in their paper, endothelium and platelets are closely interrelated with vWF and thrombin formation [145], and it is therefore plausible that the effect of polyphenols on endothelial function is one of the mechanisms underlying the association between coffee and VTE [100].

Caffeine

A number of studies have revealed that caffeine is associated with an acute increase of blood pressure, endothelial dysfunction, inflammation, impaired glucose tolerance and inhibition of insulin activity [143, 146, 147]. However, it has also been reported that chronic consumption of coffee builds tolerance to caffeine (i.e. acute negative effects appear only for the participants who do not regularly drink coffee) [143, 147]. Therefore, the favorable effects of multiple compounds included in coffee might outweigh the acute negative effect caused by caffeine intake in long-term consumers.

5.4 Strengths

The notable strengths of the present study are the large sample size of participants recruited from the same residential area and the high attendance rate (>65% in all three surveys). The inhabitants of Tromsø are mostly Caucasians and have relatively high standards of education and lifestyle factors. The incidence rate of VTE was also in accordance with those reported in other Western countries [11, 43]. Therefore it can be assumed that the study has relatively

high external validity for a Western population. Prospective study design, long follow-up period and repeated measurements of the exposure variables are further strong advantages of the present study. The VTE information was obtained from a single hospital, which is the only institute in the entire municipality, thus the completeness of the VTE registration in the studied area is presumably high.

5.5 Methodological Aspects and Limitations

Residual Confounding

Owing to the fact that the present study is an observational cohort study, the possibility of residual confounding cannot be eliminated. It can be seen from the patient characteristics of the present study that the participants who consumed high amounts of coffee had relatively unhealthy lifestyle habits such as smoking or being less involved in a regular physical activity. However, adding those factors into the model along with the known risk factors of VTE such as age, sex, BMI, cancer and pre-conditional cardiovascular diseases did not attenuate the results. There is also a possibility that the status of coffee abstainers was particularly linked to poor health. For example, persons who experienced a disease might have refrained from coffee drinking in an attempt to achieve a healthy lifestyle. Therefore, the findings might be biased by underlying diseases or poor health conditions among participants who do not drink coffee. However, coffee abstainers of the present study were younger and had lower prevalence of diseases like diabetes, history of cardiovascular events, and cancer. They also had lower levels of cholesterol and BMI and more participants were dedicated to hard physical exercise compared to coffee consumers. Despite the relatively healthier conditions

among coffee abstainers, a significant inverse associations remained in the multivariable adjusted models, indicating that the findings of the present study are unlikely to be explained by unmeasured residual confounding.

Measurements of the Exposure and Sample Size

A possible weakness regarding the methodology is the fact that our main exposure variable, coffee consumption was obtained through questionnaires. Some measurement error and misclassification cannot be avoided in self-reported measurements. However, a validation study in German adults showed a high correlation between coffee consumption reported in a food frequency questionnaire (FFQ) and a 24 hour telephone recall ($r=0.78$) [148]. Hence, questionnaires are a relatively reliable method to obtain coffee intake information.

Moreover, the probability of attaining the incorrect measurements was equal with regard to participants who experienced VTE and those who did not. The coffee consumption habit was measured at the study enrollment, thus the reported coffee intake amount was not influenced by the occurrence of the outcome event. This is called non-differential misclassification and can bias the results towards null [149]. The effect of regression dilution bias also needs to be taken into consideration for the Part II analysis, since the coffee intake information before the first VTE event was used in that analysis and was not updated until the end of follow-up. However, a strong association was revealed in both Part I and Part II analyses, and therefore the effect of misclassification and regression dilution bias on our findings is most likely minimal.

The present study lacks information about precise measurement of the coffee intake per day (size of the coffee cup), use of milk, sugar and/or other additional toppings, types of coffee other than boiled (e.g. espresso, instant coffee, cappuccino) and the amount of caffeine consumed with coffee. Moreover, we did not adjust for daily caloric intake in the present study. However, the previous Tromsø 4 study revealed almost no difference in results after adjusting with caloric intake for the subgroup aged 25-69 years [99]. Therefore, the caloric intake with or without coffee probably did not confound our findings.

The limited statistical power of the analysis of recurrence and mortality also needs to be noted. For example, only three events occurred among the patients who consumed 1-2 cups of coffee daily during the entire follow-up period in recurrent VTE analysis. Both recurrence and mortality analyses were not stratified by further categories like sex, age, provoked/unprovoked VTEs or types of consumed coffee due to the small number of incidents. Nevertheless, strong inverse associations were indicated for most of the coffee consumption categories, and the risk estimation trend was similar to the Part I analysis.

Finally, information about a previous history of VTE prior to the study enrolment might be lacking for some of the study participants. Thus, some subjects who experienced an VTE event in the past and therefore needed to be excluded from the study might still be included in our analyses. However, considering the size of study population, the possible influence of this misclassification on overall risk estimation would be negligible.

6 CONCLUSION

Overall, coffee consumption was associated with a 37% decreased risk of incident VTE. Moreover, coffee consumers had a 66% lower risk of recurrence and an 18% decreased risk of all-cause mortality after an event of VTE compared to coffee abstainers. The association was U-shaped in all analyses, suggesting that a moderate daily coffee intake (3-6 cups) may be beneficial. The study used time-varying analysis to minimize the regression dilution bias. The risk estimates were lower in present findings compared to a previous study using conventional analyses, nonetheless the differences were not significant. Because data on this topic are scarce, especially on recurrence and mortality after an incident VTE, further experimental and epidemiological studies are necessary in order to affirm the effect of coffee on the risk of VTE and to elucidate the underlying mechanisms.

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Appendix

S1 Table: Baseline characteristics (Boiled/Other coffee)

Boiled coffee: n=11,935	0 cups per day	1-2 cups per day	3-4 cups per day	5-6 cups per day	>6 cups per day
Subjects	3079	1117	2294	2647	2798
Male	1201 (39.0)	456 (40.8)	905 (39.5)	1206 (45.6)	1646 (58.8)
Age (years)	38.3 ± 13.6	49.0 ± 18.4	55.0 ± 17.4	51.9 ± 15.9	48.1 ± 13.8
Cholesterol (mM)	5.4 ± 1.2	6.0 ± 1.4	6.4 ± 1.4	6.4 ± 1.3	6.4 ± 1.3
History of CVD	76 (2.5)	90 (8.1)	264 (11.5)	209 (7.9)	152 (5.4)
Current smoker	541 (17.6)	239 (21.4)	606 (26.4)	1162 (43.9)	1855 (66.3)
Other coffee: n=13,893					
Subjects	3079	1776	3306	3173	2559
Male	1201 (39.0)	670 (37.7)	1375 (41.6)	1542 (48.6)	1548 (60.5)
Age (years)	38.3 ± 13.6	45.3 ± 15.7	47.6 ± 14.5	47.1 ± 13.0	45.8 ± 11.5
Cholesterol (mM)	5.4 ± 1.2	5.7 ± 1.2	5.8 ± 1.3	5.9 ± 1.2	6.0 ± 1.2
History of CVD	76 (2.5)	95 (5.4)	161 (4.9)	170 (5.4)	126 (4.9)
Current smoker	541 (17.6)	243 (13.7)	744 (22.6)	1150 (36.3)	1412 (55.2)

S2 Table: Sex-stratified analysis for VTE events by coffee consumption categories

	Person-years	Events	M1 HR* (95% CI)	M2 HR** (95% CI)
Female (n=15,589)				
Coffee yes/no	166727.25	267	0.69 (0.44-1.07)	0.59 (0.38-0.93)
0 cups per day	16775.13	22	REF.	REF.
1-2 cups per day	24243.02	58	0.84 (0.51-1.39)	0.73 (0.43-1.22)
3-4 cups per day	50219.77	84	0.59 (0.37-0.96)	0.5 (0.30-0.81)
5-6 cups per day	43954.77	62	0.63 (0.38-1.03)	0.53 (0.32-0.88)
>6 cups per day	31534.56	41	0.82 (0.49-1.39)	0.77 (0.46-1.32)
P for trend			0.31	0.33
Male (n=14,205)				
Coffee yes/no	145961.31	224	0.71 (0.41-1.23)	0.71 (0.40-1.25)
0 cups per day	10607.61	14	REF.	REF.
1-2 cups per day	14857.95	26	0.67 (0.35-1.28)	0.57 (0.28-1.16)
3-4 cups per day	34350.78	67	0.76 (0.43-1.36)	0.74 (0.40-1.36)
5-6 cups per day	39879.58	61	0.69 (0.38-1.23)	0.71 (0.39-1.30)
>6 cups per day	46265.39	56	0.71 (0.40-1.29)	0.74 (0.40-1.36)
P for trend			0.51	0.95

*Model1 (M1): adjusted for age. **Model 2 (M2): adjusted for age, body mass index (BMI), smoking status, physical activity, diabetes, history of cardiovascular disease (CVD) and cancer.

S3 Table: Analysis according to boiled or other types of coffee consumption categories

	Person-years	Events	M1 HR* (95% CI)	M2 HR** (95% CI)
Boiled coffee				
Coffee yes/no	101948.21	170	0.69 (0.47-1.01)	0.67 (0.45-1.00)
0 cups per day	26783.42	35	REF.	REF.
1-2 cups per day	9848.43	25	0.92 (0.54-1.55)	0.91 (0.53-1.55)
3-4 cups per day	19791.37	48	0.72 (0.46-1.14)	0.68 (0.42-1.09)
5-6 cups per day	22362.69	33	0.56 (0.34-0.90)	0.55 (0.33-0.89)
>6 cups per day	23162.31	29	0.67 (0.41-1.11)	0.67 (0.40-1.11)
P for trend			0.02	0.03
Other coffee				
Coffee yes/no	147524.52	216	0.66 (0.46-0.96)	0.59 (0.40-0.86)
0 cups per day	26783.42	35	REF.	REF.
1-2 cups per day	20308.81	33	0.65 (0.40-1.05)	0.49 (0.29-0.84)
3-4 cups per day	39090.21	65	0.65 (0.42-0.98)	0.57 (0.37-0.88)
5-6 cups per day	35188.36	53	0.69 (0.45-1.07)	0.62 (0.39-0.97)
>6 cups per day	26153.71	30	0.67 (0.41-1.11)	0.67 (0.40-1.11)
P for trend			0.23	0.33

*Model1 (M1): adjusted for age and sex. **Model 2 (M2): adjusted for age, sex, body mass index (BMI), smoking status, physical activity, diabetes, history of cardiovascular disease (CVD) and cancer.



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